VII
EUROPT(R)ODE

Seventh European Conference on Optical Chemical Sensors and Biosensors

EUROPT(R)ODE VII

Madrid, Spain April 4-7, 2004

Book of Abstracts

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Book of Abstracts

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Complutense University
Madrid, Spain

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FOREWORD

Europt(r)ode Conference series has reached its seventh edition showing the vigor of optical *chemical* sensor and biosensor research and technology. Optosensing applications to chemical monitoring now span environmental quality assurance, process control, food analysis, medical diagnostics, biosphere investigations, and even extraterrestrial research! More importantly, optical chemosensors are finally leaving the laboratory and challenging competitors in the market arena. I'm convinced that Europt(r)ode meetings have had a lot to do with technology transfer and key developments alike, thanks to multidisciplinary attendance and stimulating discussions.

Researchers, technologists, industrial scientists and students from over 25 countries are all welcome to Madrid. The following pages give an account of their latest results in the fields of new materials and principles for optical chemical sensing and biosensing, novel optoelectronics instrumentation and components for sensor development, integrated optical systems, micro-, nano- and multiplexed sensors, optosensing arrays for genomics and proteomics, and applications of optical chemical sensors including commercial developments.

The Europt(r)ode VII scientific programme has been organized around six plenary lectures and eleven invited presentations displaying the above mentioned variety of topics. In order to satisfy the widest possible audience, two parallel sessions will collect 38 additional oral presentations. I apologize (but we will be happy) if you find it difficult to make your mind up and to choose one of them to attend. Last but not least, take a look at the 132 accepted posters and you will get a fine picture of the state-of-the-art optical chemical sensing and biosensing research. Moreover, this Conference will see again the awarding of the (Second) Roche Prize for Sensor Technology to a young scientist with outstanding achievements in the field of chemical optosensing. I hope they keep on honoring the contributions of young researchers in future editions.

Obviously, such a Conference organization is not a matter of a single person. I would like to thank specially *Prof. María C. Moreno-Bondi* for her Executive Secretary role and for preparing most of this Book of Abstracts. My colleagues of the Executive Organizing Committee have contributed to make possible this prestigious event. Numerous helpful suggestions to improve its scientific programme and a thorough review of the submitted communications have been the collaboration of the Permanent Steering Committee and National Organizing Committee members. My gratitude to all of them for their valuable contribution. The Europt(r)ode VII logistics have required the special effort of our PhD and post-doctoral students which I gratefully acknowledge.

Thanks to our institutional and private sponsors, we have been able to offer a reduced registration fee to bona fide students and participants from less-favored countries. They constitute about one third of the attendees to Europt(r)ode VII in Madrid. We wish their number keeps growing in future editions so that they profit from the interaction with leading scientists and we all learn from their fresh approach to the field.

Welcome to Madrid and enjoy both Europt(r)ode VII and the capital city of Spain. We've done our best to make your stay scientifically beneficial and culturally unforgettable.

Madrid, April 2004

Prof. Guillermo Orellana

Europt(r)ode VII Chair

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SCIENTIFIC PROGRAMME

MONDAY April 5, 2004						
Room A – "Ramón y Cajal" Amphitheater				*Room B – "Prof. Botella" Auditorium*		
09:00		**Welcome**				
Opening Plenary Session Chair: O.S. Wolfbeis						
09:30	PL1	**Economical Optical Real-Time DNA Arrays – Optical Biosensors at the Onset of the XXI Century** *K. Cammann*,[a] C. Peter[b] [a]University of Münster, Germany; [b]ICB GmbH, Germany				
10:30	Coffee break					
Plenary Session Chair: G. Gauglitz						
11:00	PL2	**Confronting the Challenge of Design of Selectivity for Optical DNA Biosensors and Biochips** *U.J. Krull*, P.A.E. Piunno University of Toronto, Canada				
Session 1 Chair: H. Podbielska				**Session 2** Chair: L. Lechuga		
12:00	IL1.1	**Optical Detection of DNA-Modifying Enzymes on Immobilized Substrates** *F. F. Bier*, N. Gajovic-Eichelmann, E. Ehrentreich-Foerster, P.M. Schmidt, J. Henkel Fraunhofer IBMT, Germany		12:00	IL1.2	**Recent Development in Transducers Based on Planar Integrated Optical Waveguides** *S. Scott Saavedra,* J.T. Bradshaw, S.B. Mendes, N.R. Armstrong University of Arizona, USA
12:30	OA1.1	**Signal Enhancement of Protein Chips**, C. Preininger,[a] *U. Sauer*,[a] M. Trombitas,[a] O. Obersriebnig,[a] G. Krumpel,[b] W. Kern[c] [a,b]ARC Seibersdorf Res. GmbH, Austria; [c]University of Technology Graz, Austria		12:30	OB1.1	**Development of Deep Silicon Hollow Waveguides for Optical Sensing** *V.J. Cadarso,*[a] A. Llobera,[b] C. Domínguez[a] [a]IMB-CSIC, Spain; [b] Technische Universität Braunschweig, Germany
12:50	OA1.2	**Protein Immobilization for Multi-Channel Biosensors** C. Boozer,[a] J. Ladd,[a] S. Chen,[a] Q. Yu,[a] J. Homola,[b] *S. Jiang*[a] [a]University of Washington, USA; [b]Academy of Sciences of the Czech Republic, Czech Republic		12:50	OB1.2	**Deep Probe Optical Waveguide Biosensors with Reverse Symmetry Design for Micron Scale Biological Objects** *R. Horváth*, H.C. Pedersen, N. Skivessen, D. Selmeczi, N.B. Larsen Risø National Lab., Denmark
13:10	OA1.3	**Analytical Biosensing Based on Measurement of Biomolecule Conformation Changes Using Surface Plasmon Resonance** L.M. May, *D.A. Russell* University of East Anglia, UK		13:10	OB1.3	**Ultra-Thin Freestanding Si_3N_4 Membrane Waveguides for Application in Evanescent Field Sensing of MEMS Movements** *G. Altena*, M. Dijkstra, G. Van Elzakker, G. Venhorts, H. Hoekstra, P. Lambeck University of Twente, The Netherlands
13:30	**Lunch**					

Plenary Session Chair: T. Vo-Dihn						
15:00	PL3	**Room Temperature Phosphorescence Detection for Optical Sensors** *A. Sanz Medel* University of Oviedo, Spain				
Session 3 Chair: M. Nakagawa				**Session 4** Chair: P. Nikitin		
16:00	IL1.3	**Optical Detection in Microfluidic Systems** *A. Dybko* Warsaw University of Technology, Poland		16:00	IL1.4	**Integrated Optochemical Sensors Based on New Vis-NIR Chromoionophores** *J. Alonso,* M. Puyol, L. Rivera Universidad Autónoma de Barcelona, Spain
16:30	OA1.4	**Plasmonic Enhacement of Fluorescence for Sensor Applications** O. Stranik, C. McDonagh, *B.D. MacCraith* Dublin City University, Ireland		16:30	OB1.4	**Application of Tailored Integrated Optical Chips for Label-Free (Bio)Chemical Sensing** *R.E. Kunz*, K. Cottier CSEM, Switzerland
16:50	OA1.5	**Zwitter-Ionic Conjugated Polyelectrolytes, New Fluorescent Probes for the Recording of Biospecific Interactions** *K.P.R. Nilsson*, O. Inganäs Linköpings University, Sweden		16:50	OB1.5	**Label Free Detection of Proteins** *C. Hoffmann*, B. Schirmer, H. Benter, A. Brandenburg Fraunhofer-Institut für Physikalische Messtechnik, Germany
17:10	OA1.6	**Fluorescence Detection of Small Molecules by Fused-Ring Heterocycles** *T. Bell* University of Nevada, USA		17:10	OB1.6	**An Optical Fibre-Based System that Measures the Quality and Temperature of Food in a Full-Scale Production Environment** *M. O'Farrell*,[a] E. Lewis,[a] C. Flanagan,[a] T. Sun,[b] K.T.V. Grattan,[b] N. Jackman[c] [a]University of Limerick, Ireland; [b]London City University, UK; [c]Food Design Applications Ltd, Ireland
17:30	**POSTER SESSION "O"** + Refreshments					
19:30	End of session					
19:00	PSC Meeting (meeting room)					

TUESDAY April 6, 2004						
Room A – "Ramón y Cajal" Amphitheater				*Room B – "Prof. Botella" Auditorium*		
08:30	**Roche Award & lecture** Chair: O.S. Wolfbeis					
Plenary Session Chair: L.J. Blum						
09:30	PL4	**Genetic Engineering in Biosensing and Micro/Nano Analytical Methods** *S. Daunert* University of Kentucky, USA				
10:30	Coffee break					
Session 5 Chairs: P.V. Lambeck / B. Mizaikoff				**Session 6** Chairs: P. Caglar / Z. Zhujun		
11:00	IL2.1	**Applying Fluorescent Nanosensors to Measure the Intracellular Environment** *J.W. Aylott* University of Hull, UK		11:00	IL2.2	**Using Reversible Chemical Reactions to Optically Detect Analyte Molecules** *G.J. Mohr* University of Jena, Germany
11:30	OA2.1	**Nanosensors for Monitoring Molecular Signaling Pathways in a Single Living Cell** *T. Vo-Dinh*, P.M. Kasili, G.D. Griffin, M. Culha, D.L. Stokes, J.M. Song Oak Ridge National Lab, USA		11:30	OB2.1	**Chemiluminescence Microfluidic Chip Fabricated in PMMA for Determination of Benzoyl Peroxide in Flour** W. Liu, *Z. Zhang*, L. Yang Shanxi Normal University, China
11:50	OA2.2	**Determination of the Concentration of Living Immobilized Cells by Fluorescence Spectroscopy** *O. Podrazky*, G. Kuncova Academy of Sciences of the Czech Republic, Czech Republic		11:50	OB2.2	**Electrochemiluminescence Imaging Through an Ordered Array of Submicrometer-Sized Individually-Readable Electroptodes** Chovin,[a] P. Garrigue,[a] P. Vinatier,[b] *N. Sojic*[a] [a]Université Bordeaux; [b]Inst. Chimie de la Matière Condensée, Bordeaux, France
12:10	OA2.3	**Array Biosensor for Food Safety** *F. S. Ligler*,[a] L.C. Shriver-Lake,[a] K.E. Sapsford,[b] N. Kulagina,[a] M. Ngundi,[a] J.P. Golden,[a] C.A. Rowe Taitt[a] [a]Naval Research Lab, Washington DC, USA; [b]George Mason University, USA		12:10	OB2.3	**Identification of Food Flavor Using the Sensitized Cataluminescence-Based Gas-Sensors** *M. Nakagawa*,[a] N. Matsuo,[a] T. Okabayashi,[b] I. Yamamoto,[b] K. Utsunomiya,[b] N. Yamashita[c] and S. Terakado[d] [a,b,c]Okayama University, Japan; [d]Sibata Scientific Technology LTD, Japan
12:30	OA2.4	**Multi-Analyte Optical Sensor Chip** *O. McGaughey*, A.K. McEvoy, B.D. MacCraith, J.M. Sabattie, J. Charmet Dublin City University, Ireland		12:30	OB2.4	**Enantiomeric Separation by Polymeric Chirasil-Calix Layers with Optical Sensing Devices** *S. Busche*, M. Kasper, A. Ruderisch, V. Schurig, G. Gauglitz Eberhard-Karls-Universität Tübingen, Germany
12:50	OA2.5	**Single Molecule Surface Reactions by Confocal TIRF Microscopy** *T. Ruckstuhl,* A. Krieg, S. Seeger University of Zurich, Switzerland		12:50	OB2.5	**Frequency Domain Measurement of Room Temperature Phosphorescence Lifetimes in the Presence of Background Signals** M. Valledor, *J.C. Campo*, J.C. Viera, I. Sánchez, J.M. Costa, A. Sanz Medel University of Oviedo, Spain

13:30	**Lunch**				
Plenary Session Chair: R. Narayanaswamy					
14:30	PL5	**Past, Present and Future of Ionophore-Based Optosensing** *K. Suzuki* University of Keio, Japan			
Session 7 Chair: F. Baldini			**Session 8** Chair: J. Homola		
15:30	IL2.3	**Planar Waveguide Technology for DNA and Protein Microarrays** *M. Ehrat* Zeptosens AG, Switzerland	15:30	OB2.6	**Optical Microsystems Platforms CMOS Compatible Based on Interferometric Biosensor Nanodevices** *B. Sepúlveda*,[a] J. Sánchez del Rio,[a] F.J. Blanco,[b] A. Calle,[a] C. Domínguez,[a] A. Montoya,[c] L.M. Lechuga[a] [a]Microelectronics National Center, Spain; [b]IKERLAN S. Coop, Spain; [c]Universidad Politécnica de Valencia, Spain
16:00	OA2.6	**INDUSTRIAL PRESENTATIONS SESSION**: *Bridging the gap between research and marketing of optical chemical sensors and biosensors* • Osmetech (USA) • Ocean Optics (USA) • Interlab IEC (Spain) • Biacore (Sweden)	15:50	OB2.7	**Highly Sensitive Optochemical Sensors Based on Reactive Dyes Incorporated into Molecularly Imprinted Polymers** *K. Haupt*,[a] G. Mohr[b] [a] Compiègne University of Technology, France; University of Jena, Germany
16:15	OA2.7		16:10	OB2.8	**Polyelectrolyte Multilayer Patterning for SPR Liquid Sensing** *M. Palumbo*, M.C. Petty Centre for Molecular and Nanoscale Electronics of Durham, U.K.
16:30	OA2.8		16:30	OB2.9	**Holographic Design of Integrated Surface Plasmon Resonance Sensor Chip** *H.C. Pedersen*,[a] W. Zong,[b] M.H. Sorensen,[b] C. Thirstrup[b] [a]Risø National Laboratory, Denmark; [b]Vir Biosensor, Denmark
16:45	OA2.9				
17:00	**POSTER SESSION "E"** + Refreshments				
19:00	End of session				
21:30	**Conference Banquet**				

WEDNESDAY April 7, 2004						
Room A – "Ramón y Cajal" Amphitheater				*Room B – "Prof. Botella" Auditorium*		
Plenary Session Chair: F. Ligler						
09:00	PL6	**The Secret of Low Detection Limits Using Optodes: Engineering, Chemistry and Applications of Optodes** *U.E. Spichiger-Keller*, R. Cannas, J. Glebska, M. Linnhoff, T. Nezel, V. Ramos-Pérez, S. Spichiger, G. Zhylyak Swiss Federal Inst. of Technology, Zürich, Switwerland				
Session 9 Chairs: N. Jaffrezic-Renault / M.C. Moreno-Bondi				**Session 10** Chairs: J.D. Dakin / B. MacCraith		
10:00	IL3.1	**Fluorescent Nano-Crystals as Ultra-Bright Time Resolved Sensors** *R. Pansu*[a], N T. Ha-Duong,[a] V. Lemonier,[b] R. Méalet Renault,[a] A. Ibañez[b] [a]Ecole Normale Superior de Cachan, France; [b]Lab. Cristallographie UPR5031 CNRS, France		10:00	IL3.2	**In Situ Fluorescence Sensors for Coastal Oceans** *R. Chen* University of Massachusetts, USA
10:30	Coffee Break					
Session 9 – (continued) Chairs: N. Jaffrezic-Renault / M.C. Moreno-Bondi				**Session 10 – (continued)** Chairs: J.D. Dakin / B. MacCraith		
11:00	IL3.3	**New Materials for Optical Sensors Based on Molecular Imprinting** *S. Piletsky* Cranfield University, U.K.		11:00	IL3.4	**Biological and Screening Application of the Optical Oxygen Sensing** *D. B. Papkovsky* University College Cork, Ireland
11:30	OA3.1	**Quantum Dot Based Fluorescence Resonance Energy Transfer Nanosensors** A. R. Clapp, *I. L. Menditz*, E. R. Goldman, H. Mattoussi US Naval Research Lab, Washington, USA		11:30	OB3.1	**Automated Water Analyser Computer Supported System (AWACSS)** *G. Proll*, J. Tschmelak, G. Gauglitz Eberhard-Karls-Universität Tübingen, Germany
11.50	OA3.2	**Small and Massively Parallel Optical Sensors: Ionophore-Based Microsphere Ion Optodes** *E. Bakker*, K. Wygladacz, C.Xu, Y. Qin Auburn University, USA		11.50	OB3.2	**IR-ATR Spectroscopy for Underwater Sensing Applications** *G.T. Dobbs*,[a] P. Boezerrooij,[b] N. Pennington,[c] F. Vogt,[d] B. Mizaikoff[e] [a,b,e]Georgia Institute of Technology, USA; [c]Alcohol and Tobacco Tax and Trade Bureau, USA; [d]Arizona State University, USA
12:10	OA3.3	**Self Assembled Sedimentation Arrays Based on Luminescence Encoded Microspheres** *C. Moser*, I. Klimant Graz University of Technology, Austria		12:10	OB3.3	**BTEX Monitoring in Ground Water Remediation Applying UV Fiber Evanescent Wave Sensors** *H. Lehmann*,[a] U. Lubenau,[b] G. Schwotzer,[a] R. Willsch[a] [a]Inst. of Physical High Technology of Jena, Germany; [b]DBI Gas- und Umweltechnik GmbH, Germany

12.30	OA3.4	**A New Combinatorial Approach to Sensor Discovery and Fabrication: Where Simplicity and Effectiveness Meet** *R. Zimmerman*, D.N. Reinhoudt, M. Crego-Calama University of Twente, The Netherlands		12.30	OB3.4	**Use of Fibre-Optic Optodes for Monitoring of Sea Water: Towards and Optical CTD Probe** *A. González-Cano*,[a] M.C. Navarrete,[a] O. Esteban,[b] N. Diaz-Herrera[a] [a]Universidad Complutense de Madrid, Spain; [b]Universidad de Alcalá, Spain
13:00	**Poster Awards and Closing Address**					
13:30	**Farewell Cocktail**					

POSTER PROGRAMME

P-1 **A REAGENT-LESS FLUORESCENT SOL-GEL BIOSENSOR FOR SUPEROXIDE ANION DETECTION**

I. Pastor, R. Esquembre, E. Rico, V. Micol, R. Mallavia and C. Reyes Mateo, Universidad Miguel Hernández, Elche, Spain

P-2 **NOVEL DEVELOPMENTS IN CHROMOGENIC AND FLUOROGENIC RECEPTORS AND REAGENTS FOR CATION AND ANION SENSING**

R. Casasús, M. Comes, A. B Descalzo, B. García Acosta, D. Jiménez, J. V. Ros Lis, R. Martínez Máñez, F. Sancenón, and J. Soto.
Universidad Politécnica de València, Spain

P-3 **FLUORESCENT BIOSENSOR FOR NITRIC OXIDE BY INSERTION OF 2,3-DIAMINONAPHTHALENE IN LIPOSOMES IMMOBILIZED IN SOL-GEL GLASSES**

R. Esquembre,[a] I. Pastor,[a] C. Tormo,[b] R. Mallavia[a] and C. Reyes Mateo[a]
[a]Universidad Miguel Hernández, Elche, Spain; [b]Hospital General Universitario de Elche, Spain

P-4 **ORGANIC-INORGANIC HYBRID MATERIALS FOR OPTICAL DETECTION OF ANIONIC AND NEUTRAL SPECIES**

Rosa Casasús,[a] María Comes,[a] Beatriz García Acosta,[a] Ana B. Descalzo,[a] Gertrudis Rodríguez-López,[a] Félix Sancenón,[a] María Dolores Marcos,[a] Ramón Martínez-Máñez,[a] José V. Ros-Lis,[a] Juan Soto,[a] Luis Villaescusa,[a] Pedro Amorós,[b] Daniel Beltrán,[b] Carmen Guillem,[b] Julio Latorre[b] and Knut Rurack[c]
[a,b]Universidad Politécnica de Valencia, Spain; [c]Bundesanstalt für Materialforschung und –prüfung (BAM), Germany

P-5 **MICROARRAY COMPACT-DISC BASED METHODS APPLIED TO GENOMICS. SUPPORTS TREATMENT COMPARISON**

S. B. Morais, J. V. Mor, R. Marco-Molés, R. Puchades and A. Maquieira.
Universidad Politécnica de Valencia, Spain

P-6 **OPTICAL SENSORS FOR CO_2**

R. Cannas, G. Zhylyak, T. Nezel, U.E. Spichiger-Keller
Center for Chemical Sensors, ETH Technopark, Switzerland

P-7 **ASSAY OF LUMINESCENCE- BASED TRACERS FOR PESTICIDE IMMUNOSENSING**

J. Penalva,[a] M.A. González-Martínez,[a] A. Maquieira,[a] R. Sedano,[b] M. Carramolino,[b] E. Brunet,[b] J.C. Rodríguez-Ubis[b] and R. Puchades[a]
[a]Universidad Politécnica de Valencia, Spain; [b]Universidad Autónoma de Madrid, Spain

P-8 **RESPIRATION-BASED TOXICOLOGICAL TESTS WITH MICROPLATES EQUIPPED WITH OPTICAL OXYGEN AND pH OPTODES**

S. Arain,[a] C. Krause,[b] G. T. John[b] and I. Klimant[c]
[a]University of Regensburg, Germany; [b]PreSens Precision Sensing GmbH, Germany;
[c]University of Technology of Graz, Austria

P-9 **NOVEL FLUORESCENT RATIOMETRIC pH SENSORS WITH A MINIMIZED EFFECT OF IONIC STRENGTH**

B. Weidgans,[a] C. Krause,[b] I. Klimant,[c] and O. S. Wolfbeis[a]
[a]University of Regensburg, Germany; [b]PreSens GmbH, Germany; [c]University of Technology, Graz, Austria

P-10 **EUROPIUM TETRACYCLINE: A VERSATILE BIOSENSOR PROBE FOR TIME-RESOLVED FLUORESCENCE IMAGING APPLICATIONS**

M. Schäferling, M. Wu, Zhihong Lin and O. S. Wolfbeis
University of Regensburg, Germany

P-11 **A NEW SUPRAMOLECULAR CHIRALITY SENSOR ON THE BASIS OF ACHIRAL ETHANE-BRIDGED BIS-PORPHYRIN**

V. V. Borovkov, G. A. Hembury, and Y. Inoue
Japan Science and Technology Agency (JST), Japan

P-12 **USE OF PLANAR OPTODES IN MARINE MICROBIOLOGY-OVERVIEW OF MOST RECENT APPLICATIONS**

L. Polerecký,[a] U. Franke,[a] E. Precht,[a] C. Schröder,[b] B. Grunwald,[a] G. Holst,[c] D. de Beer[a] and I. Klimant[d]
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ABSTRACTS

ECONOMICAL OPTICAL REAL-TIME DNA ARRAYS -OPTICAL BIOSENSORS AT THE ONSET OF THE XXI CENTURY-

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After over 40 years of biosensor research their rather limited fields of application does not satisfy our expectations. The possible reasons will be discussed and an outlook will be given. This will be done by presenting an example of a recently developed optical biosensor system in my group rather than reviewing uncountable more or less exciting developments found in the literature. One important point seems to be to start as early as possible in the course of the development of new sensor systems with some really self-critical reflections concerning their possible market share. Besides the fact that only complete and patented systems (sensor + readout system + software) will earn the profit to pay back the R&D expenditure any successful device must by very competitive. In order to convince traditional analytical chemists of the superiority of sensor systems, especially of the reliability of biosensors strict and quantifiable quality factors must demonstrate their competitiveness (fit for the purpose but much more economical).

To example this, a recently developed optical DNA array chip based on an evanescence field excitation of fluorescent labeled (Cy5 and novel own optimized dyes) target molecules combined with a special integrated low cost microfluidic sample introduction (eliminating diffusion control) will be described. The latter allows a fast kinetic evaluation method. With up to 400 complementary DNA probe spots immobilized on the surface of a planar wave guide such a low density DNA chip allows a real-time recording and evaluation of the association (hybridization event) and dissociation kinetics in less than about 200 seconds. It is also a low cost chip, since only 2 PMMA and/or glass plates are glued together with a double adhesive spacer which defines the specially formed flow and measuring channel. The optical evanescence field fluorescence measuring method employing a low-cost diode laser at 635 nm eliminates any washing steps. A CCD camera and together with a special software allow a simultaneous real-time recording and evaluation of all hybridization or dissociation events on the chip. The analytical signal is the change of the fluorescence light if the sample passes the probe spot array in the measuring channel with a constant flow rate in the range of 1 to 5 µL/sec. This slope is strictly proportional to the target DNA (analyte) concentration with a dynamic range > 2 orders of magnitude. First results with Cy5-labeling indicate a sensitivity of about 10 counts/(sec • nM) resulting in a detection limit in the lower pM range. This was obtained with µm spots and µM probe concentrations. Using the novel synthesized dyes and optimized spot geometries a fM detection capability and an increased specificity seems feasible. But most important is the fact that the kinetic evaluation method used allows the production of pre-calibrated sensor chips showing each a reproducibility of s ≈ 5 %. Furthermore, the array chip can be re-used with a minor sensitivity decrease (~ 0.5 % per rejuvenation step) which renders this simple optical DNA array approach even more economically.

This low-cost DNA array was validated using real food reference samples (beef, pork, chicken, turkey, sheep, goat and mixtures of them) and for a simple and rapid detection of microorganisms with experimental 5x6 DNA array chips after an appropriate PCR amplification. Alien meat impurities as low as 0.1 % were reliably detectable. A further special advantage of this DNA-array chip lies in the fact that cross hybridizations or the known (non-Watson-Crick-interaction) mismatch possibility with the G-T couple which is difficult to detect during the course of the association process can easily be detected during the dissociation event using a more stringent dissociation buffer immediately after the hybridisation event. Since this optical chip approach is also the fundament of a whole technology platform which allows also multiple immunochemical reactions to be simultaneously followed and evaluated, increasing production numbers will further lower the production costs. Competitiveness should come besides the above mentioned advantages and evident economical reasons also from the advantage of the production of pre-calibrated immunochemical and DNA array chips needing no additional calibration step through the user.

CONFRONTING THE CHALLENGE OF DESIGN OF SELECTIVITY FOR OPTICAL DNA BIOSENSORS AND BIOCHIPS

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Biosensors and biochips can determine the presence of nucleic acid sequences in a test sample through fluorescence detection of hybridization between an immobilized nucleic acid (probe) and a nucleic acid in a test sample (target). Of particular relevance is research that addresses development of surface chemistry that can ultimately be integrated to increase speed, sensitivity and selectivity of nucleic acid diagnostic devices. One goal is to achieve substantially increased selectivity and speed of hybridization through control of the environment of the oligonucleotides that are immobilized onto a surface.[1] It is a challenge to create an environment for immobilized nucleotides that offers good structural regularity and reproducibility, where nearest neighbour interactions provide for control of selectivity, yet where the degree of hybridization does not alter nearest neighbour interactions. Using fiber optic biosensors, we are exploring the use of a "matrix isolation" method that will produce the desired environment for the probe molecules. The DNA oligonucleotide probes are polyelectrolytes with charged backbones and significant flexibility. It is possible to isolate the probe molecules by surrounding each on average with a sheath of immobilized polyelectrolyte that is not based on complementary nucleic acid. In fact, it is possible in such mixed films to use oligomers such as polyethers that are not based on nucleotide chemistry so as to tune the selectivity of ssDNA oligonucleotide probe molecules. Preliminary results suggest that it is possible to design the duplex melting temperature (T_m, or temperature at which 50% of all duplexes formed are denatured) of such immobilized systems, and that the selectivity of such systems can be improved in comparison to immobilized films of oligonucleotides.

The intention is to develop immobilization methods that enhance differences in signal magnitude generated for fully matched target nucleic acid in contrast to partially matched target nucleic acid prior to signal processing. This significantly improves confidence in results from devices that are not designed to operate with large redundancy, and makes the task of signal processing less onerous, time consuming and complex. Furthermore, control of the surface chemistry can be used to adjust the effective duplex melting temperature so that combinations or arrays of immobilized nucleic acid films in a system can be made to have similar T_m, regardless of nucleotide length and sequence. This allows for optimization of simultaneous analysis of many different interfacial hybridizations, facilitating quantitative high throughput screening capacity.

The finding that the control of the environment of immobilized single stranded probe molecules can be used to tune selectivity to facilitate detection of even single base pair mismatches provides opportunities for design of novel biochips. One new approach provides for a multi-dimensional distribution of selective chemistry at a surface, but in such a way that the coatings of probe molecules are continuous, and operate to provide gradients of selectivity in one or more directions. Such a Gradient Resolved Information Platform (GRIP), is based on a surface that is coated with a continuous gradient of density and/or sequence and/or orientation and structure of ssDNA. The location, extent of hybridization, and speed of hybridization on such a surface by a target sequence can be used to identify and quantitatively measure the target. Microfluidics is being combined with this novel form of biochip to create a quantitative sensing system that is suitable for concurrent rapid analysis of multiple nucleic acid targets.[2] Sensitivity gains can be achieved by improvement of signal capture and reduction of noise. A smaller detection area will reduce background. We are moving towards planar waveguides and areas of immobilized ssDNA of ~10000 um^2 dimensions. Time-resolved fluorescence spectroscopy can extract signals from intercalated dyes while rejecting signal from dye that is not intercalated. Preliminary work with a confocal collection system, a cooled detector, and a frequency-doubled, diode pumped passively Q-switched Nd:YAG laser source has already shown that it is possible to detect as few as 100 molecules of intercalating dye, and to detect 1000 molecules of hybridized DNA on surfaces.

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[1] J. Zeng, A. Almadidy, J. Watterson and U.J. Krull, *Sensors and Actuators* **2003,** *90*, 68.
[2] D. Erickson, D. Li and U.J. Krull, *Analytical Biochemistry* **2003**, *317*, 186.

ROOM TEMPERATURE PHOSPHORESCENCE DETECTION FOR OPTICAL SENSORS

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Photoluminescence based optical sensors are experiencing a tremendous growth in the last few years, because luminescence measurements offer an exceptional sensitivity and high selectivity. These favourable analytical characteristics make them most useful for developing sensitive and selective optical sensors for a wide variety of applications. Moreover, the availability of low to moderate cost commercial instrumentation for luminescence measurements is an aspect particularly positive helping to develop and market such optical sensors technology.

In this vein, fluorimetry has been extensively used in the development of luminescence optical sensors. However, room temperature phosphorescence (RTP) based optical sensors offer several advantages over the corresponding fluorescent ones. Most of these advantages are derived from the longer lifetimes of the triplet state (which facilitates the design of relatively inexpensive optical sensing systems based on decay-time measurements). Additionally, the larger Stokes' shifts of the RTP processes simplify the separation of the scattered excitation light from the phosphorescence emission. Moreover, RTP background from conventional solid supports used for optosensing, where the luminophor is immobilized, is usually comparatively low. All these properties point to phosphorimetry as a highly attractive and promising detection technique for the development of new optical sensors.

Therefore, the present importance[1] and state-of-the-art of RTP detection will be reviewed, while stressing the main basic requirements to obtain useful analytical signals derived from RTP measurements. RTP optosensing features will be discussed at length in this context. Despite RTP advantages, this detection mode is, of course, less widespread than the fluorimetric one, particularly for optical sensing. The main practical drawbacks of RTP include: the comparative lack of RTP dyes or indicators, RTP is more sensitive to external conditions changes and offers less intense emissions, unstability of the active phases, analyte recognition based on irreversible chemistry, etc.

The different concepts and approaches reported so far in order to overcome such limitations will be discussed. Particular reference will be given to attempts in our research group to enhance the potential of RTP optical sensors in two directions:

Firstly, trying to improve analyte recognition mechanisms for anions sensing, to use molecularly imprinted polymers for PAHs optosensing, or tailored sol-gel structures for O_2 and pH sensing in experiments associated to phosphorescence changes.

Secondly, transduction approaches alternative to intensity or lifetime RTP conventional measurements will also be reported. Particularly we will refer to our efforts investigating the potential of CdSe quantum dots for such alternative transduction purposes or the use of certain frequency domain RTP measurements (phase-shift at two optimal frequencies) to measure accurate RTP lifetimes even in the presence of overlapping high background signals.

[1] J. Kuijt, F. Ariese, U.A.T. Brinkman, C. Gooijer, *Anal. Chim. Acta* **2003**, *488*, 135.

GENETIC ENGINEERING IN BIOSENSING AND MICRO/NANOANALYTICAL METHODS

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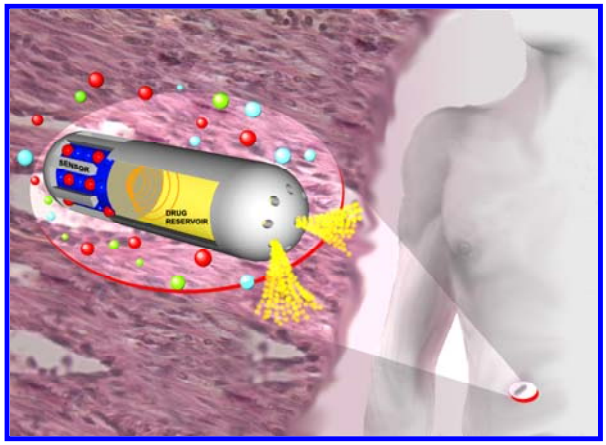
The design of instruments and techniques capable of detection, quantification, and delivery of small amounts of biomolecules is essential in the development of new bioanalytical methods. Advances in microfabrication and microfluidics have been instrumental in advancing this field. In order to detect target molecules in small volumes and microfabricated structures, it is necessary to prepare bioreagents that provide enough sensitivity for their detection. In our laboratory, we employ genetic engineering techniques to design proteins and cells that are employed in a variety of biosensing methods. We prepare modified photoproteins with altered emission characteristics for the array detection of biomolecules. Recombinant DNA methods are also used to rationally design biosensors. One approach involves genetically engineering binding proteins to incorporate signal-generating molecules into their structure and employ them to develop the biosensors. The binding proteins change conformation upon binding to their analyte ligand, which causes a change in the signal emitted that can be directly related to the amount of analyte present. In addition, whole cell biosensors are designed by genetically modifying cells to recognize a target analyte, and in response, emit a signal. These whole cell biosensors when incorporated on a centrifugal microfluidics CD platform can find applications in the high throughput of pharmaceuticals, as well as in environmental and clinical analyses. On another front, we are addressing the need for individualized therapy by incorporating genetically engineered biosensors with actuators to design accurate and reliable miniaturized implantable responsive drug delivery devices. Several strategies are investigated for the fabrication of these devices.

Miniaturized implantable drug delivery device for in vivo sensing and in vivo delivery



Microvials fabricated on plastic substrate containing small electrodes are "blasted open" electrochemically to release the drug. In addition, electroactive polymers that allow for the reversible opening and closing of the microvials are being used. A different configuration incorporates an electroactive hydrogel that acts as an artificial muscle to open the valve. An on-board biosensor monitors a stimulus that triggers the electrochemical opening of the hydrogel, thus controlling the release rate from the drug reservoir. In another strategy, a protein immobilized within a hydrogel acts as a recognition element for drugs. The integrated molecular recognition within the hydrogel allows for simultaneous sensing and actuating, thus providing with a novel approach to responsive drug delivery systems.

PAST, PRESENT AND FUTURE OF IONOPHORE-BASED OPTOSENSING

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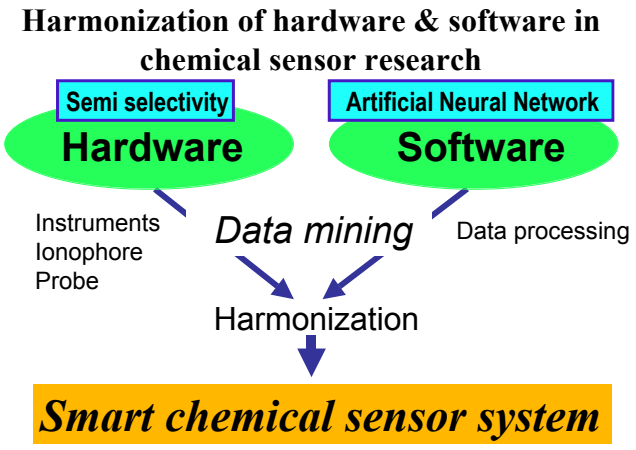
Regarding the ion optode and its system, we have designed and synthesized several ionophores that are highly selective ion-sensing molecules of alkali and alkaline earth ions. Some of the important points in the design of alkali-metal monovalent cations is not only how to prepare the fitted complex for the analyte ion as the ligand, but also how to design the poor fitting complex for interfering ions. This design concept creates ionophores for Li^+ (DD14C4), Na^+ (TD16C5) and NH_4^+ (TD19C5). These ionophores were successfully applied to the ion-sensors for serum and water quality analyzers. For designing alkaline earth metal divalent cations, a wrapping complex based on a double-armed diazacrown was used as the base-component. In this case, a charged β-diketone group is one of the selective binding sites for Mg^{2+}. The fluoroionophores for selective Mg^{2+} imaging of a living cell was obtained as the cumarine and fluorescein derivatives of the α-keto acid forms (KMG-20, KMG-104, etc). Another design was used to produce a multi-ion imaging probe for Mg^{2+} and Ca^{2+}(KMG-2) in a living cell.

Our pH optode based on a lipophilic merocyanine dye has the problem of durability. The boron dipyrometene derivative dye overcomes this problem. This optode can be applied not only to a fiber-optic device, but also to a simple color-changeable sensing film and multi-microplate devices. In this case, the virtual simulation that realizes an effective color change with our developed Digital Color Analysis method. The ion optode using an ionophore and a color changeable dye also applied to a surface plasmon resonance system using our developed absorption-based SPR method.



A micrometer-sized membrane unit in the SPR tip can measure a multi-analyte such as ions and biologically-relevant molecules with chemical, enzymetric and immnological reactions. Our recent important target analyzed ions are heavy metal and transition metal cations in which the creation of highly selective ionophores are very difficult. To solve this classical problem, we have proposed an artificial neural-network sensing system called a "Smart Chemical Sensor System".

In one ideal case, one optode sensor based on spectral information can determine multiple heavy metal and transition metal ions using this system based on the network inversion method. This smart sensor system combined with instrumentation (an easily prepared semi-selective sensor) and software (neural network analysis) opens the way to new sensors for the next generation.

THE SECRET OF LOW DETECTION LIMITS USING OPTODES: ENGINEERING, CHEMISTRY AND APPLICATIONS OF OPTODES

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Sensitivity and detection limit of various types of optodes and optical assays can be scaled from low to very high which coincides with the detection of a concentration range between 10^{-1} and 10^{-13} mol per liter. The design and physical principle of optical devices rank from traditional transmission measurements to highly sophisticated integrated planar optical waveguides where 100 attomoles of flourescently labeled DNA were detected.[1] However, not only the design but also the operating principle and the chemical mechanism of the optical sensors and arrays profoundly influence both the sensitivity and the detection limit. On the other hand low detection limits are not required for all applications. The optical device has rather to fit the requirements of the application. Since the features of optical devices are obviously defined by a number of different parameters as mentioned above, the tailoring of optodes to specific applications is feasible.

One of the most **attractive feature of optical sensors** opposite to electrochemical ones is its ability to detect neutral molecules such as gases (CO_2, NO_2, NH_3, SO_2, HCN), aldehydes, amines and alcohols based on reversible chemical reactions (Chromo- and fluororeactands).[2,3] In addition, new detection principles such as calorimetry and SAW-detectors, technologies that are addressed by CMOS-sensors showed to be promising in combination.[4] Surprisingly it is hardly made use of these sensors in industrial applications yet. Along with the variable applications in the gas phase, optodes can be applied to liquid sample as single optodes and in arrays. Reactand-based sensors frequently show a good lifetime of several months opposite to enzymatic approaches. For applications in gas sensing arrays as mentioned above, where the gas phase is transparent, it's mostly good enough to use plasticized optical polymer films in transmission or reflection mode.[5] The addition of titanium dioxide to the film rises the relative reflection intensity (I/I_o) by a factor of 1.7.

Many authors focused on internal reflection spectroscopy and evanescent wave sensors using optical fibers and planar waveguides as a more efficient measuring principle. The two designs were compared by G. Robinson.[6] However, referred to own investigations, the design of the physical transducer is only half of the truth. Chemically, the most relevant influence factor is a **high enrichment factor** described by the distribution coefficient of the analyte between sample phase and chemically active film. The enrichment factor is derived from the numerical results of experimental investigations on sensitivity of the optode under controlled conditions. In own experiments with NO_2 in the gas phase, enrichment factors in the range of 47'000 were observed. This result went along with a high selectivity of the NO_2-selective optode based on the metal complex aquacyanocobalt(III)-cobyrinate (ionophore NI 1) combined with a chromionophore ETH 5418 (Fluka Chemie AG, CH-9471 Buchs) incorporated into a plasticized polymer film.[7] The membrane composition was known to show a very specific reaction of the metal complex with NO_2^- within the polymer film. This reaction was investigated in detail in order to understand the low detection limit, and non identical behaviour in the range of ppb and ppm concentrations were noticed. Improvements in terms of lifetime had been shown based on a Fe-

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phthalocyanine complex.[8] In sum, important factors to improve the detection limit is an efficient chemical extraction process, which at least allows the sensor to regenerate under "normal" exposure conditions, and a chromophore characterized by a high absorption coefficient.

In account of lower detection limits, two further approaches provided more sensitive measurements. These are:

1. The measurement in continuous flow arrangement (fully automated instrumentation for calibration and fluidics)
2. The combination of high sensitivity inducing chromophores and a controlled immobilization process of peptides on integrated optical thin film waveguides operated in internal reflection mode.

The **effect of the continuous flow arrangement** was investigated within a project on the development of polymeric lead-selective optode membranes based on a specifically designed thiacrown ether and a chromoionophore with high pK_a.[9] The experiments showed that the enrichment factor of the optode membrane for Pb^{2+} must be $>10^5$ in the concentration range between 10^{-4} to 10^{-8} M. The mechanism was based on the principle of an ion-exchange membrane using a chromoionophore with high pK_a which is 100% protonated if no lead ions are extracted within the polymer film. A model was calculated based on the assumption that for batch response full protonation of the chromoionophore is the base line situation. At identical diameters of the Pb^{2+}-selective optode membrane and the fluidic cell, the film being by a factor of 10^{-5} smaller in thickness than the cell thickness, the sensor response is exactly the same for batch and continuous flow analysis in the concentration range between 10^{-4} to 10^{-8} M (buffered aqueous solution). In all other cases where sample cell volume and film volume approach each other, batch measurements show much lower sensitivity than continuous flow operation. Continuous flow operation extends the cell volume artifically relative to the film volume and therefore, allows to extract higher total amounts of the analyte irrespective of time under thermodynamic equilibrium assumptions.

The second approach based on **integrated optical waveguides** was investigated using an entirely new approach where the **activity of serine-proteases** on specific substrates was investigated. Specific dye-labeled tripeptides were synthesized and immobilized on the surface of the optical chips. The activity of serine-proteases such as those found in the LAL-lysate for the endotoxin test for pyrogens and in clotting tests were investigated. The hydrolysis of the dye-label was observed in the evanescent field along a propagating distance of 12 mm at the wavelength of 641 nm. First estimates allowed to conclude that the cleavage of approx. 10^5 molecules was traced. This approach will be the main topic of the presentation. More detailed informations will be provided.

The approach was not only applied to the detection of the enzymatic activity of proteases but in addition for potassium activity measurements using selective ion-exchange optodes. Furthermore, the integrated optical chips were used as a photometer in order to check the correlation between signal intensity and concentration of dye solutions and to investigate the quality of signal predictions. A considerable disadvantage of these optodes was the instability of the nileblue dyes especially in the deprotonated form. In between, a large range of nileblue and meldolablue dyes with different substituents and pK_a were synthesized, the rules of the instability were investigated and a range of more acidic dyes were proposed. Besides of its higher stability, these dyes / chromoionophores are more useful for measurements in the physiological pH-range around pH 7.4 and provide a higher resolution under these conditions. Again the detection limit and its applicability to the quantitation of potassium and lithium ions by combining these dyes with well-known ionophores was increased by chemical means.

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Invited Lectures

Abstracts

OPTICAL DETECTION OF DNA-MODIFYING ENZYMES ON IMMOBILISED SUBSTRATES

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The primary task of a microarray experiment is to detect a lot of binding events simultaneously. Most applications are transcription profiling and use fluorescence as label. Prior to the experiment, the sample has to be labeled by a suitable fluorochrome. The binding is done in a separate incubation step, the final result is taken after drying. Therefore usually microarray reader produce information on the fluorescence intensity at a given time of the binding process.

Progress towards diagnostic applications is still slow, quantitation of the results is a problem and fabrication methods up to now are not reliable enough to allow for large series production. The fluorescence intensity measured in microarray experiments represents the amount of bound analyte that depends on the concentration in the sample as well as on the affinity of the involved binding partners and time given for the binding. It is not possible to differentiate these influencing factors in the usual setup.

To overcome the limitations in microarray technology developments are under way to facilitate measurement of binding kinetics in the microarray format. Homogeneous sample flow over the whole microarray is one of the technological problems that recently has been solved in our laboratory.

Enzymatic reactions on immobilized substrates may also be observed using a flow through type of scanning device. Parallel detection and comparison of a variety of substrates or templates are now accessible in one single experiment and will be presented here. To demonstrate the power of the approach, we chose restriction endonucleases and polymerases.

Keywords: microarray, biochip, DNA-modification, enzyme activity

RECENT DEVELOPMENTS IN TRANSDUCERS BASED ON PLANAR INTEGRATED OPTICAL WAVEGUIDES

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The single mode, planar integrated optical waveguide (IOW) is an inherently sensitive geometry for attenuated total reflection (ATR) spectroscopy of interfacial samples, including recognition materials used in chemical and biochemical sensing transduction. A major disadvantage that has limited its wider use is the difficulty of measuring broadband spectra. Due to the quantized nature of light propagation in a planar IOW, conventional grating and prism couplers are efficient only over a narrow (<5 nm) spectral range at a given launch angle. Consequently, a laser source has been utilized in most IOW-based spectroscopy and chemical sensing reported to date. Several years ago, we developed a multichannel IOW spectrometer, based on an achromatic incoupler, that permitted a broadband visible ATR spectrum of a molecular film supported on a single-mode, planar IOW to be measured over a ca. 150 nm bandwidth.[1] However, the required custom fabrication coupled with the difficulty of alignment precluded wide application of this technology.

More recently, we developed a new generation spectrometer that circumvents these limitations.[2] As opposed to the previous design, this broadband IOW instrument is much simpler in design, is more chemically robust, and transmits light down to at least 400 nm. The attenuated total reflection element consists of a single mode, planar IOW fabricated by dip coating a ~300 nm thick, sol-gel composite layer deposited on a glass substrate. A commercially available prism is used as the incoupler, with an integral holographic diffraction grating acting as the dispersive outcoupling element.

The information content and molecular selectivity of planar waveguide ATR can be further enhanced by combining it with an orthogonal analytical technique, such as electrochemistry. To explore this concept, we extended the recently developed broadband technology to create a single mode, electrochemically-active (EA) planar IOW platform with a 200 nm spectral bandwidth.[3] With an estimated pathlength enhancement of ca. 10,000 relative to a transmission geometry, highly sensitive spectroelectrochemistry of surface confined films can be performed. Subtleties in the redox chemistries of adsorbed molecular films, which were difficult to monitor with a monochromatic EA-IOW,[4] are readily apparent when using the broadband coupling scheme.

These new planar waveguide techniques should have a significant impact in several areas, including spectral characterization of thin molecular films and simultaneous chemical sensing of multiple analytes.

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OPTICAL DETECTION IN MICROFLUIDIC SYSTEMS

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The concept of micro total analysis systems (μTAS) was introduced in the early 1990's.[1,2] One expected advantage of the application of the microfluidic structures was the integration of the whole analytical process, i.e. the sequential operations like sampling, sample pre-treatment, analytical separation, chemical reaction, analyte detection, and data analysis would be performed in one analytical microdevice (thus the expression: lab-on-a-chip originated). μTAS finds typical applications in biology and medicine during DNA, genome, and clinical measurement.[3]

Originally the structures were fabricated using conventional micromachining technologies based on silicon wafers. Nowadays, many polymer (polydimethylsiloxane, polymethylmethacrylate, epoxy resin) as well as ceramic materials are also utilised in μTAS design. The advantages of μTAS are evident if construction details are considered. The analysis performed in such a system requires a very small sample to be delivered, the reagents' consumption is also reduced, the separation obtained is much better than in ordinary bench-type measurements, and the time of the analysis is quite short. Nevertheless, new problems were faced by the designers like hydrodynamics of fluids in small channels, novel phenomena in very small channels, chemical resistivity of the microfluidic structure to a variety of chemical compounds etc.

The paper presents various constructions of microfluidic systems with the detection based on the application of optical fibres. The microfluidic structures were fabricated in polymer technology using poly(dimethylsiloxane) (PDMS), and ceramic material (by means of so-called Low Temperature Co-fired Ceramic, LTCC). Several different structures were designed and tested. A fibre optic chemical coupler was constructed in which the coupling efficiency between the fibres depends on the refractive index of liquid delivered to a microchannel formed by the fibres. Two polymer fibres were used to manufacture the coupler. The claddings of the fibres were removed in order to allow optical power coupling. The coupling efficiency depends also on the absorbance or fluorescence of a sample and thus the coupler can be used in various experiments. Another type of a microfluidic structure was manufactured by covering a small (diameter of 250 μm) tube with PDMS. After cross linking of the polymer the tube was removed and optical fibres were introduced forming a microspectrophotometric cell with the path length of 1 cm for absorbance measurements. Similarly, a microcell for fluorescence tests was fabricated. Based on LTCC technology a versatile optical detection system was fabricated. It consists of a ceramic support in which a microchannel was fabricated in a form of meander (length of 26 cm). At the end of the meander there are optical fibres mounted into the structure in such a way to be used in absorbance or fluorescence measurements.

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INTEGRATED OPTOCHEMICAL SENSORS BASED ON NEW VIS-NIR CHROMOIONOPHORES

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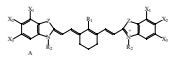
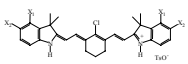
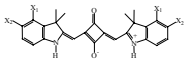
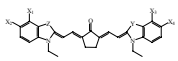
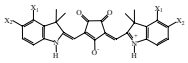
The lack of recognizing elements, whose interaction with a certain analyte gives an optical signal, and the difficulties found in their design, have lead to the use of indirect approaches, mainly based on the combination of two separated reactions, the analyte recognition and the signal generation. The whole process is driven by the electroneutrality principle in the membrane, which must be taken into account when formulating the membrane composition. The main accountable compounds are an ionophore and a cromoionophore. The later is usually pH indicator, which changes its spectroscopic characteristics as a result of the co-extraction or the exchange of ions between the bulk of the membrane and the aqueous solution. In this presentation, we report the design, synthesis and optical characterisation of different kind of chromoionophores[1-23] with appropriate features such as good solubility in common PVC plasticizers, for being used as chromoionophores in ion-selective optical membranes, and intense absorption or emission bands in the NIR, for their usage in the development of integrated optical sensors as Integrated Waveguide Absorbance Optodes (IWAOs)[4,5] or Integrated Waveguide Fluorescent Optodes (IWFOs).

Table 1. Synthesised dyes and general characteristics in ethanol

General name	General formula	pKa range	λ_{max} range (nm)	ε range (kg/mol cm)
heptametincyanine		11.0 - >13.0	678.0 - 814.5	8.5x104 - 9.3x105
nortricarbocyanine		2.8 – 5.2	780.0 - 821.0	1.1x104 - 2.7x105
norindosquarocyanine		8.3 - 12.6	648.5 - 679.5	$8.2x10^4$ - $1.7x10^5$
ketocyanine		1.7 - 4.3	717.0 - 748.5	$8.0x10^4$ - $2.8x10^5$
norindocroconine		6.5 - 9.7	759.0 - 800.0	$4.4x10^4$ - $1.7x10^5$

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The acidochromic properties (pH response characteristics) and stability of most of the obtained compounds have been determined as well as in ethanolic solution as in plasticised PVC membranes. A certain membrane composition has been optimized for each dye and its response has been first characterized in a conventional absorbance/transmittance configuration based on a flow-cell. The majority of the membranes are totally homogeneous demonstrating the good solubility of the indicators in the selected organic solvents (DOS, TOPh, DBS). As is expected, a solvatochromic shift is observed, which moves away or brings closer their absorbance maxima from the working wavelength of the commercially available light sources (LEDs emitting at 780 nm, 670nm, 650nm). On the other hand, the synthesized dyes cover a wide range of pK_a values, which allows to select the proper one according to the ionophore used for a certain application. In this way, they are being used to develop ion-selective optode membranes and then, they are being applied in IWAO platforms to develop automatic analytical microsystems. The obtained results will be presented. Furthermore, the fluorescent characteristics of some chromoionophores are being examined in order to develop new integrated optical systems based on fluorescent measurements, the so called IWFOs.

APPLYING FLUORESCENT NANOSENSORS TO MEASURE THE INTRACELLULAR ENVIRONMENT

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Techniques to measure the composition of the intracellular environment are important in elucidating the mechanisms of cell function and understanding cellular behaviour. Developments in the field of nanosensors may provide some solutions to enable quantification of analytes in the cellular environment, overcoming disadvantages, such as cell destruction and lack of spatial localisation, associated with the current methods of choice. The small size of the nanosensors, typically <100 nm, allows many sensors to be introduced to a single cell with minimal physical perturbation of the cell. The nanosensors detailed in this paper are prepared using microemulsion techniques and consist of one or more sensing components co-immobilised with a reference dye in an inert optically transparent matrix; polyacrylamide or sol-gel. The nanosensor matrix imparts two primary advantages; the sensing dye is protected from interfering species in the intracellular environment whilst simultaneously the cell is protected from any cytotoxic effects of the sensing components.

Nanosensors selective to glucose, oxygen, zinc and calcium have been prepared and characterised in terms of dynamic range, selectivity, reversibility and response time. Furthermore the cellular delivery of these sensors will be discussed, as will strategies to extend the range of analytes that can be measured.

USING REVERSIBLE CHEMICAL REACTIONS TO OPTICALLY DETECT ANALYTE MOLECULES

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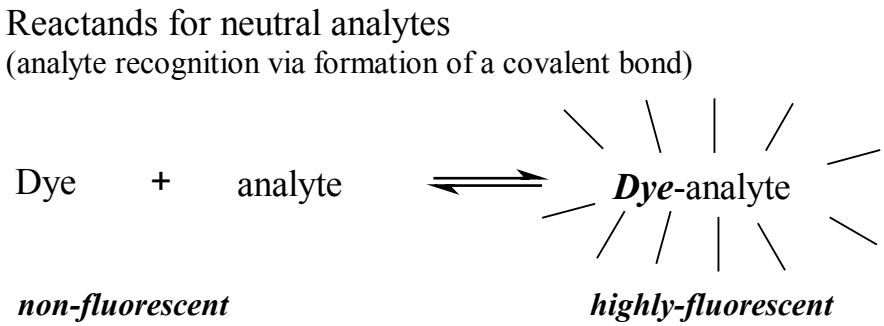
Neutral molecules are difficult target analytes for optical sensors because the interaction with indicator dyes usually is weak and signal changes are small. Frequently, enzymatic sensors are used but they suffer from poor operational stability and shelf life.

Chromo- and fluororeactands are indicator dyes that allow the optical detection of electrically neutral analytes.[1] Unlike complexing agents such as calixarenes, cyclodextrines or cyclophanes, reactands reversibly form a covalent bond with the analyte molecule. When embedded in plasticised polymers, the chromo- and fluororeactands respond to the analyte with significant colour changes due to changes in electron delocalisation or in energy transfer. Although chemical reactions are taking place in the sensor layer, response times are fast and usually in the range of minutes. Very often, appropriate choice of catalysts or sample pH can improve the response behaviour.

Reactands for analytes such as amines, alcohols, aldehydes, saccharides, as well as carbon dioxide and sulfur dioxide will be reviewed.[2,3] Methods to enhance the sensitivity of the reactands as well as the operational and shelf life of the corresponding optical sensors are discussed. Potential new applications in the areas of molecular imprinting or nanoparticle preparation will be introduced.



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PLANAR WAVEGUIDE TECHNOLOGY FOR DNA AND PROTEIN MICROARRAYS

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There is a growing need in the drug development process for more sensitive microarrays and microarray systems to be able to detect low abundant genes and proteins, as well as for highly reproducible microarrays for quantitative results, complemented by robust and easy-to-use assay procedures.

To meet these requirements Zeptosens has developed a unified platform for ultra-sensitive detection of genes and proteins. A planar waveguide based optical fluorescence excitation scheme combined with application tailored proprietary surface chemistries proved to boost the sensitivity of DNA and protein microarrays to a few hundred analyte molecules per spot on an microarray. The Ta_2O_5 planar waveguide surface is coated with a dedicated polymer or a SAM on which the oligonucleotides or proteins are spotted. The evanescent field on the surface of the waveguide, created by the propagating light in the waveguide, efficiently and selectively excites the dye-labeled analyte molecules bound to the corresponding spots of the microarray. The strength of the fluorescence signal depends on the analyte concentration and it is collected in parallel for all spots of a microarray simultaneously with a CCD based high sensitivity camera system. The automated analysis platform allows the unattended measurement of up to 300 microarrays in one run.

Several factors contribute to the outstanding performance of this system. The evanescent field excitation, the measurement in solution and the surface chemistry optimized for low unspecific binding in the assay provide images with very low background. Both the measurement in a natural environment and the dedicated surface chemistry offer a high binding affinity of the analyte and capture molecules. With a proprietary flow cell system the sample consumption can be reduced to a minimum. And the ability to measure DNA microarrays without any signal amplification steps gives a straight picture of the true gene expression profile without the bias introduced by traditional biochemical amplification (PCR).

An outlook will be given on a new application of planar waveguides using two-photon fluorescence excitation of macroscopic areas via the evanescent field. Two-photon fluorescence excitation is possible not only at a single focal point but along the whole trace of the beam guided in the waveguide structure.

FLUORESCENT NANO-CRISTALS AS ULTRA-BRIGHT TIME RESOLVED SENSORS

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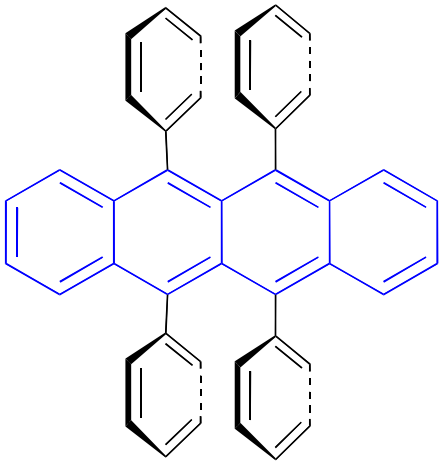
Molecular fluorescent probes are widely used for sensing ions and metabolites in cells or other complex environments.[1] But non-specific interactions of the dye with the other components of the analysed medium[2] turn this approach into a tricky trail. Imbedding fluorescent probes into polymers or gathering them into stiff crystals protect them from such artefacts. In addition, nanoparticles optimise the spatial resolution of optical detection: 20 nm. By putting more fluorophores in the voxel volume, we get more light for the same spatial resolution. But a multi-probe assembly gives an ensemble response. To see blinking behaviour and to have access to the dynamics of fluctuations around the equilibrium, we are looking for nano-sensors that will detect a single ion at their surface.

We use organic nano-crystals as fluorescent particles. They are produced by crystallisation in a sol-gel matrix. A mixture of the fluorescent dye with the silane precursors was spin-coated over microscope cover glasses. The quick evaporation of the solvent during the drying of the spin-coated layer induces a high super saturation and the nucleation and restricted growth of crystals with an average size of 100 nm.[3] The crystals are uniformly distributed as a monolayer in the thin sol-gel matrix.

Most fluorescent dyes do not emit light in the crystal due to the strong interaction between the π orbital of the neighbouring molecules. We have synthesised dyes surrounded by bulky substituants in order to reduce these interactions. To insure the sensibility of the core of the crystal to events occurring at their surface, we have chosen dyes with a good overlap between the absorption and fluorescence spectra and thus an efficient energy transfer among neighbouring molecules. Thus photons absorbed in the core of the crystal can diffuse to the surface where they can react with solutes. The balance between an efficient energy transfer and the absence of self-quenching has been tested among nine potential candidates. The most sensitive crystals are those made of Rubrene an aromatic hydrocarbon. In the case of rubrene neither oxygen nor photo-products act as inhibitor of the fluorescence in the crystal. Triplet states and some unknown impurities do kill that fluorescence. But nano-crystals have a lower probability to contain impurities and exhibit a fluorescence yield of 70%. We have some evidences that a single quencher can inhibit the fluorescence of 100 nm nanocrystals.



100 nm crystal can be easily seen one by one under a microscope. The sensitivity of the nano-crystals is shown is exemplified by the quenching of the fluorescence the adsorption of Cibachron Blue, an ATP analogue. We measure that each Cibachron Blue molecule quenches 1000 fluorescent dyes in their neighbourhood when they adsorb on the crystal surface.

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[1] O.S. Wolfbeis, *Chemical Sensing Using Indicator Dyes*, in "Optical Fiber Sensors", B. Culshaw and J. Dakin, Eds., Artech House, Boston-London, 1997, pp53-107.
[2] L. Choutteten, P. Denjean, R.B. Pansu, *Phys. Chem. Chem. Phys.* **1999**, *1(10)*, 2463.
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IN SITU FLUROESCENCE SENSORS FOR COASTAL OCEANS

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A fiber-optic fluorescence sensor system has been designed for *in situ* deployment in coastal oceans, estuaries, and harbors. The system provides a versatile delivery of UV light into the ocean environment from a variety of platforms from small day boats and barges to large oceanographic research vessels. While the current sensor system has been optimized for pyrene, a four-ring, polycondensed aromatic hydrocarbon and Environmental Protection Agency (EPA) priority pollutant, variable excitation wavelengths, full emission spectra and time-resolution capabilities, and a variety of probe tip geometries and applications may allow this versatile system to measure a variety of environmentally relevant compounds in real-time from ships or buoys. Thus this time-resolved laser-induced fluorescence (TR-LIF) system can provide high spatial and temporal resolution measurements of contaminants that were not previously possible.

The time-resolved fluorescence spectroscopy delivery system is composed of a nitrogen laser (λ=337 nm), an armored fiber optic (9 x 200 μm emission fused silica fibers around 1 x 400 μm excitation fiber) cable, a 0.25 meter spectrometer, and an intensified charge-couple device (ICCD) detector. A short optical fiber inserted into the diffuse laser beam acts as an optical trigger for a digital delay generator. The fluorescence signal is optically time-delayed (~180 ns for 50 meters of fiber) so that the ICCD can be gated ON and OFF at various time delays after the laser pulse. Fifty laser pulses are averaged to increase the signal to noise ratio. A full emission spectrum is obtained from the ICCD every 10 seconds or so.

The TR-LIF system has been deployed in Boston Harbor, Chesapeake Bay, San Diego Bay, San Francisco Bay, and most recently in the Hudson River estuary. The system has recently been incorporated into the Integrated Coastal Observation System (ICOS) which consists of an undulating vehicle (ECOShuttle, 0-50 meters depth), a hydrographic winch, and a mobile instrument laboratory. High resolution pyrene measurements from the Hudson River estuary and around Manhattan will be discussed as an application for *in situ* fiber optic sensors. Various laboratory sensors for pH, oxygen, trace metals, and endocrine disruptors have been attempted with varying success. The system has also been adopted for solid-phase fluorescence measurements for determining petroleum in contaminated marine sediments. We look forward to adopting emerging optical sensors into our deployment system to meet the need for environmental monitoring of impacted coastal oceans.

Acknowledgements. This work was made possible by close collaborations with Dr. Steven Rudnick and Dr. Bernie Gardner at the University of Massachusetts Boston and Eduardo Blanco at the University of Cadiz. This work was funded by the Office of Naval Research and MIT SeaGrant.

NEW MATERIALS FOR OPTICAL SENSING BASED ON MOLECULAR IMPRINTING

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Molecular recognition is the basis for most biological processes, such as ligand-receptor binding, substrate-enzyme reactions, translation and transcription of the genetic code and is therefore of interest for researchers trying to mimic this phenomena and use in practice e.g in separation and sensing. One of the most promising areas of biomimetics is *Molecular imprinting* which can be defined as process of template-induced formation of specific recognition sites (binding or catalytic) in a material where the template directs the positioning and orientation of the material's structural components by a self-assembling mechanism (Figure 1). Synthetic receptors prepared using molecular imprinting possess a unique combination of properties, such as high affinity, specificity, low price and robustness, which make them an attractive alternative to natural receptors, enzymes and antibodies used in biosensors.[1,2] The recent advances in rational analysis and predictive modelling permit to engineer new generation of imprinted polymers with superior characteristics and also tailor them for practical applications.[3]

This report gives a brief overview of the technology with specific emphasis on the mechanisms underlying the ability of imprinted polymers to perform highly selective functions such as recognition and transformation of a binding event into a detectable optical signal. The problems associated with the application of molecularly imprinted polymers (MIPs) in sensors are highlighted. Possible solutions to these problems are discussed and recommendations made about where commercial application of imprinted sensors seems most feasible in the near future.

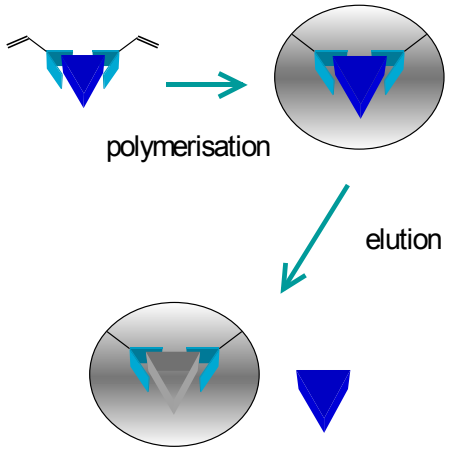

Figure 1. General scheme of molecular imprinting

Acknowledgements. The research was supported by EC (project No. IST-2001-33326). SAP acknowledges Royal Society-Wolfson Research Merit Award.

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BIOLOGICAL AND SCREENING APPLICATIONS OF THE OPTICAL OXYGEN SENSING

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Quenched-luminescence oxygen sensing is of high utility for life sciences, biotechnology and medicine. Several different approaches, sensing materials, measurement formats and complete platforms have been described in recent years. With different degree of success, they have been used in a number of applications.

Recently, our team has developed a new cell-respirometric screening technology (CRS) which is based on the use of the phosphorescent water-soluble oxygen probes.[1] CRS assays are carried out in a microtitter plate format using existing fluorescence and time-resolved fluorescence plate readers. This approach, sensing materials and new assay formats have been successfully applied to in number of applications and validated with practical targets, including different cell lines, drugs/toxicants, enzymes and their substrates, and whole organisms.[2] Further development and commercialization of this platform technology and particular applications are underway.

In this presentation, the principles of CRS technology and some representative applications will be presented, with emphasis on the following:

- Phosphorescent water-soluble oxygen probes for HTS applications
- Screening assays for cell viability and drug toxicity in standard microplates
- Low-volume, high-sensitivity platform(s) for CRS assays
- Assessment of drug metabolism and inhibition of cytochrome P450 enzymes
- Monitoring of respiration of small organisms and single cells

Critical comparison of these assays and screening systems with established assays and applications currently used in drug discovery, general cell biology and biotechnology will be given.

Acknowledgements. Financial support of this work by the Irish Research Foundation "Enterprise Ireland", grant ATRP/2002/105, and by the Irish Health Research Board, grant RP/106/202, is gratefully acknowledged.

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ORAL PRESENTATIONS

ABSTRACTS

SIGNAL ENHANCEMENT OF PROTEIN CHIPS

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Biochip technology has been revolutionizing most fields of molecular biology. One of the main challenges for further development of biochip technology is the strength of signals produced by probe-target interaction, especially for low probe and target concentrations.

In order to achieve higher signals and lower detection limits we aim at a) the optimization of chip process parameters, b) the use of gold particles as labels in surface enhanced fluorescence detection, and c) increased surface reflectivity by addition of SiO_2 and TiO_2 layers. Surface chemistries, such as porous poly(styrene-co-4-vinylbenzylthiocyanate) (PST-co-VBT)[1,2] and hydrogel-doped sol-gel which provide high immobilization capacity and appropriate density for protein attachment have been developed: PST-co-VBT was illuminated with 254 nm UV light to achieve the desired photoisomerization SCN-NCS and subsequent binding of proteins. Providing binding sites for both thiols and amines, PST-co-VBT was used for simultaneous immobilization of Au-labelled oligonucleotides and proteins. The length of the oligonucleotides, carrying the gold particles, was tuned to the distance at which resonance between the metal and the fluorescent target-protein occurred, resulting in signal enhancement. Silica-co-polymers of hydrogels were generated for protein immobilization to combine the mechanical stability of silanes with the biocompatibility of hydrogels.

Enhancement of the reflectivity by incorporation of high index refractive TiO_2-layers between two low-refractive polymer-layers or between the glass-substrate and the polymer-layer was achieved by hydrothermal synthesis of nanostructured TiO_2-particles or by Sol-Gel technology.[3] An increase of the reflection of light with desired wavelength was expected by coating a TiO_2-layer, which was characterised by a tuneable refractive index and a defined thickness, on the chip- substrate (glass) or by coating high refractive index TiO_2- layers between low refractive index layers (SiO_2 or polymer). These interference coatings were characterized by the refractive index of the layers, the film thickness and the stack design.

Process parameters that were optimized were print buffer, humidity control during arraying, slide agitation, drop size, probe and target concentration, etc. Important parameters of evaluation were immobilization capacity, signal-to-noise ratio and spot morphology. Quantitative quality control procedures were applied to compare the results from slides of different immobilization chemistry and detection technique, experiments and charges.

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PROTEIN IMMOBILIZATION FOR MULTI-CHANNEL BIOSENSORS

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The simultaneous detection of multiple analytes is an important consideration for the advancement of current biosensor technology. Currently, few sensor systems possess the capability to accurately and precisely detect multiple antigens. The work presented introduces a novel approach for the functionalization of sensor surfaces for multi-channel detection. In this work, a stable and versatile biosensor surface is prepared by site-directed immobilization of ssDNA-protein conjugates onto a complimentary ssDNA probe surface. The protein conjugates consist of an antibody chemically linked to a ssDNA target with a sequence complimentary to the surface bound ssDNA probes. The conjugates are immobilized on the surface via sequence specific hybridization, as shown in Figure 1. Two ssDNA platforms are used, both based on self-assembled monolayers (SAMs). The first probe surface used in this work is a mixed SAM consisting of ssDNA and oligo(ethylene glycol) (OEG) terminated thiols. The second platform ultilizes biotinylated ssDNA linked to a mixed SAM of biotinylated alkanethiol (BAT) and OEG via a streptavidin bridge.

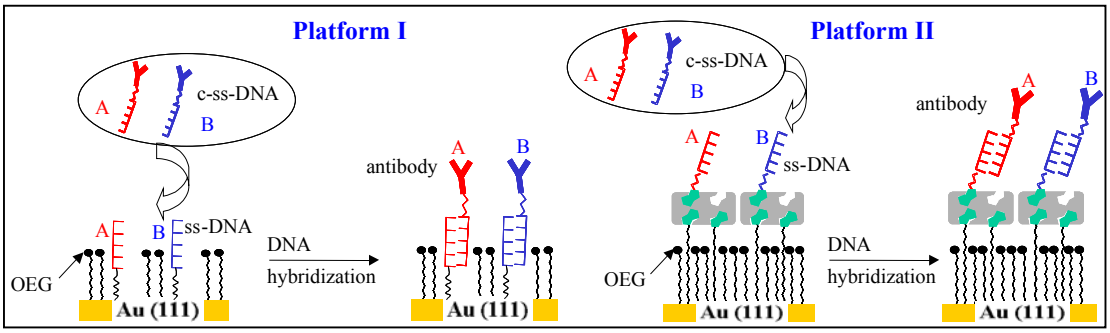

Figure 1. Immobilization of ssDNA-antibody conjugates.

Compared to standard antibody immobilization techniques, this approach offers many advantages. The exceptional specificity of DNA hybridization combined with the diversity of potential sequences makes this platform perfect for multi-channel sensors. Once a surface is patterned with the appropriate probe sequences, sequence specific hybridization will sort out the target conjugates and direct them to the appropriate spots on the surface. Furthermore, a ssDNA probe surface acts as a universal surface, with an infinite number of potential applications. The desired end use of the chip dictates its functionalization, not the other way around. Whereas a protein chip that has been pre-spotted with prescribed antibodies can only be used to detect the associated antigens, a ssDNA chip can be used to create a custom surface by immobilizing any desired protein conjugates. In addition, the DNA probe surfaces used in this work are very stable and well suited to recycling by dehybdridization of the conjugates from the surface bound probes.

In this work, we demonstrate the feasibility of using DNA directed immobilization to produce a multi-channel surface plasmon resonance (SPR) biosensor with high sensitivity and specificity. Extensive control experiments have been performed to check for non-specific binding and cross reactivity. Additionally, we have optimized both platforms for maximum antibody coverage and activity. Human Chorionic Gonadotropin (hCG), a 37 kDa hormone secreted during pregnancy, has been used to check the sensitivity of both platforms, with a measured lower detection limit of 0.1 ng mL^{-1}.

ANALYTICAL BIOSENSING BASED ON MEASUREMENT OF BIOMOLECULE CONFORMATION CHANGES USING SURFACE PLASMON RESONANCE

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A large number of biological molecules change conformation upon interaction with a specific substrate species. An obvious example is the change of conformation exhibited by haemoglobin upon binding of oxygen. In solution the changing conformation of biomolecules, particularly secondary structural conformations, can be measured using circular dichroism (CD), NMR and infrared (IR) spectroscopies. Whilst these spectroscopic techniques provide sensitive measurement of biomolecule secondary structure they are not particularly amenable for the development of sensing technologies based on analyte induced conformational changes.

Surface plasmon resonance (SPR) is a surface sensitive technique capable of measuring changes in refractive index (RI) which occur in proximity to the sensor interface. By depositing biomolecules onto the gold-coated sensor surface of an SPR instrument it is possible to measure changes of secondary structural conformation as a function of substrate concentration. The particular attraction of conformational change measurement by SPR is that the biological receptor need not have a chromophore or redox centre to report the analyte concentration. Thus, this transduction principle will significantly extend the range of biological receptors which may be used in the development of biosensing systems.

We have modified a number of biological molecules, including polypeptides, proteins and enzymes with *N*-succinimidyl 3-(2-pyridyldithiol) propionate to introduce disulfide moieties onto the molecule's surface. Such modification enables the formation of a self-assembled monolayer (SAM) of the biomolecule directly on the gold-coated sensor surface of an SPR instrument.

In our initial work, polypeptides were formulated as SAMs on SPR sensor surfaces and varying concentrations of alcohols were passed over the monolayer surface. For example, polylysine was deposited onto the sensor surface and varying ethanol concentrations (20-80% v/v) were used to change the secondary structure of the polypeptide. At high ethanol concentrations a dramatic increase in the SPR signal ($\Delta\theta$, m°) was observed.[1] Using IR and CD spectroscopies it was established that the high SPR signal occurred when the polypeptide was induced into the α-helical conformation. Similarly, the SPR signal was related to other secondary structures (including β-sheet and random configurations) of both polypeptides and the protein Concanavalin A. The intensity of the SPR signal being related to the RI of the secondary structural configuration of the biomolecule on the sensor surface.

Recently, we have further developed this sensing strategy by self-assembling urease onto SPR sensor surfaces in order to measure the changing conformational change of this enzyme as a function of the heavy metal cadmium. CD spectroscopy again showed that the urease enzyme changed conformation with varying cadmium concentrations. On the sensor surface, the SPR signal from the urease monolayer increased in intensity as a function of cadmium concentration in the range 0 – 300 mg L^{-1}. A linear relationship was observed between 0 – 10 mg L^{-1}. These data show that conformational changes of biomolecules can be measured by SPR and used to analytically determine the concentration of heavy metal ions without the need for activity assays. To our knowledge this is the first report of the direct determination of metal ions using a SPR based biosensor.

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PLASMONIC ENHANCEMENT OF FLUORESCENCE FOR SENSOR APPLICATIONS

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It is well established that the presence of metallic surfaces or particles in the vicinity of a fluorophore can dramatically increase the radiative decay rate, and consequently the quantum efficiency, of the fluorophore. This effect, which depends on parameters such as metal particle size and fluorophore-particle separation, is manifest as a substantial enhancement in fluorescence emission intensity. This presentation will focus on optimisation strategies to maximise the enhancement for important applications such as fluorescence-based biochip platforms.

Ordered arrays of metallic nano-islands were fabricated on a range of substrates by a process of natural lithography using monodisperse polystyrene nanospheres. The metal particle dimensions were tailored in order to match the plasmon resonance wavelength to the spectral absorption of the fluorophore. A range of fluorophores was investigated in order to elucidate the quantum efficiency enhancement. These included Cy5 dye, which is widely used in optical immunoassays and has a medium quantum efficiency (~0.3) and also a range of ruthenium dyes with quantum efficiencies from < 0.1 to ~ 0.6. A critical feature in the plasmonic interaction is the separation between fluorophore and metal-nanoparticle. The fluorophore-particle distance was tuned by depositing intermediate buffer layers of varying thickness on the metallic array, prior to depositing the fluorophore layer.

The morphology of the metallic arrays was investigated using SEM and AFM. Absorption and emission spectroscopies, as well as fluorescent decay time measurements, were used to elucidate the enhancement effect and its dependence on metal island morphology, fluorophore quantum efficiency and fluorophore-metal separation. By probing the fluorescent response as a function of these parameters, the nature of the interaction between metal and fluorophore was investigated. Results were correlated with existing theoretical models. The applicability of this important technique to sensor platforms, such as fluorescence-based biochips and microwell plates, will also be discussed.

ZWITTER-IONIC CONJUGATED POLYELECTROLYTES, NEW FLUORESCENT PROBES FOR THE RECORDING OF BIOSPECIFIC INTERACTIONS

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A chiral, 3-substituted polythiophene with a zwitter-ionic side chain (POWT) has previously been used for the detection of single nucleotide polymorphism (SNP) in DNA[1] and conformational alterations of synthetic peptides.[2] The detection method is based on non-covalent assembly of the zwitter-ionic polythiophene derivative and the receptor molecule of interest. Upon exposure to the analyte of interest, a conformational alteration of the polymer backbone and a change in the electronic properties of the polymer occurs, and these alterations can be detected by fluorescence from the polymer. Here we used the same principle to detect Ca^{2+}-activation of Calmodulin and the binding of Ca^{2+}-activated Calmodulin (CaM) to Calcineurin, a part of the cascade used in the intra-cellular signal pathway.

Introduction of Calmodulin will induce aggregation of the polymer chains and planarization of the polymer backbone, detected as a decrease of the intensity and a red shift of the fluorescence (figure 1a). Upon addition of Ca^{2+} the intensity of the emitted light is increased and blue shifted (Figure 1a). This is due to a separation of the polymer chains and twisting of the polymer backbone, induce by the conformational alterations of Calmodulin upon Ca^{2+}-binding. The CaM/POWT complex is then exposed to Calcineurin and the intensity of the emitted light is further increased and even more blue shifted. The ratio of the intensity of the emitted light at 540nm and 670 nm, 540/670nm, can be used as a measurement of the geometrical alteration of the polymer chains and has previously been used for the detection of biospecific interaction (1, 2). As shown in figure 1b, the ratio of the intensity of the emitted light at 540/670nm is increased upon addition of increasing amount of Calcineurin to the POWT/CaM complex.

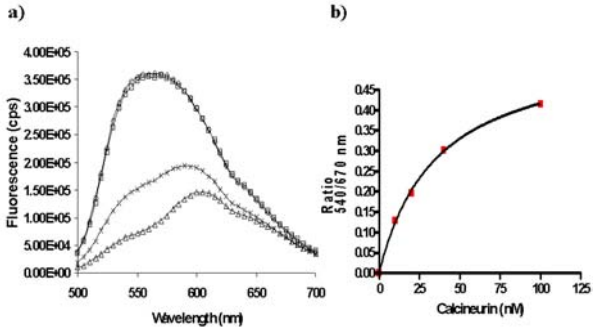

Figure 1.a) Emission spectra of POWT (□), POWT/Ca^{2+} (◊), POWT/CaM (Δ) and POWT/CaM/Ca^{2+} (×) complexes in 20 mM Tris-HCl buffer solutions. **b)** Fluorescence titration of POWT/CaM with calcineurin.

In conclusion, a novel methodology has been developed that allows fluorometric detection of a part of the cascade used in the Ca^{2+}/Calmodulin intra-cellular signal pathway. This rapid and selective method does not require any modification of the receptors or the analytes, and is based on electrostatic interactions between a zwitter-ionic polythiophene derivative and Calmodulin. We foresee that the present mechanism also may be used for detection of other biospecific interactions, and that the simplicity and the diversity of this methodology make it suitable for making inexpensive protein- and DNA-chips for rapid detection of biomolecular recognition.

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FLUORESCENCE DETECTION OF SMALL MOLECULES BY FUSED-RING HETEROCYCLES

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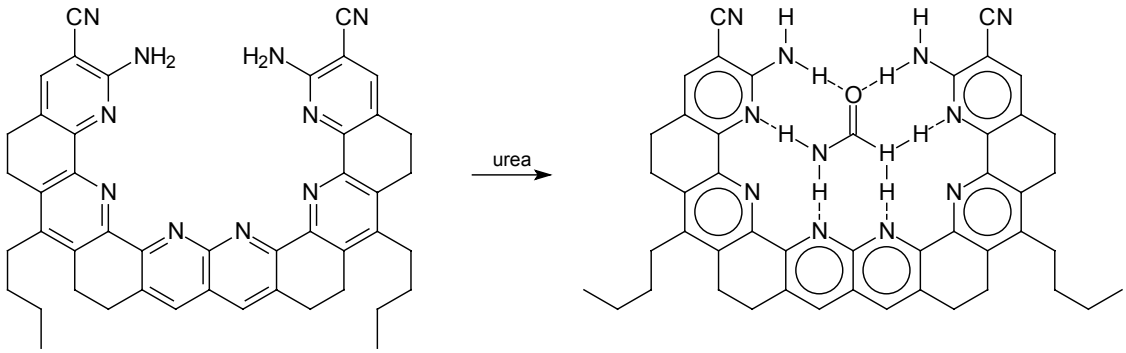
Optical sensors for organic analytes in solution require materials capable of binding the target molecule and selectively producing useful changes in light absorption or fluorescence. We are developing novel sensor materials based on the principles of hydrogen bonding, host preorganization and host-guest complementarity. Our artificial receptors generally consist of several six-membered rings fused together to form a molecular cleft lined with hydrogen-bonding sites. The guest analyte with complementary hydrogen-bond donor and acceptor groups binds to this cleft, and the binding event is signaled by a fluorophore that is an intrinsic part of the receptor structure. We have used this approach to make artificial receptors for creatinine,[1] urea[2] (see below), amino acids, monosaccharides,[3] and bicarbonate.



The receptor shown above was reported previously to bind urea with a modest (16 nm) bathochromic shift of the longest wavelength absorption band (372 nm).[2] We have now found that binding urea either in homogeneous solution or by extraction produces 50-96% quenching of the fluorescence emission (414 nm). Some new, structurally related receptors show similar fluorescence quenching effects upon binding urea. A novel approach to fluorescence detection of nerve toxins by means of fused-ring heterocycles is also presented.

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DEVELOPMENT OF DEEP SILICON HOLLOW WAVEGUIDES FOR OPTICAL SENSING

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Hollow fiber optics have been broadly used in medical and industrial applications where high-power laser guiding is required. As compared to fiber optic with glass core,[1] they present clear advantages, as could be the non–presence of Fresnel loss, a high transmission power and high mechanical and chemical durability according with the used material. Nevertheless, up to now, there has been low development of integrated optical devices based on hollow waveguides, with the exception of hexagonal-shaped hollow waveguides.[2] The combination of the features of hollow waveguides and the silicon micromechanization capability offers an outstanding flexibility for the development of integrated optical sensors, taking advantage of the high interaction efficiency between the guided beam and fluids or gases[2] under measurement. Moreover, the fabrication simplicity, the high hardness (when silicon is used), the low propagation loss and the avoid of cross-section polishing for reducing the insertion losses (when end-fire coupling is used) are only some of the most attractive properties for the development of low-cost, mass-production integrated optics devices based on hollow waveguides.

Simulations done with the Finite Difference Method (FDM) and the Finite Element Method (FEM) have allowed an optimization of the losses as a function of the geometrical parameters (depth and width) for a working wavelength of 633nm. Although silicon is absorbent for wavelengths smaller than 1.1µm, it can be clearly observed in Figure 1, how there is a decrease of the losses as the waveguide gets deeper, reaching values where the losses are clearly affordable and are even lower than other types of waveguides. This point is in agreement with the reduction of the number of reflections inside the waveguide. For the fabrication of silicon-based hollow waveguides, the most important technological step is the Deep Reactive Ion Etching (DRIE). It has been required to optimize the etching conditions so as to obtain perfectly vertical deep walls with roughness below the working wavelength. In Figure 2, a cross-section of several test structures for the fabrication of hollow waveguides are shown just after the DRIE process, where the previously mentioned verticality and low roughness are clearly observed.

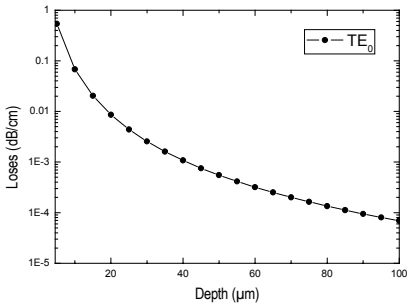

Figure 1. Losses of square silicon hollow waveguides vs depth

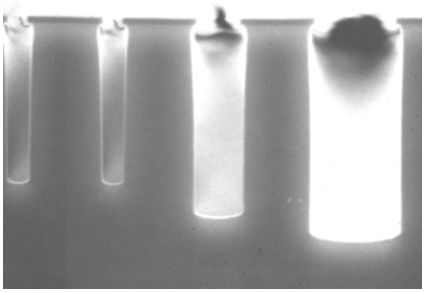

Figure 2. SEM picture of the hollow waveguides definition after DRIE technological step.

Preliminary measurements without the silicon-to-silicon fusion optimized have shown propagation losses close to 5dB/cm. At this moment, an optimization of the technology for obtaining these waveguides is under development. It is clear that once the Si-Si fusion and the wall roughness on the DRIE process are improved, a drastic reduction of the losses is expectable.

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DEEP PROBE OPTICAL WAVEGUIDE BIOSENSORS WITH REVERSE SYMMETRY DESIGN FOR MICRON SCALE BIOLOGICAL OBJECTS

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Optical evanescent wave sensors such as waveguide and surface plasmon resonance sensors have been used so far to detect biological materials at the surface of the sensor in aqueous cover media. These devices are based on the phenomenon that any change in the refractive index of the cover media shifts the effective refractive index of the surface mode. This change is detected through an evanescent optical field decaying exponentially from the surface of the sensor. Until recently, the penetration depth of this evanescent field was limited to 100-200nm. This reduced the sensitivity when aiming at refractive index changes far from the sensor surface, for example in the case of detection of bacteria or living cells that are 1-10 microns in size.

Recently a new type of waveguide design with the so-called reverse symmetry was suggested to overcome the fundamental limitations of the cover penetration depth.[1-3] In this design the refractive index of the waveguide substrate is lower than the refractive index of the aqueous cover media, i.e. lower than 1.33. With this new configuration the penetration depth can be tuned, in principle, up to infinity by simply choosing the right thickness of the waveguiding film.

In the present work a sensor with reverse symmetry is realized by depositing a thin polystyrene film onto nanoporous silica with a refractive index of 1.2. An embossed surface relief grating in the PS film is used as coupling element (see Figure 1). The presented sensor system is demonstrated for (i) refractometry of liquids, (ii) bacterial detection, and (iii) detection of the attachment and spreading of mammalian cells. Apart from a high sensitivity, the present sensor exhibit several unusual sensor effects, such as a strong change in the amplitude of the incoupling peaks and the appearance of side-peaks when the sensor is used for monitoring cell spreading.

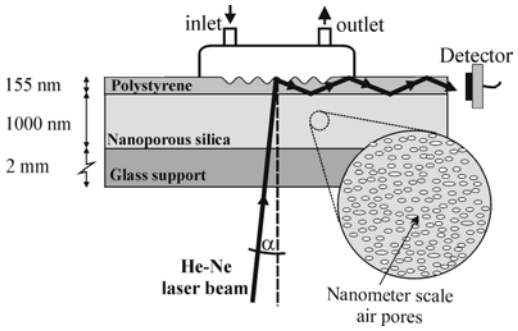

Figure 1. Deep probe sensor design.

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ULTRA-THIN FREESTANDING Si_3N_4 MEMBRANE WAVEGUIDES FOR APPLICATION IN EVANESCENT FIELD SENSING OF MEMS MOVEMENTS

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In many MEMS structures it is required to monitor some characteristics of movements of subparts of the system either during production or during operation. For probing these characteristics during in situ operation integrated optical devices are expected to offer good perspectives. This probing can be based on the phenomenon, that characteristic properties of a guided mode, which propagates through an integrated optical waveguide channel, are influenced by the penetration of bodies into their evanescent field.[1,2] However, in common integrated optical waveguides the decay length of these exponentially decaying evanescent fields is in the range 0.2- 1 micrometre only, requiring a very intimate contact of the MEMS and the optical read out head. Here we propose a waveguiding structure consisting of freestanding Si_3N_4 membranes in air showing decay lengths up to 10 micrometre. Movement of the mechanical part will result into a change of the real part (N'_{eff}) and/or the imaginary part (N''_{eff}) of the effective index of the mode. For conversion of these changes in an electrical signal these sensing structures have to incorporated into an optical read-out system, e.g. a Mach-Zehnder interferometer[3] (for read-out of N'_{eff}) or an attenuation measuring circuit (for read-out of N''_{eff}).

Several types of membrane structures have been fabricated using standard deposition, lithography and etching techniques. In Figure 1 a membrane (labelled 'A') is shown that is all-sided clamped via tapered sections ('B') on the common waveguide structure ('C'). The Si3N4 waveguiding core is thinned from 300 nm to 100 nm. On this waveguiding core ridge-type channel waveguides are present. The realized structures show smooth surfaces without any curtaining. Tensile stresses are sufficiently low to avoid also any cracking. A yield over 90% can easily be obtained.

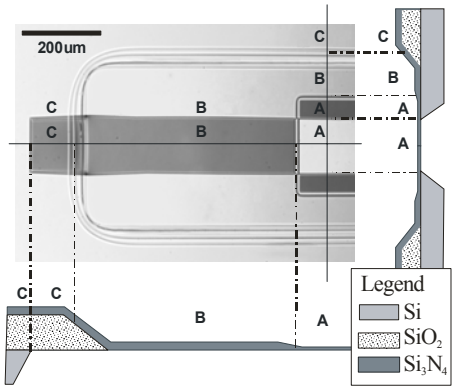

Figure 1. Micrograph (with cross-sections) of a realised Si_3N_4 membrane waveguide structure

Insertion loss measurements at a wavelength of 1550 nm were carried out on straight ridge-type channel waveguides on top of free standing Si_3N_4 membranes and on corresponding common waveguide structures. From experimental data the additional attenuation induced by the tapering and membrane formation has been calculated to be 2.6 dB for TE polarisation and 7.8 dB for TM polarization at 1550 nm.

Complete sensing systems have been produced and experimental results will be presented during the conference.

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APPLICATION-TAILORED INTEGRATED OPTICAL CHIPS FOR LABEL-FREE (BIO-)CHEMICAL SENSING

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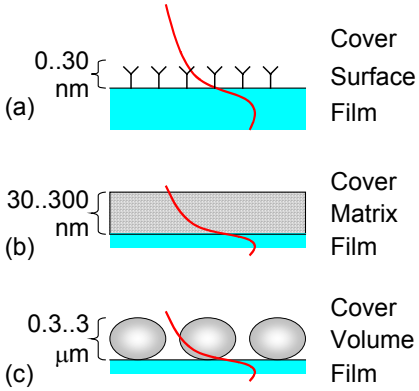
Label-free sensing is an important method for many (bio-)chemical applications in fields such as biotechnology, medicine, pharma, ecology and food quality control. This contribution deals with a sub-class of optical techniques, namely the so-called evanescent-wave sensing. Among these techniques, two have gained a major relevance in recent years. The first one makes use of the well-known surface plasmon resonance (SPR) phenomenon, while the second one uses dielectric waveguides forming an integrated optical (IO) chip for creating the evanescent wave that is used to probe the species to be detected in an analyte solution.[1]

While the driving force originally was the detection of molecular species being adsorbed to a thin recognition layer immobilized directly on the sensor chips' surface, a current trend is to extend the application range to the detection of very large molecules or even cells.[2] An other application where the task is not necessarily to define a small "probe region" near the surface, but to adapt the properties of the evanescent field to the "region of interest", as for instance in the case where a "matrix layer" of several hundred nm thickness is chosen to capture molecules and other species (e.g. ions) to be detected. Examples are chemo-optically sensitive membranes ("optrodes"), swellable polymers and hydrogel-based sensing layers.[3]

In this contribution we will present results of a numerical study that show that there exists a great potential and flexibility to tailor the properties of the evanescent field to different applications. This fact results from the many degrees of freedom that can be used for the IO chip design, in contrast to other technologies such as SPR where the material's (metal) properties limit the range of choices. The IO chips can not only be designed for achieving maximum sensitivity, but also to obtain an optimum "signal-to-background ratio" in the sense of maximizing the sensitivity in the sensing region of interest, but minimizing it in the regions of non-interest, e.g. in the bulk analyte volume.
The results will help to fully exploit the potential of IO sensor chips for future practical applications.



The following applications/configurations will explicitely be considered and discussed:
(a) ***"2D sensing layers"*** (e.g. biosensors (proteomics)) with adsorption processes occurring within some 10 nm from the chip's surface in a "surface sensing region" (see Figure a);
(b) ***"3D sensing layers"*** (e.g. hydrogel-based layers, viruses) with processes to be monitored within some 100 nm from the chip's surface in a "matrix sensing region" (see Figure b);
(c) ***"micro-volume sensing"*** (e.g. "optrodes", cells ("cellomics"), bulk micro-refractometry) with processes to be monitored within probe volumes extending to some 1000 nm from the chip's surface (see Figure c).

Results will be presented for a single waveguiding film surrounded by additional layers. All configurations will be based on using purely dielectric materials. It will also be shown that the findings will enable one to optimize grating-based sensors as well as other types, for example integrated-optical interferometers.

Acknowledgements. We gratefully thank Guy Voirin for helpful discussions.

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LABEL FREE DETECTION OF PROTEINS

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Fluorescence detection has become of great importance in various fields of biosensors in particular for DNA analysis in chip format of for the investigation of biomolecules in microplates. All these methods rely on the labelling of the molecule to be detected with a fluorescence dye. Chip based fluorescence measurement techniques have become a standard in DNA-analysis. This is due to the fact that the method is fast, highly parallel and requires only tiny amounts of the sample. The detection of proteins by use of fluorescence markers, however, has not yet been established in the same way since fluorescence labelling of proteins is much more complicated, often alters the specific function of the protein e.g. by changing the three dimensional structure of the protein and therewith the biological activity.[1]

Motivated by the deficiencies of fluorescence-labelling of proteins on the one hand and the potential advantages of fluorescence based methods on the other hand, we are presently investigating the detection of the protein auto-fluorescence in the UV spectral range for label free protein detection. The focus of our work lies in chip based methods. These are applicable in diagnosis, for receptor-ligand investigations or for the measurement of expression profiles.[2] The auto-fluorescence of proteins is mainly based on the fluorescence of the amino acids tryptophan and tyrosine, whereas the fluorescence intensity of tyrosine is about 17,8% of that of tryptophan. It was reported that a database research among $\approx 10^6$ proteins with masses larger than 10 kDa yielded that 99.5% of these proteins contain at least one of these two aromatic amino acids.[3]

We developed a protein biochip reader, which detects the auto-fluorescence of proteins. The fluorescence is excited at 280nm by the narrow bandwidth filtered emission of an HgXe arc lamp. A second interference filter at 340nm is used for filtering the fluorescence light. A high-resolution UV-enhanced CCD camera finally detects the radiation. Various chip substrates were tested with regard to background fluorescence. The system has been characterised with the well known protein streptavidin (Figures 1, 2). A direct comparison to the fluorescence of a common dye was possible by the investigation of fluorescence labelled streptavidin. We furthermore investigated binding reactions of antibody-antigen pairs and applied the system to the label free readout of electrophoresis gels (Figure 3). As bleaching is negligible at intensities in the 1mW/cm² range, longer exposure times allow for a more sensitive detection. The detection limit for streptavidin (24 tryptophan molecules) currently lies at 4000 Molecules/µm² (400pg/mm²).

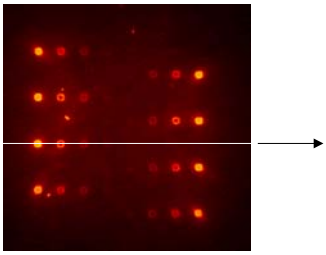

Figure 1. Array of different streptavidin concentrations on a silicon substrate. λ_{ex} = 280 nm, λ_{em} = 340 nm

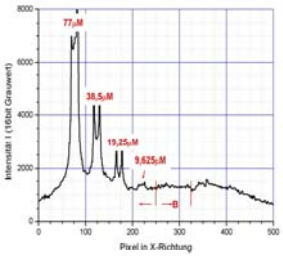

Figure 2. A line scan yields the linear dependence of the fluorescence signal upon protein concentration

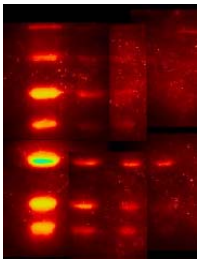

Figure 3. The auto-fluorescence of an electrophoresis gel containing 7 different marker proteins

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AN OPTICAL FIBRE-BASED SYSTEM THAT MEASURES THE QUALITY AND TEMPERATURE OF FOOD IN A FULL-SCALE PRODUCTION ENVIRONMENT

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[b]*City University, London (United Kingdom)*
[c]*Food Design Applications Ltd., Newtown, Limerick (Ireland)*

An Optical fibre based sensor system has been developed for the purpose of examining the core temperature and colour of food products online as they cook in a large-scale industrial oven. It is unacceptable for errors to occur in the cooking of large batches of various food types in industrial ovens of lengths up to 20 metres, where conveyor belts will be fully loaded with food for maximum efficiency and the cost of an error in one batch would be intolerable for the producer. There is a need for monitoring the food as it passes through the oven and determining that all the processing parameters are correct, the most important of these being the food's core temperature and the colour of the food (both internal and external).

The environment in these ovens is very hostile to electronics as the temperatures are very high (avg. 200^{o}C). The atmosphere is also very moist and saline. Space within the oven is also limited. These constraints make it difficult to place machine vision system and thermocouple systems within the oven. Another factor against machine vision systems in quite simply that they do not allow you to measure colour inside the food without cutting it open, which is not an option. This is where Otical Fibres offer a solution in that they can operate within the harsh environment, allowing all electronics to be placed outside the oven and they permit unobtrusive internal readings due to there size (the internal colour probe is only 2.5mm diameter). All these measurements can be taken online while the oven is operating.

The colour is measured using a reflective probe and recording the light reflected in the visible spectrum, which is representative of the colour. Illumination of the food is performed with 6 fibres attached to a tungsten halogen white light source. One fibre returns the reflected light to an Ocean Optics s2000 spectrometer, controlled by a host P.C.. The spectra are then saved for subsequent analysis using the Stuttgart Neural Networks Simulator (SNNS) the Neural Network Software.[1]. Examples of the use of artificial intelligence for decision-making in the food industry are plentiful[2] The optical temperature sensor scheme used in the work is based upon the results of previous research by some of the authors. It involves the monitoring of the fluorescence decay time of a rare earth material and correlating this with temperature, as discussed by Grattan and Zhang.[3] Given the temperature range under consideration in this work, it was decided to base the optical system upon the use of a thulium-doped garnet (Tm: YAG) coupled with silicon optical fibres. The fluorescent medium is excited by light from a LD light source operating at 785 nm, coupled to the active material through a silica fibre bundle. The received fluorescence emission is detected with an extended wavelength InGaAs photodiode and the lifetime data extracted using a PLD (phase locked detection) scheme reported by some of the authors elsewhere.

A broad range of products are examined i.e. fresh minced beef burgers, inside and outside, Chicken en croute, the colour of the meat inside and the pastry outside. Successful results were obtained by interrogating the spectra from the food samples with Neural Network Pattern Recognition, using a feed forward network with backpropagation, as it's learning function to determine the difference between raw, light, correct, dark and burnt stages of cooking of the various products. The core temperature was measured using an optical fibre probe which achieved an accuracy of ± 1^{o}C. These measurements are novel in the area of mass food production and it is intended to integrate the two internal measurements (Colour and Temperature) into a single point probe for future developments of this system.

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NANOSENSORS FOR MONITORING MOLECULAR SIGNALING PATHWAYS IN A SINGLE LIVING CELL

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Monitoring cellular signaling pathways inside single intact cells is becoming increasingly important fundamentally because cells in a population respond asynchronously to external stimuli. There is a need to further our understanding of basic cellular signaling processes associated with disease in order to obtain new information that is not available from population-averaged cellular measurements. A further advantage of single-cell assay is to understand the exact pathways by which signaling pathways move through the architecture of the cell. In addition, many cellular signaling pathways act on timescales of a few seconds and there is critical need for single-cell measurement techniques with similar time resolution. Not only is there a need to temporally resolve such measurements, there is also a need to spatially resolve them. For these reasons, progress in cellular physiology requires new measurement strategies at the nanoscale level applied to individual cells with great temporal and spatial resolution.

For this purpose, we have developed a new generation of nanosensors and nanoprobes combining bio-recognition and nanotechnology for in vivo monitoring of biochemical processes in a living cell.[1,2] This technique could provide unprecedented insights into intact cell function, allowing, for the first time, studies of molecular functions in the context of the functional cell architecture in an integrated system approach. This presentation describes two areas of research related to the development of nanoprobes and nanosensors for single-cell analysis and imaging: (1) nanoprobes for surface-enhanced Raman scattering (SERS) biochemical analysis, and (2) nanosensors for in vivo analysis of a single cell.

The first research approach involves the development of metallic nanostructures that can produce the SERS effect for ultrasensitive biochemical analysis. The intensity of the normally weak Raman scattering process is increased by factors as large as 10^6-10^{11} for compounds adsorbed onto a SERS substrate, allowing for ultra trace-level detection. These substrates can generally be fabricated as silver-coated nanoprobes (300-nm diameter) that are capable of enhancing the Raman signal of adsorbed compounds (Figure 1). The development of a SERS gene probe technology based on the solid nanostructures is described. Sensitive and selective detection of HIV DNA and BRCA1 breast cancer gene using the SERS technology is discussed.[3]

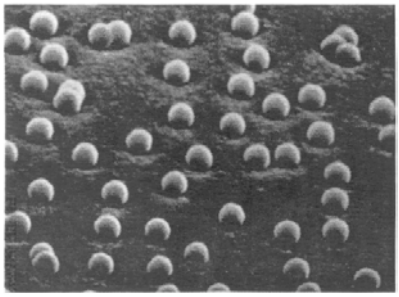

Figure 1. Silver-Coated SERS Nanoprobes

Recent advances in nanotechnology leading to the development of optical fibers with nanoscale dimensions have opened new horizons for intracellular measurements in living cells. For example, an antibody-based nanosensor was developed to monitor benzopyrene tetrol (BPT), a DNA-adduct biomarker of human exposure to the carcinogen benzo[a]pyrene. Interrogation of single cells for the presence of BPT was carried out using antibody nanoprobes for excitation and a photometric system for fluorescence signal detection. Figure 2 shows a photograph of an antibody-based nanoprobe used to measure the presence of BPT inside a single cell.

We have recently demonstrated the application and utility of nanosensors for monitoring the onset of the mitochondrial pathway of apoptosis in a single living cell by detecting enzymatic activities of caspase-9.[4] The tetrapeptide Leucine-GlutamicAcid-Histidine-AsparticAcid (LEHD) is a caspase-9 substrate. The fluorescent molecule 7-amino-4-methyl coumarin (AMC) has an emission maximum at 460 nm. Minimally invasive analysis of single live MCF-7 cells for caspase-9 activity was demonstrated using the optical nanosensor which employed a modification of an immunochemical assay format for the immobilization of LEHD-AMC. The substrate LEHD-AMC was cleaved by caspase-9 and the released

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AMC molecules were excited and emitted a fluorescence signal. By monitoring the changes in fluorescence signals, caspase-9 activity triggered by apoptosis within a single living MCF-7 cell was detected.

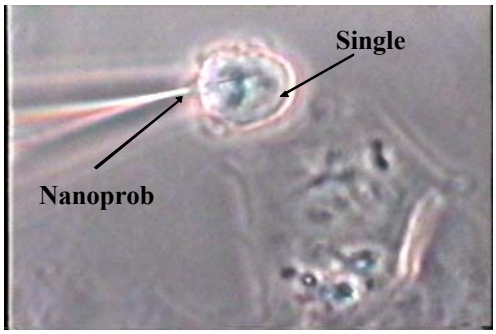

Figure 2. Photograph of Single-Cell Sensing Using the Nano-Biosensor.

These studies demonstrate the first applications of nano-biosensors for measurements of molecular processes inside a single cell. These nanodevices could also be used to develop advanced biosensing and bioimaging systems in order to study *in situ* intracellular signaling processes and to study gene expression and molecular processes inside individual living cells. Such nanoprobes open new horizons to a host of applications in biotechnology, molecular biology research, medical diagnostics and investigations of the therapeutic action of pharmaceutical agents in single living cells.

DETERMINATION OF CONCENTRATION OF LIVING IMMOBILIZED CELLS BY FLUORESCENCE SPECTROSCOPY

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The content of biomass and concentrations of dead and living cells are the most important parameters in evaluation of biocatalysts with immobilized cells. Fluorescence spectroscopy is one of suitable methods for direct monitoring of immobilized cells because it does not require (in certain arrangement) transparent environment like e.g. absorbance measurements. The aim of this work was to develop method allowing measurements of concentrations of living immobilized cells using only intrinsic fluorescence of biogenic fluorophores in cells.

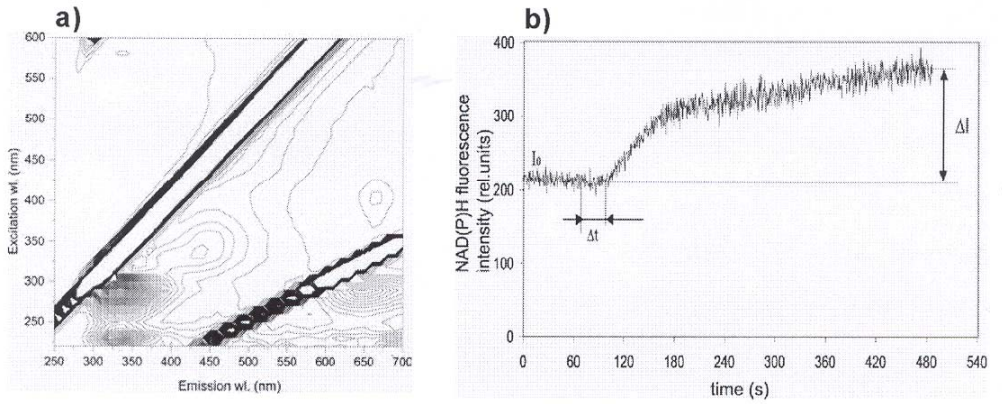
Saccharomyces cerevisiae yeast cells were chosen for experiments because their metabolism is well known and they do not require special conditions for cultivation and they have relatively high content of nicotinamide adenine dinucleotide coenzymes (NAD(p)H). Yeast cells were immobilized into alginate and into mix of alginate with prepolymerized tetramethoxysilane (TMOS). These biocomposites were placed into flow-through cuvette in front-face arrangement and perfused with aerated mineral medium. After certain time aerated medium was exchanged for medium without dissolved oxygen and changes in fluorescence were monitored. Static and dynamic approaches to measurements were used. During static measurements whole 2-dimmensional fluorescence spectra (see Figure la) were scanned and correlated with concentrations of immobilized cells. Dynamic measurements were based on measurement of changes in NAD(p)H fluorescence intensity. Following parameters were used to correlate these changes with concentrations of living immobilized cells (see Figure l b):

Δt - time delay between change of conditions from aerobic to anaerobic and the change of NAD(P)H fluorescence

Δt - the difference between NAD(p)H fluorescence intensity in anaerobic and aerobic state ratio $\Delta I/I_o$ where Io is intensity of NAD(p)H fluorescence in aerobic state

Static measurements showed that the fluorescence of NAD(P)H well represented the concentration of living immobilized cells in alginate. However, Done of intracellular fluorophores was found suitable for determination of total immobilized biomass or for determination of concentration of living cells immobilized in mixed alginate- TMOS matrix. Dynamic measurements gave good correlations between concentration of living cells and changes in NAD(P)H fluorescence intensity for both the sole alginate and mixed alginate- TMOS immobilization matrices. In case of mixed alginate- TMOS matrix the ratio $\Delta I/I_o$ well represented the concentration of living cells. Static measurements were found suitable for determination of cells immobilized into well defined and optically homogenous matrices, while dynamic measurements overcome the problems caused by more complex matrices.

Figure l: a) 2-dimensional fluorescence spectrum, **b)** parameters of changes in NAD(P)H fluorescence intensity.



ARRAY BIOSENSOR FOR FOOD SAFETY

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The food industry needs a device which can undertake rapid detection of pathogens and toxins at various stages during food processing and marketing with minimal sample preparation. A miniaturized, fully automated biosensor is described which weighs only 5.5 Kg and is designed for field use. Using fluorescence-based assays, the array biosensor is able to detect multiple targets in multiple samples simultaneously.[1,2,3] The array biosensor has demonstrated its ability to detect Staphylococcal enterotoxin B (SEB), *Salmonella, Campylobacter, E. coli* 0157.H7, *Shigella* and the mycotoxins fumonisin, ochratoxin, and deoxynivalenol (DON). Assays for the allergen ovalbumin have also been performed with pg/mL sensitivity. Analyses have been performed in buffer and in complex food matrices with comparable detection limits. A number of other assays are currently under development including biohazards such as aflatoxins, *Staphylococcus aureus* and *Listeria*.

Acknowledgements. This word was supported by the US Food and Drug Administration, the US Department of Agriculture, and the US Department of Defense.

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MULTI-ANALYTE OPTICAL SENSOR CHIP

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The major trends driving optical chemical sensor technology are miniaturization and multi-parameter functionality on a single platform (so-called multi-analyte sensing). A Multi-Analyte Sensor Chip device based on miniature waveguide structures, sol-gel materials and compact optoelectronic components has been developed. The application for this system offers significant potential for fabricating key elements of micro-total-analysis (Lab-on-a-chip) devices.

In this work, two approaches have been used in the fabrication of the miniature waveguide structures. In the first, sol-gel waveguides were fabricated using UV curable sol-gel and standard photolithography techniques. The second approach involves the fabrication of plastic waveguides by micro-injection moulding poly(methyl methacrylate), PMMA. A series of these waveguides were used to form the platform for the multi-analyte sensor chip.

Sensing films for both oxygen and carbon dioxide have been developed. These sensor films consist of a fluorescent indicator dye entrapped in a porous sol-gel matrix. The analyte diffuses through the porous matrix and reacts with the indicator causing changes in the detected fluorescence. A ruthenium dye complex is used as indicator for the oxygen sensor, and for the carbon dioxide sensor, a pH indicator, hydroxpyrene trisulphonate (HPTS) is used. The reaction between the dye and the analyte is completely reversible with no consumption of the analyte gas and no degradation of the signal after detection of different concentrations of the analyte. The properties of the sensor films were tailored to have optimum sensitivity in the concentration ranges of interest and their surface characteristics were altered to optimise adhesion to the waveguide structures.

These sensing films were deposited on top of the waveguides using stamp printing and pin printing. A single blue LED is used as excitation source for both the oxygen and carbon dioxide sensors. The fluorescence was detected at the end face of the waveguides using a linear detector array, enabling the output from all the waveguides to be detected simultaneously.

The simultaneous detection of several analytes is a major requirement for fields such as food and environmental quality control as well as biomedical diagnostics. Sensor films for humidity and temperature are currently under development and this multi-analyte chip device can be expanded to measure a wide range of analytes for many applications among which are condition control and blood gas monitoring.

SINGLE MOLECULE SURFACE REACTIONS BY CONFOCAL TIRF MICROSCOPY

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We have developed a confocal total-internal-reflection fluorescence (TIRF) microscope that generates a detection volume for analyte molecules of less than 5 aL (5×10^{-18} L) at a water-glass interface.[1,2,3] Compared to conventional confocal microscopy this represents a reduction of almost two orders of magnitude, which is important in order to isolate individual molecules at high analyte concentrations, where many biologically relevant processes occur. Diffraction-limited supercritical focusing and fluorescence collection is accomplished by a parabolic mirror objective. The system delivers TIRF images with excellent spatial resolution and detects single molecules with high signal-to-background ratio. Consequently the system combines surface biosensing and high resolution microscopy. The scheme and performance of the instrument is characterized in Figure 1.

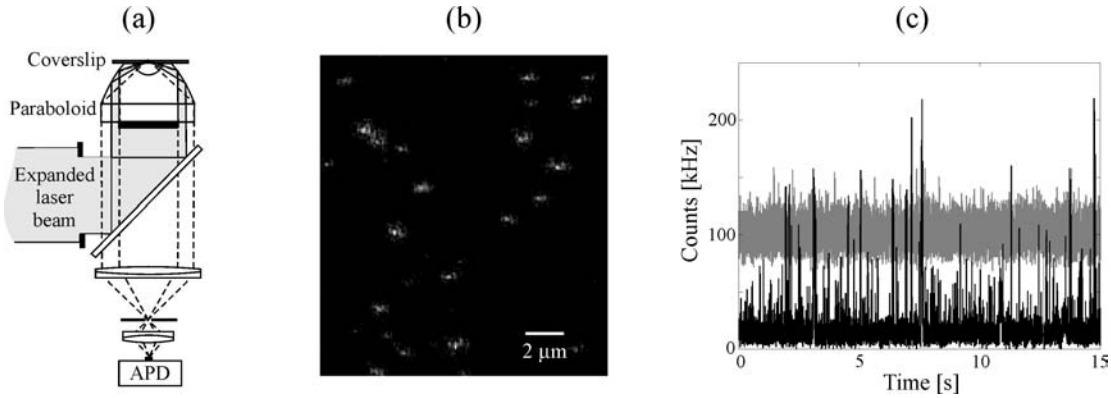

Figure 1 (a) Scheme of the confocal TIRF microscope. (b) Image of point sources by scanning the surface. (c) Tracks of the fluorescence intensity of dye molecules (EVOblue30, conc. 0.5 nM, black curve) on a coverlip showing non-specific surface adsorption and on a coverslip with strongly reduced adhesion properties (dye conc. increased to 100 nM, gray curve)

The possibility to detect single molecules at high analyte concentrations is of special importance for the study of enzymatic reactions. DNA polymerases require substrate concentrations in micromolar region in order to work efficiently. In ensemble measurements on a biosensor[4] it has been demontrated that enzymatic DNA double strand synthesis of surface-immobilized single DNA strands leads to a high incorporation yield of dye labeled nucleotides.[5] This is an important precondition towards single molecule DNA sequencing.

A major obstacle for the observation of biological processes at high concentrations on the level of single molecules is the non-specific interaction of analyte molecules with the surface. The fluorescence signal generated by adsorbing molecules is hardly distinguishable from enzymatic events. In the present work we report on our current progress at the reduction of this background and demonstrate the detection of individual double stranded DNA. Further we present first results in applying the confocal TIRF microscope in fluorescence correlation spectroscopy and fluorescence lifetime imaging.

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NINE YEARS AND 30+ MILLION SENSORS LATER: LESSONS LEARNED SCALING OPTODES FROM LAB TO PRODUCTION

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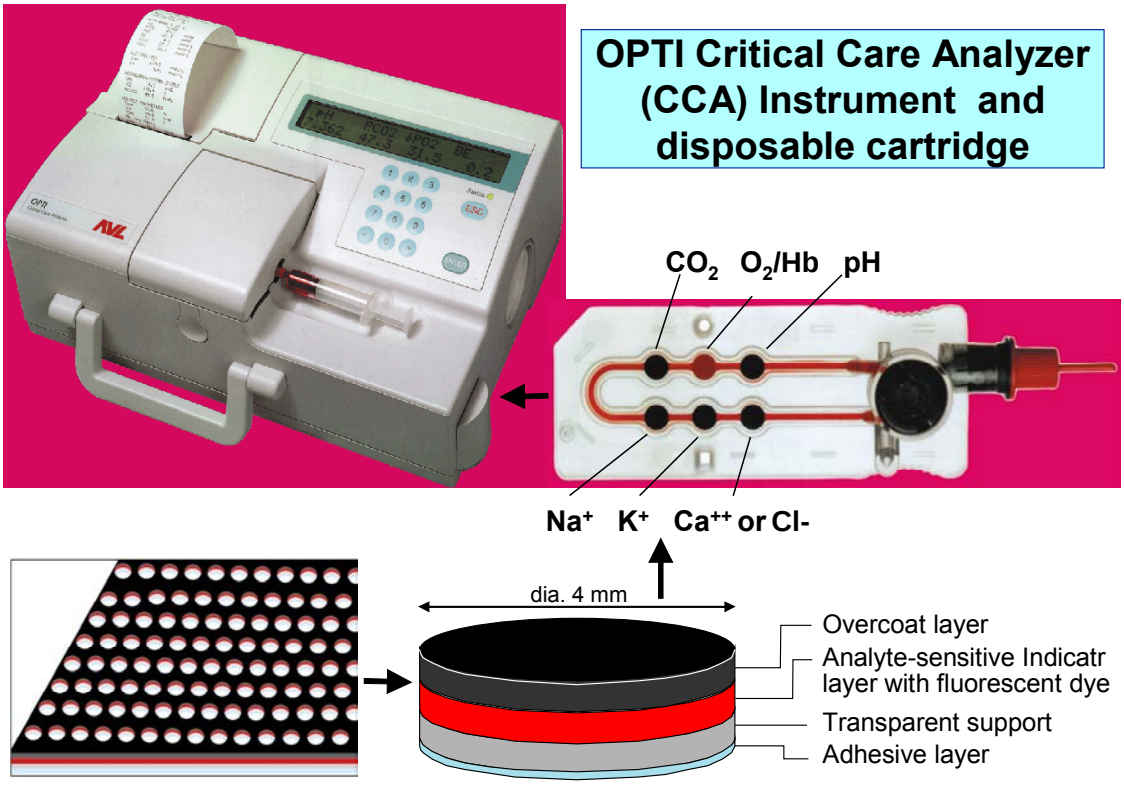
There are very few optode-based products that have emerged from the lab into large-scale production. To those of us working in the field of optodes this may seem puzzling—after all, optical sensing technology can be very attractive with its simple reagentless and connectorless elegance and flexibility, be it in a planar configuration or dipped at the end of a fiber-optic. In principle such sensors may be designed to be very simple and inexpensive to manufacture, and are quite insensitive to small variations in sensor geometry and interrogation method, as well as robust to surface fouling. This is what attracted the small Graz-based blood-gas company ***AVL Medical*** to apply this technology to the measurement of blood analytes in the late 1980's, (and later helped attract the attention of the not-so-small diagnostics company ***Roche*** to acquire ***AVL Medical*** in the year 2000). With the help of many talented scientists and engineers, AVL and Roche scaled-up the technology and in 1993 introduced a product line called "OPTI", consisting of a lunchbox-sized instrument that runs single-use disposable cassettes containing 3-6 fluorescent sensors. In this system approximately 100 microliters of whole blood is automatically aspirated into a cassette where up to eight analytes are measured in two minutes, with laboratory-grade precision, e.g. pH±0.005 and [Na+]±0.4%. Looking back today, after nine years and 30+ million sensors, we have learned a few generalizable lessons after making more than a few mistakes with the scale-up. This presentation will give a few multidisciplinary examples of unexpected or underestimated problems encountered during product scale-up which may be pertinent to future technologies and products ready to emerge from the lab.



Acknowledgements. The author would like to acknowledge the many dedicated and talented scientists and engineers who worked on the OPTI technology/system, both within the firms AVL, Roche, and Osmetech, as well as the talented outside consultants and academic researchers who initially showed us the way.

FROM LAB TO MARKET: DESIGN AND PERFORMANCE OF COMMERCIAL OXYGEN AND pH FIBEROPTIC SENSORS

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The discovery that the fluorescence of some Ruthenium complexes (as well as of complexes of Pt and other elements) is quenched by oxygen, is some 40 years old.

Why did it take so long to convert these discoveries into viable products?

The first commercial fiber optic oxygen probes were developed in the 70's and 80's for medical monitoring applications. Only after key patents expired – and under pressure from even newer technologies in the medical field – a few firms have dared enter the laboratory, environmental, and industrial market with turnkey systems of acceptable (sometimes excellent) precision, reliability, and durability.

Much work is needed to convert a laboratory observation into a prototype, and even more to convert a prototype into a viable product. In the case of optical chemical sensors, enabling technologies, beside sensor chemistry, have included optical components (fibers, filters), sources and detectors, electronics, and data processing.

The evolution of the design of Ocean Optics oxygen and pH fiberoptical sensor systems will be described in technical detail as an example to illustrate how many problems had to be solved to reliably produce instruments that satisfy the customers.

Fiberoptic oxygen sensors open new markets in application where classical electrochemical technologies are not applicable and where traditional laboratory methods (such as GC) are too cumbersome and expensive

Large corporations have invested heavily into optical sensor development, and have produced substantial IP in the form of patents and in same cases usable, inexpensive components; but they are reticent – and not optimally suited either – to develop commercial and customized solutions.

A market divided in many niches presents excellent opportunities for OEMs (Original Equipment Manufacturers) knowledgeable of their customers and capable of successfully integrating the different components of a viable product.

OPTOSEN®: HIGH RESOLUTION MULTICHANNEL TIME-RESOLVED FLUOROMETER

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OPTOSEN® system is a 4-channel time-resolved fluorometer introduced by INTERLAB IEC in 2001. This phase-sensitive fluorometer presents 4 independent optical channels of measurement, which may be employed together to carry out the measurement of a multisensed parameter or several different parameters at the same time. Depending on the application or the sample, the user can configure the working method by selecting the excitation LED intensity, the photodetector gain and the modulation frequency of the excitation source (between 20–320 kHz). Moreover, the OPTOSEN® system shows fluorescence intensity measurements not affected by the ambient light, which is a perfect complement for measuring the emission phase shift.

The emission lifetime of the luminescent sample or sensitive head is obtained from the phase shift measured by the instrument. The OPTOSEN® shows a dynamic range from 70 ns to 20 μs, offering a 10 ns resolution. Besides, this system includes analog inputs, which allow their use as data logger for external transmitters such as temperature sensors among other posibilities. In addition, thanks to the analog and digital outputs, the OPTOSEN® allows device activating or controlling while measuring. This electronic system is connected to a PC which performs the configuration and interface functions as well as the data processing. The small outer dimensions and robust box, make it ready for indoor and outdoor applications.

Figure 1. Different photographs of the OPTOSEN® system.

In a long-standing close research collaboration with the Laboratory of Applied Photochemistry (Prof. G. Orellana) and the Optical Sensors Groups (Prof. M.C. Moreno-Bondi) of Universidad Complutense de Madrid, the OPTOSEN® system has been designed to produce a line of monitors for several key parameters in environmental (water/air quality monitoring) and industrial matrices, namely oxygen, temperature, humidity, pH, hydrocarbons, ammonium, carbon dioxide, acidity and BOD. The system allows selection of the desired analyte just by changing the instrument sensor tip.

An example of environmental applications of this instrument is the BOD-OPTOSEN®. This system is based on a new BOD (biochemical oxygen demand) luminescent biosensor designed for all kind of applications such as environmental monitoring and process control of waste water (urban and industrial) and river water in remote sites and outdoor places.

BOD-OPTOSEN® device has a number of interesting qualities such as minimisation of analysis time, self-calibration, low sample consumption, long periods of monitoring between maintenance operations and a simple measurement process. Thus, the instrument can operate as a stand-alone sensor and it is suitable for field monitoring. Therefore it possesses all the required attributes of an efficient water monitoring system.

The BOD-OPTOSEN® has been installed in several places to analyse different kind of samples. This system has been successfully employed to measure BOD in the influent of a water treatment plant at Cariñena (Zaragoza), in the Madrid Manzanares river, at a SAICA monitoring booth and in the effluent of an urban water treatment plant in Madrid.

Acknowledgements. This work was carried out with financial support from Spanish CDTI (Centro para el Desarrollo Tecnológico Industrial) agency. Interlab has funded sensor research at UCM since 1997 under several Art.11/83 contracts.

BIACORE – MEASUREMENTS OF BIOMOLECULAR INTERACTIONS

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The following aspects will be tackle in the presentation with particular emphasis in the evolution from research to product marketing:

1 - How do Biacore systems work?

2 - Benefits of the technology.

3 - What can Biacore do?

CHEMILUMINESCENCE MICROFLUIDIC CHIP FABRICATED IN PMMA FOR DETERMINATION OF BENZOYL PEROXIDE IN FLOUR

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A chemiluminescence (CL) microfluidic chip fabricated in Polymethyl methacrylate (PMMA) for determination of benzoyl peroxide in flour is described in this paper. Benzoyl peroxide is a common additive in flour because of its bleaching and sanitizing properties. But excessive benzoyl peroxide in flour would do harm to the people. Benzoyl peroxide can directly oxidize luminol to produce chemiluminescence. The microchip (50×40×5mm) fabricated in Polymethyl methacrylate(PMMA) was readily produced in our analytical laboratory. It had two plates. The top plate had four reservoirs. Two reservoirs were linked with the pumps. The micro channels were on the surface of the bottom plate. The two plates bonded together. Four-drilled holes were in the top plate and they linked the reservoirs with the channels on the bottom plate at a to d. The width and the depth of the micro channel were all 200μm. Reagent (luminol) was moved through the channels using an injection pump (Figure 1). In this paper, we use a double-cross sample injection structure. The injection structure is meant for definition of specific amount of sample. Sample is introduced from c to b thus defining a sample flug in bc. (Figure 1). The sampling volume was 1μL every time. Then b is closed and luminol was pumped from a (Figure 1.) and mixed with the definite sample to produce chemiluminescence in cd. PMT was placed under the chip and chemiluminescence was detected at the point with the distance to d is 3mm. The linear range of the benzoyl peroxide concentration was 8×10^{-7} - 1×10^{-4}g mL^{-1}. The detection limit was 4×10^{-7}g mL^{-1} and the R.S.D for 11 times is 4.5% .The proposed method has been successfully applied to the determination of benzoyl peroxide in flour.

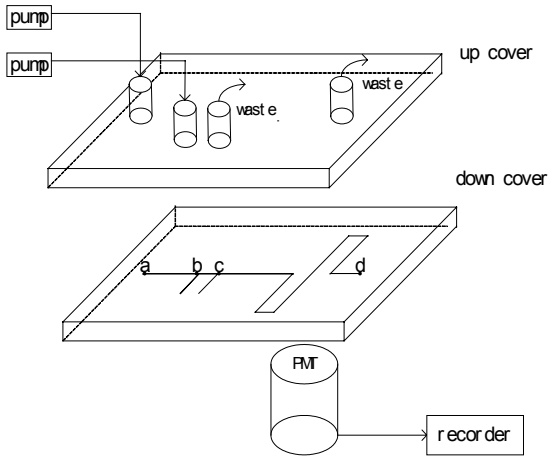

Figure 1. Schematic diagram of CL microfluidic chip for determination of benzoyl peroxide. The width and depth of the microchannel were 200μm. ab=9mm,bc=4mm,cd=22mm.

ELECTROCHEMILUMINESCENCE IMAGING TROUGH AN ORDERED ARRAY OF SUBMICROMETER-SIZED INDIVIDUALLY-READABLE ELECTROPTODES

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Coherent optical fiber bundles have been extensively used in a variety of analytical and bioanalytical applications (e.g., localized corrosion, parallel multi-analyte detection, artificial noses, DNA and cell arrays).[1]

We present a novel approach which results from the hybridization of imaging and electrochemical techniques allowing the fabrication of an ordered high-density array of 6000 opto-electrochemical individually readable sensors with sub-micrometer dimensions.[2,3] This device was fabricated by chemical etching the distal face of a coherent optical fiber bundle to produce an array of conical nanotips. The surface of the etched bundle was sputter-coated with a thin layer of indium tin oxide (ITO) in order to create a transparent and electrically-conductive surface which is insulated eventually by an electrophoretic paint except for the apex of the tip (Figure 1). These fabrication steps produced an ordered array of electroptodes with sub-micrometer dimensions which retains the optical fiber bundle architecture (Figure 2).[3]

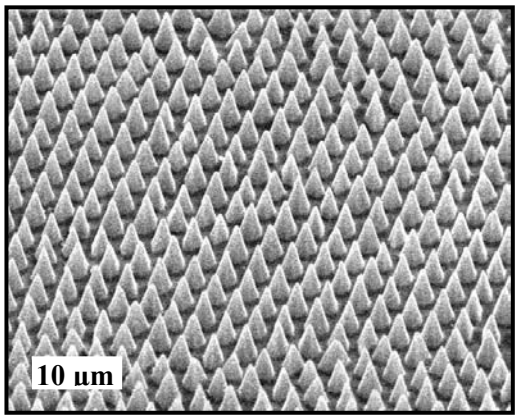

Figure 1. Scanning electron micrograph of the electroptode array

The electrochemical behavior of the electroptode array was independently characterized by cyclic voltammetry and electrochemiluminescence (ECL) experiments. Sigmoidal-shape of steady-state voltammogram indicates that the sensors are diffusively independent. This electroptode array was further studied with aqueous ECL model systems, such as tris(2,2'-bipyridine) ruthenium(II)/tri-*n*-propylamine and luminol/hydrogen peroxide. The last part of this work is devoted to the demonstration of the analytical performance of this array. We demonstrate that our electroptode array is able to generate ECL, to transmit it through the optical fiber bundle and finally to detect it. Therefore, remote ECL imaging is performed through the optical fiber bundle itself. Calibration curves were established to test the quantitative validity of this strategy. We could show that the sensors are optically independent and individually readable via the imaging fiber. Finally, ECL imaging and white-light imaging of a sample were concomitantly performed through the electroptode array.

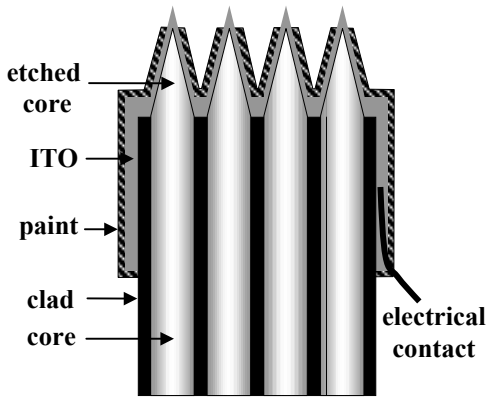

Figure 2. Schematic illustration (side view) of the electroptode array

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IDENTIFICATION OF FOOD FLAVOR USING THE SENSITIZED CATALUMINESCENCE-BASED GAS-SENSORS

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We propose a new sensor system of simple configuration to identify various food flavor. For this purpose, a sensor having higher sensitivity to various food flavor and faster response was developed on the bases of the *cataluminescence*-based gas-sensor. '*Cataluminescence*'(CTL) is chemiluminescence accompanying catalytic oxidation of combustible gases. We have reported that addition of rare-earth compound to the catalyst causes the increase in sensitivity of CTL-based sensors.[1] Now, we investigated role of the rare-earth by means of solid-surface analyzers of catalyst, a mass-spectrum analyzer of gases, thermo-gravimetric analyzer of the adsorbed catalysts, thermoluminescence and CTL measuring equipments. As a result, we found that the CTL is attributable to the recombination of carriers at Dy^{3+} center, and the sensitivity of a catalyst doped with Dy using aqueous solution of $Dy(NO_3)$□□□□□ is about ten-times as higher as that of undoped one for various flavor. Furthermore, even a thin layer of the catalyst made by screen-printing on a ceramic substrate has high sensitivity and fast response because the CTL emission results from surface-chemisorption process in a course of catalytic oxidation.

We made a working model of identification system of food flavor using the sensitized CTL-based sensors based on a new identification mechanism. Two types of the CTL based sensors (sensor A and B) made of □Al_2O_3:Dy and □Al_2O_3 catalysts, respectively, were used. These sensors are laid in a single chamber and jets of detection gas are impinged through two holes opened in a quartz window just above the sensor. Sensor A and B are alternatively heated and cooled between 100 and 500°C, and the CTL-intensities from these sensors are detected by a photomultiplier by means of the time-sharing method. During a heating cycle of sensor B, sensor A is kept at 100°C and gas is physisorbed and accumulated on the surface of sensor A, and the amount of the adsorbed molecules reflects the physisorption property of the gas. During the next cooling cycle of sensor B, sensor A is heated to 500°C and a certain proportion of the physisorbed gas is chemisorbed by getting thermal activation energy and a sharp CTL peak arises. Thus, the peak intensity (I_P) reflects physisorption characteristics of the gas. As the sensor has fast response, the CTL peak is disappeared within 30 s according to the oxidation of the pre-physisorbed molecules, and a steady-state CTL-intensity (I_{SS}) is observed by oxidation of gas transferred to the sensor surface. As the CTL of sensor B results from the excited formaldehyde HCHO produced in a course of catalytic oxidation of gas as reported previously, the CTL emission mechanism is different from that for sensor A. Thus, the ratio of I_{SS} for sensor A to B ($I_{ss}A/I_{ss}B$) reflects the chemical characteristics of the gas-molecule. Therefore, the position of a plot on a graph whose x-axis shows $I_{ss}A/I_{ss}B$ and y-axis shows $I_PA/I_{ss}A$ reflects physical and chemical characteristics of gas-molecules. Because of the linear characteristics of the CTL-based sensor, the plots are similar between a certain-type of gases of various concentrations, where Henry-type adsorption isotherm is established. Finally, we could identify ten types of food flavor belonging ketone, aldehyde, terpene and alcohol by means of a simple CTL-based sensor system consisting of only two sensors.

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ENANTIOMERIC SEPARATION BY POLYMERIC CHIRASIL-CALIX LAYERS WITH OPTICAL SENSING DEVICES

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The discrimination of chiral antipodes is considered to be one of the most difficult tasks in analytical chemistry, since the physical and chemical properties of enantiomers are identical in a nonchiral environment. Chiral recognition has to be used to perform a successful discrimination of the enantiomers. The separation of optical isomers can be achieved by different stationary phase materials, e.g. cyclodextrins[1] and chiral amides.[2] An important drawback is that GC is an off-line procedure, and hence an on-line control is not possible. Achieving chiral discrimination with sensors is fundamentally more difficult than with gas chromatography,[3] because only one theoretical plate (one absorption and desorption process) is available, whereas in GC discrimination typically results from thousands of successive absorption/desorption equilibria. The sensitive layers for the sensor application have to be chosen carefully and have to lead to clear and significant results for the discrimination of enantiomers with sensors.

Calixarenes are considered as interesting synthetic selectors because of their high thermal stability and their unique cavity-type supramolecular shape. In this work a resorc[4]arene basket-type selector containing chiral diamide groups is used as sensitive layer for the separation of enantiomers. The chiral macrocyclic selector is chemically bonded to a polysiloxane polymer. The separation of enatiomers on chiral calixarenes has been reported in electrokinetics and gas chromatography.[2] The recognition principle is mainly based on hydrogen bondings between the stationary phase and the analyte molecules.

To achieve unambiguous results we are applying two different optical transducing principles for the discrimination of the enantiomers. The first principle of detection is Surface Plasmon Resonance Spectroscopy (SPRS) which is based on the detection of changes of the refractive index of a sensitive layer. An array of Reflectometric Interference Spectroscopy (RIfS) sensors is used as second principle. RIfS is based on interference effects in thin transparent films. A light beam passing through a polymer film is reflected in part at each of the interfaces. As the reflected beams travel different optical paths, a phase difference is introduced and the interference pattern can be evaluated. By absorbing analyte molecules in the polymer film, the thickness of the film is slightly changed, this causes changes in the interference pattern.

The thin polymer layers necessary for the two sensing principles are produced by spin-coating from the dissolved polymer. The layer for the SPR measurements has a thickness of about 90 nm and the layers for the RIfS measurements are between 150 and 300 nm thick. To achieve statistical evidence and to exclude possible artefacts a nonchiral PDMS (SE-30) sensor is used as reference.

As analytes the D-(+)- and L-(-)-enantiomers of methyl- and ethyl-lactate were chosen. Further results of the separation of amino acid derivatives with the chirasil-calix polymer will be presented. With regard to the boiling point and vapour pressure amino acids with protected acid and amino groups are under investigation. All measurements will be performed with SPR and RIfS in the gas phase. The test gases are generated by temperature-controlled vaporizers. Defined vapour concentrations are obtained by computer-driven mass flow controllers. Dry air is used as carrier gas.

The aim of this work is to demonstrate the ability of our sensitive layers to clearly discriminate optical isomers. The enantiomer discrimination factors will be compared with factors measured in GC experiments. In a GC column coated with chirasil-calix the L-(-)-lactateester is eluted first. Corresponding to this results the L-(-)-enantiomer gives a weaker signal on our sensors.

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FREQUENCY DOMAIN MEASUREMENT OF ROOM TEMPERATURE PHOSPHORESCENCE LIFETIMES IN THE PRESENCE OF BACKGROUND SIGNALS

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Most of the developments on luminescence optical sensors are based on the measurement of fluorescence emission. However, room temperature phosphorescence (RTP) may offer interesting advantages over fluorescence for optical sensing including better sensitivity (the RTP analytical signal is a low-noise emission) and larger Stoke's shifts. Additionally, the relative large value of the excited state lifetime of the phosphorescence also facilitates the measurement of the triplet lifetimes. This measurement is much more robust than conventional intensity measurements (decay times are mostly independent of reagent concentrations, light losses, lamp drifts, etc.)

The frequency domain technique typically used to determine the RTP lifetimes consists on the excitation of the phosphorescent sensing material using a sinusoidal intensity-modulated light followed by the calculation of the decay time by measuring the phase-shift, α, of the emitted light. Assuming that light emitted by the sensing material follows a monoexponential function, the relation of the phase-shift and the decay time, τ, is given by the equation:[1] $\tan(\alpha) = 2\pi f\tau$

where, f, is the frequency at which the measurement of the phase-shift is performed. However, in phosphorescence measurements, a significant amount of fluorescence emission could remain sometimes and overlap with the measured phosphorescence (even if fluorescence and phosphorescence measured intensities and spectra greatly differ). In such cases the measured phase shift may be quite different to that expected, thus resulting in errors in the lifetimes calculation.

In this presentation, a new method based on the measurement of the phase-shift at two optimal frequencies is presented. The first optimal frequency is selected aiming at maximising the sensitivity of the phase with respect to the decay time. The second optimal frequency is obtained by evaluating the propagation of error when measuring the second phase-shift. The problem consists of finding the frequency where measured lifetime sensitivity to the observed error is at a minimum.

Figure 1 shows the measurement system developed to evaluate the proposed method. Al-Ferron trapped in a Sol-Gel support[2] has been used as RTP sensing material. The RTP lifetime of this material greatly depends on the oxygen concentration, while it presents a strong fluorescence emission peak at about 480nm and a RTP emission band centred at 590nm.

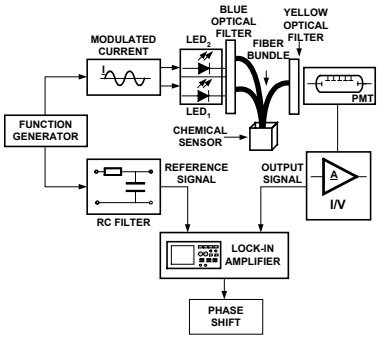

Figure 1. Block diagram of the experimental set-up.

The experimental set-up will be described and its performance for different oxygen concentrations will be presented and discussed.

Acknowledgment: Financial support from "Fundacion para la Investigacion Cientifica y Tecnologica del Principado de Asturias" (FICYT) through the project FC-02-PC-CIS01-13 is gratefully acknowledged.

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OPTICAL MICROSYSTEM PLATFORMS CMOS COMPATIBLE BASED ON INTERFEROMETRIC BIOSENSOR NANODEVICES

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There is an increasing interest in system based on micro/nanotecnologies for ultrasensitive and miniaturised biosensors. Genomics and proteomics sensing are fields where new laboratory analysis (faster, direct, multianalyte, more accurate, smaller and cheaper than conventional methods) are demanded. Integrated optical Mach-Zehnder interferometer devices fabricated with micro/nanotechnologies are highly sensitive bio/chemical sensors. Due to their high sensitivity, mechanical stability, microelectronics fabrication and their miniaturization, these devices are quite suitable for further integration in a microsystem. The integration of optical, electrical and fluidics functions on the same microsystem will render in a complete lab-on-a-chip.

To this aim, we have fabricated an integrated Mach-Zehnder nanodevice based on Total Internal Refraction (TIR) waveguides.[1] For biosensing applications the waveguides of the MZI device are designed to work in monomode regime and to have a very high surface sensivity of the sensor arm. The basis of the TIR waveguide structure is: (i) a Si substrate, (ii) a cladding of SiO_2 (2 μm thick, n=1.46), (iii) a core of LPCVD Si_3N_4 of 100 nm thickness and a refractive index of 2.00. To achieve monomode behaviour is needed to define a rib structure, with a depth of only 3 nm, on the core layer by a lithographic step. The final devices (within all its fabrication processes) are CMOS compatible. These devices have a Surface Sensivity of $2 \cdot 10^{-4}$ nm^{-1} in TE polarization. In the Figure 1 the cross section of the MZI TIR waveguide is shown.

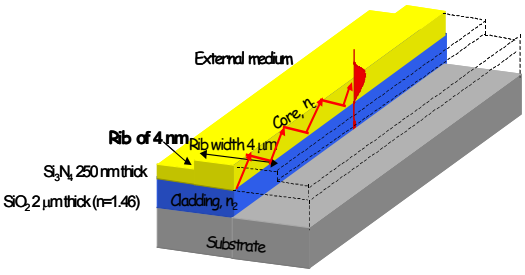

Figure 1. Cross section of the waveguide of a MZI-TIR nanodevice

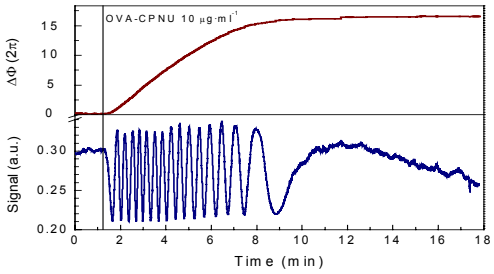

Figure 2. MZI response to the receptor immobilisation

For biosensing purposes, a layer of receptor proteins, specific to the analyte to be detected, is previously immobilised on the interferometer surface (see Figure. 2) using silanization procedures. The flow cells are specifically designed and fabricated using a novel fabrication method[22] of 3-D embedded microchannels using the polymer SU-8 as structural material. On the other hand, several modulation schemes at the reference arm (as magneto-optical compensation) is under study.

The microsystem will incorporate together the nanodevices, the flow cells, the modulation system and, in a further step, the CMOS electronics.

Acknowledgements: authors would like to thank the financial support from MCyT (project BIO2000-0351-P4-05) and AECI (project 20p/02). B. Sepúlveda acknowledges financial support from the CSIC I3P program.

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HIGHLY SENSITIVE OPTOCHEMICAL SENSORS BASED ON REACTIVE DYES INCORPORATED INTO MOLECULARLY IMPRINTED POLYMERS

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Molecularly imprinted polymers (MIPs) are synthetic receptors, which are produced by polymerising functional and cross-linking monomers in the presence of a target molecule that acts as a molecular template. MIPs may be used in the place of biomolecules as recognition elements in various analytical systems including chemical sensors.[1]

We have designed an optical sensor based on imprinted polymers. A polymerisable reactive stilben dye[2] was incorporated into the binding sites of a MIP by copolymerisation. The dye acted at the same time as a functional monomer that formed a covalent bond with the template. The MIP was synthesised in the form of a thin film on the surface of a glass substrate. Upon analyte binding to the MIP, the visible adsorption spectrum of the dye was changed, allowing for detection and quantification of the analyte. Highly selective MIPs were produced for a range of different amines. For example, benzylamine could be detected selectively at picomolar concentrations, whereas other amines such as octylamine were detected by the benzylamine-MIP only at concentrations several orders of magnitude higher. Selectivity was further demonstrated by using, as competitors, structural analogues of the analyte that do not have an amino group. Other amines such as amphetamine, benzocaine and L-tryptophane methylester were also imprinted, and could be detected selectively at low concentrations. For example, the detection limit for L-tryptophane methylester was in the low femtomolar range.

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POLYELECTROLYTE MULTILAYER PATTERNING FOR SPR LIQUID SENSING

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We have recently reported a single chip multichannel surface plasmon resonance (SPR) device for the detection of metal ions[1,2]. Here, further developments towards the realization of ordered patterns of polyelectrolyte multilayers[3] will be presented. Such patterns provide the different sensing channels within the SPR device[2]. Several approaches have been used to define the channels: (a) physically isolated patterning using flow cells; (b) polymer on polymer stamping; and (c) photolithographic-based methods. Atomic force microscopy was used in order to correlate surface topography and sensing performance of different layer-by-layer self-assembled structures.

In Figure 1(a), a plan view of one of the flow cells used for the fabrication of the different sensing channels is illustrated; a photograph of one of the sensing chips is shown in Figure 1(b). The sensing channels are deposited along the X direction, while the SPR investigation is performed along the Y direction, over a distance of approximately 15 mm.

The SPR system[1,2] in conjunction with an appropriate sensing platform might lead to an SPR "optical tongue". In such a device, several active materials are exploited in order to discriminate multi-component analytes and to eliminate any ambiguity in the sensing results. A discussion on the different sensing mechanisms used by the polyelectrolyte multilayers to detect metal ions will be presented.

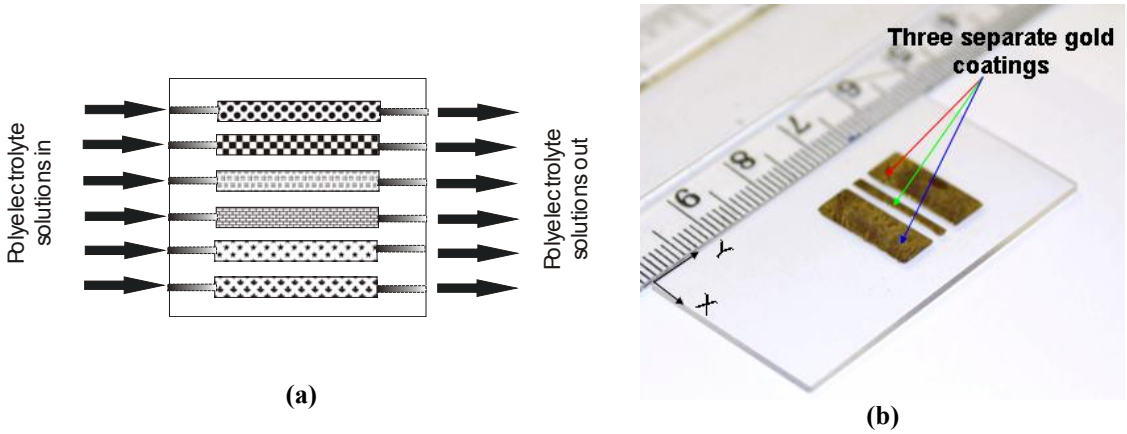

Figure 1: (a) Schematic representation (plan view) of the flow cell system used to pattern different layer-by-layer self assembled thin films; (b) Large area multi-channel single chip. The flow cell system is aligned with the gold coatings and several sensing architectures are built-up along the X direction. The SPR scan is performed along the Y direction for the entire gold coatings surface.

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HOLOGRAPHIC DESIGN OF INTEGRATED SURFACE PLASMON RESONANCE SENSOR CHIP

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A new integrated design of a surface-plasmon resonance (SPR) sensor is reported. As opposed to the conventional Kretschmann configuration, in which a laser beam is focused via a lens and a prism at a metal-coated glass slide and recollimated via a second lens to reach a CCD camera, the present design comprises a simple planar injection moulded polymerchip with focusing gratings and metal coating integrated in the surfaces of the chip. With the integrated design all light coupling and focusing is achieved without lenses or prisms and because of the very inexpensive manufacturing process the sensor chip can be made disposable.

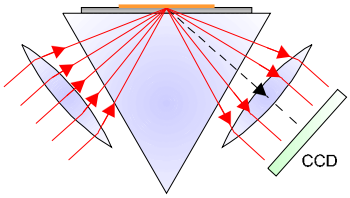

Figure 1. Conventional Kretschmann SPR sensor configuration.

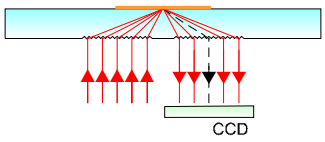

Figure 2. Integrated SPR sensor chip design.

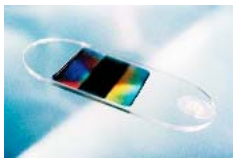

Figure 3. Injection moulded SPR sensor chip.

The two focusing coupling gratings are mastered holographically by recording the interference between two HeCd laser beams in photoresist. However, since the diffraction angles have to span the expected SPR angular range, which assumes values of up to 77 deg, the resulting holographic gratings appear to be far from paraxial. This implies that the classical holographic design techniques reported by Latta[1,2] in the early seventies are pushed far beyond the paraxial limit and therefore break down. It has therefore been necessary to develope an alternative design algorithm to achieve a sufficiently accurate chirp in the grating profiles. The new algorithm is based on matching the local grating spacing of the recording interference pattern to the desired grating spacing in the injection moulded sensor chip. This is opposed to the classical method, which is based on aberration balancing. The new design algorithm is demonstrated and is shown to have superior performance for the design of non-paraxial holograms.

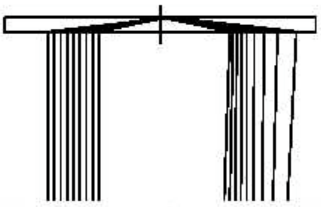

Figure 4. Ray trace of sensor chip with aberration balanced holograms.

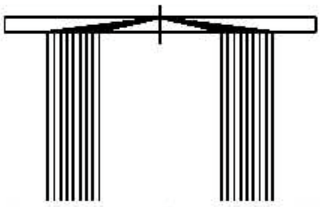

Figure 5. Ray trace of sensor chip with grating-matched holograms.

Figure 6. CCD image of SPR dip.

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[2] J. N. Latta, *Appl. Opt*. **1971**, *10*, 609.

QUANTUM DOT BASED FLUORESCENCE RESONANCE ENERGY TRANSFER NANOSENSORS

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Colloidal luminescent semiconductor nanocrystals or quantum dots (QD) possess unique attributes that make them superior to commercially available organic dyes when used for optical-based biological sensing assays. These include exceptional photochemical stability, broad excitation and size-dependent tunable photoluminescence (PL) spectra with narrow emission bandwidths (full width at half maximum of ~ 30-45 nm) that span the visible spectrum.[1] Recent studies have employed QD-protein conjugates in fluorescence-based energy transfer assays to study the photophysical properties of this system.[2] We found that the readily tunable QD emission over a wide range of wavelengths permitted effective tuning of the degree of energy overlap between the QD donor and the acceptor for a fixed dye, thus allowing control over the rate of FRET in these complexes.[2] These results were further exploited to design a prototype of a FRET-based QD nanosensor.[3] We utilized *E. coli* maltose binding protein (MBP) and targeted its preferred substrate, maltose in the nanosensor. To form the nanosensor assembly, MBP was first coordinated to the QD surface and a displaceable β-cyclodextrin conjugated-QSY9 dark quencher was allowed to bind to the sugar-binding site of MBP, resulting in FRET quenching of the QD photoluminescence (PL) by the proximal QSY9 quencher. Added maltose displaced the β-cyclodextrin-QSY9 conjugate, and increased QD emission in a systematic and concentration dependent manner. Analysis of QD PL recovery provided a measure of the dissociation constant for maltose and demonstrated sensor sensitivity and specificity. Derivatives of this sensor were developed that utilize a 2-step FRET mechanism to overcome inherent QD donor-acceptor distance limitations. Further nanosensor variants that utilize reversible modulation of QD emission are being explored. This sensing format can be applied to other receptor proteins or bio-recognition units and may facilitate development of a new generation of hybrid QD-based biosensors.

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SMALL AND MASSIVELY PARALLEL OPTICAL SENSORS: IONOPHORE-BASED MICROSPHERE ION OPTODES

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We report here on our recent efforts to develop fluorescent ion sensing particles and their use in analytical flow cytometry and in fiber optic based random arrays. Eventually, this research will allow one to greatly expand the palette of available analytes that one can assess with random array technologies and paves the way to the realization of a bead-based clinical total analysis system. Today, common electrolytes in whole and diluted blood are usually assessed with ion-selective electrodes. Consequently, the extraction and complexation chemistry of these sensors must be adapted to function in fluorescent microspheres. Such particles function according to bulk extraction properties into the hydrophobic polymeric interior, rather than according to a surface binding reaction as in most other particle-based assays. Consequently, the ion sensing chemistry developed originally for thin film based optodes by the group of Simon and others is adapted here for use in microsphere-based sensing assays.

Principles of this new bead-based sensing strategy, along with current efforts to particle manufacturing and initial flow cytometry measurements are presented. The lifetime and reduced cross-contamination of ion sensing particles is enhanced by developing plasticizer-free polymeric sensing materials that are also useful as ion-selective electrode membranes. Similarly, the covalent attachment of active sensing molecules is described, including cation-selective ionophores, anion-selective metalloporphyrins, chromoionophores, and lipophilic ion-exchangers. Such synthetic efforts are much more important with miniaturized sensing systems that with macroscopic ion sensors because of the larger surface to volume ratio and the limited reservoir of available sensing components. In some instances, the covalent attachment reduces important long term instabilities that have been observed with freely dissolved components.

Acknowledgements. This research has been supported by the National Institutes of Health and by Beckman Coulter, Inc.

SELF ASSEMBLED SEDIMENTATION ARRAYS BASED ON LUMINESCENCE ENCODED MICROSPHERES

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Sensor arrays provide an architecture for parallel multianalyte sensing. On conventional microarrays (immuno- or DNA-chips) each individual receptor is addressed by its defined position on the slide. Another way to address a receptor is to bind it onto the surface of microspheres carrying a defined fluorescent code. A number of such individual microspheres (i.e. sensors) can be randomly attached to the distal end of an imaging glass fiber bundle forming a randomly ordered sensor array.[1] It is also possible to disperse these particles in a solution and to analyze them in a flow cytometer.[2]

Here we introduce a new concept towards multiplexed analysis including advantageous features of both approaches mentioned above. The use of fluorescence-encoded microspheres of significantly higher density than water results in sedimentation in the range of minutes. Magnetic poly styrene microspheres allow even higher flexibility in mixing and sedimentation. During the sedimentation process the binding of the analyte onto the receptor modified particle surface occurs. This leads to in situ formation of randomly ordered sensor arrays e.g. at the transparent bottom of each individual well of a microplate. This in-situ formed array is then evaluated by an inverted fluorescence microscope equipped with a CCD-camera that is connected to an automated image analysis system.

We also present and characterise new encoded luminescent microspheres. They allow the use of a conventional fluorescence microscope equipped with a triggered blue light emitting diode (LED) as light source and a tuned fast triggered CCD-camera. While all common approaches require different spectral windows to identify the nature of individual particles, in our concept no change of optical filters is necessary during identification. The particles were stained with a couple of luminescent dyes showing distinguishable luminescence decay but similar (optimally identical) spectral properties. A phosphorescent ruthenium(II)-pyridyl complex (selected from a series of complexes with almost identical spectral properties but different decay times ranging from 1µs to 6 µs) and a fluorophor with a nanosecond decay were mixed in defined ratios. One fluorescence image (I_1) is recorded during the excitation (LED on) – quantifying the overall signal of both dyes - and two phosphorescence images (I_2 and I_3) are recorded during the decay of the phosphorescence dye (LED off). From these 3 images the identification of the phosphor via decay time (I_2/I_3), its concentration (I_2) and the ratio of fluorophor and phosphor ($I_1/_2$) is possible. Adding the size of particles as additional code, allows distinguishing more than 1,000 individual particle populations.

The selection of appropriate particles based on different materials, details of the encoding scheme and the image analysis are discussed. The proof of principle is demonstrated. A critical comparison with other established concepts for sensor arrays is given.

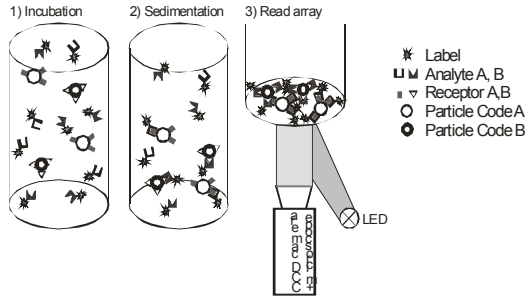

Figure 1. Schematic drawing of self assembled sedimentation array principle.

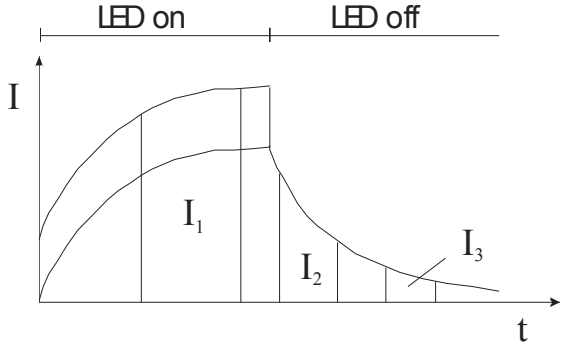

Figure 2. Encoding scheme to identify luminescent microparticles via fluorescence and phosphorescence decay (λ_{ex} = 485 nm, λ_{em} > 515 nm).

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DETECTION OF MOLECULAR RECOGNITION BY FLUORESCENCE ON SELF-ASSEMBLED MONOLAYERS ON GLASS

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The importance of the chemical sensing of ions and small organic molecules is illustrated across a broad range of areas encompassing medical diagnostics, environmental monitoring, and the food industry. Traditional design of molecular receptors as chemical sensors for small molecules and ions especially, was based on the "lock and key" principle of one receptor, one analyte.[1] Through this traditional approach, binding selectivity has been improved by focusing on geometric and functional group complementarity of the fluorophore-appended host to match that of the guest. The remarkable selectivity imparted by this technique cost significant time and effort toward the synthesis of receptors with appended transducers prior to analysis of their binding capabilities.

A possible solution is to integrate surface-confined sensing technology into the search for chemical sensing systems. One approach which has been successfully applied is the use of combinatorial chemistry to generate microarrays through such means as in situ synthesis, chemical ligation, and non-covalent bonding has resulted in a broad range of protein and small molecule microarrays on surfaces such as optical fiber tips, microbeads and microwells.[2] The majority of systems developed thus far have focused on biological applications, while the few chemical sensing systems have suffered from tedious synthesis and complex, time consuming hit deconvolution procedures.

Our lab has successfully developed a simple strategy for the generation of chemical sensing libraries for cations by combining the independent deposition of commercially available fluorophores and simple small molecules onto a self assembled monolayer on glass.[3] This allows for the in situ development of a complete sensing surface without the need for a complex interface or hit deconvolution. We found that small changes in the surface functionalities imparted a large change in the sensing properties of the surface, and that such detection was transferable to the microscale via microcontact printing and microchannels. Additionally, this strategy was equally extendable to the detection of inorganic ions, which are traditionally more difficult to sense than cations.

We now hope to extend this methodology to the more weak interactions associated with hydrogen bonding systems. This could allow us to apply the technique to such systems as base pair and carbohydrate-protein interactions, which would thus broaden the scope of the applicability of the methodology.

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AUTOMATED WATER ANALYSER COMPUTER SUPPORTED SYSTEM (AWACSS)

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European Directives and regional regulations have begun to demand on-line monitoring of water catchment areas for an ever-expanding list of pollutants. The goal of this EU funded consortium was to develop a cost-effective on-line water-monitoring biosensor that measures a variety of small organic pollutants in short-time with remote control and surveillance. Surface water, ground water and drinking water are the targets to be analysed. Even today there is very little technology for monitoring water sources in real-time and at reasonable costs. The AWACSS instrument is networked so that sources will be monitored with trend analysis and early-warning capabilities.

This newly developed fully automated optical biosensor is based on Total Internal Reflection Fluorescence (TIRF) technology (Figure 1) combined with a microfluidic setup. Laser light is coupled into a pigtailed transducer and guided down the transducer by total internal reflection following the integrated optical structures. To produce chips for multianalyte measurements, a spatially resolved surface chemistry protocol applied by the contact-free spotting technology system TopSpot was developed. Analyte-specific antibodies are labeled with a fluorescent marker. Analyte recognition is based on a binding inhibition assay (Figure 1). Upon binding of the antibodies to their specific antigen immobilised at particular spots at the transducer surface the fluorescent markers are excited in the evanescent field. The waveguide offers the possibility to measure at 32 different chemically modified spots.

With this biosensor we have shown that it is now possible to analyse for example estrone with a limit of detection below 10 ngL^{-1} without pre-concentration. For the first time it will be possible to monitor real-water samples in multi-analyte mode for up to 32 substances. Including the established auxiliary system with PNA sequences immobilised at the spots, a fast and easy exchange of the applied multi-analyte panel is possible. Furthermore, intelligent chemometric methods are implemented to take advantage of the cross-reactivity of antibodies to quantify classes of substances or to calculate single concentrations. We have proven that our immunoassays are highly reproducible and achieve good recovery rates measuring real-water samples for a variety of substances such as pesticides, antibiotics, toxins and EDCs.

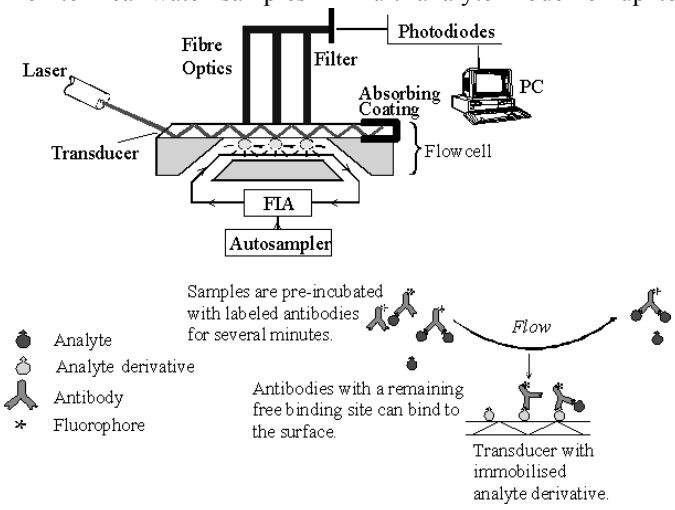

Figure 1. TIRF experimental setup and the immunoassay principle.

Consortium: University of Tuebingen: Project Management, TIRF Immunoassay and System Integration. Central Research Labs (London): Instrument Hardware Design. Siemens (Germany): Electronics, Internet Communication, and Market Analysis. Optoelectronics Research Centre (Southampton): Integrated Optical Chips. King's College London: Immunochemistry. Instit. of Chem. & Environ. Chem., IIQAB (Spain), DVGW, Technologiezentrum Wasser (Germany), Environmental Instit. (Slovakia), Water Reasearch Institute (Slovakia): User Friendly Design and Field Testing.

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IR-ATR SPECTROSCOPY FOR UNDERWATER SENSING APPLICATIONS

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IR-ATR spectroscopy has been confirmed as a capable technique for *in-situ* monitoring of organics in harsh, aquatic environments, in particular when utilizing polymer coated silver halide fibers.[1] The polymer coating enriches hydrophobic analytes while minimizing interferences from water and other hydrophilic compounds. Hydrophobic analytes present in the enrichment layer interact with the evanescent field as light propagates through the fiber producing a readily measurable signal.[2] A second-generation underwater spectrometer is currently in development to minimize the size and weight of the instrument as well as extend the operating range to several thousand meters of depth. The development of this instrument provides the potential for monitoring dissolved methane resulting from gas hydrates and a variety of other organic compounds in deep sea marine ecosystems.[3]

Monitoring analytes to depths over 1500 m establishes harsh operating conditions with pressures exceeding 150 atm and temperatures ranging from 1 to 15 deg C. Changes in either of these conditions are hypothesized to strongly influence spectroscopic measurements. Thus improved understanding of the sensor behavior at such conditions and reliable data evaluation algorithms accounting for these parameters are essential. The studies presented in this contribution focus on a variety of investigations on the influence of hydrostatic pressure on ATR spectroscopy in the aqueous phase and the resulting impact on multivariate data evaluation.

The experimental setup is based on a Bruker IRcube FT-IR spectrometer coupled to a custom-made pressure cell for investigating aqueous samples with polymer coated ATR crystals up to pressures of 20 atm. A wide range of organics dissolved in the aqueous phase are investigated along with a variety of polymer coatings including poly(trimethylsilyl)propyne, Teflon AF, and ethylene-propylene copolymer on both germanium and zinc selenide ATR crystals. Extent and rate of enrichment for organics including 1,2 dichlorobenzene, tetrachloroethylene, trichloroethylene, chloroform, and dissolved methane are discussed as functions of pressure and concentration. Furthermore, instrument miniaturization and advanced chemometric data evaluation aspects will be discussed. Results from this work and ongoing studies will lead to a more fundamental understanding of this measurement technique for future sensor development and the ability to perform reliable data evaluation in real-world deep sea environments.

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BTEX MONITORING IN GROUND WATER REMEDIATION APPLYING UV FIBER OPTIC EVANESCENT FIELD SENSORS

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An UV absorption based fiber optic evanescent wave sensor system for BTEX monitoring in groundwater remediation facilities is presented. The sensor principle, design and performance are described. Results of field tests in a remediation facility operating on a former petrol industrial site will be shown.

Benzene, toluene, ethyl benzene and xylene (BTEX) belong to the most significant water pollutions in the base of former industrial sites, gasoline stations and pipelines. Highly BTEX-contaminated ground water plumes must be decontaminated to avoid endangerments for public health and environment For this purpose the groundwater is pumped trough remediation facilities where it will be treated by air stripping and carbon filtering. To optimize the treatment process and to meet the legal limits in the cleaned water, a continuous monitoring of all process stages is required.

The common method to measure the BTEX- content in water is to investigate water samples by gas chromatography (GC). Optochemical standard methods such as photometry or fluorimetry are usually not applicable because of colouration, turbidity and the humine acid content of untreated ground water.

To measure BTEX on-line in coloured and turbid water, an evanescent field absorption sensor (EFAS) and an UV- spectrophotometric interrogation method has been developed. A special high OH-grade silica core fibre with 200µm core diameter and a 20 µm thin polydimethoxysiloxane (PDMS) cladding is used as transducer. PDMS is very suitable for solving and enrichment of non-polar BTEX molecules from aqueous environment.[1] Further, the PDMS- cladding is keeping polar compounds as metal oxides, dyes and aliphatic hydrocarbons, particles and biological tissues away from the core-cladding boundary, where the evanescent field may interact with the analyte in the cladding. The sensors are photometrically interrogated at different wavelengths between 230 nm and 350 nm for BTEX measurement and between 550 nm and 700 nm for referencing, using a Xenon light source and a low-cost miniature spectrometer.

The spectra are processed by using a new kind of signal processing algorithm, allowing to remove wideband spectral disturbances from light source as well as refractive index effects originating from the polymer fiber cladding.

Depending on the lenght of sensor fiber, BTEX in a concentration range from less than 1 mg/L up to some hundred mg/L can be detected with an accuracy better than 10%. In first field tests in an industrial ground-water remediation facility, the sensor signals have been monitored for more than half a year (Figure1).

No cross-sensitivity to small aliphatic hydrocarbons and only weak sensitivity to humine acids was found. The sensors have withstood at least for a year the harsh conditions in the main flow of an industrial ground water remediation facility, but required monthly recalibration when used in highly contaminated water.

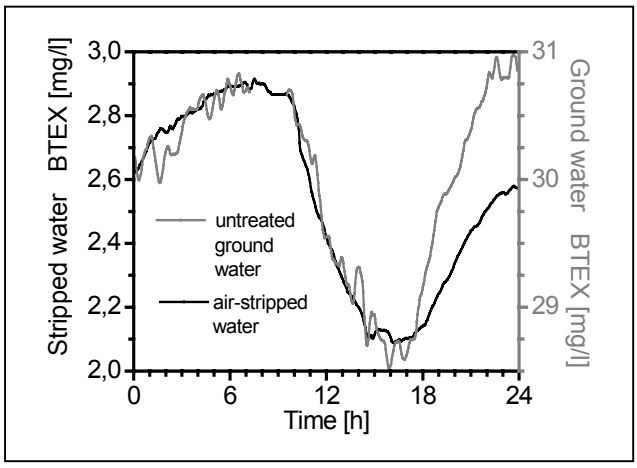

Figure 1. Fluctuations of BTEX content in untreated and air stripped water, recorded over 24 h.

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USE OF FIBRE-OPTIC OPTODES FOR MONITORING OF SEA WATER: TOWARDS AN OPTICAL CTD PROBE

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CTD* probes are very important research equipment for oceanographic ships. The use of optical sensors to replace the traditional electrical sensors is a technological challenge and a very important step in the incorporation of optical devices to environmental monitoring. The Applied Optics Complutense Group has been involved in two European research projects** whose main goal is the use of fibre-optic sensors for the real-time determination of the concentration of pollutants in sea water. As a result of our developments, the goal of an all-fibre-optic CTD probe is in the way to be achieved.

Three separate sensors have been developed, that use the same wavelength range and share some configuration features. The **salinity** sensor is based in the relationship between this parameter and the refractive index of seawater. The possibility of using SPR (surface plasmon resonance)-based sensors to determine salinity was first demonstrated by the authors some years ago.[1] The sensor was based on the deposition of a double layer (dielectric, TiO_2 – metal, Al) on a D-fibre. The sensor was optically interrogated and the measured parameter was the attenuation of the transmitted optical power for the guided mode. In recent times we have developed a new sensor based on similar principles, but using tapered optical fibres. In this case, we have deposited in an asymmetric way the layers and can use both a total output power measurement or a spectral measurement, with a LED illuminating source.[2] Based on the D-fibre sensor we have produced a fully operative optode, that has been demonstrated in real measurement conditions during a cruise in an oceanographic ship.[3]

For the **temperature** measurement, two schemes have been successfully tested. In one case, we deposit on a tapered optical fibre a thermochromic material (lophine, 2,4,5-triphenylimidazole), whose variation of absortance implies also an attenuation of the optical power transmitted by the fibre.[4] As an alternative possibility, the dependence of this transmitted power on the curvature of the fibre is used as the basis of a sensor which employs capillary metallic tubes as transducers: the dilatation or contraction of the tube induces a change in the geometry of a tapered optical fibre introduced in it.

Finally, for the **pressure** sensor we have opted for a two-FBG (Fibre-Bragg-Grating) configuration. Two FBGs are written on the fibre and, when interrogated, the spectral response of the sensor is dependent on the strain suffered by the structure. One of the gratings can be used for thermal referencing.

All the sensors are conceptually very simple and well prepared for their use as optodes in real measurement conditions. All of them use the same monomode fibre and work in the near infrared range (about 800 nm), a region for which the cost of the components is low. Their integration in the MISPEC measurement platform is easy and, in this way, their use as an optical alternative to conventional CTD probes is feasible.

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* *CTD* stands for Conductivity – Temperature – Depth. Conductivity is the electrical parameter traditionally used to measure salinity, which, in our case, is measured with the refractive index. Depth and pressure are, obviously, intimately related. What we develop could be, then, called, an STP (Salinity – Temperature – Pressure) probe.

** *SOFIE* (Spectroscopy using Optical Fibres in the Marine Environment, ref. MAS3-CT97-0157, 1998-2000); *MISPEC* (Multiparametric in-situ Spectroscopic Measuring System for Coastal Monitoring, ref. EVK3-2000-00519).

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Poster Abstracts

A REAGENT-LESS FLUORESCENT SOL-GEL BIOSENSOR FOR SUPEROXIDE ANION DETECTION

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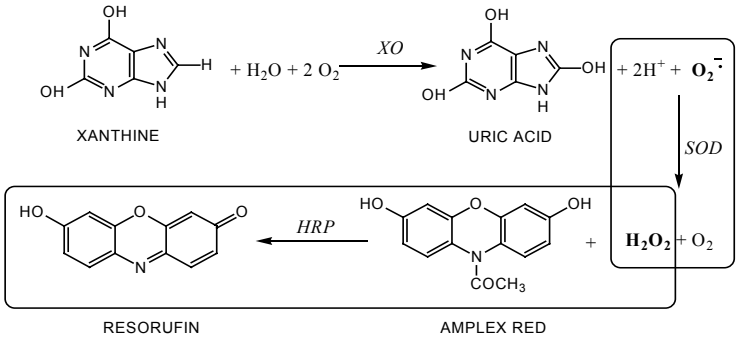
Superoxide anion, O_2^-, is produced in living systems as a reduced intermediate of molecular oxygen by either auto-oxidation processes or by enzymes involved during the normal metabolism, and its production rate is largely increased under conditions of oxygen stress[1]. This radical O_2^- is very reactive and can be attacked by other active biomolecules (e.g. NO) and scavenged by enzymes (e.g. superoxide dismutase (SOD), catalase) and antioxidant agents (e.g. ascorbate, Vitamin E). Due to these reactions, its half-life is rather short and it is difficult to obtain a reasonably accurate measurement for O_2^- concentration. Although numerous spectroscopic and electrochemical methods have been recently developed, the fact is that, to date, the measurement of the concentration of superoxide in a biological system continues to be a challenging analytical problem.

In this work, we have developed a reagent-less and ready to use fluorescent biosensor for superoxide radicals based on the simultaneous encapsulation of a coupled superoxide dismutase-peroxidase system and the probe N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) in a sol-gel matrix. In the biosensor, the enzyme superoxide dismutase (SOD) catalyzes the dismutation reaction of the generated superoxide radical with the release of hydrogen peroxide, which in the presence of peroxidase (HRP) reacts stoichiometrically with the non-fluorescent Amplex Red to generate the red-fluorescent oxidation product resorufin[2].



The high sensitivity of the Amplex red allows the determination of O_2^- on highly diluted fluids, which makes unnecessary any sort of sample pretreatment. The stability, activity and the capability to reuse the biosensor have also been studied.

Acknowledgements. Work supported by grants PI020606 (Instituto de Salud Carlos III, Spain) and EVES (Generalitat Valnciana)

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NOVEL DEVELOPMENTS IN CHROMOGENIC AND FLUOROGENIC RECEPTORS AND REAGENTS FOR CATION AND ANION SENSING

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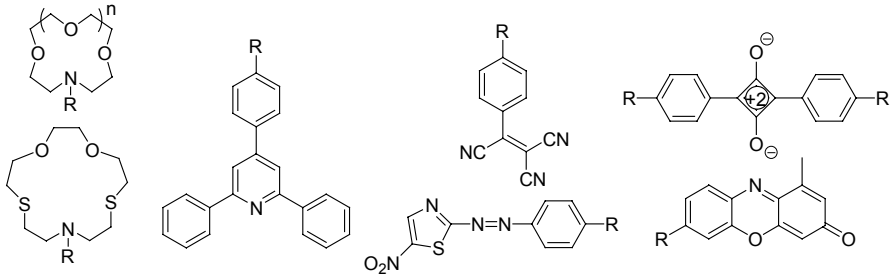
The development of molecular receptors for cation and anion sensing based on supramolecular chemistry concepts has been grown in interest in the past ten years. Three main approaches have been used in the development of these receptors namely the binding site-signalling subunit, the chemosdosimeter and the displacement approach.[1] Perhaps the binding site-signalling subunit approach has been the most used and consist in the linking, thorugh a covalent bond, of one signalling subunit and one binding site. The most interesting molecular receptors were obtained by coupling a dye (chromogenic receptor) or a fluorophore (fluorogenic receptor), as signalling subunits, to a suitable binding site. Thus the microscopic coordination event induced a macroscopic signal (change in colour for chromogenic receptors and in emission for fluorescent receptors) that allows to detect the presence of the target species.

In this sense our group has been developed a great variety of chromogenic and fluorogenic receptors for cation and anion sensing by coupling a great variety of dyes with suitable binding sites for cation (crown ethers) and anion (amides, ureas, thioureas) sensing. Azoic dyes, aniliniopyridines, tricyanovinyl benzene dyes, phenoxazinones and squaraines have been used as signalling subunits in the development of this novel receptors.



Using tricyanovinyl derivatives a three channel sensing of metal cations was achieved in the sense that trycyanovinyl moiety is a dye, and presents fluorescence emission and redox properties. Choosing the appropiate channel one can tune selectivity towards certain metal cation. Solutions of azoic dyes and phenoxazinones functionalised with the dithia-dioxa-monoaza crown ether changes its colour selectively in the presence of Hg^{2+}. Also phenoxazinone derivative present selective fluorescence intensity enhancement in the presence of the Hg^{2+} cation.[2] Squaraine dyes have been used to detect nucleophilic species (cyanide, thiols, amins) in aqueous solutions due to the electrophilic character that presents the central four membered ring.[3] Anilinopyridines presents two binding sites (crown ether moieties and pyridine ring) but the selectivity towards metal cations is very scarce. Although some of this metal complexes change their colour selectively in the presence of certain anions. Based on this unselective anilinopyridine–metal cation complexes chromogenic detection of various small monovalent anions was achieved from signalling pattern recognition. For one specific ensemble, a remarkable selective chromogenic response toward acetate was found.

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FLUORESCENT BIOSENSOR FOR NITRIC OXIDE BY INSERTION OF 2,3-DIAMINONAPHTHALENE IN LIPOSOMES IMMOBILIZED IN SOL-GEL GLASSES

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The discovery of nitric oxide (NO) as a molecular mediator of a variety of physiological processes, including blood pressure regulation and neurotransmission, has lead to an increasing interest in improved methods for detecting and monitoring this compound.[1] Measurement of NO is also essential to prove and clarify many other physiological actions in which NO could be implicated. However, it is difficult to determine this radical in biological samples due to the small concentration *in vivo* and the lability of NO in presence of O_2. In this view, a simple, sensitive and selective method of detecting NO is required. Fluorimetry is one of the promising techniques satisfying these requirements.

In this work, we have developed a fluorescent biosensor for NO based on the immobilization of the probe 2,3-diaminonaphthalene (DAN) in sol-gel glasses. The relatively fluorescent DAN reacts rapidly with NO, to yield the highly fluorescent product 2,3-naphthotriazole (NAT).[2] To get immobilization of DAN in the sol-gel matrix, the molecule was previously incorporated in liposomes of phosphatidylcholine, which were later encapsulated into the matrix.

Incorporation of DAN in liposomes was monitored from changes observed in the anisotropy, intensity and fluorescence lifetime of the probe. The phospholipid/water partition coefficient of DAN calculated from these experiments, was found to be relatively high (K_p= 600). Results show that a) DAN successfully inserts into the liposomes but remains close to the membrane surface and b) DAN incorporated in membranes is able to react with NO to yield NAT (see Figure 1).

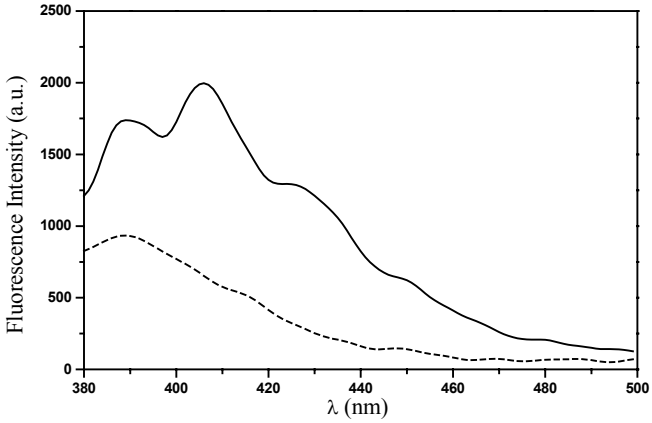

Figure 1. Fluorescence emission spectra of a) DAN in phosphatidylcholine (PC) liposomes (dotted line) and b) NAT in PC liposomes, after adition of SNAP (solid line). [DAN] = 6 μM; [PC]= 3 mM; [SNAP]= 5.4 μM ; λ_{exc}= 365 nm; T=30ºC.

Immobilization of DAN /liposome system in sol-gel slides (1 mm x 9 mm x 17 mm) was monitored by fluorescence microscopy. The ability of this biosensor to determine NO was investigated using SNAP, which spontaneously releases NO in aqueous solution.

Acknowledgements. Work supported by grants PI020606 (Instituto de Salud Carlos III, Spain) and MAT200203515 (MCyT, Spain)

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ORGANIC-INORGANIC HYBRID MATERIALS FOR OPTICAL DETECTION OF ANIONIC AND NEUTRAL SPECIES

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The development and study of new chemosensors for different chemical species has rised an increasing interest during the last years. Among them, the least studied have been those focused on the sensing of anionic and neutral species, mainly due to the fact that usually the interactions are weaker and less specific when anions and/or neutral species are tried to be detected. In this context our group has been devoted to the development of new ideas for the detection of such species mainly based on supramolecular chemistry concepts that bridge from molecular to solid properties. The design approach was inspired in the well-known characteristics of MCM41-type solids such as their very large specific surface, relative facility of functionalisation, homogeneous porosity, etc., and in the ability of different organic moieties to interact with the target species. Usually, the organic moiety is composed by two subunits as it will have to work in the same way as the molecular sensors do:[1] the receptor subunit which is in charge of the interaction with the receptor and the signaling subunit which will rise the signaling event. The anchoring of the organic moiety onto the inorganic matrix could give to the final hybrid system not only an easier handling but an apparent increase of the target concentration, the possibility of sensing target species in aqueous media with hydrophobic organic sensors, easier fabrication of final sensing devices, and the participation of the solid in the recognition process.

We have chosen the UVM-7 inorganic matrix consisting of MCM-41 mesoporous (1100 m^2 g^{-1} specific surface and average mesopore size of 32 Å) nano-sized particles tied together in micrometric conglomerates that give to the solid a noteworthy textural porosity in the range of 20 to 70 nm that might facilitate the migration of the target species through the solid. The mesoporous silica materials were prepared according to literature procedures.[2] This material has been functionalised with different organic moieties which have been chosen to fulfill the selectivity criteria towards each selected specie. In the case of anions, amines, amides, ureas, or thioureas have been chosen as the receptor unit; for neutral species specific receptor have been developed. In addition, the selectivity of the final hybrid material can also be tuned by means of the functionalization method utilized. Hence, the combination of different functionalization processes (cohydrolysis, post-synthesis grafting) on differently treated solid matrix (mesostructured, extracted mesoporous, calcined mesoporous or activated mesoporous) may give rise to a wide diversity of behaviors in the final solid.

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MICROARRAY COMPACT-DISC BASED METHODS APPLIED TO GENOMICS. SUPPORTS TREATMENT COMPARISON

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Microarray technology as applied genomics, proteomics, diagnostics, environmental, and drug discovery areas, is a research topic for which different chip-based devices have been developed. DNA microchip combines lithographic immobilization techniques with oligonucleotide synthesis to form high-density microarrays on silicon chips[1]. Another way of making microarrays is by means of high speed robotic printing or spotting onto glass slides. Fluorescence confocal microscopes or CCD cameras are most commonly used detectors. Advantages of microspot-based formats are the increase signal to noise ratios and conduct highly parallel simultaneous analysis. The microarray-related works described in the literature use a rectangular coordinate system on 7.5 x 2.5 cm glass-chips, to index individual microspots.

Despite the fact that discs have been used for the storage of digital data (e.g. magnetic floppy discs and optical compact discs) for many years, the use of CDs (CD-ROM, CD-R and DVD) compact discs as an analytical platform is new. CD-based technology is an interesting tool to apply in chemical interaction detection. Also, there is a lot of potential for overlapping/coordinating the activity of data analysis and data storage on a single rewritable disc. This combines well for performing simultaneous, multianalyte, array-based analysis in the field utilizing a CD reader instrument to detect dots with a size about 400 μm. Disc supports are also ideal to develop different sample treatments in a simple manner based on centrifugal flow[3].

We have explored the principles of compact disc-based chemical assays. This new methodology successfully combined high-density microarrays applied via a piezoelectric inkjet applicator with circular or Cartesian indexing on polycarbonate (PC), polymethylmetacrilate (PMMA) or directly over CD-disc surface.

Activation of PMMA by surface amination was reached by alkaline ethylendiamine treatment. After, cross-linking of amino-terminal oligonucleotide probes to surface was reached. Polycarbonate derivatization studies have not given conclusive results yet. On the other hand, avidine coating was considered as a general polymers treatment. Also, different working variables related with hybridization step has been studied. Figure 1 shows the hybridization results using Cy5 labeled oligos.

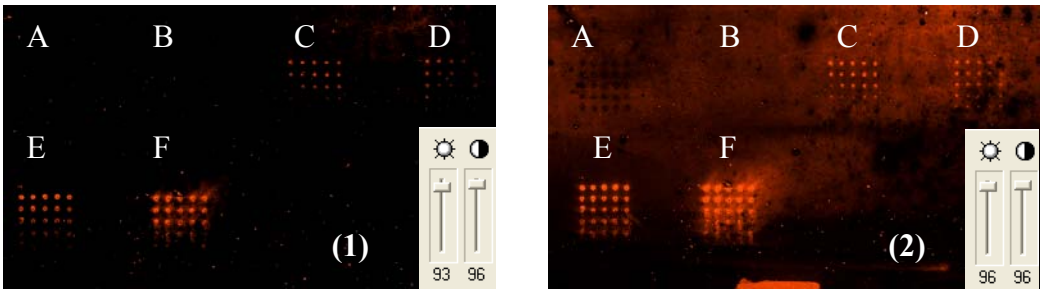

Figure 1. Hybridization assays, at different oligonucleotide concentrations (A – F, 0 – 26 μM), developed on avidine coated raw Polycarbonate (1) and Polycarbonate-CD surfaces (2).

The results are good and comparable to those obtained by the methodologies based on glass chips, suggesting that compact disc-based microarray technology competes well giving qualitative and quantitative results for simultaneous multianalyte assays.

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OPTICAL SENSORS FOR CO_2

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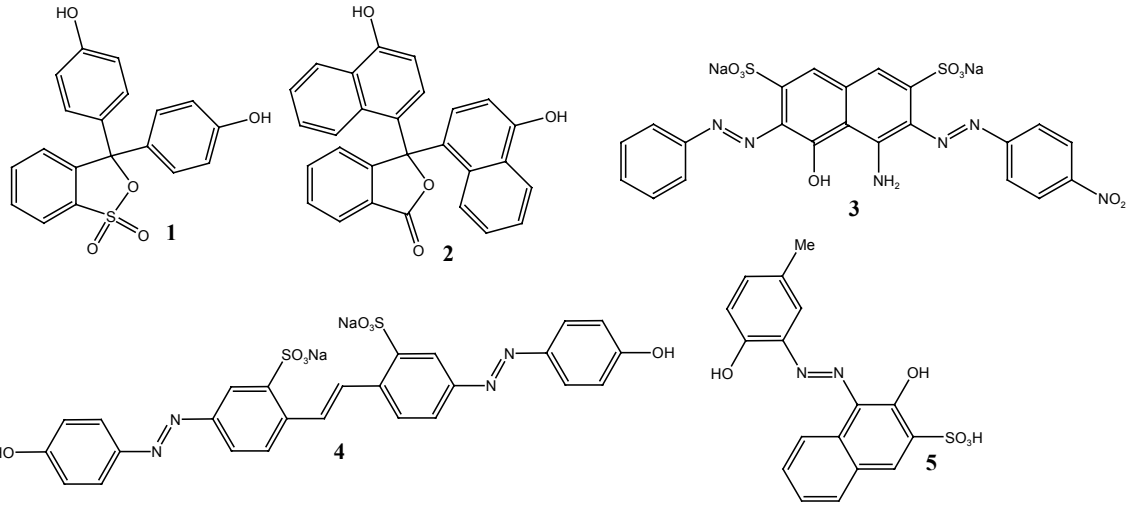
The development of optical sensors for detection of gases, ions, and molecules of physiological and biological importance has now become a well-established field of research. One gas of particular interest is CO_2 as its quantitative detection is important in such fields as medical monitoring, e.g. breath-by-breath CO_2 analysis,[1] and food packaging, e.g. screening of modified atmosphere packaging.[2]

Amongst the most recently developed optical sensors for CO_2 are the liquid gel-like, thin plastic films mounted on a solid support, usually glass. The optical sensors that our investigations are referred to go back to Mills and coworkers. They first introduced the use of an optical sensor.[3] The optical sensor constitutes of a plastic film which utilizes a pH sensitive hydrophilic indicator dye, phenol red **1**, combined with a phase-transfer agent, Q^+OH^-, to form an ion pair, Q^+D^-, which can be dissolved in a hydrophobic, plasticised polymer.

We studied a series of commercial dyes, containing different functional groups. Based on the results obtained, a strong correlation was found between the structure, the pKa and the sensitivity to CO_2, which can explain the activity of certain pH-indicator dye and why some of them failed.



The best results were obtained with the dyes **1-5**, which demonstrated sufficient sensitivity to CO_2, reversibility and stability for more than a month and which were practically inert to humidity.

The dye **2** was used as model for the investigation of the membrane reaction and to review the mechanism of the interaction between the dye and carbon dioxide, using a modified ATR FT-IR spectrometer. Based on these measurements, it was possible to derive a new mechanism.

Acknowledgements. This work was supported by CTI MedTech within Project 5562.2 SUS. This support is gratefully acknowledged.

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ASSAY OF LUMINESCENCE- BASED TRACERS FOR PESTICIDE IMMUNOSENSING

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The use of biosensors for pesticide determination in environmental matrices is prone to interferences, and sample extraction and clean-up steps are often required. For this reason, the effect of the organic solvents in immunochemical interactions has been studied and different sensors working in this media have been developed.[1,2] This finding allows the direct determination of pesticides using standard clean-up methodology. However, the sensitivity reached in organic media is lower than that obtained in aqueous media. Usually, HRP based tracers with colorimetric or fluorescent detection are used in immunoassay, but few studies have been carried out using alternative enzymes such as alkaline phosphatase (AP) with luminescence detection or labeled with chelates for time-resolved measurements.

In this work, a comparative study of different tracers for Carbaryl and Irgarol 1051 immunosensing in both aqueous and organic media is addressed. Several immunoassay protocols have been tested for both analytes using Protein A/G as support of the heterogeneous reaction, the best combination of reagents in aqueous and organic media,[3] and different tracers based on enzyme labels with fluorogenic (HPPA) and luminogenic (CDP-star™) substrates and Terbium (III) chelate for time-resolved fluorescence detection.

The biosensor with the best performances in organic media has been used for the determination of carbaryl in different water samples extracts (Table 1). It is concluded that luminescent detection using AP and CDP-star™ substrate can be applied for carbaryl determination below the MRL in drinking water. Also, based on the good RSD values obtained (4-14 %), the immunosensor seems to be a suitable tool for pesticide detection.

Table 1. Results of the analysis of water samples fortified with carbaryl (100 ng/L).

Sample	*Found Level[a] (ng/L)*	*Recovery (%)*
Mineral water	98.3 ± 12.1	98
Anoia	94.4 ± 11.0	94
Can Carné	83.6 ± 11.3	84
Pont Molins	81.4 ± 5.7	81
Pont Vilamare	95.7 ± 5.2	96
Prese Cairat	81.3 ± 3.3	81
Sallent	82.2 ± 3.4	82
S. Joan Despí	92.4 ± 10.0	92

[a]*Found level expressed as mean ± deviation standard (n=3)*
Organic media: 20% ethyl acetate-30% methanol-50% TBS buffer

It is worth mentioning that no competition was observed using the same immunoreagents but HRP or Tb chelate as labels, in both aqueous and organic media, although with other Ab-hapten combinations higher sensitivity was obtained under time-resolved mode detection.

Acknowledgements. This work has been supported by DGES Project PB98-0563. The authors thank M.P. Marco and D. Barceló for providing the immunoreagents.

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RESPIRATION-BASED TOXICOLOGICAL TESTS WITH MICROPLATES EQUIPPED WITH OPTICAL OXYGEN AND pH OPTODES

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With the increasing production of new chemicals the need for fast and low-priced toxicological tests increases as well. Standard methods are the *Pseudomonas putida* growth inhibition test (ISO 10712, DIN 38412 L8) and the *Pseudomonas putida* respiration inhibition test (DIN 38412 L27). In these tests the decrease in respiratory oxygen consumption by bacteria caused by toxic substances in wastewater is measured with oxygen electrodes and compared to the oxygen uptake in samples containing no toxic substances.

However, the use of electrodes is not applicable for fast screening tests and includes several disadvantages like possible contamination of the sample, oxygen consumption by the electrode and accumulation of bacteria on the membrane. A more convenient alternative consists in the use of oxygen-sensitive, fluorescent dyes. We used an indicator dye and an oxygen-insensitive reference dye, both embedded in a polymer and fixed at the bottom of 96 well microplates[1].

A second parameter related to respiratory activity is the pH-value. Measurements performed with sensor-coated microplates containing a fluorescent, H^+-sensitive indicator dye and an H^+-insensitive reference dye were compared to the respiratory rates measured with the oxygen sensors.

Using optical sensors embedded in microplates, the advantages of both techniques are combined. Internal referencing provides for high accuracy and reproducibility of the sensor signal. The assay is almost calibration-free, allows for high throughput screening and is compatible with existing instrumentation.

For fast testing, the respiratory inhibition test with a constant amount of bacteria is preferable to the growth inhibition test because of its much shorter measurement time. However, oxygen flux into the sample from ambient air is a main complication. It should be preferably small to achieve the best possible precision and to allow the detection of small changes in oxygen partial pressure and metabolic rates within the sample. Therefor we tested a variety of plate sealings to minimise the oxygen diffusion from ambient air.

* To whom correspondence should be adressed

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NOVEL FLUORESCENT RATIOMETRIC pH SENSORS WITH A MINIMIZED EFFECT OF IONIC STRENGTH

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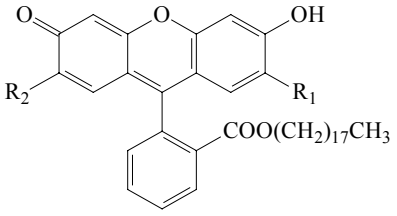
In most chemical and biological processes, the measurement and control of pH plays a major role for their success. Although the measurement of pH with conventional glass electrodes is a well-established method, there are numerous applications wherein the use of electrodes is problematic or impossible. Optical sensors, based on pH-sensitive dyes, are a promising alternative.

Optical pH determination has the fundamental disadvantage that the measured signal is depending on the ionic strength of the sample. The problem originates from the non-ideal correlation between the proton activity and the concentration of the pH-sensitive dye. The effect of ionic strength on the sensor signal depends on the charge of the indicator substance and its environment, e.g. the immobilisation matrix.

We present novel lipophilic fluorescein esters (Figure 1) with one negative charge. They are embedded in a neutral, highly proton-permeable hydrogel. The dyes differ in the substituents in 2'- and 7'-position of the xanthene structure. These varying substituents cause a pK_a shift of the indicators from 5.5 to 8.5. Their quantum yields are very high and comparable to fluorescein.

The indicators were made lipophilic by esterification of the carboxy group with a C18-alkyl chain. This results in three characteristic features: Firstly, the number of charges in the molecule structure is reduced to one negative charge. This results in a low, negligible cross-sensitivity of the sensors towards ionic strength in the range from 25 to 500 mM (Figure 2). Secondly, the modification of the carboxy group prevents lactonisation of the chromophore. Therefore, the indicators show different maxima in absorption and emission spectra for basic and acidic form which are desirable with respect to internal referencing. Thirdly, the lipophilic character of the dyes prevents their leaching from the polymer matrix. Thus, covalent coupling is not needed and eases sensor preparation.

Due to the similar spectral properties, the indicators DCFOE and CHFOE were mixed in one sensor. This results in an optical pH sensor with an extended dynamic range from pH 4.5 to 8.



DCFOE:	R1 = Cl	R2 = Cl	pK_a ~ 5.5
MCFOE:	R1 = Cl,	R2 = H	pK_a ~ 6.8
CHFOE:	R1 = Hexyl-,	R2 = Cl	pK_a ~ 7.1
DHFOE:	R1 = Hexyl-,	R1 = Hexyl-,	pK_a ~ 8.5

Figure 1. Chemical structure of the 2', 7' substituted fluorescein esters.

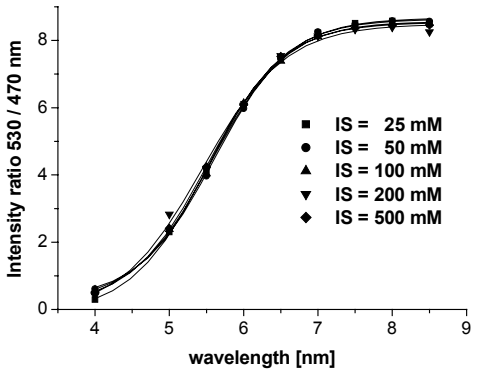


Figure 2. Calibration curves of DCFOE sensor at ionic strengths from 25 mM to 500 mM.

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EUROPIUM TETRACYCLINE: A VERSATILE BIOSENSOR PROBE FOR TIME-RESOLVED FLUORESCENCE IMAGING APPLICATIONS

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Optical imaging methods have attracted strong attention in life sciences since they find numerous applications in bioanalysis and medical diagnostics. (Immuno)histochemical staining is an important method for scanning tissue samples or cell cultures for microbiological or medical purposes, which can be combined with fluorescence lifetime imaging. On the other hand, sensor probes for the imaging of oxygen partial pressure or pH in biological samples have been developed, which can be used for the detection of skin tumor cells.[1] Europium chelates with their unusually long fluorescence decay time in the µs range have been used as labels for proteins or antibodies for applications in time-resolved fluoroimmunoassays (TRFIA).

We present now different examples of the applicability of europium(III) tetracycline [Eu(Tc)] as a molecular (bio)sensor probe for time-resolved fluorescence imaging. Based on the fact that the [Eu(Tc)] complex undergoes a 15-fold increase in fluorescence intensity on exposure to hydrogen peroxide (HP) and this effect is reversible,[2] this probe can be used as molecular sensor for the detection and imaging of H_2O_2.[3] This sensitivity and the long fluorecence decay time (~ 60 µs) of the resulting [Eu(Tc)(HP)] complex can be utilized for several applications of this reagent for luminescence lifetime imaging, all working at neutral pH:

- Imaging of glucose via planar sensor foils, prepared by coadsorption with glucose oxidase on polymer hydrogels
- Determination and direct visualization of the activity of oxidases (e.g. glucose oxidase), catalases or peroxidases
- Detection of peroxidase-conjugated antibodies which enables the time-resolved fluorimetric imaging of enzyme-linked immunosorbent assays (TRFI-ELISA) through consumption of the strongly fluorescent [Eu(Tc)(HP)] complex and formation of the weakly fluorescent [Eu(Tc)].
- Time-resolved imaging of citrate and other main intermediates of the Krebs cycle which is now possible for the first time. The assay offers their straightforward detection without the need for multi-enzyme systems.

Our self-developed imaging system, basically consisting of a LED array (405 nm), a CCD camera, a pulse generator and optical filters for excitation and emission, permits a fast data acquisition and evaluation process. The sensor probe can be adopted in microwell plate formats for high-throughput screening applications and for a parallel read-out of multiplexed samples. Different ratiometric and intrinsically referenced time-resolved imaging methods have been carried out, e.g. *Rapid Lifetime Determination* (RLD) or *Phase Delay Rationing* (PDR). The instrumental parameters were optimized, in order to obtain maximum sensitivity of the sensor, a broad dynamic range, low limits of detection and high spot homogeneity. Finally, the data acquired with help of the imaging device are compared with the experimental results achieved with commercially available microwell plate fluorescence readers.

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A NEW SUPRAMOLECULAR CHIRALITY SENSOR ON THE BASIS OF ACHIRAL ETHANE-BRIDGED BIS-PORPHYRIN

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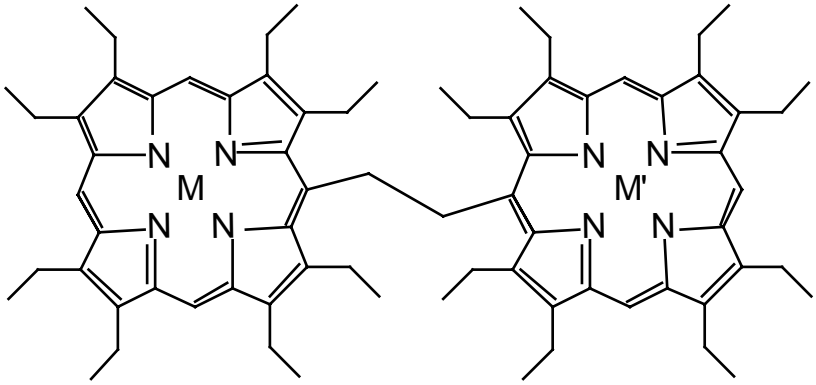
Chirality sensing is not only one of the major problems of basic chemical science but also has important practical implications in nanotechnology, medicine and pharmaceutical industries.



1. M = M' = Zn; **2**. M = M' = Mg; **3**. M = Zn, M' = 2H

Recently we have found that achiral bis(zinc porphyrin) (**1**) in which two porphyrin rings are linked by a short ethane bridge serves as an effective supramolecular chirality sensor for the determination of the absolute configuration of chiral monofunctional amines and alcohols in non polar solvents at ambient and low temperatures, respectively.[1] The sensoric ability of **1** is based on the induction of appreciable bisignate Cotton effects in the circular dichroism (CD) spectra upon interaction with chiral ligands due to screw structure formation in the resulting 1:2 host-guest complex. The sign of the induced CD couplet is unequivocally correlated with the absolute configuration of the chiral guests. Thus, when the substituent's bulkiness order at the stereogenic center α or β to the ligating group coincides with the priority rule, (*R*)-guests produce negative chirality due to formation of the corresponding left-handed twist in **1**, whilst (*S*)-guests exhibit opposite sign.

To increase further sensoric sensitivity of this chirality probe the Zn central ion was replaced with Mg to yield **2**.[2] This results in enhancement of the host-guest binding strength, and thus allows direct determination of the absolute configuration of monoalcohols at room temperature, and furthermore considerably decreases the required guest concentration.

To expand the chirality sensor's applicability to bidentate chiral compounds (diamines and aminoalcohols) a new type of monometallic bis-porphyrin **3** was prepared and effectively used in the determination the absolute configuration of the bidentate guests.[3]

Due to the non-covalent nature of the host-guest interactions the process of chirality sensing by **1**-**3** is highly dependent upon various internal and external stimuli, which can be also used as effective controlling factors to increase the sensor's sensitivity and applicability. Particularly, the roles of solvent, temperature, phase transition, stoichiometry, bulkiness and other parameters are discussed.

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USE OF PLANAR OPTODES IN MARINE MICROBIOLOGY- OVERVIEW OF MOST RECENT APPLICATIONS

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Many ecosystems studied in marine microbiology comprise boundaries through which the exchange of life-essential substances takes places between the organisms or their communities (e.g., corals, bacteria in sediments, microbial mats, etc.) and the surrounding environment (marine waters and sediments). This exchange is governed mainly by physical processes such as molecular diffusion and advection but also by life-driven processes such as bioturbation. From the nature of these processes it follows that the characteristic distance over which significant variations of the relevant elements occur is in the order of tens of micrometers to a few centimetres. The Microsensors group at the MPI in Bremen have successfully employed microsensors for many years to quantify these variations and to reveal the mechanisms of the processes causing them.

Planar oxygen optodes have been extensively used over the last number of years as a valuable tool contributing to marine research at MPI. In this contribution we provide an overview of the most recent applications of the optodes that have facilitated a better understanding of how various (micro)biological and exchange processes function by allowing us to watch them in (semi)*in-situ* conditions and in real time. The applications presented will include: visualisation and quantification of bioturbating activity of worms (*Nereis diversicolor*) in sediments, "breathing" of sediments, exchange of oxygen and nutrients between the sediment and the water column driven by the interaction between the water flow and the sediment topography (ripples), visualisation of the heterogeneous distribution of photosynthetic activity of microbial mats and photosynthetic centres in corals (see Figure 1).

The most recent developments in planar optode technology, namely the development of the hybrid pH/pO_2 and pCO_2/pO_2 sensor foils, have opened the door for a whole new series of experiments that can be carried out using the established measuring techniques developed for oxygen sensing. The first exciting results of the simultaneous imaging of pO_2, pCO_2 and pH distributions associated with the photosynthetic activity of diatoms living at the top layer of sediment will also be presented.

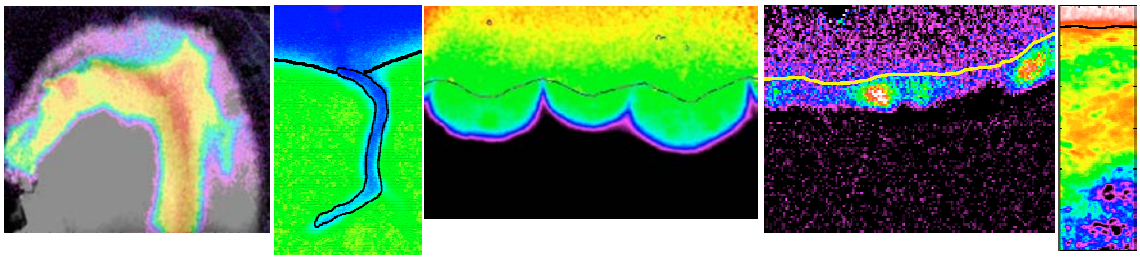

Figure 1. Examples of two-dimensional oxygen distributions in a coral (a), sediment with a burrow into which a worm pumps oxygenated water (b), sediment with ripples (c). Examples of two-dimensional distribution of the oxygen production rate in a microbial mat upon illumination (d) and the oxygen consumption rate in permeable sediment (e). The scale of the colour-coding has been omitted here as it is not necessary for the purpose of visualisation of the 2D variation of the respective quantities.

Acknowledgements. The financial support by the European project Optoden (Project-Nr. 03F0284A) is greatly appreciated.

AN INVESTIGATION OF ISOMERIC EFFECTS USING PLASTICIZED PVC FILMS FOR XYLENE EXTRACTION AND ENRICHMENT WITH ATR-FTIR SPECTROSCOPY

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Isomers are different compounds because they have different molecular structures. This difference in molecular structure gives rise to a difference in properties; it is the difference in properties that tells us that we are dealing with different compounds. The xylene isomers are primarily used as solvents. They are found in protective coatings, lacquers, enamels, rubber cements and cleaning agents. The three isomers (o-xylene, m-xylene and p-xylene) are also used individually as starting materials n the manufacture of various chemicals. Selective and simultaneous quantification of each isomer in mixtures requires that at least minute spectral difference can be observed. In the NIR, spectral difference were found to be more dominant in the 1800 – 2100 nm range compared to the 1100 – 1650 nm range.

In this study a selection of plasticised PVC phases have been investigated for xylene isomer determination using ATR-FTIR spectroscopy. The effect of the plasticiser concentration on analyte enrichment was studied in relation to: binary and tertiary mixture effects and competition with more polar analytes for enrichment into the PVC films.

It was found that when the isomers are determined as part of a mixture, the maximum absorbance at the selected wavelengths for each analyte, is reduced significantly. This is due to the competition between the analytes for space / free volume, within the polymer, i.e. m- and p-xylene were found to enrich more rapidly than o-xylene. Interesting results were found when a more polar analyte was added to the mixture. It was found that the presence of the polar analyte had the effect of increasing the ingress rate of the xylene isomers. The results show that in developing sensors for aromatic hydrocarbons, the enrichment effects due to the isomers is significant and other compounds present will also impact on sensor response.

INVESTIGATION INTO POLYMER-DIFFUSANT INTERACTIONS USING ATR-FTIR SPECTROSCOPY

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Using a polymer coated attenuated total reflectance (ATR) waveguide and Fourier-transform infrared (FTIR) spectroscopy, interactions between penetrants and a Teflon AF2400 polymer matrix were observed. The AF2400 membrane was coated onto the measuring surface of a ZnSe crystal waveguide, concentrating the target diffusant(s) within, and excluding interfering water from, the information region of the standing infrared evanescent wave. The Fickian diffusion of small molecular species into the AF2400 polymer membrane was followed through observation of distinct penetrant infrared absorption bands. During the analysis of a number of analytes (e.g. toluene, chlorobenzene, tetrachloroethylene), infrared bands caused by the action of penetrants within the AF2400 polymer matrix were observed. These bands may be attributed to the shifting of original polymer infrared bands caused by optical or molecular (e.g. electrostatic) effects. The nature/character of the observed 'interaction bands' was investigated. Through monitoring of the interaction bands an increase in the detection sensitivity was observed for the examination of toluene solutions, indicating the potential of the penetrant/polymer interaction as a novel 'smart' methodology for polymer-modified FTIR sensing.

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MULTI-COMPONENT DETERMINATION USING PLASTICISED PVC FILMS FOR ANALYTE EXTRACTION AND ENRICHMENT WITH ATR-FTIR SPECTROSCOPY

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Multi-analyte sensing has received much attention over the past few years due to the wide variety of applications it has, such as in environmental and biological analysis. Problems associated with multi-component analysis depend on the analyte type, wavelength of absorption, boiling points, dielectric constants, molecular size and shape etc. if target analytes have similar characteristics it may be difficult to distinguish between them, thus hindering their determination and quantification. Chung *et al.*[1] have shown that unique spectral features can provide information for the selective quantification of each component. Laboratory-based methods for multi-analyte determination of chlorinated hydrocarbons or aromatic hydrocarbons include NIR, IR and Raman spectroscopy, GC-MS, quartz-crystal resonators and UV spectroscopic methods. Several in situ methods have been devised and investigated for both aliphatic and aromatic hydrocarbons in aqueous and non-aqueous environments. Mass-sensitive sensor arrays[2] and fibre optic evanescent wave sensors (FEWS)[3] have been at the forefront of these investigations. This paper describes the determination of benzene, toluene, ethyl benzene and xylene (BTEX) using ATR-FTIR spectroscopy.

The results of 5-plasticised PVC films were are shown to demonstrate their potential in selective enrichment of the BTEX analytes. Plasticisers from the azelate and adipate groups have shown most promise for these analytes. Parameters such as analyte enrichment rate and maximum absorbance have been selected for comparison purposes. The results have shown that the use of plasticiser provides superior enrichment when compared with the absence of plasticiser in the PVC film. A comparison of the enrichment of aliphatic compounds and aromatic compounds is shown.

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LONG-WAVELENGTH CHROMOGENIC SUBSTRATES FOR AN ABSORPTION-BASED ASSAY FOR SERINE PROTEASES

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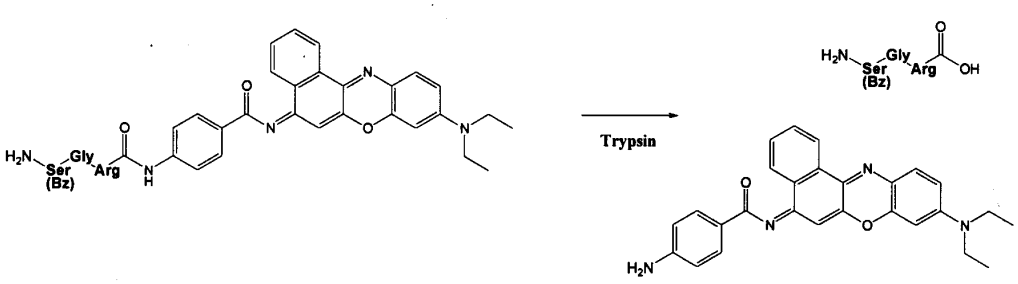
The majority of commercially available chromogenic substrates are based on p-nitroanilides systems (λ_{max} = 300 nm). These substrates release p-nitroanilin (λ_{max} = 385 nm) when treated with the appropriate protease. Since the biological background in this range of the spectrum is severely interfering, this methodology has undoubted restrictions. Therefore, peptide substrates labeled with chromophores, which absorb at higher wavelengths, are currently developed.
Novel Dyes, absorbing in the red region of the visible electromagnetic spectrum, were synthesized. Specific peptide sequences labeled with the dyes allow detection of serine proteases activity with high selectivity, at low concentration of enzyme.



Cleavage of the substrates labeled with nileblue derivatives at physiological pH was observed during this study both in solution and on an optical chip.
The substrates show an absorbance maximum at 660 nm and are supposed to show no spectral interference with constituents of blood samples or absorbing molecules from blood cells or blood plasma itself. Therefore, they are very attractive for rapid diagnosis of cancer diseases, for Point-of-Care-Testing and high throughput screening of drugs. The approach combines an integrated optical microchip with highly selective enzyme substrates in order to measure the enzyme activity.

Acknowledgements. Financial support from CTI MedTech grant #3828.2 and synthetic contributions and helpful discussions with Fluka AG, are gratefully acknowledged.

SPIN-COATED UV-VISIBLE OPTICAL SENSING DEVICES BASED ON DYE-IMPREGNATED FILMS FOR RAPID DETERMINATION OF PREDOMINANT METAL IONS IN WASTEWATER STREAMS

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Heavy metals are ubiquitous in the environment, so their quantitative determination at trace levels is very important in the field of environmental analysis. Current laboratory-based techniques used for the detection of heavy metals in water samples include atomic absorption spectrometry (AAS),[1] inductively coupled plasma emission spectrometry (ICP-ES) and anodic stripping voltammetry (ASV). These methods provide good limits of detection and wide linear ranges. They are often capable of multielemental analysis in the parts per trillion range. However, their expense and labour-intensive sample preparation procedures, limit their overall throughput. Also, these methods do not lend themselves easily to miniaturisation, which is necessary for use in a sensor design.

Optical chemosensors for the determination of heavy metal ions are receiving increased attention by researchers. The use of fibre optics in the development of chemical sensors has increased due to their simplicity, flexibility, and robustness.[2] They are capable of monitoring chemical concentrations *in-situ* and on a real-time basis. Plasticised PVC membranes with immobilised reagents have been used in the fabrication of optical heavy metal sensors. The practicability of using PVC membranes in these sensors is due to their homogeneity, ease of preparation and optical transparency. The membranes can incorporate an ion carrier, complexing agent, ionophore, ligand and chromoionophore. The development of sensors based on novel materials that are capable of interacting sensitively and selectively with a particular metal ion in aqueous solution is a subject of growing interest. Indicator dyes are used in the qualitative and quantitative analysis of metal ions. This is due to the potential availability of dye indication reaction and dye-coupled reaction for analytical measurement.[3] They form strong complexes with a large number of metal ions.

In the study described in this paper, a range of dyes have been tested by immobilising them in a plasticised PVC membrane. The membranes are investigated as a sensing phase for use in a UV-Visible spectrometry-based optical sensor. The effect of an ionophore or ion carrier in the sensing film is also investigated. The sensor will have potential for use in waste streams where particular metal ions form a significant portion of the waste streams. The potential of the films for metal speciation is also shown.

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[2] N. Mahendra, P. Gangaiya, S. Sotheeswaran, R. Narayanaswamy, *Sensors and Actuators B* **2002**, *81*, 196.
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OPTICAL FIBER pH SENSORS FABRICATED USING THE ELECTROSTATIC SELF-ASSEMBLY METHOD

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The pH sensors are utilized on a wide variety of different disciplines. A great number of their applications are related to the biomedical field where the utilization of the classic and relative voluminous pH electrodes for applications *in vivo* is inconvenient, in these cases the optical fiber pH sensors are a good alternative because they require a minimum invasive impact. The most of the optical fiber pH sensors are based on the utilization of a pH indicator that changes its color or fluorescence with pH. For the encapsulation of these indicators, different techniques have been proposed. Among others, the reagent can be entrapped on polymeric hydrogel structures, cellulosic films, sol-gel networks, incorporated in Langmuir-Blodgett films or even attached to microspheres. Unfortunately, the most of these techniques are not suitable for controlling the thickness of the coatings on the nanometer scale or for depositing materials on non-planar substrates. These are important drawbacks if the goal is to fabricate sensitive coatings on standard optical fibers where the dimensions of the coating determine in a substantial mode the sensor response, the signal level and the response time. An alternative technique that solves these issues is the so-called Electrostatic Self-Assembly (ESA) method, this technique is the most recent of the self-organizing techniques and is based on the alternating physisorption of oppositely charged polyelectrolytes.

In earlier works we have already demonstrated experimentally the fabrication of humidity, ammonia and volatile organic compounds fiber optic sensors fabricated with the ESA method. Also, other authors have proposed this technique for the possible fabrication of pH sensitive coatings[1] and, recently, we have reported the ESA method as a useful technique for the fabrication of optical fiber pH sensors[2, 3]. In this work, different optical fiber pH sensors fabricated with the ESA method that can cover the physiological blood pH range will be shown. The sensors are based on changes on the color (or fluorescence) of the different dyes. In order to obtain more durable sensitive coatings, special attention has been paid to the growing process of the coatings, this fabrication process has been studied and optimized. The durability and response of these sensors have been optimized with thermal treatments after the deposition process. The measurement range of the sensors goes from pH 4 to 8, with less than 2% of temperature cross-sensitivity (20-40ºC range). Some experimental results are shown below.

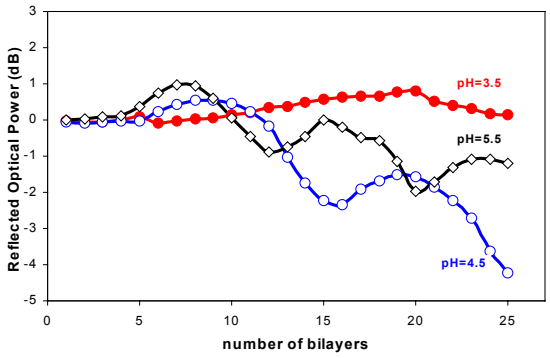

Figure 1. Curves of construction at different pH conditions. Optical power monitored at 850 nm.

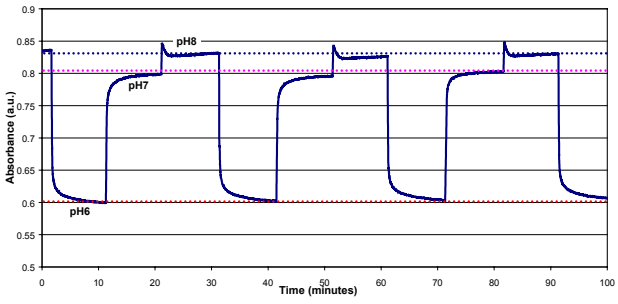

Figure 2. Dynamic response of one of the pH optical fiber sensors (colorimetric type).

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ELABORATION OF AN EVANESCENT WAVE OPTICAL FIBRE SENSOR FOR METHANE DETECTION

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It is well known that alcanes and specially methane, even at very low concentrations, is a very dangerous chemical compound for human safety and environment. So, low-cost chemical sensors are needed for monitoring natural gas leakages.

The formulation of a specific polymer for methane detection is carried out by including in a polymeric matrix with adapted optical properties (transparency, index of refraction lower than that of the fibre core) of macromolecules able to selectively trap the gas molecules, cryptophane cage A molecules were selected. This sensitive polymer is deposited by dip-coating on the PCS fibre core (provided by IREE Czech Academy of Sciences). The principle of measurement is based on the variation of effective index of refraction during the trapping of the gas molecules which induces a variation of the light intensity transmitted along fibre.

The sensitivity of the sensor was tested according to the length of the sensitive part. It decreases when the length of the sensitive part increases. The limit of methane detection reached is of the order of 2.4%. The selectivity of the sensor for various alcanes was tested (cf. Figure1). In the zone of concentration lower than 8%, it appears a strong sensitivity (6 times higher) for methane compared to that of other alcanes.

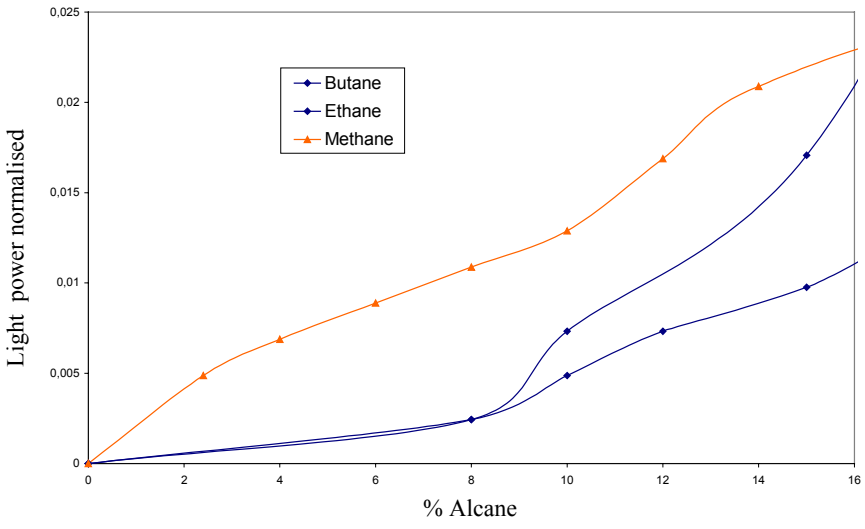

Figure 1. Sensor calibration curve for various alcanes

NOVEL CHEMICAL/BIOSENSOR PLATFORM BASED ON MULTIMODE INTERFERENCE COUPLERS

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We report on the development of a novel sensor platform based on the interference of the different modes of a strongly multimode planar wave guiding structure. This platform, which is applicable to both chemical sensors and biosensors, exploits our expertise in sol-gel-processed integrated optical circuits based on hybrid organic/inorganic materials.[1]

The structure is composed of three layers on a silicon substrate: the buffer layer (for isolation from the silicon substrate), the guiding layer and the protective layer which are deposited sequentially by spin coating. The guiding and protective layers are photo-polymerisable, which permits photo-patterning (by exposure through a mask or direct laser writing) of areas to be retained after etching or local modification of the refractive index. In the former case, this facilitates the direct writing of planar light-wave circuits (PLC's).

The circuits used in our application are multimode interference couplers[2] (MMIC's) composed of a wide planar waveguide connected to single mode waveguides used to inject or collect the light. Once light is injected in the multimode section of the device, the interference properties are such that periodically along the propagation path, the image of the injected beam is formed and the corresponding energy can be collected by a single mode waveguide placed at one of those locations. The periodicity length depends on the guiding properties of the multimode part. Thus a modification or perturbation of the index of the protective layer that lies on the MMIC structure affects this periodicity length and so the amount of energy coupled into the output waveguide. To use this property in a sensor application, a window is opened in the protective layer to access the multimode section. This window can accommodate bio- or chemo-recognition materials whereby binding of target analytes affects the local refractive index.

To prove the principle of this platform, we are currently working on the development of a humidity sensor. A TEOS-based sol-gel material is deposited on top of the waveguide core as a sensor layer, which also protect against damage from unwanted external conditions. It also provides porosity and hydrophilicity whereby the refractive index is then modified by the presence of water in the air. Those sensing properties are easily tuned by exploiting the versatility of the sol-gel process.

In this presentation, we will first present the principle of operation of the MMIC. The means of designing a sensor with the desired sensitivity or operational range will then be explained. We will describe the synthesis of the hybrid materials as well as the fabrication processes, and show initial results of the proof of principle experiments as well as device characterisation.

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DETECTION OF HEAVY METALS BY AN OPTICAL FIBRE SENSOR WITH A SENSITIVE CLADDING INCLUDING A NEW CHROMOGENIC CALIX[4]ARENE MOLECULE

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Experts estimate that industrial processes introduce up to a million different pollutants into the atmosphere and the aquatic ecosystem. Heavy metals are among these substances, although not all of them are considered harmful to humans.
The molecule of calixarene is a molecule used effectively in complexation of the heavy metal pollutants (nickel, copper…). The goal of this work is to condition a new chromogenic calix[4]arene molecule to elaborate an evanescent wave optical fibre sensor able to detect this type of pollutant.

To detect chemical species, the way followed in our laboratory consists in covering the optical fibre core by a transparent polymer whose effective refraction index is modified during absorption of the species to be detected. The chromogenic calix[4]arene molecule (figure 1) is included in a polysiloxane polymer the deposited on the silica core of a PCS fibre (provided by IREE, Czech Academy of Sciences).

The pH of solutions and the length of uncladded sensitive part were optimised (pH is around 5 and the uncladded length is 3 cm).

The limit of detection reached is of the order of 1 µM of copper(II) cations, as shown in figure 2. The limit of detection reached for cadmium(II) cations is around 10^{-4} µM.

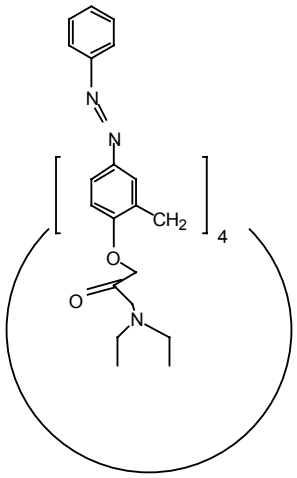

Figure 1. chromogenic calix[4]arene molecule

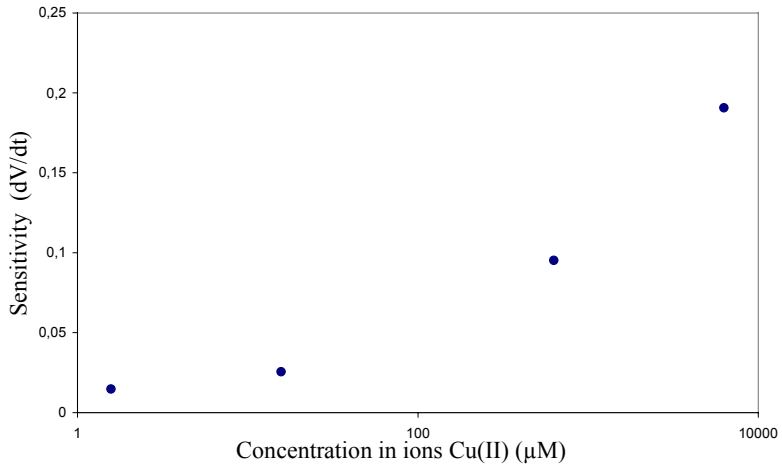

Figure 2. Calibration curve of the optical fibre sensor

NOVEL HYBRID MATERIALS FOR LUMINESCENCE-BASED SENSING

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Current sensor trends, such as multianalyte capability, miniaturisation and patternability are important drivers for matrix requirements in optical chemical sensors. In particular, issues such as stability, enhanced sensitivity and printablity are key in developing optimised sensor materials.

This study focuses on combining novel sol-gel-based hybrid matrices with engineered luminescent complexes to produce stable luminescence-based optical sensors with enhanced sensitivity for a range of analytes including oxygen and carbon dioxide. As well as optimising sensor performance, the materials were tailored to enable printed patterns and arrays on planar substrates.

Hybrid sol-gel matrices were developed using a range of precursors including methyltriethoxysilane (MTEOS), n-octyltriethoxysilane (C8TEOS), tetraethoxysilane (TEOS) and phenyltriethoxysilane (PhTEOS). Oxygen sensing and carbon dioxide sensing have been realised with these hybrid materials. Oxygen sensing is based on the luminescence quenching of the lifetime of the oxygen-sensitive ruthenium(II)tris(4, 7-diphenyl-1, 10-phenanthroline) [Ru(dpp)3], and related complexes. Carbon dioxide sensing is based on a variety of pH-sensitive luminescent complexes.

All luminophores have been selected on the basis of spectrally compatibility, enabling single wavelength LED and photodiode or CCD detection. A phase-based detection system is employed which overcomes the problems associated with intensity-based systems, thereby enhancing sensor performance.

Both C8TEOS and PhTEOS-based films exhibit enhanced oxygen sensitivity over previously developed MTEOS-based films. The microstructural features responsible for this enhancement were elucidated using techniques such as FTIR, diffusion coefficient and contact angle measurements. The long-term stability of the films has also been monitored.

The other approach to sensor optimisation involved exploiting the highly versatile family of ruthenium(II) complexes by tailoring the luminescent lifetime for enhanced oxygen sensitivity and also tuning the pH response to enable carbon dioxide sensing. Temperature insensitive complexes have also been engineered in a bid to eliminate the need for temperature correction of sensors. The behaviour of these tailored complexes has been investigated initially using the MTEOS matrix as a well-characterised model system

Print techniques, including ink-jet and pin printing, were used for patterned multianalyte sensing.

EMPLOYMENT OF ORMOCER®S FOR THE FABRICATION OF LAYERS SENSITIVE TO OXYGEN AND GLUCOSE

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Measurements of oxygen and glucose concentrations are important operations which can be often met in pharmacy, food production, biotechnology etc. For these measurements optical sensors have been tested recently.[1,2] In oxygen sensors, the detection is usually based on quenching the fluorescence of ruthenium complexes by oxygen[1]. In glucose sensors, the detection of oxygen, which is the reactant in the glucose oxidase-catalyzed oxidation of glucose, can be utilized.[2] This paper deals with the sensitivity of Ru-tris(4,7-diphenyl-1,10-phenanthroline)$^{2+}$ complex immobilized in layers of organically modified siloxanes to oxygen dissolved in aqueous solutions. ORMOCER®s, instead of the currently used Ormosils, are employed for tailoring the properties of the layers. Moreover, it is shown that the grafting of glucose oxidase onto these layers makes them sensitive to glucose in solutions.

Three types of UV-curable ORMOCER®s, based on glyceroldimethacrylate triethoxysilane (O1), methacryloxypropyl trimethoxysilane (O2) and trimethyloproprandiacrylate dimethoxysilane (O3) were used in the experiments. Siloxane backbones of the ORMOCER®s were prepared by the sol-gel method. The ruthenium complex was added to liquid ORMOCER® sols as an alcoholic solution in concentrations of 1-2% (wgt.). Then, a sol mixed of tetraethoxysilane was added and the final sols were applied on glass slides by the dip-coating method. Detection layers with thicknesses larger than 10 μm were obtained after UV curing of the applied sol layers. For glucose detection, glucoseoxidase was grafted onto the prepared oxygen-sensitive layers by using saturated vapors of glutaraldehyde.

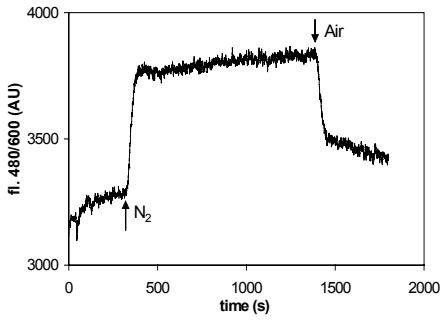

Figure 1. Time response of the ORMOCER® O2 with the immobilized Ru complex to oxygen dissolved in water

In the sensitivity determination, a slide with a detection layer was fixed in a flow cell making possible contact of the layer with aqueous solutions containing oxygen or glucose. Changes of fluorescence intensity of the layers induced by the chemicals were measured by using a HITACHI F4500 fluorescence spectrometer or by using a laboratory fiber-optic set-up. Fluorescence spectra, time response curves (see Figure 1) and sensitivity curves are shown in the paper. On the basis of these data, the performance of an extrinsic optical sensor of glucose employing ORMOCER® detection layers are discussed in the paper.

Acknowledgements: *This research was supported by the European Community (Framework V., GROWTH Programme, Contract G5RD-CT2002-00752).*

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TRACE CHEMICAL GAS SENSORS USING MID-INFRARED QUANTUM CASCADE LASER SPECTROSCOPY

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The advent of commercially-available thermoelectrically cooled distributed-feedback quantum cascade lasers (DFB-QCL) marks a turning point in the availability of mid-infrared (MIR) sources with high output power. The emission wavelength of these devices is determined by the thicknesses of alternating semiconductor layers forming the active region, instead of utilizing the actual energy bandgap of the semiconductor material. Hence, electron transitions between quantum sub-bands within the conduction band of the material, rather than electron-hole recombination is the functional principle, and allows manufacturing lasers over a wide range of wavelengths (3.5 to 19 μm) using various material combinations, such as InGaAs/AlInAs on InP substrate.

The single-mode, temperature-tunable emission provided by DFB-QCLs can be utilized for selective infrared absorption spectroscopy. Their compact size and temperature-tunable emission make them particularly well suited to trace chemical detection by absorption spectroscopy. Although current room-temperature DFB-QCLs are limited to pulsed wave (PW) operation, their potential as useful MIR source for sensors is not curbed. Hence, an alternative to larger instrumentation, such as Fourier-transform infrared (FTIR) spectrometers, is provided.

As industry grows and modern agricultural methods are increasingly adopted, demand for monitoring the impact of industrial and agricultural chemicals on the environment is steadily rising. Human exposure to trace levels of potentially hazardous chemicals in both indoor and outdoor environments is of increasing concern. Pollutants, such as volatile organic compounds (VOCs), are known to affect indoor air quality due to the widespread use of particulate materials in offices.1 As another example, cigarette smoke also contributes detrimentally to indoor environments releasing compounds such as ammonia, ethylene and nitric oxide into the air.2 As trace levels of such chemicals are sufficiently harmful to humans there clearly is a need for ultra-sensitive detection and continuous monitoring in a variety of environments.

Work detailing chemical sensing applications using a thermoelectrically cooled DFB-QCL will be presented. A number of configurations for the detection of trace amounts of environmentally relevant chemicals will be shown. The use of electronic referencing to eliminate pulse-to-pulse amplitude fluctuations in the laser emission eliminating the need for a separate reference channel and will be described. Furthermore, the effectiveness of absorption spectroscopy in open-path gas cells and hollow waveguides to serve as chemical sensors will be investigated.

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SENSITIVITY OF XEROGEL LAYERS APPLIED ON SILICA OPTICAL FIBERS TO TOLUENE DISSOLVED IN WATER

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Xerogel layers prepared by the sol-gel method have been widely investigated for the modification of properties of detection sites of optical chemical sensors.[1] They can be used for tailoring the performance of these sensors by using them for the immobilisation of optochemical transducers [1] which change their optical properties due to interaction with chemicals, or for increasing the concentration of the detected chemicals in the site.[2,3] This paper presents results of the investigation of novel approaches for increasing the sensitivity of silica optical fibers to toluene dissolved in water. For this purpose xerogel layers doped with methyl and phenyl groups and doping the layers with TiOTi groups are used with the aim of increasing the partition coefficient of non-polar toluene in the xerogel layers as well as the refractive index of the layers.

Xerogel layers were applied on segments of polymer-clad silica fibers from which an original polydimethylsiloxane cladding was removed. Input alkoxide sols based on tetraethoxysilane (TEOS), methyltriethoxysilane (MTES), phenyltriethoxysilane (PTES) and MTES with titanium tetrabutoxyde were used for layer preparation. The sols were applied by the dip-coating method and the gel layers were dried at 100 °C for 24 hours.

In the sensitivity determination, aqueous solutions of toluene were bring into contact with xerogel layers on the fibers and changes of optical properties of the layers were determined by measuring changes of the output power. In order to increase the fraction of power transmitted in the layer, the fiber was excited by an inclined collimated beam. Refractive-index changes of the cladding induced by toluene were evaluated on the basis of power changes measured at 670 nm (see Figure 1). Spectral changes due to C-H overtones as well as refractive-index changes of the layer were evaluated from spectra measured in a range of 1600-1800 nm.

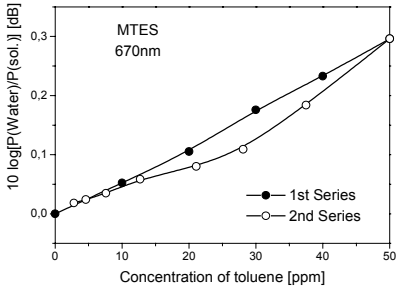

Figure 1. Sensitivity of a MTES-based layer to toluene dissolved in water

Results of experiments show that the detection of toluene dissolved in water by means of silica optical fibers with xerogel detection layers can be controlled by means of two factors. The first one is the presence of groups, which decrease the layer polarity (methyl or phenyl groups). The second one is given by the refractive index of the layer, which should be as close to that of silica as possible. Doping the layer with TiOTi chains makes possible to increase the layer refractive index, however it also increases the layer polarity.

Acknowledgements: This research was supported by the Grant Agency and by the Academy of Sciences of the Czech Republic (contracts No. 102/02/0780 and K2067107, respectively).

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PARALLEL DETECTION OF R22 AND ITS SUBSTITUTES BY REFLECTOMETRIC INTERFERENCE SPECTROSCOPY

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Since the middle of the 1970´s chlorofluorocarbons (CFC) like R22 are known to destroy the ozone layer. In the Montreal Protocol of 1987 and during the Kyoto climate conference in 1997 production and usage of these gases were severely limited worldwide. However, R22 is still in use in many countries as the restriction will not come into force before 2010 in the developed countries and not until 2030 in the developing countries.
Fluorocarbons like R134a and miscellaneous mixtures of R143a, R32 and R125 are already deployed as substitutes by now, as they do not destroy the ozone layer. Still, they have an enormous global warming potential. Therefore a recycling process for these substitutes is very important. Often, containers for recycling are not clearly defined and may include CFCs. As the fluorocarbons have to be pure in sort for the recycling process, a reliable classification and quantification of the returned substances is necessary.

Reflectometric Interference Spectroscopy (RIfS) is a fast, robust and low-cost method of detection of volatile organic compounds (VOCs) in gas phase and in liquids. The principle is based on the detection of white light interference of partly reflected beams at thin layers on a planar glass transducer. The received interference pattern includes information about the optical thickness ($n \cdot d$) of the polymer layer. As VOCs are enriched in the sensitive layer, a swelling behavior and thus a change in the optical thickness can be observed. By evaluating these changes, quantification can be achieved. The applied polymers should show a fast and reversible sorption of the gas molecules. The measurements need to be performed at a defined temperature.

The polymers utilized in this work are based on different interaction principles. Microporous layers were used to discriminate the analytes by their size. They show a time-dependent sensor response for analytes about or bigger than the mean pore size (molecular sieving effect). This impressively enhances the discrimination abilities of polymer-based optical sensors. By the use of neural networks, a quantification of mixtures with a single sensor can be achieved. As the sorption process needs not to be completed, the iteration loop of measurements can be much faster.
Rubbery polymers, such as PDMS and PUT, which use polarity differences to discriminate the analytes, show very fast sorption and desorption of the analytes. They were used for the detection of R125, as it shows a very slow sorption into the microporous polymer used in this work.
In all measurements, the analytes were mixed with dry air as carrier gas.

For measurements of different ternary mixtures of R22 (CFC) and its substitutes R134a, R32 and R143a, a glassy polycarbonate with a mean pore size of 0.1 nm^3 (Makrolon®) was applied. Using a single sensor set-up, some hundred mixtures (test and training data) in a concentration range of 0 to 10 percent by volume were measured by RIfS. The networks were trained by the training data and afterwards the concentration of the test data was predicted.

For mixtures including R125 an array consisting of microporous and rubbery polymers was used. As even small amounts of R22 can be reliably detected, sufficient purity of fluorocarbons for the recycling process can be accomplished.

SILICA-BASED OPTICAL FIBERS WITH TAILORED REFRACTIVE-INDEX PROFILES IN THE REGION OF 1.46-1.52 FOR EVANESCENT-WAVE CHEMICAL DETECTION

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Polymer-clad silica (PCS) fibers have been extensively used for the development of fiber-optic sensors operating in the visible and near-infrared spectral regions. However, these fibers offer high sensitivity to changes of the refractive index and/or light absorption coefficient of their cladding only in a narrow range of the refractive index close to that of silica. Several approaches have been developed for overcoming this limitation[1]. Only few of them, however, are based on the employment of polymer-coated glass (PCG) fibers with the core refractive index different from that of silica. Such fibers have been drawn from preforms of soft optical glasses and used for the investigation of polymer curing[2]. This paper presents PCG optical fibers with cores composed of highly doped silica with refractive indexes above that of silica. These fibers include fibers with step-index (SI) as well as inverted-graded (IGI) refractive-index profiles[3]. It is shown that the sensitivity of these fibers to refractive-index changes of their cladding can be tailored by controlling their refractive-index profiles.

Preforms for fiber drawing were fabricated by the MCVD method. The preform cores were prepared of silica doped with germanium and phosphorous oxides under optimized heat-treatment conditions preventing thermal stress. Examples of refractive-index profiles of the prepared preforms are shown in Figure 1. The silica parts of the preforms were removed by etching in a solution of hydrofluoric acid. Fibers were drawn from the etched preforms at temperatures optimized to the composition of the core and coated with a polydimethylsiloxane (PDMS) cladding.

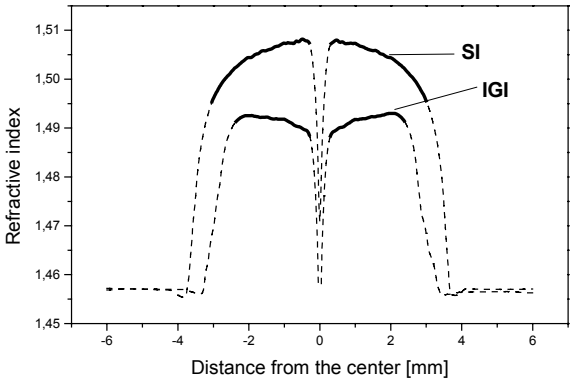

Figure 1. Refractive-index profiles of SI and IGI preforms doped in the core with GeO_2 and P_2O_5

The sensitivity of the prepared fibers was tested in immersion experiments, in which the PDMS cladding was removed from fiber segments in a length of about 4 cm. This part was immersed in liquids with different refractive indexes and changes of the cladding refractive index were monitored through changes of the output power of the fiber excited by an inclined collimated beam. Angular distributions or temporal changes of the output power were determined, from which sensitivity curves were derived. Experimental results discussed in the paper show effects of fiber preparation on the sensitivity of the fibers. It is shown that this approach makes possible to tailor the fiber sensitivity if the cladding refractive index is in a range from 1.457 to 1.52.

Acknowledgements. This research was supported by the Grant Agency of the Czech Republic (contract No. 102/02/0780).

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ON-LINE MONITORING OF pH IN SHAKE-FLASK FERMENTATIONS

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In bioprocessing, pH is one of the most important parameters for the culture growth and regulation. Therefore, there is an utmost need for its online monitoring (and possibly control). However, millions of fermentations are performed yearly in shake-flasks without any monitoring, or few data points are acquired through sampling. It would be beneficial for the biotechnology and the life sciences to be able to perform pH measurements in shake-flasks (as well as in all transparent containers for cell and tissue culturing) online. This goal is achievable if an in-situ sensor is used. Such a sensor must neither change the hydrodynamic conditions inside the tank, nor to allow the media autofluorescence to interfere with the measurements.

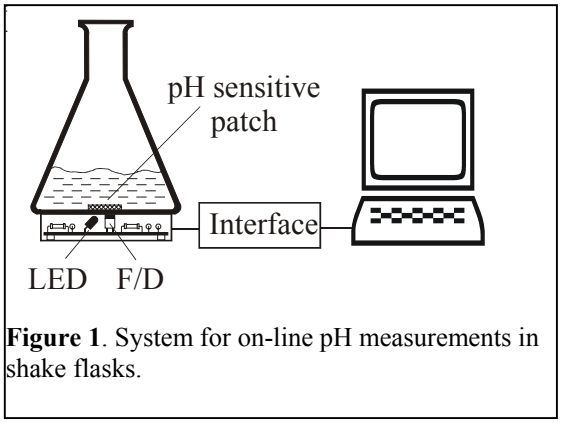

Figure 1. System for on-line pH measurements in shake flasks.

In this work, the performance of a system that utilizes sterilizable, pH sensitive peel-and-stick patch was investigated. The patch utilizes immobilized dihydroxypyrene disulfonic acid, covalently bound hydrogel matrix.[1] The use of a ratiometric dye allowed avoiding the common problems, associated with fluorescent intensity measurements. The patch was interrogated using all-solid-state ratiometric microfluorometer (Figure 1). The system with the current sensor is useful for pH monitoring in the range between 6 and 9. Strategies for elimination of the influence of LED's spectral variation were developed. Different methods for sensor sterilization (steam sterilization in autoclave, ethanol, ethylene oxide, gamma sterilization) have been tested, all with positive outcome. Two different materials for the sensors' protective layer and their influence on the sensors' response time have been investigated. Finally, the system was used for continuous on-line monitoring of shake flask fermentation. The performance of the sensors and the system, as well as strategies for their optimization are discussed.

Acknowledgements: This work was supported by the NSF Grant BES 0091705 and unrestricted funding from Du Pont, Fluorometrix, Genentech, Merck, and Pfizer.

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pO_2/pH AND pO_2/pCO_2 HYBRID OPTODES FOR 2D-SENSING IN MARINE SYSTEMS

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In marine microbiology, sediments are an important class of ecological systems containing a diverse population of organisms. The sediment/water interface is a vastly studied (micro)environment where many important processes such as the exchange of nutrients by diffusion, advection and bioturbation, primary production of organic matter by photosynthesis, and others, take place. The development of tools that would enable the monitoring of these processes is therefore an important prerequisite in marine research. Recently, the use of oxygen planar optodes has proved to be very beneficial in understanding a variety of processes in marine sediments. In these processes, the production/consumption of oxygen is closely interrelated with the change of other important parameters, such as pH and pCO_2. The possibility to monitor all three parameters simultaneously and in two dimensions would therefore greatly enhance the methodology that is used in the study of various marine systems.

The presented planar hybrid optodes were designed and optimised for the simultaneous monitoring of pH/pO_2 and pCO_2/pO_2 distributions in marine environment. In both optodes all indicators were dissolved in the same layer and spread onto a polyester foil. In the case of the pCO_2/pO_2 optode the sensor was coated with black silicone as an optical isolation and an additional proton barrier. The pH/pO_2 optode comprised a synthesised fluorescein derivative as the pH indicator with a pKa value of 8.3.

The hybrid optodes were excited with pulsed LEDs. A fast gateable charge-coupled device camera was used for the detection of the emitted fluorescence. The simultaneous determination of both parameters facilitated by the hybrid sensors was performed by combining two measuring schemes (Figure 1): With the *shark-fin* scheme (*t-DLR* scheme)[1] the ratio A1´/(A2+A3) is obtained which is not only a function of pH or pCO_2, respectively, but still contains the pO_2 information. The oxygen detection was in both cases accomplished by employing the *rapid lifetime determination (RLD)* technique. This gives the ratio A2/A3 which is a pure function of pO_2. A calculation method is presented which allows for a correction of the t-DLR images from the oxygen indicator luminescence so as to obtain an image directly related to the measured pH/pCO_2.

The hybrid optodes were applied to monitor the photosynthetic activity of micro-organisms (diatoms) living at the top of marine sediment. *Figure 2* shows the experimental setup (A) and an example of a DLR image obtained by the pH/pO_2 optode (B).

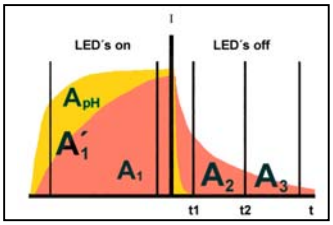

Figure 1. RLD/DLR calibration scheme with the luminescence integrals of the O_2 indicator (dark area) and of the pH/CO_2 indicator (light area)

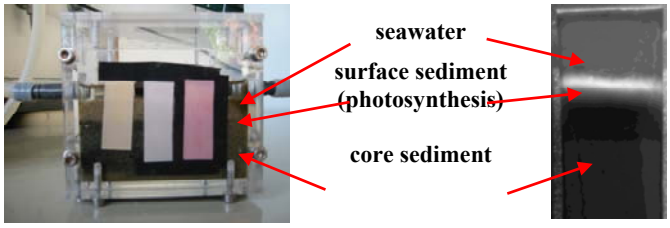

Figure 2. **A**: Sensor strips fixed in an aquarium with sediment; **B**: DLR image of the pH/pO_2 optode with a strongly enhanced signal in the area with photosynthetic activity due to increased pH and pO_2.

Acknowledgements: This work was kindly supported by the BMBF (Project-Nr. 03F02848).

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OPTICAL BIOSENSING OF BIOAVAILABLE IRON IN THE SOUTHERN OCEAN USING A SOL-GEL ENCAPSULATED SIDEROPHORE

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The primary production of organic carbon by phytoplankton in the oceans is central to the maintenance of the oceanic food chain, to the cycling of contaminants and to the air-sea exchange of carbon dioxide. This latter process has a profound affect on the climate. Recently, it has been shown that the supply of iron is the chemical limitation of phytoplankton growth particularly in the Southern Ocean.

The recognition of the role of iron poses major challenges for environmental life scientists. It is important to understand the factors regulating iron concentrations in the oceans and in turn how phytoplankton react to, and themselves regulate, the concentrations of iron. In order to develop such an understanding, instruments capable of rapidly measuring iron at the extremely low concentrations (*ca*. 0.05 nM) observed in natural waters are required. This presentation will report on the development of a fluorescence based biosensing system which is capable of measurement of bioavailable iron in seawater at such low concentrations.

Bacteria require iron for growth. Under iron deficient conditions, bacteria excrete low molecular mass iron chelating compounds known as siderophores into the extracellular environment. The Fe(III)-siderophore complex is then transported into the bacterial cell via specific acceptors prior to enzymatic reduction.

In order to achieve our measurement goal of detecting bioavailable iron in oceanic waters we have grown the bacterium *Paracoccus denitrificans* in culture, under iron stress conditions, to induce the production of the siderophore parabactin. Parabactin comprises a spermidine backbone to which three catecholate derivatives are attached. The catecholate moieties provide the sensing characteristics of parabactin as they are inherently fluorescent. Upon binding Fe(III), parabactin forms a cyclic complex and the characteristic fluorescence is quenched.

Parabactin has been purified from *P. denitrificans* cultures and characterized using NMR and MS. The purified parabactin has been encapsulated in a tetramethyl orthosilicate (TMOS) sol-gel thin film. The parabactin sol-gel film was spin-coated onto a glass support and then incorporated in a flow-cell. The sol-gel encapsulated parabactin thin film has been characterized for the fluorescence based biosensing of Fe(III) in seawater samples. For multiple use, the encapsulated parabactin can be regenerated by lowering the pH of the flowing solution. At low pH the chelated iron is reduced to Fe(II) which readily disassociates from the parabactin complex giving the free parabactin which enables further measurements to be made. A full analytical assessment of the parabactin biosensor for the analysis of bioavailable iron has been made. The biosensor has been used to analyse seawater samples collected from the Southern Ocean and shown to be capable of the direct determination of Fe(III) in the pM concentration range.

A NITROAROMATICS CHEMICAL SENSOR BASED ON FLUORESCENT TWEEZERS THIN FILMS

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A. Introduction

Nitroaromatics are volatile compounds with a notorious odour, which can be found in land field gas. The monitoring of these compounds is extremely important from an environmental point of view.

Conjugated molecules have received considerable attention as the active material in fluorescence based chemical sensors.[1,2] According to binding studies performed in,[3] to detect ppm level of the nitroaromatic A, the fluorescence changes of original conjugated molecules as molecular tweezer B were examined.

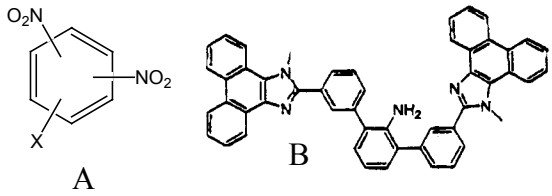

Figure 1. Nitroaromatic A and conjugated molecule B

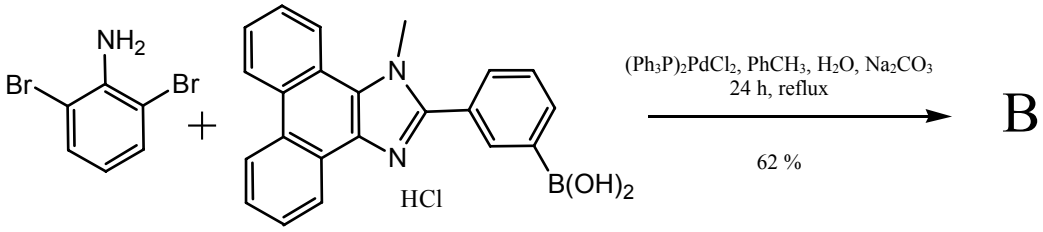

Figure 2 : *Synthesis of Tweezer B*

B. Experimental

The synthesis of the molecular tweezer has been performed as shown in Figure 2. Thin films were deposited onto glass substrates by the way of thermal deposition. The intensity of PL was then recorded by a spectrofluorometer in a flow stream in which the gas composition was alternated between N_2 and a nitroaromatic vapor in N_2.

C. Results

Figure 3 shows the variation of the intensity of fluorescence of a thin film of B exposed to N_2, 1 ppm of A and to N_2 again. An evident decrease of the intensity collected is observed while a slow reversibility of the NAC interaction appears. This quenching of fluorescence is a well-known phenomenon in the fields of NAC sensors.[4] This also confirms the strong binding of electron deficient aromatic molecules with molecular tweezers.[3]

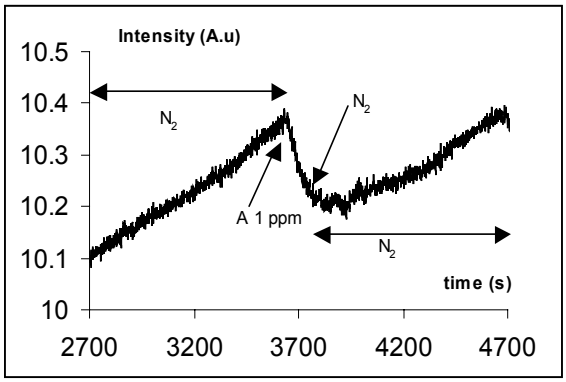

Figure 3. Effect of nitroaromatic A on the intensity of fluorescence ($\lambda_{em\ Max}$ = 450 nm) of B

D. Conclusion

The nitroaromatic optical sensor reported in this paper is based on an original conjugated molecule. The sensitivity is promising (1 ppm). Our work in now focusing on testing other tweezers whose structure is close to

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OPTOCHEMICAL FIBER BRAGG GRATING SENSORS BASED ON EVANESCENT-FIELD INTERACTION USING THIN-FILM TRANSDUCERS

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Fiber Bragg grating (FBG) sensors are usually applied for measurement of temperature and strain. Exposing them via evanescent field interaction to a surrounding analyte, they measure also changes of its refractive index nA. This sensor effect has been demonstrated for FBG's in etched D-shaped fibers.[1] In the paper presented, planar side-polishing technique of the fiber and subsequent deposition of sensor-specific transducer layers are applied for extending this spectrally encoding and network-capable optochemical fiber optic sensor technology to a broad range of liquids, and to absorbed vapors and gases and bio-molecules as well.

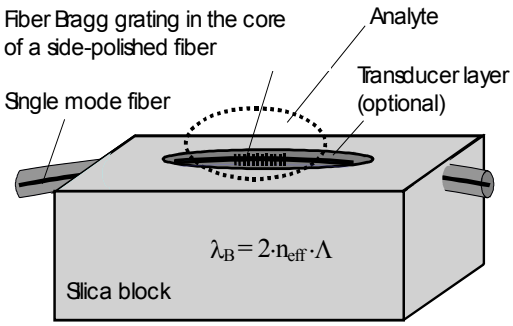

Figure 1. Scheme of the evanescent wave fiber Bragg grating sensor.

The optical fiber is embedded in a slightly curved groove, it is side-polished to a residual cladding thickness in the central part of about 0 .. 0.5 µm, and a FBG is inscribed in this area (see Figure 1). The shift of the Bragg wavelength ΔλB due to different analytes is measured using a broadband light source in the 800 nm wavelength region and a compact CCD based polychromator for read-out of the reflection spectrum.
Between liquid analytes can be differentiated measuring the increasing Bragg wavelength with increasing refractive index of the liquid. An additional high-refractive overlay between fiber core and analyte is shifting the most sensitive refractive index range from petrol products to the lower values of aqueous salt solutions.

The interaction of the guided light in the FBG with a surface plasma wave on a thin gold layer yields highest sensitivity to analytes (70 nm per refractive index unit) near to surface plasma resonance. According to the short range of the evanescent field of a surface plasma wave, this sensor allows to detect the adsorption of monomolecular layers, e.g. biochemical reactions.[2]
Application of a palladium transducer layer on side-polished fibers allows the detection of hydrogen concentrations in < 0.1 .. 4% range via monitoring the decreasing complex refractive index of Pd with increasing absorption of H_2 (result in Figure 2). Other sensor-specific transducer layers for measuring relative humidity (porous SiO_2) or pH value (special polymers) are in preparation.

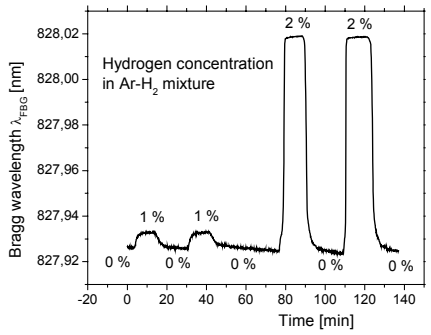

Figure 2. Experimental result of TM mode Bragg wavelength response due to hydrogen absorption in a Pd transducer layer.

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A δ FORM sPS BASED FIBER OPTIC REFRACTOMETER FOR CHEMICAL DETECTION OF VOCs.

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The combination of suitable sensitive materials and sensing techniques is the key points for the designing of microsensors based detectors. Sensitive materials are requested to collect target molecules selectively and reversibly. Polymers, exhibit high sorption capability while they usually lack in selectivity however, because of their low cost, easy processing techniques and the possibility to be deposited on several substrates revealed to be reliable materials a sensing elements in chemical detection. For these applications polymers are usually in rubbery and glassy forms. Semi-crystalline polymers, instead, show very low sorption capability because diffusion of penetrants is allowed only in the amorphous regions while the more compact crystalline domains are excluded. Nevertheless a very interesting exception is represented by the Syndiotactic Polystyrene (sPS) which is a polymorphic material that in one of its crystalline morphologies, the δ form, contains a nanoporous structure such to allow the penetration of low molecular weight compounds whose size and shape well fit the nano-cavities.[1] It was shown that, in this case, at low activity of analyte, the adsorption in the crystalline domain occurs at a much larger extent with respect to the amorphous regions.[2] This makes this material a very attractive from a technological point of view.

In this work a polymeric film fiber optic refractometer based on the sorption capabilty of δ form sPS toward volatile organic compounds was tested. In particular sorption tests were carried at very low chemical activities of Volatile Organic Compounds (VOCs), in order to analyze a range of concentrations where the sorption occurs preferencially in the crystalline phase and, at the same time, to evaluate the resolution of the optical sensor.

The choice of an optoelectronic approach based on the use of fiber optic technology seems to be suitable in light of the several advantages associated to this class of sensors such as immunity to electromagnetic interference, the dual functionalities related to their capability to serve as transducers and sensing data transportation systems.[3] The sensor head consists in the distal end of a standard silica optical fiber of 9μm core dipcoated with a thin film of δ phase syndiotactic polystyrene. Reflectance measurements were carried out by ligthing the sensing fiber with a superluminescent diode (40nm bandwidth) operating at 1310nm. A 2x2 in fiber coupler provides the necessary connections between light source, sensing interface and two receiving channel: one for reflected signal detection and the other one for power monitoring in order to obtain well compensated intensity measurements. The light source has been externally amplitude modulated at 500Hz and the sensor outputs have been retrieved by using a dual channel lock in amplifier. A special feedthrough provides the necessary ingress of the probe into the test stainless steel chamber operating at controlled vapor environment and temperature.

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STUDIES ON THE USE OF SILICONE FOR DETECTION OF AROMATIC HYDROCARBONS IN WATER EMPLOYING NEAR INFRARED SPECTROSCOPY

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The detection of aromatic hydrocarbons in superficial water and groundwater is of great interest as these compounds can indicate a contamination by petroleum and its derivatives, such as gasoline or diesel fuels. The techniques employed to perform this determination are usually based on gas chromatography (GC) or high performance liquid chromatography (HPLC). Solid phase extraction (SPE) is frequently employed in conjunction with these chromatographic techniques, for the pre-concentration of the hydrocarbons from contaminated water. Despite its good performance, the SPE procedure requires re-extraction of the contaminants with an appropriate solvent before injection of the sample into the chromatograph. Solid phase micro-extraction (SPME) has been proposed to have advantages over SPE, as the extracted contaminants can be directly injected in the gas chromatograph. Although advantageous, these methods require sophisticated instrumentation, therefore, present difficulties in field monitoring. On the other hand, optical sensors can be easily miniaturised for field applications. Methods for determination of aromatic hydrocarbons in water are usually based on the evanescent waves,[1,2,3] in which the organic compounds are extracted by the silicone cladding of an optical fibre, changing the properties of the propagating wave. One of the disadvantages of these methods is related with the use of very long optical fibres to improve the detectability.

Considering these aspects, the present work proposes the use of a silicone rod for detection of aromatic hydrocarbons in water, using near infrared (NIR) spectroscopy. Sensing phases of polydimethylsiloxane (PDMS) with different thicknesses were prepared from Silicast T-2 (Dow Corning). Several rods (different heights and diameters) were cut and adapted to the transflectance probe of a Luminar 2000 Brimrose Spectrophotometer for measurements from 850 to 1800 nm. Deionised water was separately contaminated with known amounts of benzene, toluene, m-xylene and ethylbenzene for evaluation of the PDMS sensing phase. A 500-mL closed reactor with constant stirring was employed to perform the measurements in order to prevent lost of the hydrocarbons to the environment. A rod with a diameter of 3.2 mm and height of 10 mm showed the shortest response time and the highest detectability. Experiments showed that an equilibrium state is obtained after 90, 180, 360 and 405 minutes for benzene, toluene, ethylbenzene and o-xylene, respectively. However, measurements made after inserting the probe in contaminated water for 30 minutes (benzene and toluene) and 100 minutes (ethylbenzene and m-xylene) provided signal intensities high enough to determine these compounds in water, presenting linear responses up to 360, 290, 100 and 80 mg L^{-1}, with detection limits of 7, 10, 8 and 5 mg L^{-1}, respectively. The PDMS sensing phase showed reversible response, as it returns to the original spectrum after placing the probe in air or distilled water. The precision was evaluated by performing blank measurements with different rods, providing a relative standard deviation of 0.5%. The sensor was evaluated for determination of contamination of water by Brazilian gasoline type A (without ethanol), gasoline type C (with 25 % of anhydrous ethanol), and diesel fuel. The normalised first derivative spectra obtained during 45 to 60 min. of exposure were submitted to a Principal Component Analysis, which was able to classify the water in two distinct groups, contaminated by gasoline or contaminated by diesel fuel. The results obtained in this work indicate that the PDMS sensing phase can be useful for detection of aromatic hydrocarbons in contaminated water.

Acknowledgements. Authors are grateful to CAPES/PROCAD, FINEP/CTPETRO, CNPq and FAPESP for financial supports.

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CONFINEMENT EFFECTS ON POLYSTYRENE THIN FILMS GLASS TRANSITION

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Recently much attention has been focused on the properties of thin polymer film in light of the important and growing role they play in the high-tech industries, specially in microelectronics and optoelectronics. Among all the physical properties, one the most important parameter to know for the correct polymer processing, and future use of the polymer manufactures themselves, is glass transition temperature.[1] This is why in the recent years many techniques like Ellipsometry, X-Ray Reflectivity, Acoustic Wave Spectroscopy have been used for the detection of glass transition in thin polymer films.

When polymers are in confined geometries as in the case of thin film cast on a substrate, interfacial interactions can induce molecular ordering at the interface affecting the segmental mobility and thus the glass transition.

This work, in particular, aims to measure, in the case of thin polymer film cast on a plane glass surface, shifts in the glass transition temperature as the thickness of the polymer layer is decreased. To this aim, thin films of atactic Polystyrene with thicknesses ranging between 20 and 200nm were cast on the cleaved tip of a standard optical fiber of 9μm core, and T_g was determined upon cooling down the films from 150 to 25°C. As sensing mechanism a fiber optic refractometer was used to carry on measurements of reflectivity as the temperature is lowered with at rate of 5 °C/min. In Figure 1 is reported a schematic of the optoelectronic set-up.

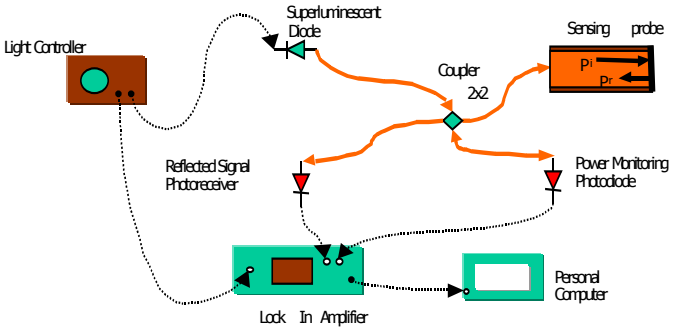

Figure 1. Basic scheme of the optoelectronic sensor set-up

Reflectance measurements were carried out by ligthing the sensing fiber with a superluminescent diode (40nm bandwidth) operating at 1310nm. A 2x2 in fiber coupler provides the necessary connections between light source, sensing interface and two receiving channel: one for reflected signal detection and the other one for power monitoring in order to obtain well compensated intensity measurements. The light source has been externally amplitude modulated at 500Hz and the sensor outputs have been retrieved by using a dual channel lock in amplifier.

The optoelectronic sensor used for this work, being based on the fiber optic technology, revealed to be a very suitable choice for this kind of application as it can serve both as transducer and as sensing data transportation systems[2] in addition to other advantages that are associated to this class of sensors such as immunity to electromagnetic interference, the possibility to be used in adverse conditions, the low cost and the low intrusivity.

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AN ORGANOPALLADIUM-PVC MEMBRANE FOR SULPHUR DIOXIDE OPTICAL SENSING

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Nowadays, there is great interest and necessity to develop methods for gaseous sulphur dioxide measurements in air samples. Sulphur dioxide monitoring is essential for air pollution control because, in the atmosphere, SO_2 is actively involved in physicochemical processes such as the formation of aerosols, clouds and acidic precipitations.[1] Sources of environmental contamination by sulphur dioxide include the paper and pulp industries, petroleum refineries, roasting of non-metallic ores and the incineration of solid waste, particularly hazardous and medical wastes.[2] Although many optical sensors have been described for the detection of several variables, such as relative humidity, oxygen, carbon dioxide, oxides of nitrogen, only a few can be found for detection of sulphur dioxide.

This work describes the development of a sensing membrane for determination of sulphur dioxide, in which an organopalladium complex was immobilised in a poly(vinyl chloride) thin film plasticised with ortho-nitrophenyloctylether (o-NPOE). The palladium complex $Pd_2(dpm)_2Cl_2$ (dichloro bis (diphenylphosphine) methane dipalladium I) was synthesised as previously described.[3] The membrane solutions were prepared by dissolving a total of 100 mg of the components ($Pd_2(dpm)_2Cl_2$, PVC and o-NPOE) in 1.0 mL of tetrahydrofuran (THF). Several membranes were prepared, using 20, 25 and 30 % of PVC; 1, 5 and 8 % of palladium complex and enough o-NPOE to make a total of 100 mg. The sensing membranes were obtained by manual deposition of 10, 15 and 20 μL of the solution onto cellulose acetate films, which were left to dry for 24 hours and then stored in a dessecator sheltered from ambient light. The membrane was placed in an acrylic flow cell, whose bottom was covered with a reflective aluminium foil to improve signal intensity. The common end of a bifurcated optical fibre bundle was also adapted to the flow cell and placed 1 mm from the membrane. Reflectance measurements were performed from 400 to 800 nm, after exposing the membrane to 0 to 500 ppm_v of sulphur dioxide in air, at a flow rate of 500 mL min^{-1}.

The membrane composed of 20 % PVC, 72 % o-NPOE and 8 % palladium complex showed the best performance and the film prepared from 20 μl of solution provided the highest signal. Measurements made at 530 nm allowed a linear response range up to 300 ppm_v, with a detection limit of 3.5 ppm_v. By employing a flow rate of 500 mL min^{-1}, response times ($t_{90\%}$) of 3 and 2 min were obtained for reaction of the membrane with SO_2 and for cleaning with dry nitrogen, respectively. Although the palladium complex also reacts with carbon monoxide in solution, this interference was not observed for concentrations up to 1000 ppm_v of CO. The membrane showed a lifetime of ca. 2 months when stored in a dessecator.

The results presented above indicate that the $Pd_2(dpm)_2Cl_2$ – PVC membrane can be employed for detection and continuous monitoring of sulphur dioxide in air, as it presents fast and reversible response.

Acknowledgements. FAPESP is kindly acknowledged for the financial support (process 01/14215-1). FLA is grateful to FAPESP (process 01/06778-6) and IMRJ thanks CNPq (process 303690/2002-0) for fellowships.

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DESIGN OF A COPPER(II) OPTODE BASED ON IMMOBILIZATION OF DITHIZONE ON A TRIACETYLCELLULOSE

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Heavy metal ions represent a major environmental problem and their detection is necessary. Copper is one of several heavy metals which is essential for life, but has a high toxicity to some organism. Due to its toxicity to bacteria, increased concentrations of copper restrict the self-purification ability of rivers and seas and reduce the power of biological reprocessing systems in water, the critical limit being 1mg kg^{-1}.[1]

Recommended procedures for discontinuous determinations of copper include photometric methods[2] having detection limits down to 1µg Kg^{-1}, flame atomic absorption with detection limit 1.5µg Kg^{-1}, graphite furnace atomic absorption with detection limit 0.06 µg Kg^{-1} and voltammetry with detection limit 0.002 µg Kg^{-1}.[3]

In this report the characterization of an optical sensor membrane is described for determination of copper (II) based on immobilization of dithizone on a triacetylcellulose membrane. This optode has a linear range of 30 -1270 ng mL^{-1} (0.48-20 µM) of Cu^{+2} ions with a limit of detection of 6 ng mL^{-1} (0.1µM) at wavelength of 610 nm. The response time of optode is within 5-8 min depending on the concentration of Cu^{+2} ions. The selectivity of optode to Cu^{+2} ions in the presence of other metal ions including Pb^{2+} , Mg^{2+}, Zn^{2+} , Ni^{2+} , Co^{3+} , Mn^{2+} , Cd^{2+} , Bi^{3+} , Fe^{3+} , Al^{3+} is good, with Hg^{+2} ions as the main interference. The life time of the optode film was determined by adding buffer solution (pH 2) in the cell including film. The signal was recorded at wavelength of 610 nm over a period of time about 8 h. No significant loss of the indicator occurs during this time.

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CALCIUM OPTICAL NANOSENSORS

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Calcium plays a vital role in cellular communication, development and differentiation. Concentrations of free calcium are highly heterogeneous within different cells, ranging from nanomolar to micromolar in the resting cytoplasm and endoplasmic reticulum, respectively. While a variety of methods are available to measure calcium in the cellular environment, long term monitoring and control of spatial resolution remain challenging.

Sensor size is a critical factor when making intracellular measurements as physical disruption of the cellular membrane can lead to spontaneous cell lysis. Production of nanosized sensors with volumes less than 1ppm of a typical cell enables *in-vivo* measurement of cellular analytes without compromising cell viability. Probes encapsulated by biologically localised embedding (PEBBLEs) are nanosized (20 – 300 nm) optical sensors fabricated by microemulsion polymerisation. Encapsulation of fluorescent reporter and reference dyes within the nanoparticle permits production of multicomponent, highly selective and extremely sensitive ratiometric sensors.

This paper discusses the production and characterisation of optical calcium nanosensors based on the entrapment of calcium dyes within a polymeric acrylamide matrix. Sensors exhibit a dynamic calcium range similar to the free dyes used and have a neutral surface charge. The sensors were not prone to either ionic or protein interference to the same extent as the free dyes.

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ELECTROCHEMILUMINESCENT DETECTION OF ACETYLCHOLINE USING ACETYLCHOLINESTERASE IMMOBILIZED IN A BIOMIMETIC LANGMUIR-BLODGETT NANOSTRUCTURE

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The performances of biosensors or other bioelectronic devices mainly depend on the properties of the bioactive sensing layer and the quality of the association to the transducer. The achievement of new biospecific membranes as organized ultrathin films, directly interfaced with the transducer and inserting biomolecules in a functionalized and orientated position, may open an original way in the development of new biooptoelectronic devices.

This study deals with the possibility to detect acetylcholine using acetylcholinesterase (AChE) orientated at the surface of an organized and miniaturized biomimetic Langmuir-Blodgett (LB) nanostructure by means of an electrochemiluminescence (ECL) device (Figure 1).

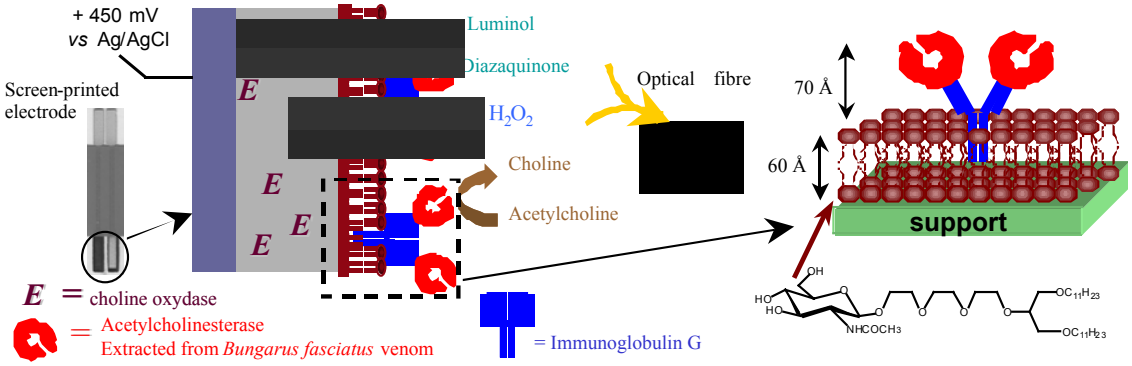

Figure 1. Screen-printed electrode associating a bioactive Langmuir-Blodgett nanostructure.

The highly organized proteoglycolipidic nanostructure including a non-inhibitor monoclonal antibody directed against the AChE monomer has been designed using an adapted combination of liposome fusion at an air/buffer interface in conjonction with the Langmuir–Blodgett technique,[1] This procedure has been previously demonstrated to be efficient to allow the antibody to adopt a preferential functionnal orientation in the lipidic matrix, both to sequester the hydrophilic enzyme in an orientated position at the surface of the nanostructure and to maintain the enzyme activity for more than several months.[2]

The intimate contact of this nanostructure with a performant optical device previously developed in our group,[3] allows to detect acetylcholine at a concentration of 10^{-6} M.

The association of such a LB nanostructure with an ECL device, first reported to our knowledge, appears to be promising to achieve miniaturized sensors and for further development in macroarray systems with the insertion of different biocatalytic elements.

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A SIMPLE ONLINE MONITORING SYSTEM FOR CONTINOUS SENSING OF GLUCOSE IN BODY FLUIDS AND CULTIVATION MEDIUM

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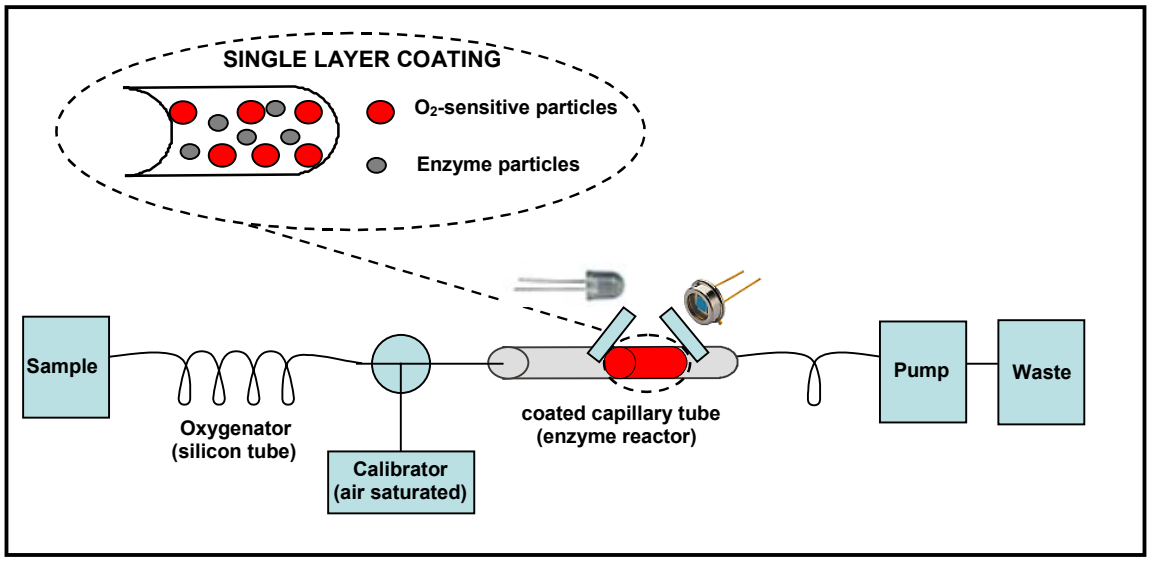
The development of sensors for continuous determination of glucose in physiological liquids is an important goal in the treatment of diabetes. Glucose sensors based on the enzymatic conversion of glucose are still the main focus of the research interest of most groups due to their high selectivity to glucose. A few research groups have been working on alternative non-enzymatic receptor based methods for glucose sensing. The majority of sensors are based on the electrochemical detection of hydrogen peroxide produced by the reaction of glucose oxidase with glucose. Optical enzymatic sensors often use oxygen optrodes as transducer. They have the advantage to be simple and inexpensive but they lack on the problem of variabel oxygen content in the sample. Especially at low levels of oxygenation the use of oxygen sensors as transducer is crucial.

Here we present a simple flow through system that can be used for online monitoring of glucose in body fluids or cultivation media. The major elements of such a system are the oxygenator (providing a constant and adjustable pO_2 level in the measuring cell) and a disposible wall coated enzyme reactor with an integrated calibration free oxygen optode element. A thin wall silicon tube with a low inner diameter is preferably used as oxygenator allowing a fast and reliable oxygenation of small sample volumes. The sample have a defined oxygen level (preferably air saturated) if it reaches the enzyme reactor. The disposible enzyme reactor is a glass capillary coated with a single layer containing the immobilised enzyme and the oxygen sensitive element. GOX and the pO_2 sensitive luminophore were dispersed as particles in a thin and rigid polymer layer. In presence of glucose the pO_2 level in the sensing layer drops, increasing the luminescence decay time of the incorporated pO_2-sensor.

The dynamic range can be easily tuned either by varying the flow rate or the length of the enzyme reactor. Flushing the oxygenator with a gas mixture with a low oxygen content, very low detection limits of glucose could be reached. The analysis system is flexible (useful for other oxidase substates as lactate) and easily to install. A cascade of oxygenators and enzyme reactors allows multianalyte analysis.

The characteristics of the monitoring will be presented, discussed. The performance of the monitoring system for glucose sensing in interstitial liquids is shown and compared with other commercially available instruments.



DIRECT IMMOBILIZATION IN PDMS FOR DNA CHEMILUMINESCENT BIOCHIP. DETECTION OF SINGLE BASE MUTATION IN P53 SEQUENCE

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p53 tumor suppressor gene is well known as a transcription factor of cell regulation, and mutation of *p53* gene is the most common phenomenon in carcinogenesis and tumor progression. Sensitive and rapid detection of such mutation could then help to diagnose early cancer development and then increase the success of the treatment. In the present system, the study was focused on a particular codon (273) localized in the exon 8 of the *p53* gene. This codon was shown to be a "hot spot" in esophageal adenocarcinoma.[1] A one-pair base mismatch in this sequence (C**G**T →C**A**T) lead to the exchange of an arginine by an histidine with a significant loss of *p53* activity.

Codon 273: TTGAGGTG**CGT**GTTTGTGCC

The present study shows how a micro-array type analytical system could be obtained, on the basis of a powerful and well characterized arraying system,[2,3] and used to detect such point mutation. The wild type sequence, including the codon 273, is immobilized at the surface of carboxylated latex beads. Those beads could be subsequently transferred, in an addressed manner, at the surface of a PDMS (polydimethyl siloxane) block. The array obtained exhibits spots of latex beads, presenting a high density of the particular DNA sequence due to the 3D immobilization procedure (Figure 1). The hybridization of biotinylated complementary oligonucleotide 20mer, at different temperature enables the distinction between the wild and the one-base pair mismatch sequences. Moreover, DNA sequence amount close to 10^7 molecules could be detected with hybridization time as low as one hour.

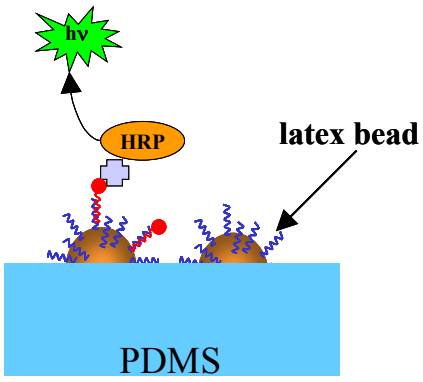

Figure 1. Representation of the PDMS based chemiluminescent p53 biochip. HRP: Horseradish peroxidase.

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BIOSENSOR BASED ON SURFACE PLASMON INTERFEROMETRY INDEPENDENT ON VARIATIONS OF LIQUID'S REFRACTION INDEX

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Several methods of Surface Plasmon Resonance (SPR) registration are proposed and realized in biosensors. The most popular from them are Kretschmann configurations using angular and wavelength interrogation. Exploiting of a phase measurement at SPR permits to increase the sensitivity of SPR sensors by one[1] or even two order of magnitude (using abrupt phase jump near SPR)[2] in comparison with ones which use angular and wavelength interrogation.

But there is a general problem for all previously mentioned methods of SPR registration. It is an undesirable sensitivity of the SPR to changes in the refraction index (RI) of the liquid due to variation of the liquid temperature, composition and so on. For example, the water temperature change by 1 ^{o}C gives the RI change about 10^{-4}. So potentially very high ultimate sensitivity of SP phase detection becomes meaningless without comprehensive temperature and composition stabilization. There is therefore a need for such a method of the phase registration which will be high sensitive to the properties of a layer deposited on the SPR supporting film, but will be insensitive to the variations of the RI of the liquid.

We present a surface sensitive optical sensor based on registration of the phase of surface plasmons by such a method of the phase detection that makes it possible to essentially eliminate an undesirable sensitivity to variations of refraction index of the liquid. This objective has been achieved by detecting the interference between the surface plasmon wave and a bulk wave propagating at grazing angle just above the surface. The proposed interferometric method also does not suffer from vibrational noise.

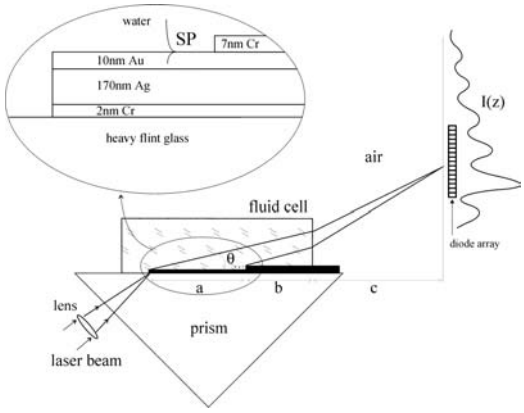

Figure 1. The scheme of the experiment. On the insert the structure of the metal films is shown.

We have shown theoretically and experimentally[3] that the proposed method is insensitive on variations of refraction index of a liquid due to temperature, composition and other changes. Suppression of these undesirable variations of liquid's RI is more strong for the small variations. For example, according to theoretical estimation the total improvement factor (TIF) in comparison with ordinary Kretschmann configuration is TIF=4000 in the case of the RI deviation from 1.327 to 1.33 (this corresponds to water cooling down by 20 ^{o}C), and TIF=40000 in the case of the RI deviation from 1.3270 to 1.3273 (this corresponds to water cooling down by 2 ^{o}C). From other hand this method holds high surface sensitivity of phase measurements. We used the deposition of a self-assembled monolayer (SAM) of 2-mercaptoethanol to demonstrate the surface sensitivity of our method. The registered phase shift was appropriate to the deposition of a thin layer with thickness d=(3.1±0.1) Å (for n_d=1.5).

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BIOCHROMIC FILMS BASED ON THE BACTERIORHODOPSIN FOR CHEMICAL SENSORS

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[c]*Cranfield University (England)*

Photochromic bioorganic materials, to which bacteriorhodopsin (BR) concerns, cause interest in connection with opportunity of creation functionally new electronic optical elements. In photosensitive film structures on the base of BR, under action of light there is a photochemical cycle of reactions, during of which the BR molecule passes from one intermediate condition in another, which is characterized by displacement of absorption bands. The addition into the structure of filmmaker material halogen-, nitrogen-, and sulfur substances in various combinations allows modifying of photosensitive and temporary characteristic of a photocycle over a wide range.

In the given work opportunities of influence of external vapor-gases environmental media onto the parameters of a photocycle of the BR films is investigated, that would give the preconditions for creation of working long time and in a real mode of time miniature optical vapor sensors, due to good reversibility of which BR has.

With the help of electron microscopy the distribution of nanosize fragments BR in the water soluble matrixes (gelatin, polyvinyl alcohol etc.) is investigated, is shown that obtained film have developed meso- and microporosity, that allows to take participation in change of optical parameters under influence of an environmental medium, not only top layers of a film but all film volume.

The results of research of influence of vapors of water and ammonia onto the parameters of a photocycle are given. Is shown that depending on relative humidity within the limits of 20-97 %, the magnitude of absorption in films is changing on 20-30% depending on chemical composition, and the temporary characteristics of a photocycle can vary up to three orders. The presence of ammonia similarly influences parameters of a photocycle. And in both cases these changes are absolutely reversible.

Fiber and integral optical sensors with a sensitive element on a base of BR films are offered and their characteristics are given.

DEVELOPMENT OF Cy5 – BASED OPTICAL IMMUNOSENSOR FOR VETERINARIAN DIAGNOSTICS

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An optical immunosensor was developed for veterinarian diagnostic applications, in order to quantify a monoclonal anti-goat IgG (MAGIgG) present in a sample. The quantification is based in the measurament of the Cy5 fluorescence of the detection antibody (MAGIgG-Cy5), after the competition with the MAGIgG present in the sample, for binding to goat IgG (GIgG) covalently immobilised in controlled pore glass (CPG) beads,[1] inside a 30 µL flowcell. The approach here presented consisted in a model system for veterinary diagnostics for ruminants, in order to quantify antibodies against different pathogenic agents, which can be modified for various diseases by using different immunoreagents. Reutilization was studied by using different pH's solutions,[2] from those, phosphate buffer pH 2 dissociated 95 % of the antigen-antibody complex leading to a decrease of 5 % in the original signal after a regeneration cycle. Photobleaching of free and coupled Cy5 was characterised and analysed. The results show a significant decrease in photobleaching in the presence of CPG,[3] therefore this physical phenomenon under the used conditions does not significantly affect the fluorescence signal acquisition. Thereafter, sequential and simultaneous competitive protocols were performed to detect MAGIgG in samples. In both cases, a detection range up to 0.11 mg mL^{-1} of antibody was obtained. The results show that the developed immunosensor is suitable for quantification of antibodies, in a fast, low cost and reproducible manner.

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CHITOSAN THIN FILMS AS AN OPTICAL BIOSENSOR PLATFORM

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Heavy metal detection continues to be a high priority as elevated levels of metals have been isolated from soil and drinking water in residential areas. As heavy metals have been demonstrated to have carcinogenic properties at high concentrations, they continue to be an important analyte for careful monitoring of environmental levels. Currently, there are many laboratory-based, metal ion specific sensors on the market. An ideal metal ion sensor for the residential analysis of water would be an inexpensive, rapid, color-based, dipstick test for heavy metal salts in waste and drinking water. The dipstick would have different panels of generic and sensitive films that change colors in response to the presence of heavy metal ions in water.

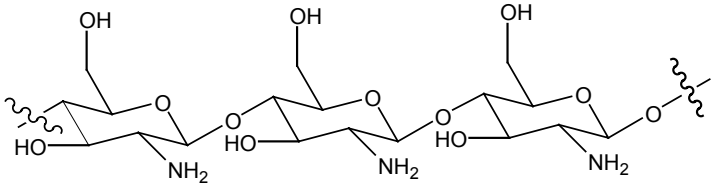

Figure 1. Chitosan

Chitosan, a β(1→4) linked 2-amino-2-deoxy-α-D-glucopyranose biopolymer, was chosen for the dipstick films due to its ability to adsorb metal ions in solution. Previous work[1] identified crosslinkers and plasticizers that would create rugged colorless chitosan films. Colored thin films of chitosan, the soluble form of chitin, crosslinked with Resimene and poly(allyl amine) hydrochloride (PAH) crosslinked with gluteraldehyde demonstrated that these films changed their thickness and color in response to all metal ion solutions tested, therefore creating a generic sensor platform.[2] The generic sensing films of crosslinked chitosan and PAH were, however, found not to be dose dependent, with all metal ion solutions tested generating similar thickness and color responses.

Modifications of the chitosan backbone and varying the crosslinker are a means of selectively tuning the thin colored film's sensitivity towards different analytes. For example, colored films of chitosan and PAH, crosslinked with hexamethylene 1,6-di(aminocarboxysulfonate), HDACS, were selective for Cr(VI) and Cu(II)/Cu(I) salts respectively over all other metal ions tested including two varieties of Cr(III). The films were tested in various pH's and environmental samples to distinguish the effects of pH, from the response to heavy metal ions.

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PREPARATION AND APPLICATION OF SPHERICAL POROUS GLASS IN AN OPTICAL IMMUNOSENSOR FOR THE DETECTION OF TARGET ANTIGENS

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Inorganic supports materials have been show to be excellent carriers for the immobilization of biomolecules and also to have good physical properties. Consequently silica based supports, like glass fibres, waveguides or controlled porous glass are largely utilized in biosensors.

The aim of this work is to determine the potential of a recently commercialized spherical silica support for protein immobilization and study it suitability as imunoassay support in an optical biosensor. This type of spherical support presents advantages over the conventional irregular shaped porous glass. The general goal is to maximize the amount of immobilized antibodies in this support, therefore characterization of its capacity has been studied, using bovine albumin as a model protein.

This study was conducted with spherical porous glass TRISOPERL® (Schuller GmbH, Johns Manville Company, Germany) using 4 different types: 47.8, 55.7, 102.6, 108.8 nm of average pore diameters. The native surface of the material has been first silanized and functionalized, in order to allow protein immobilization. 3-aminopropyltriethoxysilane was used for the silanization protocol, in order to obtain an alkylamine support. Afterwards, the surface was further functionalized to obtain two different reactive groups (aldehyde and isothiocynate) capable of link to the amine groups of the proteins. The aldehdye functionalized support has shown better efficiency for protein immobilization. The amounts of protein/support obtained (around 15 mg/g) are comparable to the described in literature for other porous glasses.

The antibodies used in the immunoassay has been previously characterized and tested in ELISA. Also elution of the immunocomplexes has been tested by different buffers (organic solvents and different pH), with encouraging results (more than 85%). Finally the spherical support has been tested directly in an optical biosensor (distal phase). Fluorescent labelled antibodies have been used for the immunoassay, which has been monitorised and quantified by the sensor detector. The results obtained showed that the use of spherical glass support improve the arrangement inside the flow cell (package), lower the light scattering and improve the reagents distribution along the immobilized antibodies. Those characteristics provide more stability and reproducibility to the optical biosensor.

PHOSPHORESCENT OLIGONUCLEOTIDE PROBES FOR DNA DETECTION

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Mono-functional p-isothiocyanatophenyl derivatives of Pt(II)- and Pd(II)- coproporphyrin (PtCP-NCS and PdCP-NCS) were used for labelling of synthetic amino modified oligonucleotides with high yields (70-100%) and purity of resulting conjugates.[1] Corresponding single- and dual-labelled oligonucleotides were used to design a number of phosphorescent probes for DNA detection, both in the solid-phase and separation-free formats of hybridisation assays.

5'-PtCP-labelled oligonucleotides have been shown to function as efficient primers in PCR amplification reactions to generate both double stranded and single stranded PtCP labelled product. The product has been detected with pM sensitivity directly from the agarose gel by time-resolved fluorescence. Detection off the solid phase has also been achieved, using an immobilised probe that captures a labelled target. In this case, an oligonucleotide probe linked to the surface was used to capture PtCP-labelled target.

Model systems employing pairs of complementary oligonucleotides, one labelled with metallo-coproporphyrin and the other with a quencher dye, showed strong and specific proximity quenching of PtCP label upon hybridisation in solution. Examples of quenchers used are Cu(II)-coproporphyrin and QSY-7 giving ~92% and ~95% quenching respectively.[2] Dual-labelled probes, with the phosphorescent reporter label at one end and a selected quencher at the other, were designed and used to detect synthesised target and PCR product in solution. Applications of these dual labelled probes for the detection of PCR product in a closed tube format are currently under development.

Acknowledgements. Financial support of this work by the Irish Research Foundation 'Enterprise Ireland', grant IF/2001/008, and by the Irish Higher Educational Authority is gratefully acknowledged..

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NON-DESTRUCTIVE MEASUREMENT OF RESIDUAL OXYGEN LEVELS IN PACKAGED FOOD USING THE OPTICAL OXYGEN SENSING

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Packaging under modified atmosphere is widely used with various foods, the residual oxygen being an important determinant of food quality and shelf life for such products. Knowledge of the actual levels of oxygen in each pack during its shelf-life provides important information about the integrity of packages, the effectiveness of packaging process, operation of the packaging machine and indicates quality changes in the product. By far, residual oxygen was mainly measured by destructive methods.

Recently, an optical oxygen sensor system was developed by our team for non-destructive measurement of oxygen by luminescence quenching. The oxygen analyser, which operates with disposable solid-state sensors placed inside packs and a portable external detector, was tested in several trials with various types of packaged foods to non-destructively measure residual oxygen in packs and determine food quality. [1,2]

Several small-scale industrial trials were carried out with several food producers with different types of food and packaging conditions. In particular, batches of MAP meat, sliced hams, convenience foods (beef lasagne packaged under sous vide conditions), cheddar cheese were monitored for residual oxygen. Various methods of sensor incorporation in packs and migrations studies with the sensor components were carried out, to secure high reliability of sensor use and its safety.

The optical oxygen sensor system was shown to provide valuable information about performance of the packaging process, product storage conditions and food quality in a convenient and cost-efficient fashion and non-destructively. It can reliably identify improperly packed samples and be used for early prediction of product deterioration. Efficiency of packaging was seen to be higher for the cheddar cheese with low levels (< 0.5%) over the over product shelf life (4 months at +4ºC). Unacceptably high levels of oxygen were found in beef lasagne (5-20%). Lipid oxidation and microbial growth were seen to correlate with the levels of oxygen.

Acknowledgements. This research has been part funded under the Irish Food Sub Programmes of the Operational Programme for Industrial Development which is administered by the Department of Agriculture, Food and Forestry and supported by National and EU funds, grant DAFF/00/R&D/C/822.

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A FIBER-OPTIC HYDROGEN GAS SENSOR BASED ON THIN FILMS FABRY–PEROT INTERFEROMETER

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A new optical-fiber hydrogen sensor has been presented. The sensor utilizes layered sensing structure. This structure is made at the end of multi-mode optical fiber as a sensing element.

Thin films of Pd exhibit a change in their optical properties when exposed to hydrogen. A new sensor element consisting of a layered structure metal/dielectric/metal has been developed for optical detection of hydrogen. This sensing structure composed of a combined Pd and a-WO_3 thin film stack on Au thin film has been tested. A structure uses a thin a-WO_3 film as a resonant cavity and is acting as a Fabry-Perot interferometer.[1]

The gasochromic mechanism in amorphous tungsten oxide films has been used. Chemochromic materials, such as tungsten oxide, can reversibly react with hydrogen in air. This interaction display significant changes optical properties of.[2,3] Sensor structures utilize thin films of these materials applied to a sensor at the end of an optical fiber have been used to detect low concentrations of hydrogen gas in air. When the Pd/a-WO_3 films are gasochromically coloured by exposure to diluted hydrogen gas,[2] interference peak light has change position.

Change of optical constants of resonant cavity due to gasochromic coloration in a-WO_3 films is directly related to the double injection of hydrogen ions and electrons.[2] Optical cycling tests confirm that the Pd/a-WO_3 films can not be bleached without oxygen. The sensor presents good sensitivity for low hydrogen concentration and the stability of parameters in room temperature.

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MODELING TEMPERATURE BEHAVIOUR OF PHASE-FLUORIMETRIC OXYGEN SENSORS USING PHYSICAL MODELS OF LUMINESCENT ACTIVE MEDIUM

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The use of phase-fluorometric sensors operating with disposable sensing elements for the determination of oxygen concentration is becoming increasingly popular in biomedical, environmental and packaging applications. The detector measures the phase shift of luminescent signal with respect to excitation, and this parameter is then converted into oxygen concentration using sensor calibration and special calculation algorithms. Recently, we described a new approach for such calculations, in which parameters of sensor model were determined on the basis of the initial calibration and then used for calculation of oxygen concentration.[1,2] Different physical models of active luminescent media, including the traditional double exponential and single-exponential model with non-linear solubility of oxygen, and also distributed models, were tested with real sensors and calibrations to optimise the accuracy of oxygen determination. Sample temperature should also be taken into account in such fitting algorithms, as temperature has strong effect on sensor calibration and it can vary broadly in some cases such as environmental and packaging applications. Taking into account variation of temperature, sensor models giving the best fitting of calibration data can be different.

In this study the two common tasks were considered: 1) to find the optimal model for the sensors working at known temperature (constant or measured separately) within the defined range; 2) to find the optimal model for the sensors working at variable temperatures, using a set of calibrations obtained at several temperatures. The second task is applied to the following two cases: a) when sample temperature is unknown or it may vary during the measurement; b) when the accuracy of oxygen concentration determination is determined by temperature variation..

This modeling approach was applied to the oxygen sensors based on platinum(II)-octaethylporphin-ketone and porous support material, which are currently being used in packaging applications.[3] The calibrations were performed with six standard gas mixtures (oxygen balanced with nitrogen) ranging 0 - 21 kPa at four temperatures: 5 15, 25 and 30 ^{o}C using the developed phosphorescent phase detector working at modulation frequency of 2.6 kHz. For each of the above cases, several common physical models of active medium (discrete and distributed) were analysed to identify the best one. These were: one-parametric Rayleigh distributed model (approximation accuracy at each temperature better than 0.25 kPa): two-parametric Extreme Value distributed model (accuracy better than 0.1 kPa) and three-parametric double-exponential model (accuracy better than 0.04 kPa). Model parameters were determined by minimising absolute mean error of approximation, which reflects the agreement with calibration data and follows the application requirements. For the best model, temperature behavior of parameters was analised. Comparison of two modeling approaches, including achievable accuracy of calculation of oxygen concentration and requirements for temperature accuracy accordingly different oxygen accuracy for best models is given.

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DESIGN AND APPLICATION OF BIOLOGICAL ADDRESSABLE MICRO- AND NANOSENSORS

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In modern life science, medicine and analytics there is a permanent and increasing demand for simple, sensitive, fast and selective analytical devices which should be small, portable and have no need for modification of the test matrix. Very popular is the use of nano- and microspheres as a tool for bioanalytical applications. Combination of optical sensor technology and microsphere approaches was first presented by Kopelman et al. They designed so-called PEBBLE-sensors for the intracellular determination of oxygen, glucose, pH and selected ionic species as potassium, calcium, or zink. [1,2]

Here we present a new class of functional nano- and microspheres with diameters from 100 nm up to 10 µm. These particles contain pH- or pO_2 sensitive luminophores and are individual optodes. In contrast to the PEBBLES cited above, the surface of the spheres is modified with biomolecules as specific receptors. This allows binding such nano- and microoptodes onto specific sites in heterogeneous living systems (e.g. cell membranes). Site specific monitoring of oxygenation or pH-conditions in complex biological systems becomes possible with such particles as tool.

Strategies to prepare addressable pH- and pO_2-sensitive microspheres were shown and different methods for receptor immobilisation methods will be compared.

The application potential for the new adressable sensor spheres is discussed. Examples for application in immuno- or oligonucleotide analysis as well as in enzyme screening are presented.

Additional multifunctionality of such sensor particles can be achieved by using magnetic sensing spheres. This will open the goal for completely new screening applications.

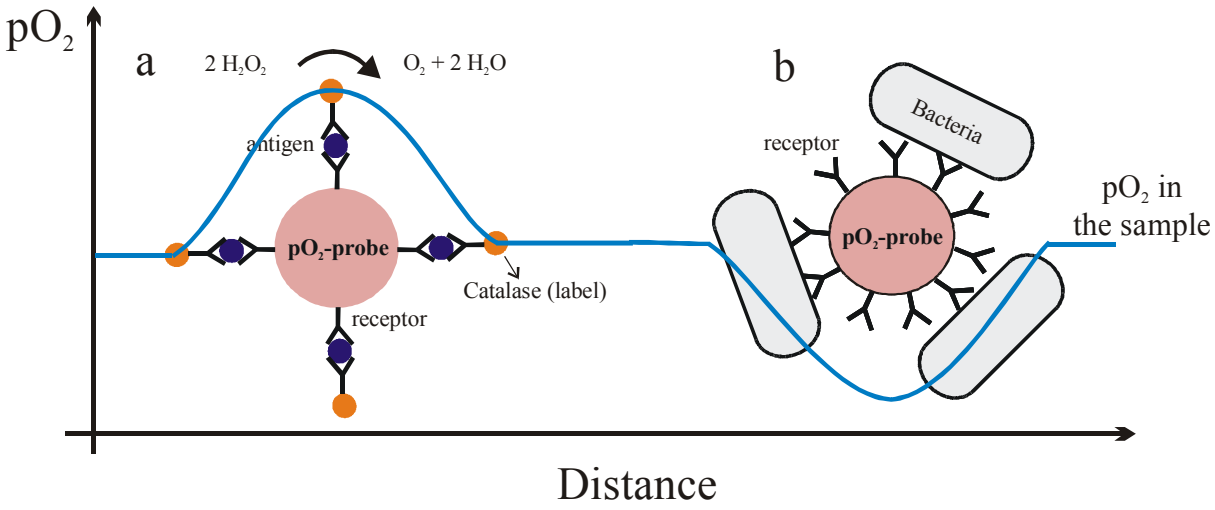

Figure 1. Use of pO_2 sensitive microspheres in immunoassays (a) (cathalase as label) and screening the activity of enzymes expressed in bacteria cells. (b); local oxygenation level around the microspheres is detected.

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AN OPTICAL FIBRE NITRIC OXIDE SENSOR FOR BIOLOGICAL SAMPLES

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Since the identification of nitric oxide as a biological signalling molecule it has been realised that NO is involved in the regulation of blood vessels, communication in the brain, and in immunological defence against invading organisms.[1] Since this discovery numerous analytical techniques have been applied in the development of NO chemical sensors.

Currently work is being undertaken at DIAS (UMIST) to develop an optical fibre sensor using various colorimetric reagents which interact with NO producing a change in optical response that is measurable in the UV/VIS region of the electromagnetic spectrum. Following the successful immobilisation of the copper complex of eriochrome cyanine R (ECR) in silicone rubber[2] further work was carried out using the copper complex of 2,9-dimethyl-1,10-phenanthroline (dmp) to detect nitric oxide. It was first reported in 1996 that nitric oxide can reduce $Cu(dmp)_2^{2+}$ and this is accompanied by an increase in absorbance at 454 nm.[3] Initial studies have been carried out in physiological media, followed by immobilisation of the complex in various matrices, including silicone rubber and nafion.

The ultimate goal is to produce a reagent scheme which can detect NO in sub-micromolar quantitites. In solution $Cu(dmp)_2^{2+}$ could detect 0.44 μM NO. Immobilisation in nafion produced a reversible response to NO gas with a detection limit of 0.465 ppm (~ 15 μM). This compares well with the sensitivity of other optical fibre sensors for NO.[4]

Further to this work the selectivity of the sensor was investigated with respect to other gases in the mammalian body e.g. O_2, CO and NO_2. Biological interferents such as nitrite, hydrogen peroxide, peroxynitrite and superoxide were also investigated as they may interfere via reaction with NO or by reacting directly with the reagent. These results will be reported in this poster presentation.

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MATHEMATICAL MODEL FOR SENSOR FILMS BASED ON CHEMICALLY MODIFIED ENZYMES

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In recent years our research group has studied the design and characterisation of sensor films based on the immobilisation of covalently bonded enzymes to fluorophores whose fluorescence changes during the enzymatic reaction. This metodology has been applied to the design of a sensor film for glucose based on the immobilisation of glucose oxidase chemically modified with a fluorescein derivative on polyacrylamide.[1] Measurements were performed by flow injection analysis. The peak height is related to glucose concentration. In this work a mathematical model which relats the analytical parameter with the hydrodinamic and chemical parameters of the system is presented.

The expression that justifies the relationship between the peak heigth and glucose concentration is as follows

$$\frac{I_m - I_o}{I_o} = \frac{KK_1K_gK'[G]_o}{K_2K_o[O_2]_o + K_1K_gK'[G]_o} \qquad (1)$$

where I_m is the fluorescence intensity at the maximum, I_o is the inicial fluorescence intensity, $[G]_o$ is the glucose concentration, $[O_2]_o$ is the oxygen concentration, k_1 y k_2 are the kinetic constants of the glucose oxidase reaction, k_g y k_o are the constants that depend on the diffusion coeficients and the limit liyer thickness for glucose and oxygen respectively and K' is a constant that depends on the hydrodinamic parameters of the FIA system such as flow rate (Q), sample volume (V_m) and flow cell volume (V) througth the following expression:

$$K' = (1 - \exp(-V_m/V)\exp(-(Q(t_m - V_m/Q))/V)$$

being t_m the time at which the fluorescence maximum is reached.

The expresión (1) implies that the process was limited by the glucose and oxygen diffusion througth the sensor film. In order to verify this fact the ascorbic acid in neutral form diffusion througth the sensor film as a glucose diffusion model was studied; ascorbic acid produces quenching on the fluorophore covalently bonded to glucose oxidase. The model predicts that peak heigth does not depends on the glucose oxidase concentration which is in accordance with the experimental results.

Finally, a study of interferences has been developed in order to apply this methodology to glucose determination in clinical samples.

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OPTICAL TEST STRIP FOR CITRATE: DESIGN AND CHARACTERISATION

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Different strategies have been devised for anion recognition. The most important concern: a) ligands that utilize hydrogen bonds, electrostatic interactions or both to coordinate with anions and b) metal containing ligands.[1] A synthetic receptor from the first group for citrate in a competitive media, such as water, was studied by Anslyn et al.[2] taking into account design aspects such as preorganization, hydrogen bonding, and charge pairing. The receptor incorporates the recognition groups (guanidinium group embedded in aminodihydroimidazolium groups), used for carboxylic acid recognition, in a 1,3,5-triethyl-2,4,6-trimethylbenzene motif to achieve preorganization. A binding constant in water of 6.9×10^3 was reported. Later, a competitive indicator method in solution for the determination of citrate using xylenol orange and methylthymol blue as indicator molecules was proposed.[3]

We are interested in developing test methods for anions using a test strip format to obtain in-situ information in a simple and inexpensive way. Here, we use the described synthetic receptor to prepare an optical bulk membrane containing all necessary components for extraction and recognition of citrate dissolved in a homogeneous plasticised polymeric membrane. The host-guest recognition process of citrate by the ionophore I is coupled –through an electroneutrality condition- to the coextraction of a reference ion, a proton, which is reversibly complexed by chromoionophore C, which acts as a transducer of the recognition process. In contact with an aqueous solution containing citrate (X^{3-}), the following coextraction equilibrium holds in the test strip, characterised by the constant Ke. The three guanidinium groups of the receptor molecule are protonated and the receptor acts as a chloride salt.

$$\overline{IH_3^+Cl_3^-} + \overline{C} + H_2X^- + H^+ \leftrightarrow \overline{IH_3^-X^{3-}} + \overline{HC^+Cl^-} + 2HCl$$

The prepared single-use test strip consists of a rectangular strip of an inert polymeric material with a circular film adhered on its surface. This circular film is the sensing zone, and is composed of a poly(vinylchloride) membrane plastisiced with tributyl phosphate that incorporates the above-described citrate receptor and lipophylized Nile blue as a proton selective chromoionophore. From the fit to the mathematical model it is possible to calculate for log Ke the value 6.37.

The present study includes the following features: synthesis of receptor, study and characterisation of the sensing reaction, construction and properties of the sensor membrane, study of the reaction with citrate -- especially selectivity and response time, analytical parameters of the test strip and analytical application to natural waters.

When the sensor is introduced into 10ml of a buffered solution containing citrate at pH 4.0, without stirring, equilibrium is reached after 40 s, changing the colour of the membrane from red to blue. The absorbance of the strip measured at 660 nm is used as the analytical signal. The test strip responds linearly in the range 7.7×10^{-3} M to 8.3×10^{-4} M. The reproducibility intermembrane is 5.7 % (as RSD) and the intramembrane is 6.0 % (as RSD) in the middle of the calibration range.

This method was applied to different types of beverages and pharmaceutical formulations with acceptable results validated against an enzymatic method for citrate.

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OPTICAL SENSORS BASED ON THE REDOX PROPERTIES OF POLYANILINE

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During last years, the research on optochemical sensors has mainly focused on the study of new supports materials on which stability of the immobilised reagents is achieved. Optical sensors based on conducting polymers have thus become a promising solution. Some of this compounds, obtained after chemical polymerisation, display optical properties in the Vis-near IR region (being compatible with LEDs and diode lasers, the most frecuently used excitation sources in optical sensors) which change with the chemical reaction. They combine the advantages of optical sensors (long distance measurement and freedom from electrical interference being the most important), with those of the conducting polymer (the polymer itself acts as both indicator and support so that no leaching into the solution is observed).[1] One of these conducting polymers is polyaniline (PAN), widely employed in electrochemical sensor development because of its stability, conductivity and pH sensitivity. In the case of optical sensors, the variation with pH of PAN and substituted PAN films have been used, apart from pH determination, for acetic acid and ammonia determination.[2] Another important application of PAN films is as the base of optical biosensors (i.e. saccharides determination and enzyme immobilisation).

Bearing in mind these advantages, and taking into account that PAN exists in three different oxidation states, which display different UV-Vis spectrum, a new application of chemically polymerised PAN films is presented, based on its redox properties. PAN films are obtained by chemical polymerisation of iron(III)chloride and aniline during 30 minutes. In this conditions, the emeraldine base form (partially oxidised) of PAN is obtained. Working in a pH buffer solution, a study of the effect of several compounds with redox properties shwoed that changes in the analytical signal were due to the formation of the reduced PAN (leucoemeraldine). The response of sulphite[3] and ascorbic acid has been studied in this way (Fig. 1). The method has permitted the determination of sulphite in wine samples and the determination of ascorbic acid is being studied in real samples. Other reductor compounds are also being studied.

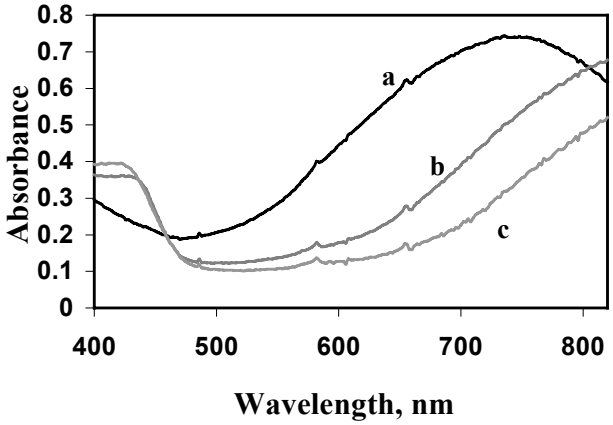

Figure 1. Spectra of PAN films obtained after exposure to (a) buffer solution of pH=1, (b) sulphite in buffer solution of pH=1, (c) ascorbic acid in buffer solution of pH=1.

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PHOSPHORESCENT SENSOR FOR CARBON DIOXIDE DETERMINATION BASED ON RESONANCE ENERGY TRANSFER

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Carbon dioxide is usually a by-product of many industrial processes and is believed to aggravate the greenhouse effect in our environment. Medical diagnosis and treatment of critically ill patients in intensive care units and operating theatres often requires monitoring[1] the CO_2 partial pressures of arterial blood. Consequently, the determination of CO_2 is of considerable importance in environmental and biomedical analysis and analytical chemistry. Considerable effort has been devoted over many years to the development of new techniques to measure carbon dioxide concentration. In general, sensing CO_2 is moderately difficult because of the inertness of this molecule. In contrast to a variety of cations and anions, fluorescent probes which are directly sensitive to CO_2 are not known. Carbon dioxide does not act as a collisional quencher of luminescence, and chelators for CO_2 are not known.[2] Because of the difficulties in direct detection of CO_2, most sensors have relied on coupling the partial pressure of CO_2 to the pH changes in a buffer solution.

The most used scheme is based on a pH sensitive dye that has its acid-base equilibrium regulated by means of a lipophilic quaternary ammonium hydroxide incorporated into a hydrophobic membrane. The absorbance or luminescence intensity is the analytical parameter and suffers from the usual drawbacks for intensity signals. The use of ratiometric signals can overcome drifts and change in intensity. The measurement of luminescence lifetime turns out to be insensitive to signal drift, but the number of fluorescent pH indicators with adequate pKa values is rather limited. One approach for using coloured indicators in lifetime-based sensors is resonance energy transfer (RET), [3] in which the excited-state energy from the initially excited donor is transferred to an acceptor. Sensors based on this concept convert colour changes into decay time information. The co-immobilization of a pH indicator and an inert fluorescent dye, whose emission band overlaps the pH indicator absorption band, makes it possible to prepare CO_2 sensitive membranes. The use of long-lifetime luminophors permits the design of RET-based sensors for low-level carbon dioxide determination compatible with low-cost instrumentation.

The use of long-lifetime donors has the drawback of quenching molecular oxygen. Molecular oxygen acts as an interfering factor and also limits the long-term stability of the CO_2 sensors due to reactive singlet oxygen formation, which causes photobleaching of the indicators. Sensors described for CO_2 usually show a pronounced influence of oxygen. Different strategies have been devised to minimise the oxygen cross-sensitivity, for example incorporating the luminophore into polymer nanobeads[4] or polyacrylonitrilo beads[5] of low oxygen permeability.

We present an optical sensor for carbon dioxide measurement which is unaffected by oxygen. It is based on lifetime measurement using RET from a platinum porphyrin complex included in a poly(vinylidene chloride-co-vinyl chloride) copolymer acting as a luminescent donor to the pH indicator thymol blue as acceptor. The present study includes these features: selection of donor and acceptor compound, choice of oxygen barrier polymer, membrane preparation and sensor performance.

Linear calibration plots over the range 0 - 0.6 % CO_2 were found. The recovery behaviour in the gas phase gives response times of t_{90}= 35 s going from 0% to 5% CO_2 and t_{90}= 115 s going from 5% to 0% CO_2.

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DETERMINATION OF PHOTOSYNTHETIC HERBICIDES BASED ON AN OPTICAL FIBRE SENSOR

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Photosynthetic herbicides are one of the classes of herbicides that are used most widely. These chemicals can be highly toxic to human and animal health in low concentrations. Therefore disposal of rapid screening techniques for photosynthetic determination is necessary. Biosensors are of particular interest for the monitoring of photosynthetic herbicides because they have a common biological activity which can be used for their detection. These substances inhibit the light-driven electron transfer system in photosynthesis.

In this work, a new method for photosynthetic herbicide determination is presented based on the interaction of these compounds to the isolated reaction centre of the purple bacteria *Rhodobacter sphaeroides*. This reaction centre is a membrane-bound pigment-protein complex that catalyses conversion of light energy into chemical energy. Following light excitation of RC, electrons are transferred from a bacteriochlorophyll dimer through a number of cofactors to a ubiquinone Q_A and then to another ubiquinone Q_B. Decay of the reaction centre from the excited state to the stationary state is possible from both ubiquinones. Photosynthetic herbicides affect to the proportion of both decay pathways since these herbicides are able to compete with Q_B for the binding site in the reaction centre. Reaction centre has an absorption band at 860 nm which is less intense for the excited form than for the stationary form. Therefore, the concentration of photosynthetic herbicides can be determined by following the temporal changes in absorption at this wavelength.

An optoelectronic system has been designed to both excite the reaction centre and monitor the temporal changes in absorption. Two optical fibres connect the measuring cell to a diode at 860 nm and a hybrid photodetector. The connection between the optoelectronic unit and a laptop is via a National Instrument DAQ card 1200 data acquisition board. Lab View software is used for driving the optoelectronic unit and processing all the collected data.

Transient absorbance signal depends on the time according to a two-exponential model where τ and A are the time constants and the amplitude of the return to the stationary state from Q_B (subscript 1) and Q_A (subscript 2).

$$A_t = A_1\left(e^{-t/\tau_1} - 1\right) + A_2\left(e^{-t/\tau_2} - 1\right)$$

A_1 value depends on the herbicide concentration (Figure 1). A mathematical model has been developed in order to obtain an analytical parameter for determining herbicide concentration.

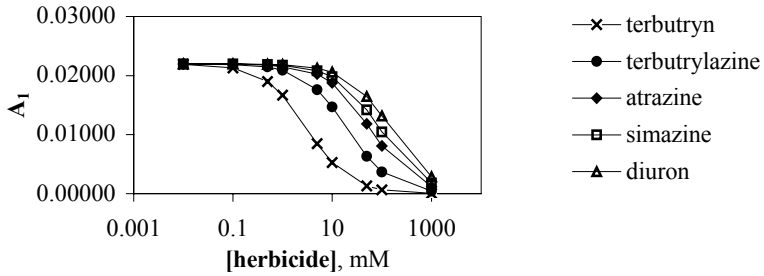

Figure 1. Effect of the concentration some photosynthetic herbicides on A_1 value

DEVELOPMENT OF A FLOW-THROUGH ROOM TEMPERATURE PHOSPHORESCENCE OPTICAL SENSOR FOR THE DETERMINATION OF 1-NAPHTYLACETIC ACID

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1-Naphthaleneacetic acid (NAA) is a naphthalene derivative which has been widely employed as plant growth regulator to prevent the premature fall of fruits and as fungicide for controlling diseases on fruits. This phytohormone must be added in a concentration of 20-100 mg L^{-1} in the spraying solution to be effective. Due to this, trace amounts in soil, surface and underground waters are expected as a result of agricultural processes.[1]

The most widely employed analytical procedures for the determination of NAA are absorption spectrophotometric[2] and fluorimetric methods.[3] The latter ones are more sensitive, but its lack of selectivity should be overcome using a previous separation technique, like HPLC. However, this compound shows also a native phosphorescence, which could be exploited for the development of a phosphorimetric flow-through optosensor.

Thus, the development of an optical sensor for NAA determination, based on the measurement of its intrinsic phosphorescence, is presented in the present work. The proposed system is based on injecting the sample, containing the phytohormone, which is then on-line immobilized onto a solid phase packed into a conventional luminescence flow-cell placed inside a phosphorimeter, RTP emissions from the packed sensing material were continuously monitored. Several solid supports have been tested and a polymeric non ionic exchanger (Amberlite XAD-7) was finally selected as solid support. After ensuring a stable RTP signal from immobilized NAA, the analyte was eluted from the packed resin before the next injection.

Several experimental variables, including the type and size of the solid supports, type and concentration of the heavy atom perturber, concentration of sodium sulphite as deoxygenate agent and regenerative system, flow rate and valve injection volume, and measuring conditions, including the gate and decay time, detector voltage, and excitation and emission slits were studied and optimized.

Analytical performance characteristics of the method and its applicability for real sample analysis will be also discussed.

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AN IMPINGING JET-TYPE GAS-FLOW CELL FOR A GAS-SENSOR SYSTEM USING THE CATALUMINESCENCE

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A gas-sensor system needs fast response and high sensitivity to detect the sudden change in atmosphere and stability independent on the position of establishment. We have reported the *cataluminescence* (CTL) -based gas-sensor that has fast response and high sensitivity because of the detection mechanism based on the chemiluminescence accompanied with catalytic oxidation of combustible gas. However, its characteristics depend on the fluid mechanical situation around the sensor because the rate of catalytic oxidation depends on the rate of the transfer of gas molecules to the catalyst surface. In this report, we propose a new gas-flow cell for the CTL-based sensor that realizes fast response, high sensitivity and stable characteristics. We investigated the fluid dynamical property around the CTL-based gas-sensor,

Three types of gas-flow cell were investigated, i.e. the A type; natural convection type, the B type; laminar flow type and the C type; impinging jet type. Through the measurements of rate of heat transfer, catalytic oxidation and the CTL-intensity as a function of the flow velocity of gas through the cell, we found that the C-type sensor has the fastest response, the highest sensitivity and the best stability. The configuration of the flow-cell is as follows: We used a planer sensor tip (3 x 3 x 0.15 mm^3) with the printed platinum ribbon-heater and a printed catalyst-layer on both sides of a ceramic substrate, respectively. A quartz window with a hole of diameter D was placed at H mm below the catalyst layer. A jet of sample gas was impinged to the catalyst layer through the hole with a velocity u. The CTL intensity was measured by a photomultiplier placed under the window. We measured the heat and mass transfer from/to the sensor tip to find out the best value of D, H and u. Semi-empirical equation for the impinging jet is given by Hrycak[1] as follows:

$$Nu = 1.95\left(\frac{2r}{D}\right)^{-1.23} \cdot \mathrm{Re}^{0.7} \cdot \mathrm{Pr}^{0.33} \quad .....(1)$$

where Nu is the Nusselt number, Re is the Reynolds number, Pr is the Prandtl number, D is the hole diameter, r is the radial distance from stagnation point. A free jet exit from a hole is impinged perpendicularly to the sensor plane, and stagnation flow spreads along the plane to the radial direction as wall jet. Experiment of heat transfer from the sensor showed that the flow-velocity dependence of the heater power to keep the sensor at a constant temperature of 450^{O}C agrees with theoretical curves estimated from Eq. 1 in the flow-velocity region over u_C for various values of D. The deviation in the region below u_C results from the effect of natural convection. The rate of mass transfer from gas phase to the catalyst surface is also given by similar equation to Eq. 1, and the CTL intensity as a function of the flow velocity showed similar behavior to heat transfer. Finally, we obtained the best value of D=2 mm, H=2 mm and u=0.5-2 m/s, which shows fast response, high sensitivity and good stability. Under these conditions, the CTL intensity of the C-type sensor was about ten-times as high as that of the A-type sensor, and its response time was less than 5 s.

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AN INTRINSIC FIBRE OPTIC CHEMICAL SENSOR BASED ON LIGHT COUPLING PHENOMENON

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The phenomenon of light coupling between fibres is widely utilised in optoelectronics e.g. for the construction of fibre optic couplers. Fibre optic couplers are finding applications in telecommunication and sensor technology. Commercially available fibre optic couplers are made by heating and pulling two fibres up to a point, where these fibres are fused together and they form a region where the optical power introduced into one fibre can be split to the second one. Various types of optical fibres can be used for the preparation of a coupler governing technology which should be applied and its final properties.

The paper presents a construction of an intrinsic fibre optic chemical sensor based on the use of polymer fibres. Two optical fibres were used to build a device which can be called as a fibre optic chemical coupler. The coupling efficiency of optical power depends on the refractive index of liquid delivered to a microchannel formed by the fibres.

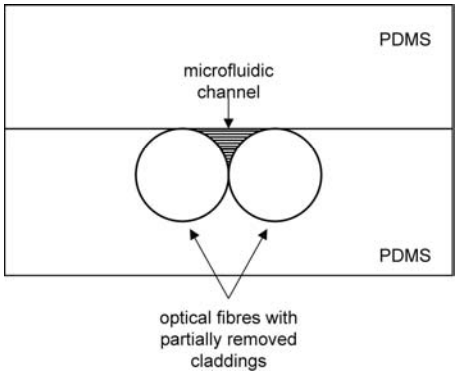

Figure 1. Schematic cross section of a sensor.

Two polymer optical fibres made of poly(methylmethacrylate) were put together, and were attached in a special mould placed on a glass plate. The poly(dimethylsiloxane) (PDMS) was mixed with a crosslinking agent, degassed, and poured over the mould. After the polymerisation, the mould and the glass plate were removed. As a result, we have obtained a PDMS plate with the fibres on the surface. To make the coupler working, it was necessary to remove PDMS from the space between fibres as well as to remove the cladding of the fibres. In our preliminary works we have done it mechanically by means of a sharp razor. In this work we have developed and described a chemical etching of the cladding of the fibres. Various chemical solvents were tested i.e. acetone, a solution of acetone in water, chloroform, ethyl acetate, methanol, tetrahydrofurane .

Preliminary measurements with a coupler were done with a structure in which two fibres were put together in parallel. The results presented in this work were obtained when the fibres were bended in order to maximise leakage of optical power from the fibre.

The structure was tested as a sensor for refractometric measurements. However, the coupling efficiency depends also on the absorbance of the liquid so it can be used in spectrophotometric experiments. The device combines the advantages of both fibre optic sensor and microfluidic structure.

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TAILORING SOL-GEL MATERIALS STRUCTURE FOR PH AND OXYGEN SENSING

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Solid supports play an important role in the development of optical sensors as they have an important influence on the quantum efficiency of the immobilized luminophor. During the last decade the use of sol-gel materials as solid supports for optical sensing has experienced a notable growth due to their advantages in terms of high rigidity, long-term stability, chemical inertness, optical transparency, low intrinsic fluorescence, etc.

Measurement of pH is important in various fields of science and technology and is routinely performed using glass electrodes. However, during the last decade, optical sensors have been developed to complement the glass electrodes for pH measurements under various circumstances. In general, pH optical sensors are based on pH-induced, reversible changes in optical properties (e.g. absorbance, reflectance, fluorescence, phosphorescence) of an immobilized dye. From the typical sigmoidal response showed by these sensors only a narrow range of the curve can be usually taken as linear (2-3 pH units). Attempts to overcome this problem, include the use of mixtures of various pH indicators[1] or one indicator with multiple steps of acid dissociation[2] in order to extend the pH range. Tuning the apparent pKa of a luminescent pH indicator by surfactants co-immobilization is a new emerging approach to overcome this problem. This approach, combined with sol-gel technology, has allowed us to develop new pH sensing phases based on mercurochrome fluorescence emission, each of them covering a different range of the pH scale.

On the other hand, dissolved oxygen sensing has become a topic of crucial importance in water pollution control. The amount of oxygen dissolved in water is an indicator of the quality of the water (a decrease in this amount usually indicates the presence of organic waste). The Clark-type amperometric electrode is still the most common sensor for oxygen sensing. However, it requires uniform sample stirring and consumes oxygen, its response can be affected by exterior electromagnetic fields and it suffers from interferences (e.g. hydrogen sulphide). So, in the last years, efforts have focused on the measurement of O_2 using optical sensors,[3] which are most frequently based on dynamic quenching of the luminescence of an indicator. Sol-gel based oxygen optical sensors usually suffer from some limitations such as low accessibility to water (resulting in long response times) or leaching of the indicator, so a thorough optimisation of the sol-gel structure must be carried out in order to achieve a robust design. In this vein, a comparison of the analytical performance of different phosphorescence oxygen sensing phase, depending on structure modifications of the sol-gel employed as solid support, will be presented.

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ANIONS OPTOSENSING BY ROOM TEMPERATURE PHOSPHORESCENCE – ENERGY TRANSFER

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Research on luminescence optical sensors for "at real time" analytical control of the quality of the environment (atmosphere, natural water, etc) has experienced a growing interest during the last decade. In this vein, fluorimetry has been extensively used for the development of sensitive optical sensors for numerous chemical species. However, it should be pointed out that room temperature phosphorescence (RTP) may offer some important advantages over fluorescence. RTP provides a highly sensitive, low background, selective and time discriminated luminescence emission whose measurement could be used to develop innovative approaches for photoluminescent detection. Moreover, combination of solid surface RTP with flow injection analysis (FIA) strategies can be advantageously exploited, since the packing into the flow cell allows simultaneously to preconcentrate and to detect the analyte[1]. This approach provides the advantages of FIA systems (e.g. it is easy to renew reagents on the solid support, irreversible chemistry can be applied for sensing purposes, sample pretreatments can be integrated in the flow systems, etc) and allows high sensitivity for detection.

Despite these advantages, very few analytical systems based on RTP have been reported so far2. Probably, the most important drawback limiting the development of RTP methods is the lack of suitable indicators able to selectively detect the analyte. In this vein, we have shown previously that energy transfer (ET) phenomena from a RTP phosphor insensitive to the analyte to a suitable analyte-selective dye indicator could be used as a general strategy to develop RTP methods for analytes unsuitable for RTP conventional analysis3. Provided that there is spectral overlap of the "absorption spectrum of the analyte dye (acceptor)" with the "emission of an inert phosphorescent molecule (donor)", colour changes can be converted into sensitive RTP information.

The analytical potential of this strategy will be demonstrated for the development of different RTP optosensors for the selective determination of a variety of anions (including bromate, cyanide, orthophosphate and sulphide) of great importance in environmental control.

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MOLECULAR IMPRINTING POLYMERS FOR LUMINESCENT OPTOSENSING OF BENZO[a]PYRENE

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Molecular imprinting has attracted considerable attention as a way to create artificial selective recognition sites in synthetic materials since they combine the advantages of easy design with physical and chemical stability and durability[1]. In this process functional and cross-linking monomers are co-polymerized in the presence of the target analyte (the imprint molecule), which acts as a molecular template. After removal of the template a recognition site in the polymer remains which retains shape and functionality complementary to the molecule which was imprinted. MIPs have found application as stationary phases for chiral separations and solid phase extractions, as "in vitro" synthetic antibodies mimics or as catalysts in chemicals reactions. Moreover, molecular imprinting technology has recently started to be employed as a tool to prepare solid supports for luminescent optical sensors, enabling the development of robust and selective devices[2].

Polycyclic aromatic hydrocarbons (PAHs) are powerful chemical carcinogens which are derived from coal tar pitch, mineral oil, tobacco smoke, grilled food, etc. For this reason, their detection and monitoring has become an important problem and this has led to the development of new and faster analytical methods, offering improved selectivity and sensitivity. In particular, Benzo[a]pyrene is one of the most carcinogenic PAHs, included both in the United State Environmental Protection Agency (EPA) and in the European Community (EC) priority list of pollutants. Because it is formed when gasoline, garbage or any animal or plant material burns, it is usually found in smoke and soot. This molecule also associates with dust particles in the air and is carried into water, soil and onto crops.

Methods for detecting PAHs are numerous today and include gas chromatographic techniques, electrochemical detection and fluorescence. Actually, the native luminescent properties of the PAHs and their derivatives have been widely exploited for analytical purposes. However, the major disadvantage of direct fluorimetry detection is the lack of selectivity as most of the PAHs derivatives exhibit similar luminescence properties.

In this work, results on development of new luminescence optosensors for BaP based on the measurement of its native luminescence emission after its selective immobilization onto a MIP-based solid support will be presented. Comparative analytical performance characteristics of the proposed system and potential applicability of the optosensor for real sample optosensing of PAHs will be discussed.

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NEW OPTO-CHEMICAL AMMONIA SENSOR WITH DETECTION RANGE FROM 1 PPM TO 200 PPM

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The determination of gaseous ammonia is of great importance in many fields of sensor applications (gas warning devices, measurement of process gas, air quality inspection, biotechnological processes and refrigeration technique). A new opto-chemical sensor for gaseous ammonia was developed with a detection range from 1 ppm to 200 ppm. This ammonia sensor offers a series of advantages as no ammonia consumption, modular construction, low operating costs and low power consumption and no influence of electro-magnetic fields.

Different ammonia sensitive layers were developed and tested for the opto-chemical detection of gaseous ammonia. Indicator dyes were immobilized in sol-gel-matrices and deposited by dip-coating onto glass slides. The developed ammonia sensitive layers PH were characterized by sensitivity (Figure1), reaction times and reproducibility. The sensitive layers have the following properties:

- high sensitivity and short reaction times in the concentration range 1ppm ... 200 ppm;
- reproducibility and long-term stability of the indicator in the layer system more than one year;
- ammonia durability in the case of concentrations >>200 ppm;
- negligible influence of humidity in the range between 30 % r:H. and 70 % r.H.

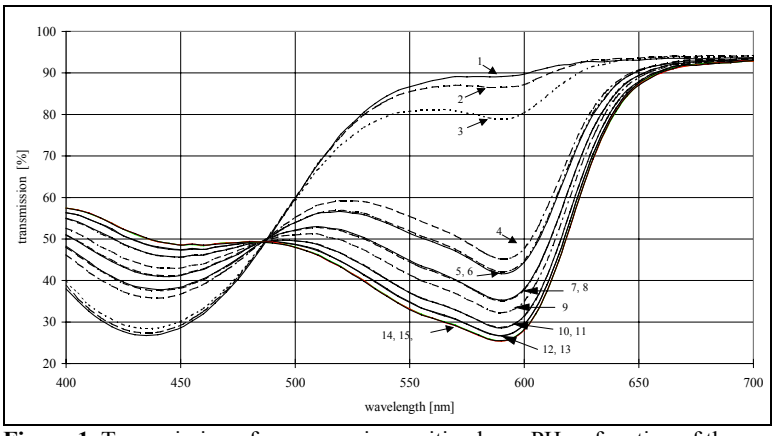

Figure 1. Transmission of an ammonia sensitive layer PH as function of the ammonia concentration (measurements every 60 seconds, ammonia concentrations see Table 1).

Table 1. Ammonia concentrations for the transmission curves in Figure 1.

	NH_3 [ppm]		NH_3 [ppm]
1	0	9	54
2	< 10*	10	80
3	< 10*	11	84
4	24	12	108
5	28	13	110
6	28	14	132
7	44	15	136
8	46	16	134

*: residue of ammonia

The sensor itself is based on the new hybrid integrated emitter-detector-unit MORES of CiS IMS. Two LED's with 450 nm and 590 nm emission wavelengths as well as a planar array of silicon-diodes were integrated in such a unit. These unit was used to measure the reflectance of the sensitive layers. The distances between the emitter-detector-unit MORES and the sensitive layer as well as between sensitive layer and diffuse reflecting back side (PTFE) were optimized to yield high photocurrents. The measured photocurrents are very well reproducible. The detection wavelength λ_1 = 590 nm is more appropriate for the concentration range 1 ppm - 80 ppm (MAK value 50 ppm). With the detection wavelength λ_2 = 450 nm it is possible to measure ammonia concentrations up to 200 ppm.

SYNTHESIS AND EVALUATION OF MOLECULARLY IMPRINTED POLYMERS FOR TETRACYCLINES OPTOSENSING

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Tetracycline antibiotics have been employed extensively as bacteriostatic and antibiotic drugs due to their high activity against nearly all gram-positive and gram-negative bacteria. Methods for measuring the concentrations of tetracycline antibiotics include spectrophotometry, HPLC, electrochemical detection and fluorescence. Actually, the native luminescent properties of the tetracyclines and their derivatives have been wide exploited for analytical purposes. However, the major disadvantage of direct fluorimetry detection is the lack of selectivity as most of the tetracyclines derivatives and other similar antibiotics (e.g. anthracyclines) exhibit similar luminescence activity.

In this vein, molecularly imprinting is attracting a wide interest as a viable method for the production of materials capable of selective molecular recognition. MIPs have found application as stationary phases for chiral separations and solid phase extractions, as in vitro antibody mimics, as catalysts of chemicals reactions and more recently as recognition elements in sensors[1]. In MIP technology, the recognition sites are tailor-made in situ by self-assembly of functionalised monomers and templates followed by copolymerisation with cross-linkers to form a polymer network. After polymerisation, templates are subsequently extracted from the imprinted polymer, leaving "recognition sites", which are complementary to the analyte. Therefore, luminescence selectivity could be undoubtly improved by the use of MIP phases.

The combination of Flow Injection Techniques and solid substrate luminescence optosensing detection in an aqueous carrier, used as a continuous flow stream, will be exploited for the study of the analytical potential of new MIP phases developed for selective tetracyclines optosensing based on luminescence detection.

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DEVELOPMENT OF A FLOW-THROUGH PHOSPHORESCENCE OPTICAL SENSOR FOR THE DETERMINATION OF THE PLANT GROWTH REGULATOR BETA-NAPHTHOXYACETIC ACID

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Naphtyloxiacetic acids (NOA) is a kind of useful plant growth regulator, specially for grapes, apples and tomatoes,etc. It is important to develop the analytical methods for determination of this compound.

In the present work, we develop an optical sensor for β-NOA based on its phosphorescence properties. Among the optical techniques, the selectivity attained in phosphorimetry is much larger than that expected in spectrophotometry and fluorimetry, as only a few compounds phosphorescence in contrast to the number that absorb radiation or have fluorescence emission.

In this methodology, a solid support (commercial resin) is placed inside a flow cell in the light path of the excitation beam and the phosphorescence signal for β-NOA is continuously monitored. It provides high sensitivity and selectivity (owing to the selective sorption and preconcentration of the phosphorescence species) as well as a low consumption of both, reagent and sample.

The system consists in the direct sample injection together with a carrier stream, and the posterior analyte elution from the sensing zone with a regenerative agent. Using as carrier a solution containing $TlNO_3$ and Na_2SO_4, we measure directly the analyte phosphorescence and we use the peak heigh as analytical signal.

The optimum instrumental parameters (detector voltage, decay time, gate time, and slits), experimental conditions (type and size of solid supports, concentration of sodium sulphite as deoxygenate agent, type and concentration of heavy atom perturber, medium pH, and regenerative agent), and flow injection values (flow rate and injection volume) were carefully evaluated.

The proposed method to determine β-NOA in environmental samples presents a very low detection limit and analytical sensitivity.

Acknowledgements. Authors acknowledge the financial support of the FPU Grant of Ministry of Education, Culture and Sport (Ref. AP2002-1033), and to Projects PPQ2000-1291-C02-C01 and MAT2003-09074-C02-01 of Ministry of Science and Technology.

ROOM TEMPERATURE PHOSPHORESCENCE OPTOSENSOR FOR AFLATOXIN DETECTION

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Aflatoxins are naturally occurring genotoxic carcinogens produced by species from Aspergillus flavus group. These mycotoxins are produced by contaminant moulds growing on foodstuffs such as corn, peanuts, cottonseed, almonds, cashews and pistachio nuts. The development of new analytical methods for the detection of aflatoxins has gained much global attention in recent years, owing to the adverse health and economic effects produced by the presence of these mycotoxins in food and feed commodities.

The native luminescent properties of aflatoxins and their derivatives have been widely exploited for analytical purposes. In fact, several fluorimetric techniques have been already developed for their determination. Additionally, it was observed that in a rigid medium, these compounds also exhibit analytically useful phosphorescence emission which could be useful for the development of novel analytical techniques for their determination.

Room temperature phosphorescence (RTP) may offer some important advantages over fluorescence. RTP provides a highly sensitive, low background, selective and time discriminated luminescence emission whose measurement could be used to develop innovative approaches for photoluminescent detection. Moreover, combination of solid surface RTP with flow injection analysis (FIA) strategies can be advantageously exploited, since the packing into the flow cell allows simultaneously to preconcentrate and to detect the analyte.[1]

In the present work, a novel room temperature phosphorescence (RTP) method for the optosensing of aflatoxins has been developed. Optimization of some experimental conditions including the nature and size of the solid supports used for aflatoxin immobilization, nature of the heavy atom donors, the carrier composition and its pH, or the concentration of sodium sulfite used as oxygen scavenger. Finally analytical characterization and potential applicability of the proposed optosensor for aflatoxins rapid analytical control will be discussed.

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STUDY OF NOVEL FLUORESCENT CYANINE-BASED DYES IN PLASTICIZED PVC MEMBRANES TO DEVELOP INTEGRATED DEVICES

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During the last years, the work in our research group has been focused on developing new analytical instrumentation, which combines integration and miniaturization. With this in mind, we have been optimizing optical membranes, which include chromoionophores or fluoroionophores with changing optical properties in the NIR region. The main advantages of working in this spectral region are a lower background absorbance from the solution matrix and the possibility of using available and inexpensive light sources and photodetectors. Besides, as a future perspective, microtechnology and fiber optics are feasible to be used, with the final purpose to develop optical miniaturized sensors.

In order to dispose of chromoionophores or fluoroionophores with adequate optical properties when dissolved in a bulk-optode membrane, a wide variety of cyanine-based dyes has been synthesized in our group. [1,2,3]

Five types of dyes have been previously studied in absorbance mode. Spectroscopic characterization in ethanolic solution and in plasticized PVC membranes has been carried out, including pH calibration curves, pKa determination in both media, reversibility and photostability. Afterwards, they have been applied as the key component of the recognition system in integrated optical sensors (IWAOs).

Some of the dyes show fluorescence characteristics, which can be exploited to obtain fluorescent bulk optodes and then, can be applied in integrated optical sensors based on fluorescence measurements. Therefore, they have been previously evaluated in ethanolic solution to determine their maximum emission wavelengths and to set their pH-indicator properties. Among the fluorescent dyes, those presenting the most intense fluorescence peaks when excited at the laser emission wavelengths (650 and 670 nm) have been immobilized in plasticized PVC membranes for further studies. Their emission wavelength, their calibration curves and their pKa have been determined in a conventional fluorimeter. After that, fluorescence has also been followed in a lab-made device as the intermediate step before the construction of a miniaturized device. It basically consists of a laser as the light source (650, 670 nm) coupled to an optical fiber, a specific optical filter depending on the emission wavelength, lenses to focus the emitted light and a PIN photodiode as the detector.

Results show the suitability of the assayed dyes as fluorescent pH-indicators for the future development of integrated fluorescence devices and, when combined with certain ionophores, their appropriateness to obtain fluorescent ion-selective bulk optodes.

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SURFACE-MODIFIED CdSe NANOCRYSTALS AS LUMINESCENT PROBES FOR ANION SENSING

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Inorganic compounds with nanometer dimensions display properties unlike the bulk material due to electronic confinement effects and are called today nanoparticles. Luminescent semiconductor nanocrystals, such as quantum dots, have generated great interest in fundamental research and optoelectronic or analytical applications in recent years. Compared with organic fluorescent dyes, the quantum dots exhibit some novel advantages, such as large fluorescence quantum yields, narrower and gaussian emission spectra (~30 nm, FHWM), tuneable maximum wavelength of emission by controlling the quantum dot size, shape and preparation procedures and they are less susceptible to photobleaching.[1]

All these features characterising the most popular quantum dots, along with the possibility of modifying their synthesis with various sizes and high luminescence quantum yield, make the applications of quantum dots of increasing importance. Since the first reports using modified water-soluble core-shell quantum dots as fluorescence labels to stain biological samples, many publications have appear reporting applications of such nanostructures as indicators of different biological processes which occur in biosystems, (e.g. detection of specific receptor-ligand interactions in cells as DNA hybridisation, fluoroimmunoassays and recognition of biotoxins, etc.). Although most developments have been performed within the biological science, as a result of the already demonstrated advantages of using quantum dots instead of conventional fluorescent dyes, some attempts have been also done using quantum dots as a new class of luminophores for use in chemical sensing.[2] So far, there are few reports in the literature of chemical sensing of small molecules and ions with quantum dots, via analyte-induced changes in their photoluminescence.[3]

Although optical sensing of anions has been a research topic of permanent interest for many years based on chemical or supramolecular chemical recognition interactions, methodologies for anion sensing using quantum dots or nanocrystals as luminophores have not been reported yet. In this presentation, we will report the synthesis of red CdSe quantum dots, modified with tert-butyl-N-(2-mercaptoethyl)-carbamate (BMC), and their application as selective fluorescent probes of cyanide and iodide anions. The luminescence from the synthesized nanocristals was dramatically increased after photo-activation under sunlight exposure and could be sensitively quenched by cyanide or iodine ions.

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REFINEMENT OF A MATHEMATICAL MODEL FOR FICKIAN DIFFUSION TO ENHANCE POLYMER-MODIFIED SENSOR PERFORMANCE.

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The implementation of mathematical modeling to achieve improved sensor response times and increased sample throughput without compromising performance is described. Using an experimental system based upon polymer-modified internal reflection infrared spectroscopy, the value of multi-disciplinary collaboration within the field of chemical sensor development is demonstrated. Through refinement of an existing Fickian mathematical diffusion simulation,[1] quantitation of analyte species was permitted using reduced data sets. For example, the environmentally significant chlorinated hydrocarbon species, 1,2,4-trichlorobenzene displayed a system equilibration time of greater than 2800 seconds. However, mathematical adaptation of the Fickian diffusion model, permitted consistent, quantifiable results for the compound with sensing times as brief as 800 seconds.

Key words: Multi-disciplinary Approach, Mathematical Simulation, Diffusion Behaviour, Polymer Enrichment Membrane, Sensor Development/Optimisation.

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FLUORESCENCE IMAGING OF PHASE MORPHOLOGY EVOLUTION IN EPOXY/POLYSILOXANE THERMOSETS

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Epoxy thermosets are commonly used in the adhesives, coatings and polymer matrix composites fields. The epoxy/amine hardener pair is usually modified with third components to improve the overall performance for specific applications. It has been recently proposed[1] the use of polysiloxane multifunctional hardeners to increase toughness and thermal resistance and to decrease water up-take of epoxy systems. Siloxane compounds and epoxy resins are mutually insoluble and because of the large differences in surface tension, they phase separate forming large domains. The use of reactive polysiloxanes, where the polysiloxane is chemically bonded to the epoxy component partially solves the problem. The reactive mixture, which is initially heterogeneous, becomes a transparent and partially homogeneous solid as curing proceeds. The final morphology becomes fixed near the gel point of the curing system and the microstructure at the gel point is an unknown function of the composition and curing trajectory.

In this work, the evolution of the morphology at the early stages of the curing process will be followed by fluorescence imaging techniques using labeled constituents. As polyfunctional hardener, a synthetic poly(3-aminopropylmethylsiloxane) (PAMS) will be used. PAMS will be labeled with the dansyl moiety (DNS). Three different epoxy resins with varying degree of thermodynamic compatibility with the hardener will be used: Diglycidylether of bisphenol A (DGEBA), hydrogenated DGEBA (HDGEBA) and 3,4-epoxycyclohexyl-3'4'-epoxycyclohexane carboxilate (ECC). Poly(methylmethacrylate), polybutadiene and polystyrene will be used as third component modifiers.

In DGEBA/PAMS-(DNS) systems, the initial reactive mixture shows dispersed epoxy domains in a polysiloxane matrix, but in 2 minutes at 60ºC the system undergoes phase inversion and an epoxy matrix with dispersed polysiloxane domains can be observed. After one more minute a co-continous morphology develops. These three steps are shown in Figure 1.

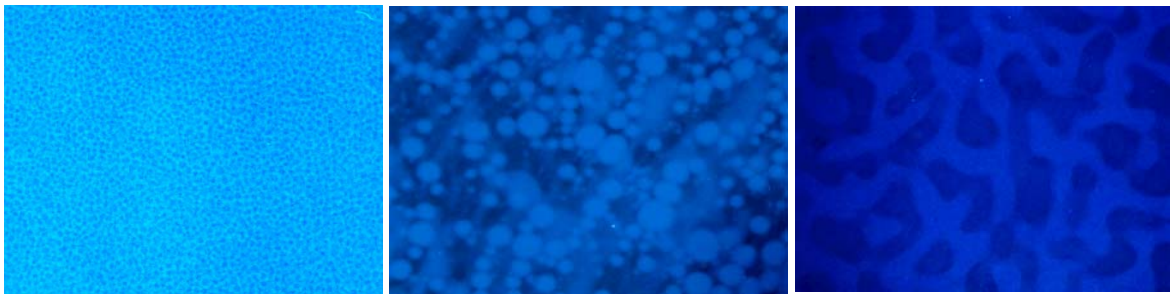

Figure 1. Morphology evolution in DGEBA/PAMS-(DNS) system. Initial morphology (left), after 2 minutes (center) and after 3 minutes (right) at 60ºC.

DGEBA epoxy resin shows high reactivity and initial incompatibility with the polysiloxane hardener, while HDGEBA shows higher initial compatibility. ECC epoxy resin shows a very low reactivity against PAMS[2] but its initial compatibility is high. Results are discussed in terms of the curing trajectory, the presence of a polymeric modifier and the nature of the epoxy component.

The fluorescence imaging technique used in this work allows a better understanding of the complex phenomena appearing during the curing of advanced thermosets.

Acknowledgements: The authors would like to thank the Comisión Nacional de Ciencia y Tecnología for financial support (MAT2000-0391-P4-02 and MAT2002-03210).

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FACTORS AFFECTING THE DIFFUSION OF HALOGENATED COMPOUNDS INTO POLYMERIC MEMBRANES

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The detection of residual solvents is an important area of study due to their impact on the environment and on human health. The main aim of the work is to develop a sensing technique capable of evaluating the diffusion of several aqueous phase trihalomethanes (THMs) using polymer-modified ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infrared) spectroscopy. THM's are formed through reaction between free chlorine and organic matter in water.

The polymer coating serves to concentrate the organic analyte close to the measurement region, which improves the overall sensitivity of the sensor. Factors affecting the diffusion of analytes into polymeric materials are therefore essential for the development of a selective and sensitive sensor. Polymer/ water partition coefficients of the analytes under investigation were estimated using an automated fluid handling system based on Sequential Injection Analysis (SIA) technology. These partition coefficients determine the enrichment of the substances into the polymer layer from the aqueous phase. Analyte solutions were brought into contact with a polymer layer of defined volume, and after equilibration, the concentration in the aqueous phase was determined by purge-and-trap gas chromatography (P&T-GC).

Results will be presented from our polymer-modified ATR-FTIR system, showing the quantification of the rate of diffusion of each analyte based on a binary Fickian diffusion type model. Single and multicomponent diffusion coefficient values have been established to date for multiple THMs using several polymers including polyisobutylene (PIB), ethylene/propylene copolymer (EPco) and Teflon® AF.

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NEAR-INFRARED DYES USED AS SOLVATOCHROMIC POLARITY PROBES

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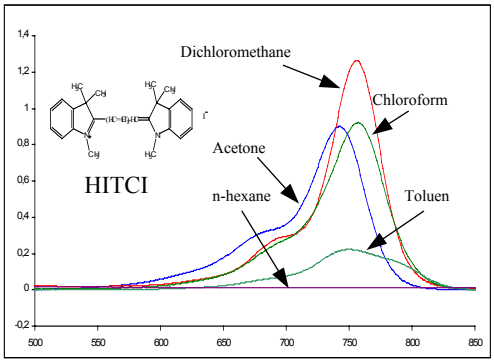
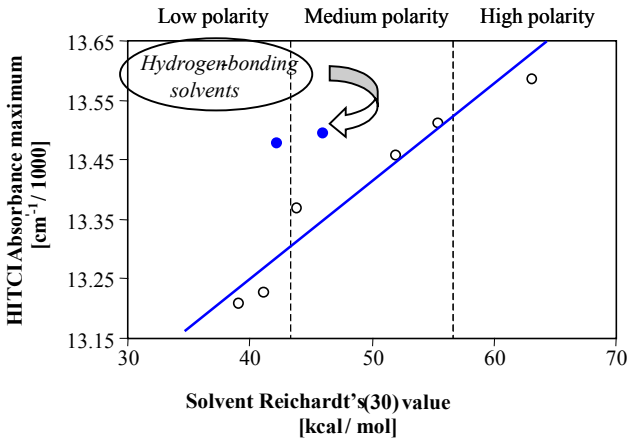
NIR absorbing and fluorescent probes that are highly sensitive to solvent polarity, pH, local viscosity and/or electrostatic fields are of invaluable interest in the study of homogeneous, heterogeneous and biological media. A major bottleneck in the utilization of NIR absorbing and fluorescent dyes for many applications is the limited number of probes with adequate optical properties for this region of the spectrum. In particular, there are relatively few reports about the role of hydrogen bonding with the solvent in the deactivation of fluorescent excited states.

Such probes, whose spectral characteristics are sensitive to hydrogen bonding, are of importance in the design of sensors. In this communication we report the NIR absorbing and/or fluorescent properties of several NIR absorbing dyes in media of different polarity/hydrogen bonding properties. Large shifts in the absorption spectra induced by the solvents indicate that some of the NIR dyes studied can be used to probe microenvironmental polarities and in the design of improved materials that generate intense colour in the presence of solvents.





SICK HOUSE SYNDROME GAS MONITORING SYSTEM BASED ON NOVEL COLORIMETRIC REAGENTS FOR THE HIGHLY SELECTIVE AND SENSITIVE DETECTION OF FORMALDEHYDE, TOLUENE AND XYLENE

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Formaldehyde (HCHO), toluene and xylene emitted from the furniture and walls in rooms are important species in atmospheric and environmental chemistry, and injures the eyes, nose and respiratory organs, and causes allergies. These effects are called the sick house syndrome. We designed and synthesized novel colorimetric HCHO-sensing molecules (**KD-XA01**), which possess an enaminone structure, and developed a hand-held instrument to monitor indoor HCHO gas using **KD-XA01**. This sensing molecule produced a speedy color change from colorless to yellow under mild conditions, which was caused by the fact that the enaminone structure in the reagent reacting with HCHO to give a lutidine derivative. This reaction took place not only in the solution phase but also in the solid phase (on surface of the cellulose paper). To take advantage of this phenomenon, a handy and rapid monitoring system has been developed for detecting indoor HCHO gas using a highly sensitive and selective detection tablet constructed from porous cellulose paper that contains silica gel as an adsorbent, **KD-XA01**, and phosphoric acid under optimum conditions. This instrument detected the surface color change of the tablet from white to yellow, which was monitored as a function of the intensity of the reflected light illuminated by an LED (475 nm). The response was proportional to the HCHO concentration at a constant sampling time and flow rate; 0.05 ppm HCHO, which is under the standard value set by the World Health Organization, was able to be detected in 5 min. The detection limit was 0.005 ppm. This monitoring system was not affected by carbonyl compounds such as acetaldehyde and acetone, alcohols, hydrocarbons and typical gases such as carbon monoxide, carbon dioxide, nitrogen dioxide, etc., which contribute to the measurement of the correct HCHO concentrations. It was possible to monitor the HCHO gas in the room of a new apartment and school using this instrument; the response values were in good agreement with those obtained by the standard DNPH method.

In the case of monitoring xylene and toluene, **KD-TX01** was designed and synthesized, which possessed a formyl group. After the reaction with toluene or xylene, the original colorless solution smoothly changed to a red solution and an absorption band appeared between 440 nm and 550 nm. The absorbance at 475 nm was plotted as a function of the toluene or xylene concentration. As a result, this calibration curve provided a good straight line to obtain a precise toluene or xylene concentration. **KD-TX01** was not affected by HCHO, acetone, benzene and other aromatic compounds.

The highly sensitive detection of HCHO, toluene and xylene using **KD-XA01** and **KD-TX01** is widely applicable as a convenient detection method.

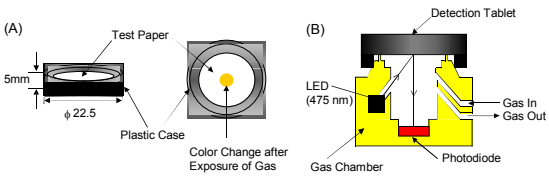

Figure 1. Schematic representation of the detection tablet (A) and the optical location to detect the reflected light from the tablet (B).

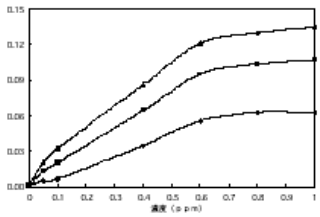

Figure 2. Relationship between response and HCHO concentration at various sampling time.

LABEL FREE SPECTRAL CORRELATION BIOSENSORS

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Label-free optical methods for detecting biological and chemical interactions are widely used to investigate kinetics of reactions, measure affinity constants between biological agents, monitor reactions in real time using fewer operations as compared with conventional assays. We proposed a simple and robust method of direct optical detection of reactions on a surface,[1,2] which used a simplest microscope cover glass as a sensor chip without deposition of any metal or dielectric films.

In this paper, we further develop the spectral correlation method and extend it for a new multi-channel biosensor variant, present results on adaptation of the method for epitope competition analysis of different monoclonal antibodies and its employment as an efficient tool for optimisation of surface interfaces of a number of biosensors, in particular, a magnetometric biosensor based on magnetic beads counting.

The developed Spectral Correlation (SC) biosensors are based on measurements of a correlation signal of two interferometers in an original optical scheme. In the first scanned Fabry-Perot interferometer, its base, i.e. distance between two mirrors, is periodically changed by a peizoelectric driver. The second interferometer is a simple microscope cover glass plate (biochip) with different recognition spots or flow channels. We use interference between two beams: the first beam reflected from the bottom surface of the glass slide and the second one reflected from the upper surface of a biological agent layer deposited onto the glass slide. The interference result depends on the phase thickness of the sensitive layer. During a reaction under study, some components of the solution adhere to the surface of the biomolecular layer whereas the other ones detach from it. This leads to a change of the phase difference between the interfering waves. While scanning the base of Fabry-Perot interferometer, the signal recorded by a photodetector periodically changes. The change of phase of this correlation signal is used for measuring the change of thickness of the biological layer on the glass surface.

This method can be easily extended to detect reactions in either a big array format such as the 1536-well ELISA format or a small micro-array with the reaction spot up to 1 - 10 microns. The major hardware and data processing techniques are the same, and it is only necessary to adjust proper optic elements to obtain array images. A micro-array biosensor has been designed to register chemical and bio-agent binding in real time in 280-micron spots. A technique of immobilization of different antibodies on such spots has been developed. The SC biosensor was also used to control all steps of antibody immobilization on different spots on the glass surface.

The SC imaging setup was developed based on a CCD camera for real-time measurements of change of thickness of biological agents over whole surface of a biochip. Such setup can be named "immunoscope" and can find wide application where resolution in depth of conventional optical microscope is not sufficient and/or recording reactions in real time is required.

The SC biosensors were successfully used to develop and test different interfaces for antibody immobilization in flow mode on glass surfaces. The devices were used to check functionality of antibody on glass depending on different storage conditions of the biochips and quantitative evaluation of biochip degradation process. The method was successfully applied for epitope mapping of different monoclonal antibodies and optimisation of selection of pairs of antibodies ("capture" and "tracer" antibodies) for sandwich immunoassay for a magnetometric biosensor based on magnetic labels.

The developed spectral correlation method provides an information signal that is independent of the refractive index of the solution, has high sensitivity at pm range of biological layer thickness change (or ≈ 1 pg/mm^2 protein binding detection), wide dynamic range and is compatible with multi-channel biosensing systems. The method can be applied in medicine and ecology, e.g. for high-throughput screening, testing samples for presence of toxic or infection agents, detection of the DNA hybridisation, testing of pharmaceuticals, etc.

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IMMOBILIZED MICROALGAE ACCOPLED TO FIBRE OPTICS: A FIRST APPROACH FOR TOXICITY ASSESMENT

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Immobilized microalgae have been accopled to an oxygen sensible membrane composed by a luminiscent indicator for fibre optics ($[Ru(dip)_3]Cl_2$). Different eucariotic and procariotic microalgae stocks were used at their full exponential phase growth *(Synechococcus* spp*., Nannochloropsis gaditana, Isochrysiss galbana, Chaetoceros* sp*., Tetraselmis chui., Rhodomonas salina, Phaeodactylum tricornotum).* The emission measurements of sensitive detection to the phase have been made using a forked bundle of optical fibres (Dolan Jenner E-472) adapted to prototype with a 420nm LED as radiation source, an interference filter of 420nm for the excitation channel and a cutting filter of 570nm for the emission channel.

The microalgae were immobilized by filtering the stock on a glass fibre support (Whatman GF/F) and then added to the oxygen sensible membrane (Ru(II) compound). The results obtained using different microalgae show that an increase in the phase is always followed by a lineal decrease of the oxygen concentration. It was associated with a respiration effect based on a difference in time response. Likewise, these results were compared to others obtained using a known concentration of toxic compound. Comparing results show a slope change related to microalgae sensibility, to the toxic compound, and to its concentration.

The best response was reported by cyanobacteria *Synechococcus* sp. (CC strain). Regarding to the eucariotic group *Tetraselmis chui, Phaedactylum tricornotum* and *Chaetoceros* sp., showed similar responses. These preliminary tests inform about a wide range of possibilities to determinate toxicity using this device. With a necessary optimization of the measurement system these analysis could be extended to photosynthetic activity such the described respiration effect.

TWO NOVEL INTEGRATED OPTICAL SENSOR TYPES BASE ON CHEMICAL INDUCED CHANGES OF MODAL FIELD

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Chemical IO sensors make use of materials of which the optical properties depend on the concentration of a specific measurand. The IO read out system can then detect a change in refractive index, absorption or luminescence of this material.[1] Most of the IO-sensors which detect a change in refractive index use the dependency of the effective index on the index of the sensing material and this effective index change is generally measured using interference effects or mode coupling. The novel sensor types introduced here are also refractive index type sensors, but unlike other sensors, they use the shape of the mode profile to measure the refractive index of the sensing material. It is therefore not necessary, like in other refractive type sensors (based on interference) to use a narrow band light source.

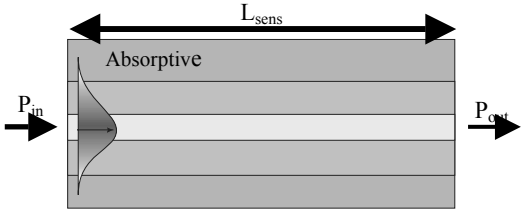

Figure 1: Top view RIMA sensor

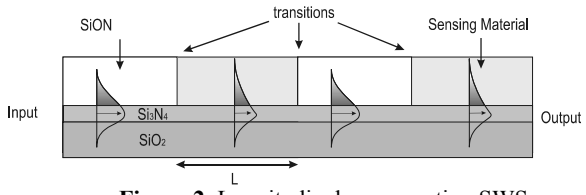

Figure 2: Longitudinal cross-section SWS

The first novel sensor type measures the refractive index by modal attenuation (RIMA sensor). The structure consists of a monomodal channel waveguide, in which the chemo-optical transduction layer is applied as a cladding material. At both sides of the channel, starting at a distance d from its sides the core layer is made absorptive (see Figure 1). A change of the refractive index of the transduction layer will result into a change of the field profile and thus into a change of the fraction of the modal power that propagates through the absorptive regions. As a consequence the modal propagation losses will become a measure of the refractive index change. Thus in this structure refractive changes are measured directly as changes of the modal attenuation. This principle has been worked out for the case of immuno sensing. Calculations show that for the change in effective immuno layer thickness a resolution of 10^{-4} nm should be feasible.

As for the second novel senor type: in Figure 2 a longitudinal cross-section of the sensing part is depicted: a ridge type wave-guiding channel consisting of two types of segments: segments with SiON as cladding and segments with a chemo-optical transduction layer as cladding. A guided mode is launched into the first segment of the waveguide. Due to the difference in field profiles of both types of segments not all guided mode power launched into this segment will be transferred to the guided modes of the adjacent segment. As a consequence at the transition radiation is generated. The amount of lost guided mode power of course depends on the difference in refractive index Δn between the cladding and the sensing material and hence is a measure for the concentration of the measurand. At one transition the chemically induced loss of guided mode power is very small; however, with a few thousand transitions a quite sensitive sensor can be obtained. Calculations showed that a resolution of 5.10^{-7} should be feasible.

Both principles have been thoroughly theoretically evaluated. For the resolutions analytical expressions have been derived. The expressions consist of the product of two quality factors, one related to the properties of the peripheral equipment (light source and opto-electronic detection system) and one related to the sensing structure. Both factors clearly show the criteria for optimizing the resolution.

A first version of the SWS has been realized using SiON technology and has been characterized. A good correspondence between theory and experiments has been obtained. Fabrication of a second segmented waveguide sensor is in progress. This SWS is optimized for high resolution and will feature a micro fluidics system, an optical reference channel and will operate with a more advanced optical detection scheme. Results will be presented at the conference

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TEMPERATURE AND INFLUENCE OF SALINITY ON THE RESPONSE OF AN OXYGEN SENSOR ($[Ru(dip)_3]Cl_2$) FOR ITS OPTICAL APPLICATION.

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The responses of optical oxygen sensors have been studied for estuarine and sea water at different temperature and salinity values from 5 to 35ºC and from 0 to 40. Oxygen concentrations are obtained by a sensible oxygen membrane composed by a luminiscent indicator for fibre optics ($[Ru(dip)_3]Cl_2$). The emission measurements of sensitive detection to the phase have been made using a forked bundle of optical fibres (Dolan Jenner E-472) adapted to a prototype with a 420 nm LED as radiation source, an interference filter of 420nm for the excitation channel, and a cutting filter of 570nm for the emission channel.

The oxygen concentrations resulting from the fibre optics are compared to those from samples measured using the Winkler`s method (with a precision of ± 0.1 μM) and concentrations registered by two conventional polarographic sensors (WTW, CellOx 325, with a precision of ± 0.5 μM). The ensemble data record is the result of two different experiments. The first studies were conducted using a thermostatized reactor (± 0.01ºC) at fixed and known salinity. At each temperature and salinity, the oxygen concentration is ranged from very low values to saturation (with nitrogen or oxygen injection). A different set of results were obtained during scientific cruises aboard of Research Oceanographic vessels, where the different oxygen sensors were set up underway and in combination with the thermosalinometer (S, T, ...) located in the vessels.

The set of oxygen concentrations obtained at different temperature and salinity values from the optical sensor were compared to those resulting from the classical methods (Winkler and polarographic sensors). The average resulting deviation from this comparison is about 0.5%. The highest deviation value was about 1% and associated with high salinity and low temperature values. The set of data allows a temperature and salinity correction of these oxygen concentrations, allowing the use of these optical oxygen sensors with acceptable precision to determinate oxygen concentrations in natural estuaries, littoral and oceanographic waters.

DETECTION OF MOLECULAR RECOGNITION BY FLUORESCENCE ON SELF-ASSEMBLED MONOLAYERS ON GLASS

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The importance of the chemical sensing of ions and small organic molecules is illustrated across a broad range of areas encompassing medical diagnostics, environmental monitoring, and the food industry. Traditional design of molecular receptors as chemical sensors for small molecules and ions especially, was based on the "lock and key" principle of one receptor, one analyte.[1] Through this traditional approach, binding selectivity has been improved by focusing on geometric and functional group complementarity of the fluorophore-appended host to match that of the guest. The remarkable selectivity imparted by this technique cost significant time and effort toward the synthesis of receptors with appended transducers prior to analysis of their binding capabilities.

A possible solution is to integrate surface-confined sensing technology into the search for chemical sensing systems. One approach which has been successfully applied is the use of combinatorial chemistry to generate microarrays through such means as in situ synthesis, chemical ligation, and non-covalent bonding has resulted in a broad range of protein and small molecule microarrays on surfaces such as optical fiber tips, microbeads and microwells.[2] The majority of systems developed thus far have focused on biological applications, while the few chemical sensing systems have suffered from tedious synthesis and complex, time consuming hit deconvolution procedures.

Our lab has successfully developed a simple strategy for the generation of chemical sensing libraries for cations by combining the independent deposition of commercially available fluorophores and simple small molecules onto a self assembled monolayer on glass.[3] This allows for the in situ development of a complete sensing surface without the need for a complex interface or hit deconvolution. We found that small changes in the surface functionalities imparted a large change in the sensing properties of the surface, and that such detection was transferable to the microscale via microcontact printing and microchannels. Additionally, this strategy was equally extendable to the detection of inorganic ions, which are traditionally more difficult to sense than cations.

We now hope to extend this methodology to the more weak interactions associated with hydrogen bonding systems. This could allow us to apply the technique to such systems as base pair and carbohydrate-protein interactions, which would thus broaden the scope of the applicability of the methodology.

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A PROTOTYPE REAGENTLESS REGENERABLE BIOSENSING SYSTEM

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Biosensors capable of specifically detecting chemicals, toxins, and bio-agents in their environment will help alleviate the current worldwide security and food/water quality assurance demands which remain largely unmet, as well as impacting healthcare and environmental monitoring concerns. We present an approach for self-assembling a modular biosensor on a surface that combines the desirable properties of being reagentless as well as regenerable after a sensing event. This biosensor consists of two co-functional or interacting entities; 1, a surface-immobilized antibody/enzyme/receptor or other molecular biorecognition element and 2, a surface-tethered modular multifunctional molecular assembly or arm that contains a point of surface attachment, a hybridizable flexible linker, and a signaling dye label which is proximal to an analog of the target analyte that interacts with the biorecognition element. As a prototype sensor we utilize the *E. coli* maltose binding protein (MBP), a well-characterized member of the bacterial periplasmic binding protein superfamily as the biorecognition entity and target its preferred substrate maltose. The MBP is site specifically dye-labeled prior to use. The multifunctional tethered modular arm designed to function in concert with this MBP biorecognition element contains a point of attachment (biotin), a hybridizable flexible linker (DNA), and a dye label which is proximal to a recognition element, beta-cyclodextrin (β-CD) which in this case interacts specifically with the MBP. These 2 entities are self assembled on the neutravidin surface of a microtiter well and when MBP binds the β-CD of the linker arm their close proximity results in fluorescence resonance energy transfer (FRET) between the dyes. Addition of the target analyte, maltose, displace the β-CD altering FRET in a concentration dependant manner. More importantly, this sensor can be washed free of analyte and regenerated for another sensing event. Several different sensor variants with different binding characteristics are assembled and tested. This prototype demonstrates that this sensor design meets almost all the criteria of an ideal biosensor. An ideal biosensor would be easily assembled, incorporate biological specificity with integrated target binding induced signal transduction, function in a reagentless mode, be regenerable, allow for variants with multiple binding affinities for sensing over many orders of analyte concentration and yet be designed in such a way that the same sensing technology could have general applicability to a variety of targets and eventually be incorporated into a self contained device.[1-3] The applicability, modularity and areas that such a sensing system can impact are discussed.

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CONFINED FLUORESCENT SENSITIVE SURFACES ON GLASS, MADE BY MICROCONTACT PRINTING AND INTEGRATED INTO MICROCHANNEL WALLS

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It has already been shown in our group that a funtionalized Self-Assembled monolayer on glass can act as a fluorescent sensor entity for both either anions and cations.[1] The simple funtionalization of a glass surface with an amino terminated silane creates an reactive surface ready to be funtionalized. The glass surface then comes to be a big unique scaffold for the pieces of a fluorescent sensor, coordinating functionalities and fluorescent molecules. When the analyte interacts effectively with the surface, the fluorescence of the system can be altered. The interaction is then translated into a measurable optical signal by the fluorophore. The fluorophore acts as a read-out system, so no additional labels are required for the transduction mechanism. The funtionalised glass surface itself comprises the whole sensor system, and due to its covalent nature, cleaning and regeneration are possible. Such an effective system is the result of mixing of simple building blocks, which makes the new methodology suitable for the integration in a combinatorial approach where the building blocks are small molecules which supply functionalities to the surface. The maximum profit of the combinatorial approach is reached when the methodology allows high through put techniques for fabrication (array formation) and screening of the properties of the array. We present here for the first time, the production of patterned fluorescence metal ion sensitive monolayers on glass made by micro contact printing (μCP), and the fabrication of the first ion sensing monolayer integrated on the walls of a microchannel. The layers presented are sensitive for 10^{-4} M concentrations of the analyte. Lower detection limits up to 10^{-6} M concentrations have been observed but not yet studied systematically. μCP is a soft lithography technique that allows us to deliver the building blocks (molecules) of the sensing system into a few square microns area. Additionally the incorporation of the sensing system in the microchanel walls directs the production of those sensing surfaces in the microscale. Both techniques might allow in the future the fast systematic and effective fabrication of chemo sensing monolayer arrays.

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REVERSIBLE HYDROCARBON MONITORING WITH LUMINESCENT Ru(II) INDICATORS AND A FIBEROPTIC PHASE-SENSITIVE FLUOROMETER

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Hydrocarbons form a class of pollutants for which numerous sensing schemes have been developed. Analytical methods based on extraction with an organic solvent and IR absorption are the most widespread. Direct determination using evanescent wave NIR spectroscopy or acoustic wave measurements are alternative methods.[1] Detection limits and interfering substances are critical features. Contamination of the soil, groundwater and water due to leaking fuel from storage tanks, vessels and pipelines is a serious environmental problem. Therefore, in order to warn of such discharges, a rapid system for detection of hydrocarbons in water is desirable. In this work, a new luminescent indicator-polymer conjugate which is able to *reversibly* detect and quantify both aromatic and aliphatic hydrocarbons in aqueous samples, is presented. The detection limit and analytical performance when the sensing head is interrogated by a commercial fiberoptic phase-sensitive fluorometer demonstrate that the tailored OPTOSEN® system is able to monitor these contaminants under real conditions.[2] The luminescent indicators used to fabricate the hydrocarbon-sensitive layers are Ru(II) complexes, molecularly engineered dyes currently used for a variety of optical sensors.[3] Among several complexes tested, $[Ru(nbpy)_3]^{2+}$ was finally selected.[4] Its photophysical properties (I_{em} and τ) in different solvents have demonstrated its suitability as indicator dye. The sensor composition was optimized, evaluating its response when the complex was immobilized in silica, cellulose, cellulose-silicone composites, C_{18}-silica, Nafion® and PTFE. Among the various combinations studied, the $Ru(nbpy)_3^{2+}$/PTFE pair was chosen for its analytical validation in the laboratory using standard hydrocarbon solutions.

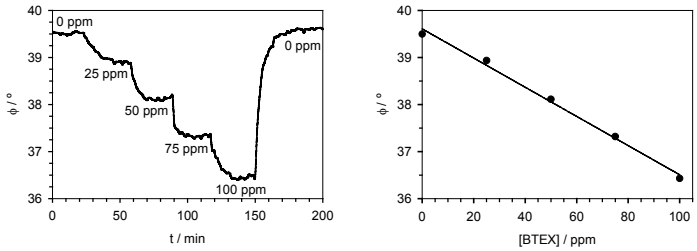

Figure 1. Response to BTEX and calibration curve of the $Ru(nbpy)_3^{2+}$/PTFE sensor, displayed as luminescence phase shift as a function of the hydrocarbon concentration in water (0–100 ppm range).

The influence of the indicator concentration, sample temperature and oxygen level on the sensing layer response to BTEX (benzene, ethylbenzene, toluene and xylenes) and aliphatic hydrocarbons has been investigated. A representative trace of the optical sensor developed related to the standard solution concentrations and the calibration curve obtained thereof, is depicted in Figure 1. A BTEX detection limit of 5 ppm and a dynamic range of 5–1000 ppm have been routinely obtained. The sensor is even more sensitive to petrol in water. Current validation in the field is under way using the OPTOSEN® system.

Acknowledgements. In addition to Interlab, S.A. funding, support from the Spanish Ministry of Science and Technology PROFIT Programme (grant no. FIT-050000-2001-32) has been received.

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[2] The OPTOSEN® system is a multichannel fiberoptic monitor specifically developed, manufactured and marketed by INTERLAB IEC (Madrid, Spain; www.interlab.es) for environmental monitoring of chemical parameters and process control. LAP–UCM has developed the sensitive tips for this system under contract with Interlab.
3 G. Orellana and D. García-Fresnadillo, in: *Optical Sensors: Industrial, Environmental and Diagnostic Applications*, Springer, Berlin-Heidelberg, Germany, 2003; pp 309-358 ($C^{h.\ 13}$).
[4] *n-bpy* stands for 4,7-dinonyl-2,2'-bipyridine.

EFFECTS OF SOL-GEL MODIFICATION OF MICROSTRUCTURE FIBERS ON THEIR SENSITIVITY TO GASEOUS TOLUENE

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Microstructure fibers (MSFs) represent a novel type of silica optical fibers in which the cladding region consists of a grid of air holes in silica runnig along the length of the fiber. The core is created of pure silica or doped silica and it may also contain an extra central air hole.[1] Microstructure fibers offer new possibilities for the study of non-linear optical effects[1] as well as for the development of fiber-optic chemical sensors.[2] In the latter case, detected chemicals filled in the air holes modify optical properties of the cladding region, which leads to changes of the propagation of light waves in the fiber and consequently to changes of the output power. This paper presents results of the investigation of sensitivity of MSFs of the "grapefruit" type to gaseous mixtures of toluene. The sensitivity is evaluated on the basis of spectral or refractive-index changes of the fiber cladding induced by toluene. Effects of the modification of the fiber sensitivity through the application of thin hydrophobic xerogel layers onto the walls of the air holes are shown in the paper.

An example of cross-section of MSFs used in the experiments and obtained by transmission optical microscopy is shown in Figure 1. The fibers with a diameter ranging from 250 to 400 μm coated with a jacket of UV-curable acrylate were prepared in the Institute. For sensitivity tests fiber segments with a minimum length of about 5 cm were used. The sensitivity of some segments was modified by the application of a thin xerogel layer onto the hole walls. Alkoxide sols based on methyltriethoxysilane were used for the layer application. A column of a sol passing through the air holes with a velocity controlled by a vacuum pump was used for this purpose.[3]

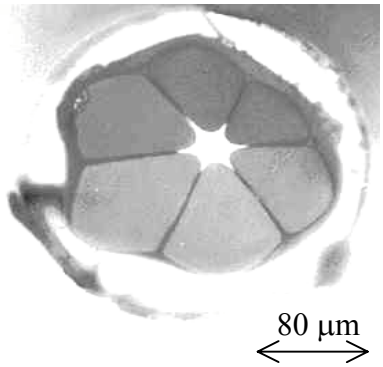

Figure 1. Cross-section of a MSF

In the sensitivity determination, mixtures of toluene in nitrogen were passed through the air holes by using a special glass chamber and angular, temporal or spectral changes of the output power were measured. The fiber was excited by an inclined collimated beam. Refractive-index changes of the cladding induced by toluene were evaluated on the basis of temporal and angular power changes measured at 670 nm. Spectral changes due to C-H overtones as well as refractive-index changes of the cladding were evaluated from the spectra measured in a range of 1600-1800 nm.

The results of experiments show that the unmodified fibers as well as the fibers modified by the xerogel layers are sensitive to gaseous toluene in the cladding holes. In experiments performed at 670 nm, an increase of the output power was observed with the unmodified MSFs, while an output power decrease occurred with the modified fibers. On the basis of the toluene spectra measured around 1700 nm it is shown that the observed changes of the output power can be explained by the presence of toluene in the holes. Experimental results showing the effect of the gas flow velocity, pressure in the chamber, fiber diameter and sensing length on the sensitivity and response of the microstructure fibers to toluene are also discussed in the paper.

Acknowledgements. This research was supported by the Grant Agency of the Czech Republic (contract No. 102/02/0779).

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AMMONIUM SENSING WITH LUMINESCENT Ru(II) INDICATORS AND A FIBEROPTIC PHASE-SENSITIVE FLUOROMETER

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Ammonium sensors are currently being installed in many waste water treatment plants to monitor the influent/effluent streams and the biological processes in aeration tanks (nitrification). In this way, compliance with legal requirements is achieved and the oxygen required to remove nitrogen and phosphorus by the active sludge treatment is carefully controlled, so that energy is saved. Automatic NH_4^+ monitors based on spectrophotometric detection (at 660 nm) of a green indophenol complex are commercially available; water samples feeding the measuring system are continuously ultrafiltrated. Electrochemical sensors for reagent-free ammonium determinations using NH_3-permeable membranes or NH_4^+-selective PVC membranes are also marketed for on-line analysis of waters. However, long-term stability, selectivity and precision are an issue in real sample monitoring. On-line ammonia analyzers based on FT have recently been introduced too.

Fiber-optic ammonium sensors have also been developed. Many of them use colorimetric or fluorometric pH-sensitive indicator dyes, either dissolved or immobilized onto a hydrogel and separated from the aqueous sample by a gas-permeable barrier (e.g. PTFE). Fluorescence detection is intrinsically more sensitive and selective than absorption or reflectance. If *lifetime*-based emission sensors, rather than *intensity*-based devices, are fabricated then the advantages of emission techniques become enhanced since lamp and detector fluctuation/drift or indicator leaching/bleaching are avoided. However, instrumentation for nanosecond emission lifetime determination is expensive and fluorescent indicators do not display often a change in their excited state decay profile with pH. This is a consequence of their emission arising exclusively from either the basic or acidic form of the excited state, being the pH-dependent fluorescence a result of their *ground* state acid/base equilibrium (e.g. HPTS-based pH fluorosensors). Luminescent Ru(II) polypyridyls have carried fiberoptic oxygen sensing on to commercial applications.[1] Their strong absorption of blue light, far-shifted red emission, excellent photochemical and thermal stability and almost diffusion-controlled O_2 quenching, together with μs excited state lifetimes, have made them the indicators of choice for O_2 monitoring. Therefore, it is desirable to have NH_4^+ sensors based on Ru(II) dyes in order to benefit from the advantages and instrumentation already developed for optical O_2 monitors. This paper describes the development of such sensors using pH-sensitive Ru(II) indicator complexes and the OPTOSEN® system, a multichannel fiberoptic luminometer specifically developed, fabricated and marketed by INTERLAB IEC (Madrid, Spain; www.interlab.es) for environmental monitoring of chemical parameters and process control.

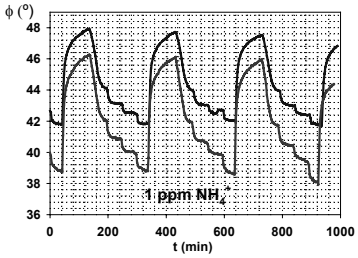

Figure 1. Two-channel response to 0–1 mg L^{-1} NH_4^+ (in 250 ppb steps) of the $[Ru(phen)_2(F_{15}phen)]^{2+}$/hydrogel/$NH_4Cl$ (10 mM)/PTFE sensor, displayed as luminescence phase shift as a function of the analyte concentration in water after addition of a small amount of 0.05 M NaOH to the sample. (F_{15}phen stands for 5-perfluorooctadecanamide-1,10-phenanthroline; different indicator concentrations in each channel)

The effect of (i) the indicator nature and concentration; (ii) the nature, concentration and pH of the internal electrolyte; (iii) the indicator support; (iv) the ammonia-permeable membrane, and (v) the sample temperature and ionic strength on the sensor response to NH_4^+ has been investigated (e.g. Figure 1). A detection limit of 10 ppb NH_4^+ and a phase shift up to 8.2º for a 0–1 ppm analyte concentration change have been obtained. Field validation is currently under way using the OPTOSEN® system.

Acknowledgements. In addition to Interlab, S.A. funding to UCM under contract, support from the Spanish Ministry of Science and Technology PROFIT Programme (grant no. FIT-050000-2001-32) has been received.

1 G. Orellana and D. García-Fresnadillo, in: *Optical Sensors: Industrial, Environmental and Diagnostic Applications*, Springer, Berlin-Heidelberg, Germany, 2003; pp 309–358 (Ch. 13).

THE SENSOR ARRAY CHIP OF HB BASED ON CHEMILUMINESCENCE IMAGE

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The determination of hemoglobin in human blood is an important biological index to diagnose many kinds of diseases .The International Committee for Standardization in Hematology (ICSH) recommends the cyanogens high iron Hemoglobin (HiCN) as a Standard. But it is not available to determine the low-grade hemolysis

In this paper, a sensor array chip for determination of hemoglobin in human blood has been developed based on chemiluminescence reaction of luminol and hydrogen peroxide catalyzed by hemoglobin and enhanced by p-iodophenal. The p-iodophenal can also slow down the rate of the chemiluminescence reaction into a slow one. The luminol and p-iodophenal encapsulated sol-gel were spotted at an array of 25 spots at the surface of the PMMA substrate. 1μL of hydrogen peroxide and 1μL of blood sample is added to each of the 25 spots .The CL intensity of all spots is measured simultaneously with CCD camera with 4 min. exposure. The image captured is shown in Figure 1.

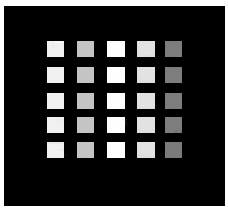

Figure 1. The chemiluminescence image on the Hb sensor array chip with difference amount Hb.

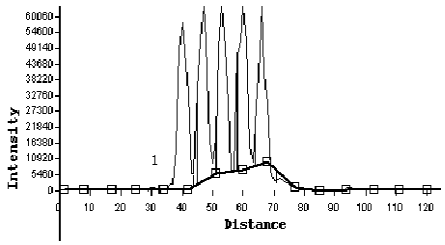

Figure 2. Repeatability of one of the vertical five points.

The present method has been successfully used for the determination of hemoglobin in whole blood and the evaluation of hemolytic anemia .For the former work, after the destruction or dissolution of red blood cells; the hemoglobin released has been determined. For the latter one, the serum is firstly separated from the hemolytic sufferer's whole blood, and then the hemoglobin in the serum can be determined with the present method. The quantity of hemoglobin in serum can be used as the index to the evaluation of hemolytic anemia. The results of hemoglobin in whole blood are compared with those obtained from the standard method (HiCN). The method has potential advantages of simplicity and high sensitivity with detection limit of 1.5 ng.The relative standard deviation is less than 5% showed by Figure 2.

INVESTIGATION OF THE FLUORESCENCE OF ISO-ALPHA ACIDS IN COMBINATION WITH LANTHANOIDES: TOWARDS A BITTERNESS SENSOR FOR BEER OR WORT

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Iso-alpha acids (IAA) are the main substances creating the bitter taste of beer. They are formed during the wort boiling from the alpha acids of the added hop or hop-extracts. The concentration of IAA in the beer determines its bitter intensity and is one of its main quality parameters being directly judged by the consumer. Hence, the determination of the bitterness of beer and wort, resp., during the production process is an important part of the quality management in brewing. The measurement of bitterness or IAA, resp., is usually done by (i) a liquid-liquid extraction using iso-octane with subsequent measurement of UV absorbance, or (ii) various, not standardized HPLC-methods.

Both methods are easily performed in the lab but cause considerable problems in a near-process or on-line application. A bitterness sensor comprising e.g. a short measurement time, little manual handling, minimised use of glassware and both low purchase and current costs could improve the quality management and the economic result of brewing.

Generally, optical measuring methods could meet these demands. However, their use is not straightforward, especially due to the low IAA concentrations in a complex sample matrix. However, combining IAA and europium ions leads to the formation of a complex showing a strong fluorescence. This effect was described by Tomlinson et al.[1] in 1995 who published some preliminary results of their measurements with IAA-solutions and their trials to apply the method to beer samples. Since then no further detailed investigations of this interesting phenomenon have been published.

In our poster we will report the results of our extended research about fluorescent IAA-complexes aiming to the development of a bitterness sensor for IAA in the brewing process. We found that only a few lanthanoids (i.e. Eu, Tb, Dy, Sm) are able to form strongly fluorescent complexes with IAA. Detailed investigations about the influence of pH and lanthanoid concentrations on the fluorescence intensity allowed the determination of optimal conditions for the fluorescence measurements in IAA solutions. The cross sensitivities for the main compounds present in beer or wort, resp., were checked, showing low values for the tested compounds (for example ethanol, sugars, proteins, amino acids, carboxyl acids) in the concentration ranges usually found in beer.

The problems of transfering the method from pure IAA solutions to beer samples will be discussed from a new point of view by taking into account the high UV absorbance of beer. Its influence can obviously not avoided even when measuring fluorescence with a front surface device.

Having in mind that sometimes only a qualitative proof of hopping (and hence of bittering) of the beer wort is needed we will finally present a new simple method that allows a very fast, easy, cheap and reliable qualitative proof of the presence of IAA in beer or wort (patent pending).

Acknowledgment: This work was financially supported by the Kultusministerium des Landes Sachsen-Anhalt (FKZ 0040KB/0502T) and the Wissenschaftsförderung der Deutschen Brauwirtschaft e.V. (R386)

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THE PERFORMANCE OF A MICROCHIP-BASED FIBER OPTIC DETECTION FOR THE DETERMINATION OF VARIOUS IONS

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Absorbance-based measurements have been a challenge in CE-based separations and micrchips due to the micro-scalar dimensions of the capillaries and the short path lengths involved.[1] This study describes the design and production of a microchip, which contains a borofloat glass bottom layer and poly-dimethylsiloxane (PDMS) top layer with a sapphire ball lens and, the use of this microchip as a sensor for the determination of Ca^{2+} ions in aqueous solutions using optical fibers

Arsenazo III (1,8-dihydroxynaphtlane-3,6-disulfonic acid-2,7-bis [(azo-2)- phenylarsonic acid]) has been used as a calcium-binding reagent because of its high affinity for the free calcium ions in the sample.

The maximum absorbance of Arsenazo III - Ca^{2+} complex was achieved at pH 9. A calibration curve was plotted for Ca^{2+} ions within the range of 1.25 x 10^{-4}-5.0 x 10^{-2} M Ca^{2+}. The absorbance of complex was measured at 668.13 nm at pH 9. The dynamic linear range was obtained in the range of 1.25 x 10^{-4} and 2.5 x 10^{-3} M for the Ca^{2+} ions. Relative Standard Deviation (RSD) of the method was found to be 5.12 % for the Arsenazo III-Ca^{2+} complex.. Detection limit of the method was calculated as 8.50 x 10^{-5} M. The effect of various ions such as Mg^{2+}, Ba^{2+}, Sr^{2+}, Na^{+}, and K^{+} on the formation of the Arsenazo III-Ca^{2+} complex has also been investigated.

When the ratio of Mg^{2+} / Ca^{2+} is equal to 5 and higher, the absorbance value increases, because Mg^{2+} has a positive interference at this level.

The calibration curve and linear range for Arsenazo III-Mg^{2+} complex was also determined. The linear range was obtained in the range of 2.5 x 10^{-4} and 1.25 x 10^{-3} M for the Mg^{2+} ions.

This detection technique has been applied to Ca^{2+} determination in urine samples.

Acknowledgements: The authors are grateful to LUNA Innovations and TUBITAK (Scientific and Technical Research Council of Turkey) for providing grants to this project.

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SELECTIVITY OF THE *PSEUDOMONAS FLUORESCENS* HK44 BIOSENSOR

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Pseudomonas fluorescens HK44 (HK44 further) is a genetically modified microorganism harbouring the pUTK21 plasmid, derived from the NAH7 plasmid[1] which codes genes for the naphthalene degradation pathway divided into two operons. Both operons are positively inducible by salicylate, therefore little of their activity is present constitutively and a huge increase in activity is observed after induction. The *Vibrio fischeri luxCDABE* gene cassette coding bioluminescence[1,2] was inserted into the *nahG* gene of the salicylate operon, thus becoming inducible by salicylate or naphthalene. Bioluminescence is emitted at the range 420-600 nm and is oxygen-dependent.[3] (not strongly oxygen dependent; see reference below).

The sensors for multiple determinations of salicylate and naphthalene were fabricated by entrapment of HK44 into 1 mm thick layers from prepolymerised tetramethoxysilane. The silica layers containing 10^7 cells/gram were stable for at least 8 months and 50 induction cycles.[2]

The aim of this work was to test 25 possible inducers of bioluminescense (3 structural analogues of naphthalene, 21 analogues of salicylate and 1 intermediate of naphthalene degradation pathway) in order to answer these questions: 1. Does the compound induce bioluminescence? 2. What is the minimal inducing concentration? 3. What is the toxicity of the compound for HK44? 4. What is the relationship between the compound structure and induction of bioluminescence?

Layers were immersed in medium containing possible inducer for 3 hours and the intensity of bioluminescence was measured. Tested concentrations were 0.5 mg/l – 5 mg/l – 50 mg/l and 500 mg/l. The compound toxicity was evaluated by comparing bioluminescence induced by salicylate (0.05 g/l) before and after the experiment.

Results showed that HK44 based biosensor was very selective. Only a few compounds were found to induce bioluminescence, however compared to salicylate several orders higher concentrations were required. This is probably the result of lower affinity of these compounds to proteins involved in the induction process. Low induction activities of the compounds directly oxidizable to salicylate (e.g. salicylaldehyde) were also observed. We suppose that abiotic oxidation to salicylate occurs during the experiment. The harmful effect was observed at concentrations of 50 mg/l and 500 mg/l of minority of tested compounds. The intensity of bioluminescence, which is a complex process involving many factors (transport mechanisms, diffusion through membrane, adsorption on the matrix etc.) was correlated to hydrophobicity, polarity, molecular size and position of functional groups in order to find any relationship to the structure.

Acknowledgements. This work was supported by the Czech Grant Agency grant no. 104/01/0461 and Ministry of Education, Youth and Sports of CR grant no. OC 840.10.

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VARIATION OF THE LIGHT TRANSMITTANCE OF POROUS GLASSES AFTER SOLVENT EXPOSURE IN DISPOSABLE CHARCOAL CARTRIDGES

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We studied the change of the optical properties of porous glasses in the presence of solvent vapours. The studied porous glasses were primarily (multimode) air clad optical fibres with a numerical aperture approaching one. The most obvious observed change is a drop of the light transmission when solvent vapours are adsorbed in the pores of the porous glass. At high solvent vapour concentrations transmittance drops to zero for an optical path length of about 8 centimetres in the porous optical fibre. Measurements of the light intensity that is transmitted by a porous optical fibre thus allows of its use as a solvent vapour sensor. Using the equilibrium transmittance after exposure we evaluated the sensitivity of porous glass sensors to be a few thousands of ppm. This sensitivity as well depends on the physical characteristics of porous fibres such as their length or their radius of curvature, but also on the physicochemical characteristics of the detected solvents, mainly their vapour pressure and their index of refraction. The drop of transmittance is at least partly ascribable to a variation of the light scattering properties of the porous glass due to its heterogeneous nature. As an example, light scattering is easily observed on a porous glass rod when white light is injected at one of its end: the rod looks bluish when observed by the sides and it looks yellow-red when observed by the end. This optical phenomenon is the same one that explains why sky is blue and sunsets are red.

Studies of the sensor response were carried out with porous optical fibres inside and outside activated carbon cartridges. At equilibrium, sensitivity is the same for both mediums but the temporal response curves are very different. When surrounded by air only, the transmittance decreases abruptly immediately after the fibre is exposed to solvent vapours (transient change) and transmittance reaches the equilibrium state thereafter. The transient change is explained by a fast impregnation at the circumference of the fibre that causes a loss of guidance and possibly creates clusters of solvent saturated pores that scatters light highly. This transient change is very sensitive to solvent vapours but it only lasts few seconds.

With activated charcoal as the surrounding medium, the transmittance first increases and then decreases to the same equilibrium value. In the case of fibres surrounded by activated carbon, the signal enhancement cannot be caused by the solvent, which is adsorbed by the activated carbon at the cartridge entrance. It is caused by the drying of the porous fibre because activated carbon also adsorbs the humidity of the air. Thereafter the signal remains about constant until, the activated carbon in front of the fibre becoming solvent saturated, the vapour finally reaches the optical fibre and its signal decreases slowly to the equilibrium value.

Consequently the presence of charcoal around the optical fibre causes the fast, highly sensitive, transitory transmittance change to disappear. This happens because the vapour adsorption in the porous fibre is much slower when the fibre is surrounded by charcoal. This causes the porous glass to remain homogeneous during the adsorption regime; therefore reducing clusters light scattering and guidance losses. At all events, its disappearing causes the porous glass solvent sensor to be less sensitive when inserted in a charcoal cartridge.

Independently of these results, the sensitivity of porous optical fibres and glasses can be increased by lengthening the optical path in the glass. However this kind of sensitivity enhancement is also effective for humidity and a hydrophobic coating on the porous glass surface must be considered.

THE EXTENDED STUDY OF COLOURED INTERMEDIATES OF PCB DEGRADATION BY *Pseudomonas species 2*

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The cells P2 were used for construction of whole cell optical biosensor for detection of PCBs. The sensor was based on measurement of production of yellow metabolites by immobilized bacteria in presence of Delor 103 (commercial mixture of PCB containing 50% congeners with 3 chlorine atoms in molecule). The aim of this study was to identify conditions of development of other coloured metabolites that was observed during biodegradation of biphenyl and PCBs by these cells.

Pseudomonas species 2 is an indigenous bacteria originally isolated from long-term PCB-contaminated soil in Czech republic. Biphenyl act as a carbon source and it is essential for induction of PCB degrading enzymes, because PCBs are metabolised only by cometabolism. This microorganism produce colour intermediates by degradation of PCBs and biphenyl.

The yellow intermediate is the third product of biphenyl degradation pathway, this product was determined, it is 2-hydroxy-6-oxo-6-fenylhexa-2,4-dienoic acid (HOPDA). Its chlorinated derivates are produced by degradation of PCBs. There are no coloured intermediates produced by degradation of only biphenyl in case of biodegradation with cells P2 immobilized on porous glass SIRAN. In the case of immobilized cells on SIRAN cultivated with PCBs (D103) the yellow coloration was produced.[1]

Biodegradations of PCBs or only biphenyl at the presence of methanol produce orange intermediates. The orange intermediates were firstly observed in the course of degradation of PCBs by P2 encapsulated into alginate beads coated with a silica layer. Methanol (0,7% vol.) is evolved during formation and aging of the silica layer.[2]

The orange intermediates were also produced by free cell degradation of biphenyl. The intensity of orange colour was depended on concentrations of biphenyl and methanol in medium. We determined that the optimal conditions for production of orange intermediates were 5g/l of biphenyl and 1-2% vol. of methanol. Methanol is a solvent of biphenyl and can act as stress factor on metabolism of microorganism. In experiments with addition of ethanol and butanol (2% vol.) yellow and orange coloured intermediates appeared with ethanol but colourless or slightly yellow coloration were observed with butanol. Additional experiments using glycerol (1%, 0,5%, 0,1% a 0,02% vol.) as a carbon source instead of biphenyl, confirmed that for appearing of orange compounds the presence of biphenyl or compounds with biphenyl skeleton was necessary.

The intensity of orange colour was higher in experiments with D103 in comparison with control with biphenyl. Unfortunately the dependence of orange colour intensities on D103 concentrations was reproducible only at D103 concentrations > 150 ppm.

The orange intermediates are very polar compounds with high extinction coefficient ($\varepsilon = 30000$) and their isolation and structure determination has not been finished yet. Trihydroxyfenyl pyridine, phenyl pyridine, dimethylhydrazide of benzoic acid, pyridine carboxylic acid and trimethylindane were identified by GC-MS analysis.[2] Dihydrodihydroxytrichlorobiphenyl and tetrahydrodihydroxytrichlorobiphenyl were identified as silylderivatives.[3] Individually these compounds are colourless, but coloured complexes of these compounds with metals were described.[4] By separation on TLC on silicagel plate four differently coloured spots, from yellow to red, were isolated. These separated colours, were further analysed by HPLC/NMR. Sensitivity and selectivity of the whole cell optical biosensor based on production of coloured intermediates by immobilized cells will be improved by detail knowledge of the structure of all colour metabolites arising by degradation of PCBs.

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FLUORESCENCE-BASED CONTINUOUS-FLOW SENSING SYSTEM FOR β-ESTRADIOL MEASUREMENT USING A MOLECULARLY IMPRINTED POLYMER

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The effects of chemicals that disrupt the normal endocrine functions in animals have been increasing concern in recent years. Screening methods for detecting potential estrogenic chemicals in wildlife are currently being developed. Among the various categories of endocrine disrupting chemicals (EDC,s), the natural hormone 17β-estradiol is one of the most potent estrogenic compounds. Several procedures have been used for the determination of 17β-estradiol, involving different analytical techniques, such as SPE-HPLC, SPE-GC-MS, radioimmunoassay, etc., requiring preconcentration methods to reach low detection limits.

On the other hand, molecular imprinting is one of the most intensively developing area in the field of synthetic receptor chemistry, focusing in the achievement of new materials with highly selective ligand properties toward target molecules.

In this work a single method for continuous-flow determination of 17β-estradiol based on the measurement of its native fluorescence was developed using a Flow Injection Analysis (FIA) system with a Molecularly Imprinted Polymer (MIP), capable of retaining 17β-estradiol, packed in a microcolumn. Chemical and physical variables affecting the retention and elution efficiency have been studied.

The resulting method provide a rapid, sensitive and selective tool for the determination of this compound at low ppb levels in analytical interest samples.

CONFOCAL SUPERCRITICAL ANGLE FLUORESCENCE (SAF) MICROSCOPY

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A novel microscope for the selective detection of surface bound fluorescence is presented. By exclusive detection of the fluorescence emission above the critical angle of refraction (supercritical angle fluorescence, SAF) the observation volume at a water/glass interface is restricted to the extreme vicinity of the surface. With an expansion of the detection volume into the aqueous solution of only ~100 nm the suppression of background fluorescence from unbound analyte is even more efficient than achieved with common biosensors based on total-internal-reflection fluorescence (TIRF). By collecting the SAF-signal with a parabolic glass lens we have demonstrated highly sensitive kinetic measurements of biochemical reactions on standard glass coverslips. [1,2]

In order to accomplish multiplex experiments, e.g. the simultaneous recording of a multitude of surface reactions, we use the SAF collection methology in a confocal scanning microscope. A single aspheric lens of high numerical aperture focuses the laser beam to a diffraction-limited spot on the coverslip surface. This lens is embedded within a parabolic glass lens as shown in Figure 1. With this new optical element the fluorescence signal is captured below the critical angle (by the asphere), as well as above the critical angle (by the parabola) with two independent detection channels. Therefore it is possible to distinguish between the fractions of surface-bound and unbound analyte molecules.

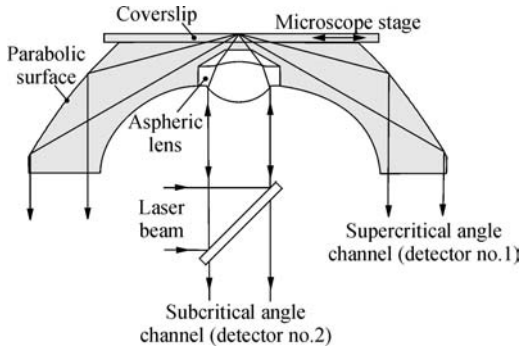

Figure 1. Scheme of the confocal SAF microscope.

The coverslip is mounted on a microscope stage for reading out the fluorescence from expanded surface areas. The system is applicable for high resolution imaging as well as for the readout of large biochips. Scanning large areas with a focused beam usually evokes the need of automatic control elements to adjust the laser spot to the designated position at the surface. Due to the special design of the excitation optics, the focus keeps at the plane of the surface even when scanning large areas, obviating the requirement of any real time control.[3]

By using pulsed laser excitation and time correlated single photon counting (TCSPC) we introduce the excellence of fluorescence lifetime microscopy (FLIM) into the field of surface selective fluorescence detection. The combination of both techniques offers high spatial and temporal resolution and therefore is of great potential for surface biochemistry. We will present the first results of the new microscope.

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STRUCTURAL STUDIES OF MIP SYNTHESIS FOR BIOSENSORS BY SCANNING ELECTRON MICROSCOPE (SEM)

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Molecular imprinting is an emerging technique for preparing artificial antibodies that have potential applications in affinity based separations, biomimetic sensors and assays.

The general principal of molecular imprinting is based on such a process where functional and cross-linking monomers are copolymerised in the presence of a target analyte (the imprint molecule) which acts as a molecular template. This procedure can be accomplished via either reversible covalent bonding or non-covalent interactions between monomers and imprint molecules.

The characteristics of the molecularly imprinted polymers (MIPs) are chemical stability, high selectivity and ease preparation, but the prediction and control of the binding properties of these materials are not fully understood.

Molecular imprinted polymers are structurally amorphous in nature and have a distribution of binding sites. This research focuses on the steric and geometrical aspects of recognition in non-covalent imprinted polymers, in particular, preparation/synthesis, behaviour and characterization for recognitive of digoxin polymers. Scanning electron microscope (SEM) has been used to investigate the surface structure of molecularly imprinted digoxin and to quantify pore size and surface roughness. A comparative study of different structures between the polymer imprinted with the analyte and its control polymer is described. Different particle size were studied in order to avoid possible ruptures of the recognition sites during the synthesis. Also the structural variations that could occur by the different synthesis conditions such as monomers used (MAA, 2-vinylpyridin, etc.), the nature and volume of porogens (ACN, chloroform, etc.) and also polymerisation process (UV, bath at 65ºC) was studied. The final surface polymeric structures after extraction by both soxhlet and microwave extraction process were also performed.

INCREASING INFORMATION CONTENT OF SPR BIOSENSING THROUGH ADVANCED DATA PROCESSING

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Surface plasmon resonance (SPR) affinity biosensors measure refractive index changes induced by the capture of target molecules by bimolecular recognition elements immobilized on the SPR sensor surface. Typically, SPR is observed as a dip in the angular or wavelength spectrum of light and the binding-induced change in the refractive index is related to a shift in the position of the SPR dip. Conventional data analysis methods rely on determining the SPR dip position by calculating its minimum position or other closely related quantities such as a center-of-mass of the dip. These approaches fail to discriminate whether the SPR dip shift originates from a refractive index produced by analyte-biomolecular recognition element interaction (specific response) or from a change in the bulk refractive index due to sample composition or temperature fluctuation. This paper presents a novel data analysis approach based on statistical analysis of whole SPR spectra and demonstrates that this method makes it possible to discriminate surface refractive index changes due to analyte capture from background bulk refractive index changes.

The presented data analysis method consists of two steps. In the first step, the SPR spectrum is assumed to behave like a product of a Lorentzian function and a low-order polynomial. Then, parameters of the model function are determined using the least squares fitting technique. In the second step, the obtained parameters are linearly projected onto surface and bulk refractive index changes.

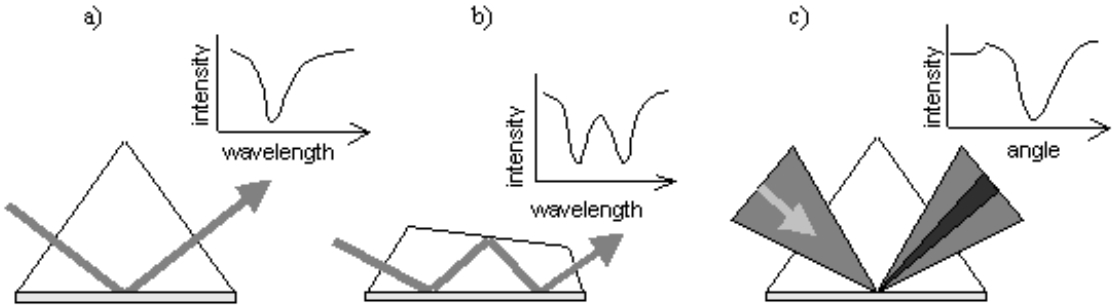

Figure 1. SPR sensor configurations and corresponding spectra.

We have evaluated the developed data analysis method in combination with three different configurations of SPR sensors based on the attenuated total reflection (ATR) method – a) SPR sensor with wavelength modulation, b) dual-channel SPR sensor with wavelength division multiplexing, and c) one-channel SPR sensor combined with a critical angle refractometer. We demonstrate that the developed data processing method allows discrimination of surface and bulk refractive index changes in each of these SPR sensor configurations. In addition, the method is shown to improve accuracy of the determined values over conventional data processing methods.

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CONTINUOUS FLOW FLUORESCENT DETECTION OF BENZODIAZEPINES USING SELECTIVE SYNTHETIC RECEPTORS

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In the past few decades, the interest for a group of compounds, included in the general designation of abuse drugs has been increased. The use of this class of compounds follows two different ways: first, the consumption of some of these substances without any control, which traffic is punished by laws; and second, the legal use in the medical treatment of certain diseases.

1,4-benzodiazepines are a chemical family of compounds included within the denomination of abuse drugs, which medical use is widely spread both in Spain and other countries.

In the present research we have developed a new analytical technique for the detection of some derivatives of benzodiazepines, in order to obtain a simple, rapid, and selective method for the routine analysis of this compounds.

With this aim we carried out the study, production and characterization of a flow-through fluorosensor, using synthetic molecular receptors selective for these analytes, based on molecular imprinting technique (MIT).

A flow-injection system with fluorometric detection was prepared in order to derivative the analytes in continuous, by hydrolysis reaction of benzodiazepines in acidic media at room temperature. This reaction was carried out in alcoholic media, using sulphuric acid. In all cases, an intense fluorescence was observed.

The analyte nitrazepam was used as template to obtain the molecularly imprinted polymer (MIP). Others reagents were methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as crosslinking monomer, acetonitrile as porogen, and an initiator of the reaction. The polymerisation was performed using ultraviolet radiation at a temperature of 0ºC. The polymers prepared with this protocol were placed in a microcolumn and introduced in the flow-injection assay (FIA) system, where the analyte was selectively retained in continuous. After the elution of the analyte and the following derivatization, without any interference of matrix of the sample, the fluorescent signal was recorded.

The physical and chemical parameters involved in the process of retention, elution, and derivatization in continuous were studied and optimised.

A NEW SENSOR BASED ON SURFACE PLASMON RESONANCE IMAGING

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Surface plasmon resonance (SPR) has been widely used in optical biosensors. It has been applied to analysis of biomolecular interactions (*e.g.* antigen-antibody, protein-DNA, DNA-DNA) as well as detection and identification of chemical and biological analytes. Recently, development of high-throughput SPR sensing devices has been pursued in research laboratories worldwide to provide a new generation of tools for highly parallelized biomolecular interaction analysis. SPR imaging has received a great deal of attention as it allows for spatially-resolved observation of SPR across entire sensing surface. As the SPR imaging relies on intensity distribution, its performance is limited by effects such as light source intensity variations, low image contrast and crosstalks among neighboring areas of sensing surface.

We report a novel approach to SPR imaging which overcomes these effects by combining sensing surface patterning and polarization contrast. In this approach, surface plasmons are excited by means of attenuated total reflection using a prism coupler. Polarization of incident light is adjusted using an input polarizer so that the light is linearly polarized at +45 degrees to the plane of incidence. Upon reflection from the base of the prism coupler the light beam passes through an output polarizer the transmission axis of which is set to -45 deg. If an SPR chip attached to the prism base contains an SPR-active structure which does not cover the whole surface but only defined small spots, only light reflected from the SPR-active spots passes through the output polarizer, while the light from other areas is extinguished. Therefore the resulting image consists of bright spots on dark background which eliminates crosstalk from neighboring sensing areas and provides a high-contrast image.

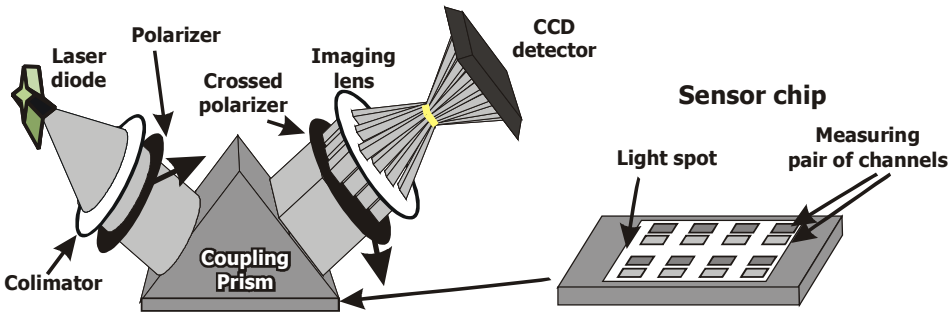

Figure 1. SPR imaging with polarization contrast and patterned chip.

On the sensor chip, there are two types of SPR-active spots (with and without a special polarization-changing underlayer) which exhibit opposite sensitivity to refractive index changes. We pair one of each to generate a normalized signal that is independent of light source intensity fluctuations. This approach also makes it possible to dynamically adjust the CCD exposure time to enhance the signal to noise ratio.

We present theoretical analysis of the proposed SPR sensor configuration, taking into account typical light levels and realistic detector shot and dark noise and demonstrate that the proposed approach provides better resolution than conventional SPR imaging. We present experimental results illustrating the potential of this approach for multichannel biosensing.

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CANTILEVER BASED PROBES FOR SCANNING NEAR-FIELD OPTICAL MICROSCOPY MANUFACTURED IN SEMICONDUCTOR TECHNOLOGY

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Conventional optical microscopy is probably the most widely used method for sample characterization, particularly in the life sciences. From imaging cells down to finer and more detailed structures such as cell organelles — or even individual biological macromolecules — the need for increased resolution is indisputable. However, the spatial resolution achievable with conventional microscopy is limited by the diffraction limit — to roughly the half of the wavelength of light used. One of the solutions to overcoming this limit is the use of Scanning Near-Field Optical Microscopy (SNOM).

SNOM features a different approach to recording the optical signal of a sample, using a scanning setup similar to common methods such as Atomic Force Microscopy (AFM) or Scanning Tunneling Microscopy (STM). Instead of force (as in AFM) or current (as in STM), light provides the key interaction between probe and sample in SNOM. Light is forced through a tiny aperture of about 50 nm formed by a metallic coating and establishes an optical near-field that interacts with the sample. When this near field is employed , the resolution of this method is comparable to the aperture diameter — in the nanometer range.

However, in the life sciences, SNOM is far from routinely used for increasing optical resolution, in contrast to, e.g., AFM, which is widely used for high-resolution imaging in material sciences. The main reason SNOM is not more widely used is the complicated and expensive fabrication of the SNOM probes: Conventionally these probes are made from optical fibers which are pulled or etched — one by one — to form a tip with a nanometer-sized aperture. Afterwards each fiber tip is individually covered with a metal layer and subjected to, e.g., focused ion beam techniques to form the aperture. The process is expensive and fails to lead to reproducible probe production, due to the need for individual handling of each probe.

To overcome these problems we are developing a fabrication method for SNOM probes based on semiconductor technology, providing all the concomitant advantages of a batch-process-based production. Starting from a silicon wafer substrate, the probe uses a cantilever design similar to the well known AFM probes: The tip is formed at the end of a lever (Figure 1) whose bending is used as the signal for the distance control feedback as in standard AFM modes. However, our probes are made from silicon nitride (lever) and silicon dioxide (tip) so that the measuring light can be coupled into the tip from the backside. The tip itself is formed by isotropic etching of glass (Figure 2). A metal coating covering the tip forms the nanometer-sized aperture and is fabricated by electron beam evaporation followed by selectively opening the very end of the tip. These processes are designed in such a way that they are applied to the wafer as a whole and hence to a few hundred probes simultaneously, leading to low-cost and reproducible sensors.

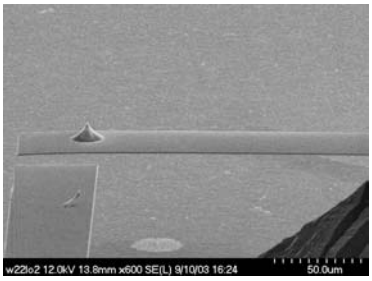

Figure 1. SEM image of an optically transparent silicon nitride cantilever (300 µm by 50 µm) with a glass tip of about 15 µm in height.

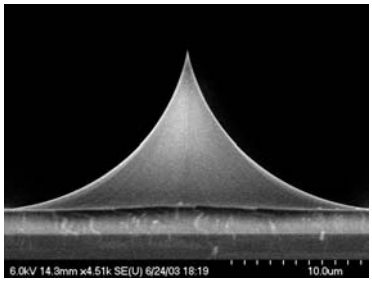

Figure 2. SEM image of a glass tip fabricated by isotropic glass etching. The very end of the tip forms a curvature of less than 30 nm in diameter.

We will present the design of these probes, their fabrication process, and preliminary evaluations of the setup, with particular emphasis on comparison to conventional fiber-based setups.

RICH INFORMATION FORMAT SURFACE PLASMON RESONANCE SENSOR BASED ON ARRAY OF DIFFRACTION GRATINGS

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In principle, a surface plasmon resonance (SPR) biosensor is a thin film refractometer, which measures refractive index changes induced by a biomolecular interaction at a surface of the sensor. SPR biosensors allow observing biomolecular interactions directly without the use of labels, which makes them very useful for characterizing and quantifying biomolecular interactions. In order to fully exploit potential of the SPR method in emerging important areas such as proteomics, a new generation of high throughput SPR sensor platforms needs to be developed.

We report a new SPR sensor platform for high throughput screening of biomolecular interactions based on an array of diffraction gratings. The diffraction gratings are an alternative to traditional prism couplers and offer numerous advantageous features such as potentially low-cost mass production by replication methods (e.g. polymer injection molding) and straightforward integration with microfluidics for fluid handling. The sensor consists of a reader and an SPR chip, see Figure 1. The SPR chip contains a two-dimensional array of miniature gratings which form individual sensing channels; the typical number of sensing channels is in the order of hundreds. In the SPR chip reader, monochromatic light beam is focused on a row of diffraction gratings exciting surface plasmons at certain angles of incidence. The excitation produces a dip in the angular spectrum of light reflected from each grating. A beam splitter is used for separation of incident and reflected light beam. The reflected light beam with the angular spectra is projected on a CCD camera. Rows of diffraction gratings are read sequentially by scanning the light beam across the SPR chip by means of a cylindrical lens mounted on a motorized linear stage.

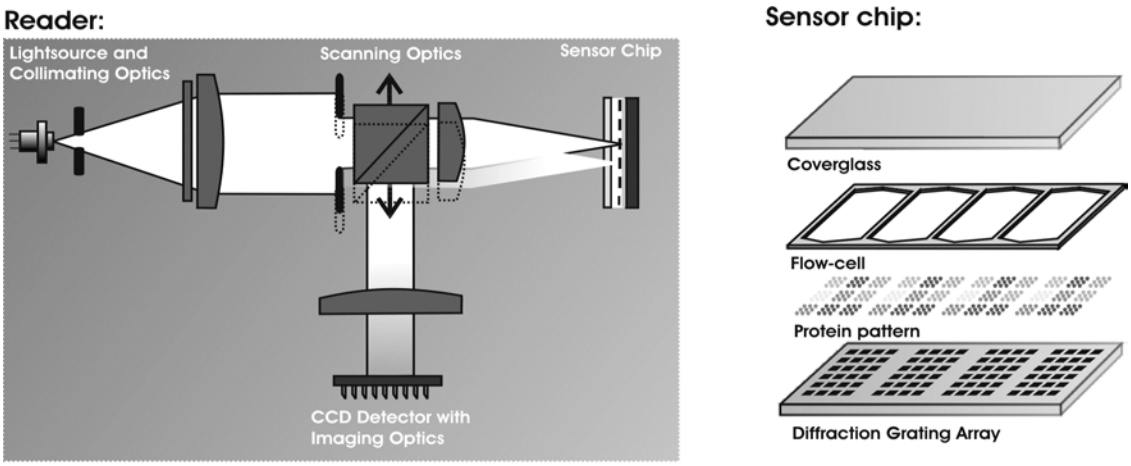

Figure 1. Scheme of the SPR chip with two-dimensional array of diffraction gratings and the SPR chip reader

A laboratory prototype of the SPR sensor platform comprising the SPR chip with 280 sensing channels, multi-channel fluidic system for sample distribution and SPR chip reader was built and characterized. The resolution as good as 10^{-6} RIU was achieved at a chip readout time of 5 seconds. It is expected that this platform will be further optimized and combined with methods for protein patterning to yield a new biosensor technology for parallelized study of biomolecular interactions.

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AN OPTICAL SENSOR FOR ANTIOXIDATIVE CAPACITY BASED ON IMMOBILISED CHROMOGENIC RADICALS

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Understanding the chemistry of free radicals and antioxidants is gaining importance, particularly in the areas of clinical medicine and nutrition science. Free radicals are known to cause damage to lipids, proteins and nucleic acids and hence are implicated in a number of pathological interactions and degenerative diseases in living organisms, including cancer, Alzheimer's and Parkinson's disease. Antioxidants act as free radical scavengers and can prevent the damage caused by oxidative reactions. Hence, the evaluation of the antioxidative activity of biomedical or food samples provides useful clinical and dietary information.

A number of different optical-based tests for the determination of antioxidant capacity have been previously devised using a variety of chemical and biological mechanisms. In general, the activity of antioxidants present in the sample is related to their ability to scavenge long-living chromogenic or fluorogenic free radicals. Some of these optical assays have been further adapted for use in commercial test kits and instruments using solution-based chemistries.

The aim of this work is to study the feasibility of developing an optical sensor test strip having an immobilised chemistry format with which to assess the antioxidative capacity of samples. For this purpose, a number of stable chromogenic radicals, including 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,6-di-tert-butyl-α-(3,5-di-tert-butyl-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy (galvinoxy free radical), were immobilised into a polymer matrix and screened for their suitability as indicators of antioxidative status. The spectrophotometric characterisation of polymer films containing immobilised free radicals was performed, and the effects of the immobilisation of the free radicals on their stability and reactivity towards standard antioxidants were studied.

TWO PHOTON FLUORESCENCE SENSORS BASED ON RESONANT GRATING WAVEGUIDE STRUCTURES

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The basic configuration of a grating waveguide structure (GWS) consists of a substrate, a polyimide waveguide layer and a grating layer on top. At a specific wavelength and angular orientation of the incident beam, the grating waveguide structure (GWS) "resonates", where the rediffracted beam destructively interferes with the transmitted beam, and most of the incident light is reflected. Specifically, a very sharp decrease of the transmitted light is observed in the spectrum of the illuminating pulse. For our purposes the most important feature of GWS is the enormous optical field enhancement that can be achieved at the grating surface, despite the low coupling efficiency. The GWS has a number of attractive features for two photon fluorescence (TPF) applications and biosensing. In particular, they have high finesse so they enable the detection of minute changes of refractive index, they are compact and robust so as to simplify experimental setups, and are relatively easy to fabricate so they can be readily incorporated into widespread biological and chemical applications.

Some representative experimental results are shown in Figure 1. Figure 1.a shows the TPF as a function of wavelength for different excitation wavelengths, obtained with a GWS on which TMR was deposited. Near the resonance the TPF intensity increases strongly, reaching its maximum at 844 nm, indicating a strong field enhancement. The peak intensity is about ten times larger than the background. Close to the maximum two photon absorption of TMR, λ_{max}= 850nm, the fluorescent signal was reduced by a factor of two. For wavelengths at the FWHM of the resonance bandwidth, the measured fluorescence decreased by approximately 20%. These results indicate that the maximum TPF intensity is observed only under resonant conditions. Figure 1.b shows a comparison of the experimental TPF results with the GWS at resonance to those with a prism on which TMR was deposited. As evident, the results with the GWS are better by at least a factor of three.

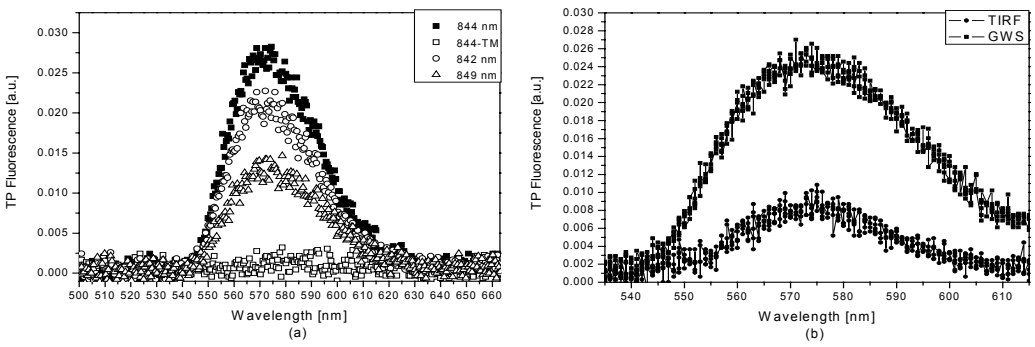

Figure 1. Two Photon Fluorescence signal with GWS and a prism (a) TPF for different excitation wavelengths. Filled squares denote excitation wavelength at resonance (λ=844 nm, TE); empty squares out of resonance (λ=844 nm TM); triangles maximum two photon absorption (λ=849 nm TE-mode); circles lower limit of the FWHM (λ=842 nm, TE). (b): Two Photon Fluorescence signal for the same excitation wavelength of 844 nm obtained using a GWS and a prism. Squares denote GWS enhanced signal; circles prism in TIR.

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EXAMINATION OF LIGHT DISTRIBUTION FROM SOL-GEL BASED OPTODES OF FIBEROPTIC SENSORS

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Chemical optical sensors belong to the family of sensors characterized by wide applications possibilities. One of the requirements for such sensors is suitable matrix enabling the entrapment of photosensitive chemical compounds. Many researchers use the sol-gel materials, since they can be porous, mechanically stable and chemically inert, and can posses the necessary optical properties.[1] Sol-gel optodes may be used for sensing of various compounds, gases, organic, as well as inorganic substances.[2,3,4] It is important to know the profile of light distribution at the sensor tip, since it informs how much light can be coupled into the sensor. Second, the knowledge about the energy density allows designing the optode with specially tailored distribution of photosensitive dye in order to avoid problems of photobleaching.[5,6]

The sol-gel films were prepared from silicate precursor TEOS (tetraethylortosilicate 98 % from Aldrich) mixed with solvent – 96 % ethyl alcohol in acid catalyzed hydrolysis. The suitable amount of detergent Triton X-100 (Aldrich) was used. The optodes matrices were produced with various ratios R=5, 15, 20, 32, 40, 50, whereas R denotes the number of solvent moles to the number of TEOS moles.

In these studies the HCS low OH, CFO 1493-12 optical fibers from LaserComponents were used (fused silica core ϕ_1=400 μm, with HCS cladding ϕ_2=430 μm; with ETFE buffer diameter 730 μm). They feature high transmission values from UV to IR (200-2400 nm). First, the optical fibers were cut into 200 cm sections and the external jacket was mechanically removed on the distance of 1,5 cm. Next, the cladding was removed by hot torch. The residuals were removed with linen cloth and washed with ethyl alcohol. The dip-coating method was applied to cover the bare fibers with sol-gel material.

The angular light intensity distribution was examined in order to find out the influence of the optode matrix type on the light intensity near the fiber end. The light beam from LEDs with maximum at λ=470 and 635nm was coupled into the optical fiber and guided to the fiber end with attached optode. The photodetector (photomultiplier tube - PMT H5702-50, Hamamatsu Photonics K.K.) was placed on a circulating arm at the distance 1cm from the fiber output and connected to the voltammeter. The graphical representation of measured data informs about the angular intensity distribution. The same experiments were repeated for matrices with entrapped Ru(bpy). Additionally, the intensity of luminescence signals were measured in order to check the influence of matrix type on performance of sol-gel optodes.

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NOVEL OPTO-CHEMICAL SENSORS FOR NON-INVASIVE OXYGEN MEASUREMENT IN TRANSPARENT PACKAGES OR CONTAINERS AND THEIR PERFORMANCE DURING APPLICATION

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Novel opto-chemical sensors, which have been developed at JOANNEUM RESEARCH, allow the measurement of oxygen inside transparent packages or containers in a non-destructive way. The sensors are based on the principle of luminescence quenching and are suitable for applications in research (packaging development and food research), industry (for on-line quality control of vacuum and modified-atmosphere packaging) and throughout the whole distribution chain for leak inspection.

The opto-chemical oxygen sensors consist of an O2-sensitive element, which has to be included into the package, and of an opto-electronic read-out instrumentation, which allows the measurement from a distance of a few centimeters, in presence of ambient light and of known luminescence backgrounds (e.g. from the packaging material). The O2-sensitive element consists of an O2-sensitive dye, which is dissolved in a polymer material. The read-out instrument measures the change of the luminescence lifetime via a phase measurement technique.

By selecting the proper O2-sensitive element and adjusting the optical components, it is possible to select the oxygen concentration range of interest. A sensor for oxygen partial pressures in the range of 0-20000 Pa (with a resolution better than 20 Pa below 1000 Pa and of 100 Pa above 1000 Pa) was developed for vacuum and modified-atmosphere package inspection. Another type of sensor for applications where high sensitivity is required, is suitable for the range of 0-1000 Pa (with a resolution better than 2 Pa over the whole range). Typically, such sensors are required for investigating the O2 permeability of plastic packaging materials. The response times of the sensors are in the order of 1 s. The performance of this technique for different applications esp. for food safety is demonstrated in a competitive way to the commonly used invasive methods. Furthermore very interesting applications for this sensor type are presented in applications where the invasive methods failed up to now.

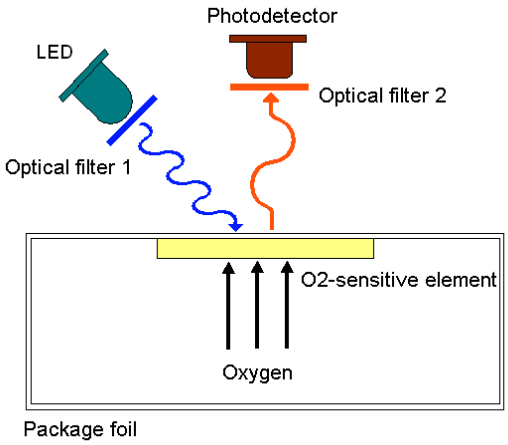

Figure 1. Example for one measurement set-up.

SURFACE PLASMON RESONANCE (SPR) BIOSENSOR DETECTION FOR FOOD SAFETY

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In the United States, the Council for Agricultural Science and Technology estimates that as many as 9000 deaths and between 6.5 and 83 million illnesses are caused annually by microbial foodborne diseases. The World Health Organization estimates that nearly two million children die worldwide due to microbiologically contaminated food and water. There is a real world need for fast, sensitive, and reliable analytical methods for detecting food contaminants from both inadvertent food contamination and deliberate terrorist attacks. This research focuses on developing a surface plasmon resonance (SPR) biosensor technology[1] capable of quantitative and simultaneous detection, identification, and monitoring of multiple analytes in complex media. Different surface chemistries have been developed to detect various analytes. This study demonstrates surface chemistries and detection assay formats for detection of *Escherichia coli* O157:H7 (large analyte) and domoic acid (small analyte).

Large corpuscular analytes such as bacteria are difficult to detect and quantify at low concentrations without time consuming amplifications (i.e. culturing, PCR). In this study, *E. coli* O157:H7, an important food contaminant, was detected down to concentrations of 10^4 cfu/ml with a SPR biosensor. Monoclonal antibodies to the LPS and it O polysaccharide moiety on the membrane of the *E. coli* O157:H7 were immobilized to a mixed self assembled monolayer (SAM) of alkanethiols on a gold sensing surface using EDC/NHS linking chemistry. Three methods for preparing *E. coli* samples were compared; untreated, heat killed / soaked in 70% ethanol, and detergent lysed with detergent lysed samples having the lowest concentration detection. The sensor sensitivity established by the detection limit, 10^4 cfu/ml, is two orders of magnitude more sensitive than standard ELISA and previously reported SPR detection of bacteria[2] [2]. This study addresses the challenge of low detection limits, specificity to identify *E. coli* O157:H7, and compensation for interferences such as temperature fluctuations, sample composition variations, and non-specific adsorption of non-target molecules.

Low-molecular weight analytes, such as drug molecules and toxins, often need to be detected and analyzed in developing drugs and controlling food safety. In this study an inhibition assay is used to detect the low molecular weight analyte domoic acid (DA), a marine toxin which is a causative agent of amnesic shellfish poisoning (ASP). In implementing the inhibition assay the analyte, domoic acid, was immobilized to a mixed SAM of amine and hydroxyl terminated polyethylene glycols (PEG) on a gold sensing surface using EDC/NHS chemistry. A fixed concentration of antibody solution is incubated with varying concentrations of analyte prior to injection into the SPR sensor. SPR sensor detects unreacted antibodies binding to analytes immobilized on the sensor surface. Our results showed that there is no non-specific adsorption on surface. SPR responses increased as concentrations of antibody are increased. A lowest detection limit of 0.1 – 1 ng/mL was achieved when an antibody solution of 1.25 μg/mL was incubated with varying DA concentrations. The effects of antibody concentration and flow conditions on detection limits will be discussed.

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INVESTIGATION OF PRIMER ELONGATION AND DYE-SURFACE INTERACTIONS IN REAL-TIME

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Polymerases are one of the most important enzymes in biochemistry and a lot of research has been done for understanding how they work. The synthesis of complementary DNA strands *in vitro* is a central step in all existing DNA sequencing procedures (Sanger, Pyrosequencing, Exonuclease digestion). Inspired by the high reaction rates of polymerases efforts are directed towards base-specific real-time single molecule detection of nucleotide incorporation. In these techniques, either a template molecule or the enzyme is fixed to a surface, where the DNA polymerase specifically incorporates fluorescently labelled nucleotides. Such an approach could possibly replace existing DNA sequencing techniques in the future due to their advantage of higher speed.

Modified polymerases which work faster or with an increased incorporation yield of labelled nucleotides (dNTPs) would be helpful, e.g. for PCR and labelling reactions. Despite these efforts there is still a lack of methods for detecting directly the facility of incorporation of tagged dNTPs by polymerases.

The SAF biosensor allows the detection of the efficiency of polymerases directly and in real time.[1] The obtainable data is useful to compare the efficiency of different enzymes, to investigate the influence of different labels and to spot Single Nucleotide Polymorphisms (SNPs).[2,3] The latter application could prove extremely expedient, as single mismatches are detected within few minutes, thus distinctly faster than with common hybridization methods.

A hindrance for the detection of single incorporation events is the measured background caused by surface adsorbed labelled dNTPs. Therefore we investigated different pairs of surface coatings and dyes to minimize the adsorption.

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TIME-RESOLVED FLUORESCENT IMAGING OF OLIGONUCLEOTIDE AND PROTEIN MICROARRAYS

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Major contributions to the increasing pace of discovery in molecular biology, in particular the completion of the human genome project and the establishment of proteomics as an independent scientific discipline and advances therein, have been made by the invention of nucleotide microarrays, also called gene chips, and the successful transition of this technology to RNA and protein chips.[1] In order to complete the even much greater task of setting up a complete library of proteins and other cellular components, their abundancies and interactions, detection methods of largely superior sensitivity and accuracy than the fluorescence intensity-based imaging methods mainly employed on standard gene chips are needed.

Over the past decade increasing attention has been drawn to the development of lifetime-based, time-resolved and time-gated fluorescent imaging. In principle, those screens are largely superior to intensity-based ones in terms of accuracy, because lifetime is completely or mostly independent of concentration and several optical phenomena which usually cause noise in intensity-based measurements. Even more importantly, using the Rapid Lifetime Decay (RLD) or related schemes,[2] along with suitable long-lived dyes, all short-lived fluorescent noise of biomolecules is referenced out, or not recorded at all. This technique also opens a new dimension by allowing to distinguish biomolecules labeled with dyes of different wavelengths or fluorescent lifetimes. Time-resolved screens can be performed either in the time or in the frequency domain, where the frequency domain is best suited for inclusive detection of ns decaying fluorescence. Although widely known and perceived for its potential, since its invention applications of time-resolved fluorescent imaging for scanning microarrays have suffered from cost, complexity and performance issues.[3] We do, however, think that those teething problems have been overcome.

We present a number of fluorescent lifetime imaging schemes based on protein-binding Ru-tris-bipy-ridyl-related compounds e.g. ruthenium-(5-isothiocyanato-1,10-phenanthroline)-bis-2,2'-bipyridine)$^{2+}$. We also have developed different time-resolved imaging screens on either conventional or streptavidin-coated microplates with Ru-labeled oligonucleotides or proteins and transferred the results to microarrays on glass microscopy slides using amino-terminated monolayers prepared from N-(6-aminohexyl)-amino-propyltrimethoxysilane (AHAPS). We have coupled protein and oligonucleotide probes via an active ester (NHS) functionalized monolayer or the biotin-streptavidin system. The protein binding capacities and stabilities were compared to commercially available epoxy and aldehyde microscopy slides.

Progress in instrumentation, in particular the availability of multiple windows, allowing to apply identical or different RLD schemes up to 16 times in one measurement, and extended integration times, have been used to enhance performance. Results are given and compared to state-of-the-art intensity-based measurements. Furthermore, the high-throughput screening capabilities of all systems were analyzed.

In conclusion, time-resolved fluorescent imaging is shown to be a viable alternative in microarray technology and the unmatched accuracy of lifetime-based screens may be a key criterion in further research and development. Multi-label time-resolved imaging experiments on biomolecules will extend the possibilities even more. The recent expansion of glass slide based chips into areas as diverse as peptide or proteom arrays, immunoassays or cell-based microarrays creates new fields of application for time-resolved measurements.

SENSITIVITY EVALUATION OF A MULTILAYERED SURFACE PLASMON RESONANCE BASED FIBER OPTIC SENSOR: A THEORETICAL STUDY

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Surface Plasmon Resonance (SPR) technique has been a useful tool for sensing a wide range of chemical and biochemical parameters. Gold and Silver are the two metals which are extensively used in SPR phenomenon. Gold shows a higher shift of the resonance angle when the sensing layer index is varied. In addition to it gold is chemically stable. Silver, on the other hand, displays narrower response curve but has a poor chemical stability. Recently a new structure of resonant metallic film based on bimetallic layers[1] (gold as outer one) on prism base has been suggested which combines advantages of both gold and silver with outer gold layer protecting silver against oxidation. In the present work the above bimetallic structure has been applied to optical fiber instead of using a coupling prism (Figure 1). In an optical fiber a single selected guided ray as well as all the guided rays can be launched. We have studied the sensitivity of such an optical fiber SPR sensor and have compared with prism based SPR sensor for different ratios of silver and gold film thickness. The effects of fiber parameters such as numerical aperture and core diameter and sensing layer parameters are also studied. Self Assembled Monolayers (SAM) like thiol (instead of gold) can also be used to protect silver film against oxidation. We have also studied its effect on the sensitivity of the fiber optic SPR sensor. A third layer of materials like ZrO_2 is applied to gold/thiol[2] layer. Its effect on the operating range of the sensor has been studied for both, single ray and all guided rays launched in the fiber. It has been shown that the third layer increases the operating range of the sensor.

The optical absorption that occurs due to imaginary part of the refractive index of the sensing layer is generally not considered for SPR based sensor. Kurihara and Suzuki[3] were the first who introduced it to prism based SPR sensor. Later, we applied this to optical fiber based SPR sensor.[4] In the present study we have extended the multiplayer structure to absorption based fiber optic SPR sensor. This, in turn, introduces various other parameters like concentration, maximum absorption wavelength etc. corresponding to sensing layer. The influence of such parameters on the sensitivity of the sensor has also been studied followed by their optimization for the best possible sensor configuration.

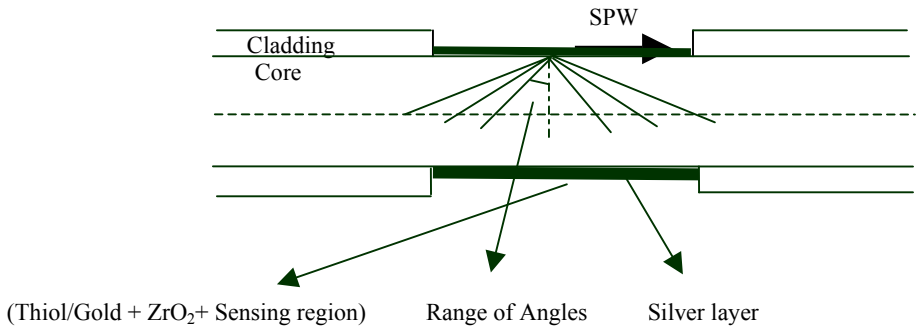

Figure 1. Fiber optic multilayered SPR sensor.

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ANALYSIS OF DDT USING A HOME-MADE SURFACE PLASMON RESONANCE BIOSENSOR

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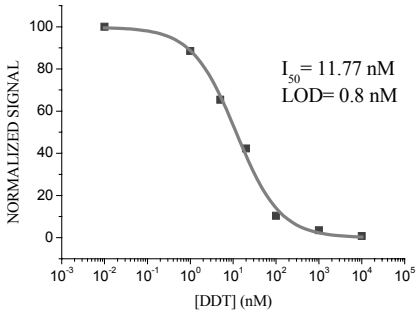
Environmental concern about the potential of some organic pollutants, such as pesticides and industrial chemicals, to cause adverse effects on human health and wildlife has arised in recent years. Among these compounds, the use of DDT (Dichloro-diphenyl-trichloroethane) as organochlorine insecticide, has been widely restricted due to its persistence and accumulation in the food chain.

The utilization of SPR biosensors for the detection of environmental pollutants is based on the principles of solid-phase immunoassays. These assays require the use of antibodies (monoclonal or polyclonal), which are the key components of all immunoassays, since they are responsible for the sensitive and specific recognition of the analyte. The application of immunoassays to environmental monitoring also involves the design of hapten derivatives of low molecular weight molecules, such as DDT, to determine the antibody recognition properties. Once hapten synthesis and monoclonal antibody production have been accomplished, the use of SPR biosensing technique provides a real-time monitoring of binding interactions without the need of labelling biomolecules.

Immunoassays developed to determine DDT were inhibition tests based on the conjugate coated format, in which a 10 µg/mL concentration of BSA-DDT in 10 mM acetate buffer, pH 5.0, was immobilized on the sensor surface. The competitive heterogeneous assay required the incubation of a mixture of the analyte (DDT) and the antibody (LIB-DDT5.25), before binding of the free remaining antibody to the immobilized conjugate.As it corresponds to binding inhibition immunoassays, the SPR



signal provided by the sensor was inversely proportional to the analyte concentration in the DDT-antibody mixture, and standard points fitted to a sigmoidal equation. A standard curve (see Figure 1) was obtained by averaging four individual standard curves normalized by expressing the SPR signal (SPR_{signal}) of each standard point as the percentage of the maximun response [100 x ($SPR_{signal}/SPR_{signal,max}$)].

Figure 1. Normalized average standard curve for the DDT SPR immunoassay.

The sensitivity of the immunoassay, expressed as the analyte concentration that reduces the assay signal to 50% (I50) of the maximum signal, was 11.7 nM. The inhibition curve obtained with the application of the conjugate-coated format allowed the detection of DDT from 2.2 to 59 nM (I80- I20), assuming this range as the operative working range of the assay. A limit of detection of 0.8 nM was also calculated from the calibration curve, as the analyte concentration for which the normalized signal was 90% of the maximum one.

Acknowledgements. This work has been supported by MCyT (project BIO-2000-3051-P4-05) and CSIC-I3P program.

A NEW PORTABLE FIBER OPTIC SENSOR FOR DETERMINING AND QUANTIFYING BENZO[A]PYRENE IN DRINKING WATER

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The need for performing the analytical process "in situ" and "at real time" in many different fields such as medicine, biochemistry, environmental control, etc, has promoted the development and application of chemical sensors in such areas. In particular, luminescence optical sensors research has experienced an important growth during the last decade[1] as a result of their excellent capabilities such as the high sensitivity and selectivity or the possibility of using these sensors coupled to fiber-optics which allows performance of remote analysis.[2]

Benzo[a]pyrene (BaP), one of the most carcinogenic polycyclic aromatic hydrocarbon compounds, is also considered to be an indicator of the presence of other PAH's in water. [3] Because it is formed when gasoline, garbage or any animal or plant material burns it is usually found in smoke and soot. This compound combines with dust particles in the air and is carried into water and soil and onto crops.

The European Union and World Health Organization (WHO) have laid down that in assessing the quality of water for human consumption six PAH's (fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene and indeno[123cd]pyrene) must be tested for and that the maximum total concentration of these six PAH's is 200 ng L^{-1}, whilst the more carcinogenic BaP may not exceed 10 ng L^{-1}, with a relative standard deviation of 25%.[4]

In this communication a portable fiber optic fluorescence sensor for determining and quantifying benzo[a]pyrene (BaP) in tap water is presented. The sensor is based on the measurement of the native fluorescence intensity of BaP after its "on-line" immobilization in a flow system (home-made probe) onto a non-ionic exchanger resin (Amberlite XAD 4) placed in the tip of a portable optode.

A bifurcated optical fiber probe, which has twelve 200 µm fiber for the excitation and a 600 µm for recollecting the fluorescence emission, was used to transport the emission light from a 380 nm LED to the flow cell and recollected the fluorescent emission and transported it to a portable spectrometer equipped with a 2048 CCD detector, a slit of 500 nm and a 600 line/mm grating (200-800 nm blazed in 500 nm).

The sensor will be evaluated for the determination of very low levels of BaP in drinking water from Granada City and different villages around it. The results will be compared with those obtained with other alternative methods, demonstrating that the sensor technology is increasing and could displace other more complex and expensive techniques in routine laboratories.

Acknowledgements. The authors gratefully acknowledge the financial support of the Spanish Ministerio de Ciencia y Tecnología (Project PPQ2000-1291-C02-01) and a grant from the Consejería de Medio Ambiente de la Junta de Andalucía (Agreement no. 1870).

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PORTABLE PROTOTYPES OF SURFACE PLASMON RESONANCE BIOSENSORS. APPLICATIONS IN THE ENVIRONMENTAL CONTROL

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We have developed three portable Surface Plasmon Resonance (SPR) biosensor systems. The mechanical, optoelectronic and computational characteristics of each one will be described. The first one is based on the classical approach for SPR sensors. The second one incorporates a linear photodetector array and the third one, bidimensional arrays (miniaturised CCD) for detection. Each SPR system has been based on the same conception: a local acquisition and data processing in an embedded system, using the computer only for software control and presentations of the results. The three biosensors have been designed over the same architectural scheme, shown in Figure 1.

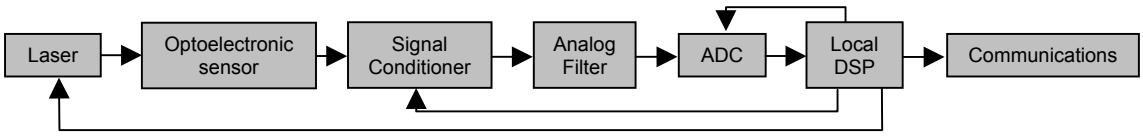

Figure 1. Conceptual blocks scheme for SPR prototypes

In this way, the prototypes are self-consistent and can be connected to any PC, digital agenda or can incorporate its own LCD screen. The final size of each prototype will allow for portability of the devices to different places. A communication module based on GSM/GPRS has been also designed (in the future this will be in UMTS) and will be incorporated to the device. The differential characteristics of the three prototypes are described in the Table 1.

Table 1. Summary of SPR prototypes characteristics.

Characteristics	*Single*	*Linear Arrays (2x16)*	*Bidimensinal Arrays (1032x1288)*
Automation	None of them	Partially automation	Full automation
Mechanics	High	Low	Low
Multibiosensor	No	2	>2
Signal-Noise Ratio	Very good	Good	Poor
Signal Processing	Low	High	Intensive
ADC Resolution	12 bits	16 bits	10 bits
Calibration	Manual	Automatic	Automatic
Connectivity	Serial	Serial , TCP/IP	Serial, USB, TCP/IP

A large number of applications for the SPR sensors ranges from clinical, industrial control processes, veterinary field to food industry or environmental monitoring, avoiding expensive, complex and time-consuming procedures. The usage of these portable SPR biosensors to environmental monitoring, as a field analytical method, can reduce the time and cost of environmental pollutants detection, allowing the performance of in situ measurements. We have used the systems herein described for detection of the environmental pollutants DDT, Carbaryl and other endocrine disrupters. One of our main objectives is to implement the SPR devices at specific localizations (i.e. water treatment plants) for on-line and in-situ detection of the environmental contaminants.

MODELLING OF SENSITIVITY FOR SENSORS BASED ON SURFACE PLASMON RESONANCE

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The computer modelling is carried out for parameter of sensitivity of optoelectronic sensors, using phenomena of surface plasmon resonance (SPR). The SPR-sensor's physical model is described in view of probable modifications of sensitive surface with a dielectric layer. The variants of optimisation of sensitivity for SPR-sensors, designed on the principle gold - dielectric are considered. With the purpose of comparison of processing techniques two methods of SPR-curve mathematical treatment - traditional with estimation of sensor's response as result of the shift of the curve minimum and proposed, using calculating of derivative in the SPR-curve half-width point are compared.

A number of works for modeling of SPR-sensors sensitivity is devoted.[1]-[3] In the all mentioned works a parameter of sensitivity for particular cases is described. More universal is the proposed variant of modeling for multilayer system with gold, dielectric layers and layer of investigated molecules with thickness dmol, adsorbed on the gold or dielectric surface. SPR-curves with a sufficient accuracy are simulated using the formalism for matrix of scattering in the multilayers structures.[4] The proposed model of the sensor's structure theoretically allows to consider system with n-layers. For study of parameter of sensitivity the original computer program, allowing step by step variation of the parameters of complex index of refraction N as well as the thickness of layers in the wide range of technologically possible values was developed The results of modelling reveal, that sensitivity of considered model of SPR-biosensor changes depending on parameters of multilayer structure in the significant range of values, especially for layers of biomolecules with a large size. Maximal theoretical sensitivity of SPR-sensor without a dielectric layer should be observed with thickness of a gold layer approximately 70-80 nm with minimal possible value of absorbtion index kAu. The index of refraction nAu don't reveal noticeable influence on sensitivity .

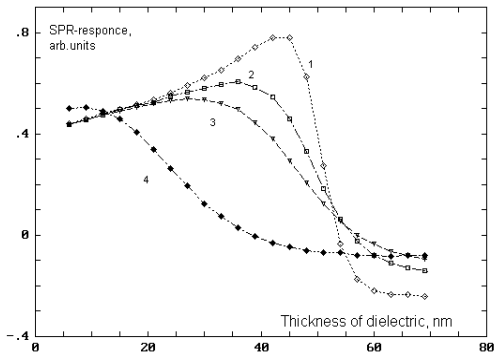

Figure 1. Dependence of SPR-response on the index of absorption and thickness of dielectric layer (N_d = 1.45). 1-for registration of biomolecules layer (N_{mol} = 1.46, d_{mol}=2nm). 1-(k_d=0.0i), 2-(k_d=0.02i), 3-(k_d=0.04i), 4-(k_d=0.2i).

In case of model with a dielectric layer the more complex dependence on SPR-response from thickness and index of refraction of a dielectric layer is observed. Sensor's sensitivity with presence of a dielectric layer (N_d = 1.45) for all thickness of biomolecule's layer grows fluently up to some critical thickness of a layer, and then sharply (Figure 1) decreases. Exception is the case, when value of index of absorption kd of dielectric increases up to 0.2, were sensitivity decreases with presence of a dielectric layer of any thickness.

The presented results of modelling allow not only to find optimal parameters of SPR-sensors, but also to specify researched parameters of biological objects, due to direct dependence of SPR-response on values of dielectric constants and thickness of layers structure. Actually, it is necessary to have a number of specific nomograms for calculating of the SPR-response which are taking into account real values of materials, that applied.

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FLUORESCENCE RESPONSE FROM POLYSTYRENE LABELLED WITH ANTHRACENE TO STUDY ITS THERMAL TRANSITIONS

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A fluorescent group (fluorophore) is always affected by its immediate surroundings. Its fluorescence response can be clearly perturbed if there is any physico-chemical change in the site where the fluorophore is immersed (rigidity, polarity …).[1] Due to this, changes in the free volume of a polymer matrix (for instance when a glass transition is taking place) in which the fluorophore is located should modify the number and type of interactions between the polymer and the dye and therefore, changes in the fluorescence response of the fluorophore should occur.[2345]

In this work, thermal transitions of polystyrene, PS, were studied as a function of molecular weight (M_w) and thermal history using steady state fluorescence of anthracene chemically bonded to the polymer chain ends. PS of three different molecular weights ($5\cdot10^3$, $2\cdot10^4$, $1\cdot10^5$) and polydispersity, $\Gamma(M_w/M_n)=1.03$ (SEC characterization using a calibration with PS standards) were synthesized by anionic polymerization with high vacuum techniques according to literature.[6] Subsequently, the polymer samples were labeled with the fluorescence anthracene moiety by an end-capping reaction using vinylanthracene. Fluorescence spectra of anthracene for each PS sample were collected as a function of temperature in cycles of heating and cooling at 0.5 °C/min and 5 °C/min.

Different photophysical parameters were selected in order to study thermal transitions in the PS samples: i) the average wavenumber of the fluorescence band, $\langle\nu\rangle$,[7] ii) the integrated intensity, I_{int},[5] iii) the intensity ratio of the second and third emission bands in the vibrational structured fluorescence spectra of anthracene, I_2/I_3, and iv) the intensity ratio of the low- to high-intensity changes (LHIC)[8] in the fluorescence spectra of anthracene. In every case, a sharp change in the photophysical parameter was observed at a certain temperature which is clearly different as a function of PS molecular weight and thermal history. In fact, very pronounced hysteresis phenomenon in the heating-cooling cycles was observed when the photophysical parameters are represented as a function of temperature and whose width varies with the thermal treatment rate. Finally, conventional DSC experiments were performed and their results were compared with those obtained by fluorescence.

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ROBUST OPTICAL MOLECULAR SENSOR ARRAY FOR ASTROBIOLOGY APPLICATION

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A key objective within astrobiology for future planetary lander missions is the detection of biomarkers – molecules whose presence indicates the existence of either current or extinct life. In general, past and current approaches to the challenge of biomarker detection have utilised or proposed spectroscopic and/or chromatographic instrument packages that have a number of features that limit their desirability including: (i) often complex molecular species cannot be identified unambiguously, (ii) detection of a limited range or class, of molecules for a given single instrument and (iii) mass and power consumption limitations.

Recent developments in the micro-fabrication of sensor arrays, micro-fluidics and micro-electromechanical systems, artificial molecular recognition systems (biomimetics) and methods to integrate the aforementioned components makes possible the realisation of a compact, robust artificial molecular-recognition sensor array for biomarkers, especially organic biomarkers, as an alternative to the approaches described above.

Molecular imprinted polymer (MIP) technology provides a path towards cheap and robust synthetic receptors that brings many advantages when compared with natural biological receptors. Indeed, robustness, cost, stability and regenerabiltiy are very often properties lacked by natural enzymes, protein or DNA when used for biosensing.

Optical evanescent wave sensing has been widely reported in the biosensor community. Briefly, an evanescent wave is created when a light beam irradiates an interface between two optically transparent media of different refractive index (RI) at a critical angle, impinging from the medium of higher RI. In this work we studied surface plasmon resonance (SPR), where the evanescent wave is created at a dielectric-metal interface and propagates along the metal's surface and penetrates some 100nm above the interface, into the immobilised biochemical sensitive layer. At a critical angle, the irradiating light is totally absorbed to create the surface plasmon, and this is observed as a sharp minimum in the intensity of the reflected light. This evanescent wave is very sensitive to changes in refractive index occurring at this region, and the critical angle will change accordingly, which allows for the precise monitoring of binding events occurring at the surface.

We describe the initial work on the integration of such biomimetic receptors as ultra-thin films (<100nm) for surface plasmon resonance (SPR) detection and fluorescent sensing towards the development of a final micro-array platform able to perform multi-analyte detection. We achieved spatial immobilisation of the MIP elements by two different approaches, later compared, namely ink-jet printing and removable photomasks. Following deposition of the MIP mixture (template dansyl-l-phenylalanine, functional monomers: methacrylic acid and 2-vinylpyridine, cross-linker: ethylene glycol dimethacrylate, in acetronitrile) the polymerisation was photoinitiated by exposing the array device to UV light and the resulting sensing device assessed.

SPR is a very sensitive detection system, usually applied to the detection of macromolecules (DNA, proteins, etc.). However the sensing of small organic molecules such as amino acids, essential in an astrobiology context, can be more challenging. We therefore also present results on new detection approaches based on dye displacement and derivatisation of target molecule for enhanced detection.

Finally, the robustness of such film exposed to simulated space environment (UV, gamma rays) and space mission specifications (acoustic vibration, sterilisation procedure) is presented.

ON-CHIP UV-VIS DETECTION USING LIQUID-CORE WAVEGUIDING WITHIN A 3-D ARCHITECTURE

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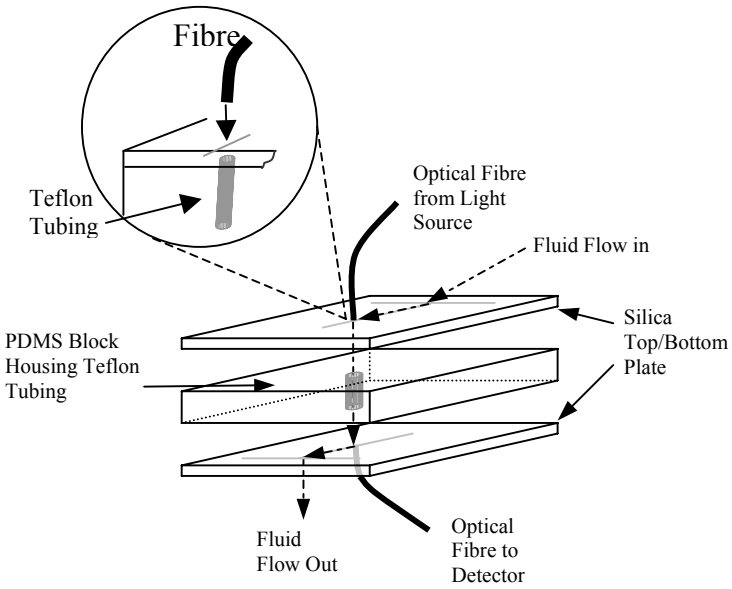
On-chip detection and measurement of analytes by UV-Vis. spectrophotometry has been found to be challenging, particularly with respect to the small sample pathlengths available in miniaturised systems. This paper details the application of liquid-core waveguides to a lab-on-a-chip based system, enabling an on-chip channel to be utilised as an optical waveguide and therefore increasing the pathlength of the on-chip detection system. In contrast to the majority of reported UV-Vis. detection systems for lab-on-a-chip applications where the detection pathlength is in the order 50-750 □m our device has a detection pathlength of 5 mm, configured in a 3-D architecture. The use of a three dimensional detection system that utilises the vertical plane of the chip as well as the horizontal plane, enables easier and more efficient coupling of the light source and detector to the chip.



The work reported in this paper demonstrates the use of a liquid-core waveguide in conjunction with three-dimensional chip architecture to aid the effective coupling of the light source into and out of the liquid-core waveguide and the extension of the sampling pathlength for absorbance measurements. This approach ensures the effective use of the probing light beam and increases the sensitivity and reproducibility of the on-chip measurement as evidenced by the on-chip spectrophotometric analysis of crystal violet. Results confirming the waveguiding effect and the subsequent spectrophotometric analysis of crystal violet producing a linear calibration with reproducibility (<2.4% RSD) and limits of detection (<1.3 μM), while maintaining a detection volume of ≤ 1μL, will be presented.

APPLICATION OF OPTICAL FIBRE SENSORS TO RESPIRATORY PLETHYSMOGRAPHY

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An array of long period gratings in a progressive three-layered fibre, working in conjunction with a derivative spectroscopy interrogation technique, is used to reconstruct the shape changes of a resuscitation manikin during simulated respiration.

Long period gratings (LPGs) are photoinduced fibre devices that couple light from the core of a single-mode optical fibre into the cladding at discrete wavelengths and thereby generate attenuation bands in the transmission spectrum of the optical fibre core. Over the last few years the fibre LPG has found applications in the field of sensing, through their sensitivity to changes in strain (□), temperature (T), the surrounding refractive index (n_s), and bend/shape, which induce corresponding changes in the spectral transmission profile.

We are developing a novel multiplexed sensor network based on LPGs, which is capable of monitoring the shape changes in the torso for monitoring of the human respiratory function. LPGs written into refractive index-insensitive, progressive three-layered fibre have been embedded into a supporting construct consisting of a carbon fibre ribbon encapsulated into a low temperature curing silicone rubber. The sensor has then been attached to a resuscitation-training manikin during simulated respiration. A derivative spectroscopy interrogation technique has been implemented, incorporating an SLED light source illuminating a series of in-line fibre Bragg gratings via a circulator, each attached to PZT extender to produce a series of wavelength-modulated sources.

This arrangement has been used to detect the curvatures experienced by the LPGs, and the data used to create a 3-dimensional model of the torso of the manikin, and its changes during respiration. The sensing/multiplexing scheme does not require an OSA, and has the potential to be constructed in a compact form for portable application. Data will be presented at the conference to show the effectiveness of the sensing/multiplexing scheme for shape reconstruction with low crosstalk between sensors.

THIN FILM SOL-GEL MATRIX FOR IMMOBILIZATION OF ACETYLCHOLINESTERASE AND CHROMOIONOPHORE FOR DETERMINATION OF PESTICIDE

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A thin film optical biosensor for the detection and determination of pesticide dichlorvos was developed. The sensing bioactive material was a two-layer membrane. A H^+-selective chromoionophore (ETH 5294) was used as an optical transducer (pH indicator) that was immobilized in the first layer of sol-gel matrix. The membrane was in contact with an outer sol-gel layer that immobilized acetylcholinesterase (AChE). AChE biosensor based on pH indicator as colorimetric optical transducer was utilized as immobilized in sol-gel,[1] covalently immobilized on porous glass[2] and immobilized on aminopropyl glass.[3] But, none of them was using the same matrix as for both immobilizations. The sensor operated in a static mode at room temperature and the percentage changes of absorbance for the immobilized ETH 5294 served as an analytical signal. The pH response range was obtained with linear response between pH 5 – 10 (r^2 = 0.9834) for the immobilized ETH 5294 in first layer. The calibration curve was obtained for substrate acetylcholine chloride (AChCl), with linear range between 60 – 90 mM and the AChCl alone didn't have effect to biosensor. Optimization of the biosensor response time towards AChCl was done and the biosensor showed an optimum incubation time at 15 minutes. Inhibition plots obtained for organophosphate (dichlorovos) pesticide exhibited linear ranges 1 – 10 ppm. The detection limit of the method was 1 ppm.

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INTEGRATED OPTICAL REFRACTOMETER WITH A DIRECT DIGITAL OUTPUT

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An integrated optical refractometer with a simple direct digital readout based on Generalized Mach-Zehnder interferometer (GMZI) is proposed and numerically analyzed. GMZI is multiple arms-multiple outputs interferometer, widely used in optical telecommunications for wavelength division multiplexing[1] (WDM) and optical switching, that with a suitable design, permits to realize highly sensitive, and wide measurement range, integrated optical refractometer useful in chemical and biochemical sensing.[2] Figure 1 shows a schematic representation of the sensor. It is composed of 1x2 power splitter and two GMZI. Each GMZI consists of two multimode interference (MMI) couplers connected by a waveguide array. The first MMI coupler is operating as 1×N power splitter, dividing the input power equally in the N monomode waveguides of the array. The waveguides represent the sensing arms of the interferometer, and the sensing pads are obtained by removing the upper claddings of the waveguides. The second MMI coupler is an N×N combiner and is the key element of the GMZI. This MMI distributes the power incident at each of its inputs to all the outputs where interference occurs between the relatively delayed optical fields. By suitably choosing the lengths of the sensing pads, it is possible to achieve constructive interference for N different refractive index values of the external liquid n_o, at different outputs of the combiner. So, as the refractive index of the external medium changes, the output intensity moves through the different outputs. In particular, if each single output is connected to a comparator with a reference level of about 0.41, it happens that, for each value of the external medium refractive index, only one output is at high level. The resolution obtained using a single GMZI is about $\Delta n/(2N)$, where $\Delta n = n_{max} - n_{min}$ is the refractive index measurement range. So, if N assumes typical values as 8, 10, etc… only low resolution can be obtained when wide measurement range is required. This problem can be avoided using two GMZI. The first (GMZI1) has a measurement range Δn whereas the second (GMZI2) has a measurement range $\Delta n/N = (n_{min}+\Delta n/N) - n_{min}$. The GMZI2 permits to obtain a resolution of $\Delta n/(2N)$,[2] but with a periodic response of period $\Delta n/N$. However, this ambiguity is removed by the GMZI 1. In fact, in each period only one output of the GMZI1 is high. In figure 1 the digital sensor response against external medium refractive index n_o of the sensor is depicted for N=8, n_{min}=1.3300 and n_{max}=1.3822. With the thick line are reported the outputs of the GMZI1 whereas with thin lines are reported the outputs of the GMZI2. The resolution is about 3e-4. As an example for n_o= 1.3569±3e-4 (see arrow) the outputs at high level are the number 8 and the number 16.

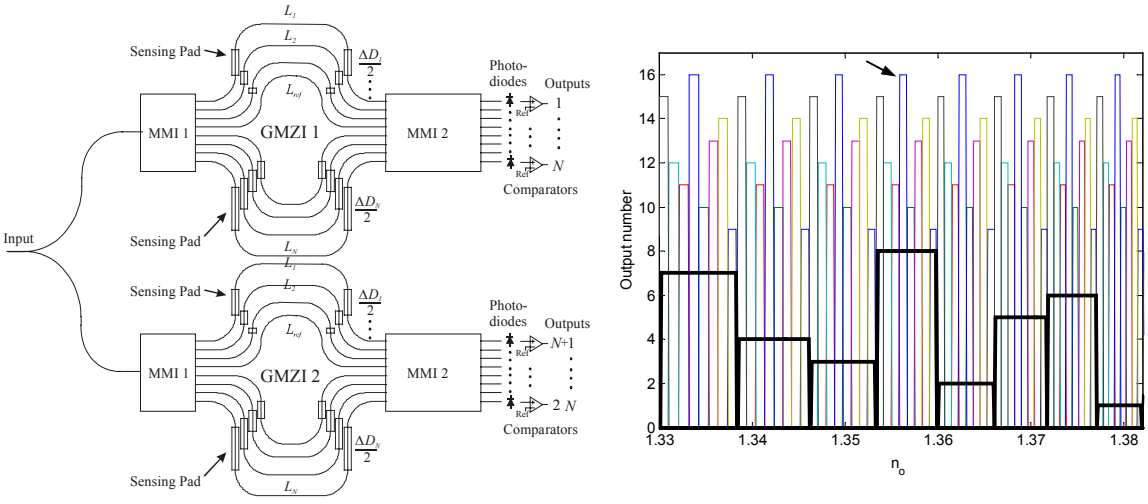

Figure 1. Schematic view of the GMZI sensor (left) and sensor output (right).

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EVANESCENT WAVE IMMUNOSENSOR FOR THE DETERMINATION OF THE PESTICIDE TRICLOPYR

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The immobilization of Triclopyr ([3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) antibody in the core of an optical fibre (plastic clad silica, core diameter 200 □m and clad 400 □m, numerical aperture 0.66) allowes an optical surface sensing. The evanescent wave generated by by total internal reflexion can excite fluorescent labelled antigen (fluorescein labelled Triclopyr).

Polysterene layers ware made by means of dip-coating from solutions of 7% polymer (w/w) in tetrahydrofuran.[1] Polystirene coated fibres were immersed in a solution of Triclopyr polyclonal antibody[2] in phosphate buffer pH 6.8.

The fibre containing the immobilized antibody was exposed to antigen-labelled and sample containing Tryclopyr. The signal, fluorescence intensity, was monitored and plotted against concentration of sample.

Instrumental settings were accomplished by using modular components of an Aminco SLM-48000S spectrofluorimeter, provided by a 450 W Xenon lamp, monochromator (λ=485 nm) and light beam sinusoidally modulated by a Pockel cell. Evanescent wave generated in the treated fibre were collected in a photomultiplier and separated of the background signal.

Keywords: Evanescent wave; immunoassay, Triclopyr

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LIQUID SENSOR BASED ON HOLLOW CORE ANTIRESONANT REFLECTING OPTICAL WAVEGUIDE

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In this work, we present a novel integrated optical refractometric sensor, based on a hollow core ARROW (AntiResonant Reflecting Optical Waveguide) waveguide.[1] The key feature of the proposed sensor is the use of high-order optical interference, differently from conventional refractometers like Mach-Zehnder interferometers. The main characteristic is that an analyte refractive index variation causes a shift in the resonance wavelength of the light.

The hollow core ARROW (AntiResonant Reflecting Optical Waveguide) waveguide is composed by two halves joined by silicon-to-silicon direct wafer bonding (Figure 1 left). One of the two halves of the waveguide is realized by anisotropic etching of <110> silicon wafers. Etching results in a square cross-section. On both the halves, two dielectric layers of silicon nitride and silicon dioxide are deposited on them. The light is confined inside the core region, where the refractive index is lower than the one in the surrounding media, by the two cladding layers designed to form a Fabry-Perot antiresonant cavity.

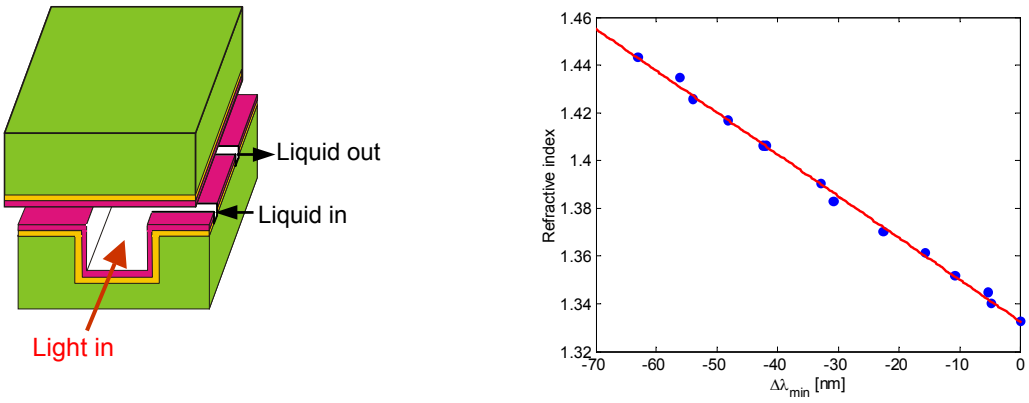

Figure 1. View of the proposed sensor (left) and experimental measurements (right).

Since this waveguide can confine the light in the fluid (air, gases and liquid) used as core, it can be employed as a sensor where, due to the antiresonant confinement of the light, minute changes of the fluid filling the core, in terms of its refractive index, will result in a variation of the transmitted spectra of the light. In fact, the waveguide transmittance has a minimum when the first cladding optical thickness is at half-wavelength. Using a one dimensional model, the transmittance minimum is $\lambda_{\min} = 2d_1\sqrt{n_1^2 - n_{eff}^2}$, where n_1 and d_1 are the refractive index and the thickness of the first cladding layer respectively, and n_{eff} is the effective refractive index of the fundamental mode (given by $n_{eff} = n_c\sqrt{1-(\lambda/2n_c d_c)}$, with n_c and d_c the refractive index and the thickness of the core).

Experimental characterization of the sensor confirms the 1-D and 2-D numerical analysis. The measurements have been performed with a 2cm long hollow waveguide filled with different acqueos liquids (see Figure 1 right). A comparison of the trasmitted spectra (detected by a CCD Spectrometer) shows a linear downshift of the minimum as the refractive index of the core (i.e. the liquid under analysis) increases. In particular, experimental results show a resolution of $1.8\cdot10^{-3}$ nm^{-1}.

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FABRICATION OF AN OPTODE FOR CAPSAICIN DETERMINATION

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Capsaicinoids are group of pungent compounds found mostly in capsicum fruits, the structure of which is acid amides of vanillyamine and C_9-C_{11} branched chain fatty acid.[1] Of these groups, capsaicin and dihydrocapsaicin are major component of most capsicum species and have been considered as a major indicator of the chilli product qualities. An accurate determination of the level of the capsaicin has become important because of the increasing demand by consumer for food, and increasing use in pharmaceutical.[2] The sensor development for capsaicin determination, which involved an electrochemical method, has been report before this.[3] In this research, a novel optodes for capsaicin determination using a Gibbs reagent immobilized on XAD-2 copolymer based on reflectance spectrophotometer has been fabricated. The sensor response was found regenerable by using buffer pH 2 solution as regenerating solution. The sensing materials have a response time of 2 minutes and optimum response was obtained at pH 11. The sensor produced a linear response for capsaicin concentration in the range of 0 -120 ppm with detection limit of 1.09 ppm. The calculated relative standard deviation (RSD) of the repeatability and reproducibility of the method are 2% and 4% respectively. An established method (HPLC) was used to validate the fabricated sensor.

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DIFFUSE REFLECTANCE ANALYSIS OF SKIN LESIONS

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Differential clinical diagnosis of pigmented skin lesions may be a difficult problem, even for experienced clinicians. One of the initiatives toward the development of a more reliable dermatological diagnosis tool is the application of spectroscopy related techniques to study several skin pathologies.[1,2,3] Among these techniques we consider the diffuse reflectance spectroscopy,[2] also called elastic scattering spectroscopy,[3] which has the potential to provide spectra from the studied tissues containing information about their morphology and composition. It is based on the evidence that the presence of malignant cells should somehow alter the optical characteristics of epidermis with respect to a healthy one.

In this work we present the results of optical diffuse reflectance measurements performed on different healthy skins and skin lesions. The study was performed on 40 patients with the skin pigmented lesions described in the Table 1, using a general purpose portable system. The measurement system consists of a portable visible near infrared spectrometer (550-1100 nm), a tungsten-halogen lamp and fibre optics reflection probes, adapted for the use in this study.

Table 1. Lesions studied, listed by histological type.

Histological diagnosis	No. of cases	No. of spectra
Intradermal naevus	11	23
Compound naevus	11	34
Lentigo	8	18
Sebhorrhoeic keratosis	7	1
Sebhorrhoeic pigmented keratosis	2	4
Junctional naevus	2	3
Spitz naevus	1	2
Melanoma	1	4

The reflectance spectra were collected for all patients and lesions according to the next procedure: healthy skin in the inside and the outside upper arm, the lesion itself, and skin surrounding the lesion. Every spectrum was obtained from the lesion centre, but in some cases, following the guidelines of the dermatologist, we also measured different points inside the lesion, which presented morphological or colour disparities. Since the reflectance spectra were displayed in real time, it allowed for accurate positioning of the probe on the lesions. Once the displayed spectra were stable, 40 spectra for each lesion were sampled (representing a compromise between noise reduction and maximal sampling time), and their average spectrum was stored for subsequent analysis.

The spectra resulted after data processing show in-homogeneities among lesion groups classified by histology. Also we observe differences among the same kind of lesion, which could indicate variations in morphological structure of the tissues. A statistical study was carried out to analyze the system capability for improving the sensitivity and the specificity with respect to the dermatologist criteria. These results contribute to the development of a specific sensory system for the clinical diagnosis of melanocytic lesions in vivo.

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ACCURATE SALICYLIC ACID (SA) SENSING AT LOW VISIBLE WAVELENGTH USING ARTIFICIAL NEURAL NETWORK (ANN)

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Optical fibre salicylic acid (SA) sensor is fabricated based on immobilisation of copper(II) acetate on Dowex-50x8. The reflectance spectra of the sensor were measured by using an optical fibre spectrophotometer. A back-propagation (BP) Artificial Neural Network (ANN) was used to analyse the response of the sensor developed. There are more and more researchers trained and optimised their sensors with ANN recently.[1-3] The results showed this SA sensor has dynamic response from 0.02 – 0.30 g/L. It has a maximum absorption at 400 nm and stable for more than 50 hours. A network with 10 hidden neurons gave the best result and scores a correlation and summation of squared error (SSE) skill of 0.9994 and 1.2006 x 10^{-4} g/L respectively. It was tremendously accurate in predicting the response of the optical fibre ASA sensor by giving low average predicted error (1.5400 x 10^{-3} g/L).

Acknowledgements. Scholarship of National Science Fellowship (NSF) towards Han Chern Loh from the Ministry of Science, Technology and Environment (MOSTE), Malaysia is greatly acknowledged.

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A NOVEL SALICYLIC ACID (SA) OPTICAL FIBRE SENSOR FABRICATION

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Optical fibre salicylic acid (SA) sensor is fabricated based on immobilisation of ferric(II) nitrate on Dowex-50x8. SA form a stable purple complex with ferric(II) nitrate at pH 2.45.[1-3] The reflectance spectra of the sensor were measured by using an optical fibre spectrophotometer. The results showed this SA sensor has a maximum absorption at 526 nm. The useful dynamic response is range from 0.01 to 2.00 g L^{-1}. It is stable for more than 50 hours.

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THE DETERMINATION OF THIN BIO-MOLECULAR FILM STRUCTURE AT HIGH RESOLUTION USING DUAL POLARISATION INTERFEROMETRY

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A novel method for the analysis of thin biological films, called dual polarisation interferometry (DPI), is described.[1] This high resolution (<0.01 nm), lab-based technique allows the thickness and refractive index (density) of biological molecules adsorbing or reacting at the solid-liquid interface to be measured in real time (up to 10 measurements per second).

The immobilisation of antibodies at the sensor surface by a number of different methods has been followed and the surface structure determined. The layer structure was found to vary according to the immobilisation and blocking protocol used, and a clear link between antibody activity and surface structure found.

Specifically immobilised antibodies were found to form relatively thick layers, approaching the antibody length and consistent with a more upright orientation. These also showed higher activity than layers that were chemically (non-specifically) cross-linked or physisorbed to the surface and which formed thinner layers.

Whilst likely relationships between immobilisation and blocking protocols and surface structure can generally be proposed, the utility of the DPI technique for studying protein interactions at a sensor surface is demonstrated in the ability to determine in each instance what the surface structure actually is. This removes ambiguity from the measurement and provides a clearer understanding of the likely processes occuring.

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HYBRID KNOWLEDGE REPRESENTATON (HKR) AS A NOVEL SOFTWARE SENSOR FOR SALICYLIC ACID (SA) DETERMINATION

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This paper focuses on the development of a hybrid knowledge representation (HKR) as a software sensor or smart sensing system in salicylic acid (SA) determination. The sensor that developed was an optical fibre sensor and ferric(II) nitrate was used as a colour developer. Recently, many researchers[1,2] have turned their attention to software sensors since these sensors can either replace the hardware sensors or be used in parallel with them to verify whether the hardware sensor are drifting and provide redundancy. Some researchers even trained and optimised their sensors with Artificial Neural Network (ANN),[3-5] a type of knowledge representation. Hybrid method has high potential to be used for representing knowledge in knowledge-based systems. It is based on the combination of a few popular methods used nowadays, i.e. genetic algorithm, frame-based, rule-based and uncertainty factors. A hybrid approach is more preferred than other singular approaches as it has more advantages. The implement of this approach in sensor system will be discussed.

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EXPERIMENTAL SENSING OF CO_2 AND CH_4 GASES USING THE COSM CORRELATION SPECTROSCOPY METHOD AND COMPARISON WITH SIMULATED PREDICTIONS FROM THE HITRAN DATABASE

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This paper describes the first experimental results on the detection of carbon dioxide and methane gases using the complementary source modulation (CoSM) method, the topology of which is shown in Figure 1. We also compare the results on each gas with theoretical simulations, using gas absorption data derived from the HITRAN database. The CoSM method[1,2] involves complementary modulation of two light sources, light from one of which is passed through a reference cell of the gas to be measured (target gas) before combining it with a fraction of the light derived directly from the other source. The light intensities of each beam component in the combined beam are equalised and then the beam is then used to probe the measurement cell for the target gas. This restores an intensity modulation in the combined beam if the target gas is present in the measurement cell. The method is self-referenced against intensity changes and has advantages of high selectivity without the problems of speckle interferometry associated with laser sources.

There is a particular interest in such sensors, which can use optical fibres coupled to a passively coupled sensing head. CO_2 and CH_4 are important gases which have reasonably strong absorption bands in the 2.0μm and 2.3μm spectral regions respectively. That of methane in the 2.3μm band is less suited for propagation over fibre lengths greater than a few metres, so the somewhat weaker absorption band at 1.6μm is more suited to sensing over extended fibre networks. Experimental detection results for these gases, using the CoSM method will be presented for the first time, and comparisons made with the theoretically predicted values for the modulation index, signal/noise ratio and detection sensitivity. In our measurements, we will examine the use of compact near-IR LED sources, at wavelengths compatible with fibre remoted operation, but for the theoretical results we will also consider the performance that might be achieved with free-space optics, without the light losses associated with coupling in and out of optical fibres.

Figure 1: Schematic of a fibre compatible implementation of a CoSM correlation spectroscopy system.

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DEVELOPMENT OF FIBER OPTIC HYDROGEN SENSORS FOR TESTING NUCLEAR WASTE REPOSITORIES

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We developed sensors to supervise the hydrogen concentration close to the high radioactive wastes deposited in the underground laboratories of HADES (SCK-CEN Mol Research Centre in Belgium), in the context of the European project "Safety and Operational Monitoring of Nuclear Waste Repositories with Fiber Optic Sensing Systems (SOMOS)". In the environment of these deposits the concentration of hydrogen much be keep between 0-4%. Low concentration levels are required to know corrosion problems of canister and high levels mark the deflagration threshold of the ternary mixture "H_2-H_2O-air". At 250 meters depth, the temperature is between 20 and 70 ºC, the humidity is about 100% (clay), and the sensors should work for periods of long time.

Study of three different sensors has been carried out:

A: The own optical fiber as sensor element. The spectral absorption of the optical fiber imbibed in H_2 2% concentration, has been studied from 1150 to 1640 nm wavelengths. Several wavelengths suffer attenuation for H_2, being the 1245 nm absorption line very effective in order to evaluate the hydrogen level. Figure 1a shows the time dependence of the attenuation produced by H_2 2% concentration in 4 km long fiber at 1245 nm wavelength. The attenuation due to H_2 in fiber is complete reversible, but the OH attenuation increasing (1390 nm) is irreversible. Wavelength of 1315 nm is not sensitive for low level of H_2 and is suitable to be used as reference wavelength.

B: Pd semitransparent film sensors. In this case a 18 nm Pd thickness film has been deposited on a SiO_2 substrate. Figure 1b shows transmittance changes of 5% for 1.4% H_2 concentration. The sensibility of this sensor is independent of the wavelength in a wide range of wavelengths (1200 to 1600 nm), showing similar time constant and sensitivity for lasers of 1300 and 1550 nm.

C: Pd-coated fiber Bragg gratings (FBG). When a Pd film absorbs hydrogen it expands because Pd converts to PdH_X which has larger volume. When FBG Pd coated absorbs H_2, the mechanical expansion stretches the fiber and causes a change in the period of the FBG. In order to enhance the stress produced by the H_2 absorption low diameter FBG must be used (20–30 μm), that we getting etching the FBG in 48% HF solution. Figure 1c shows the response of the sensor for different H_2 concentrations levels.

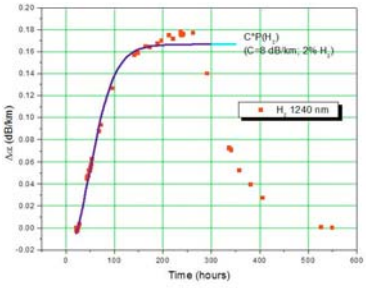

Figure 1a. Time dependence of attenuation coefficient in a fiber for 2% H_2 concentration at 1245 nm wavelength.

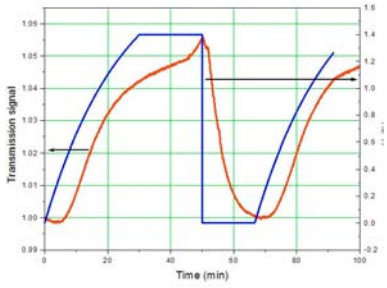

Figure 1b. Transmittance time dependence of a 18 nm Pd film against the H_2 concentration

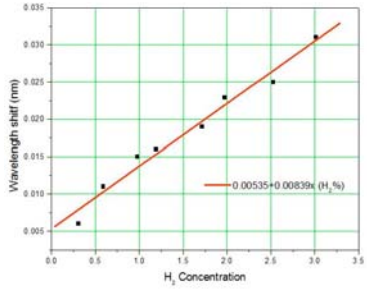

Figure 1c. Wavelength variation of a Pd-coated FBG against H_2 concentration

Details of fabrication techniques, setups used, the most relevant results and conclusions will be presented.

Acknowledgements. This work is supported by the EU project SOMOS (FIS5-2001-00106).

SPECTRAL NEPHELOMETRY FOR THE GEOGRAPHIC CLASSIFICATION OF ITALIAN EXTRA VIRGIN OLIVE OILS

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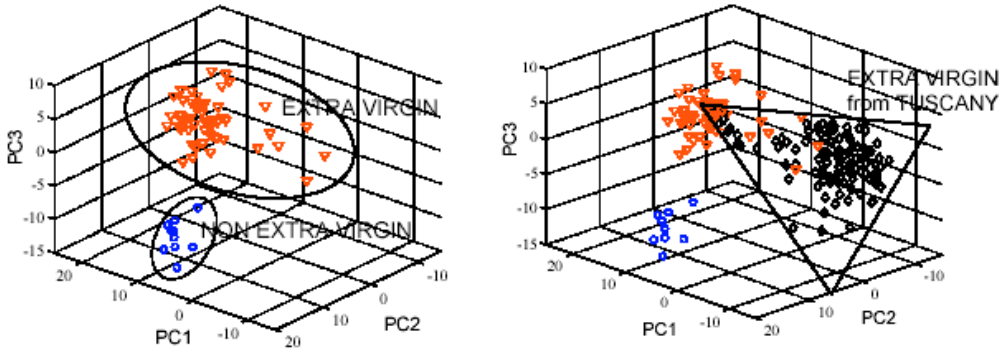
The taste and fue appearance of extra virgin olive oil is influenced by meteorological conditions and by harvesting methods, fue latter being related to local traditions. The extra virgin olive oil produced in Italy is known worldwide not only for its characteristic taste, but also for the distinctive nutritional benefits deriving from it. such as low fat and high anti-oxiding contento Consequently, methods and technologies for fue qualification and certification of Italian oils are envisaged, for the protection of both producers and consumers.

Absorption spectroscopy and multi-angle scattering measurements performed in the visible spectral range, namely "spectral nephelometry", is a novel optical technique that provides combined monitoring of color and turbidity. It has been recently used for characterizing edible oils, especially for clustering different categories of oils such as non-extra virgin, extra virgin and single-cultivar.[1]

The absorption spectra of the oil sample at different angles are measured. Four white light sources are used, which span fue 450-630 nm spectral range. A miniaturized optical fiber spectrometer serves as detector. The sources, positioned at 0°, 30°, 60° and 90° with respect to fue detector, are sequentiaUy switched on to measure, in addition to fue transmitted spectrum, fue scattered spectra at fue given angles. Spectral data are processed by means of fue Principal Component Analysis (PCA), thus providing coordinates that identify fue oil sample in a 2D or 3D map, namely fue "nephelometric map". Consequently, when many samples of oils are analyzed, fue map is populated by points, each of them representing fue individual identity of an oil, as far as its color and turbidity are concemed.



The new concept of colour and turbidity measurements together with PCA data processing made it possible to discriminate and cluster different categories of edible oils, also taking into account fue geographic area of origino Many samples of extra virgin olive oils from fue ltalian regions ofTuscany and Calabria were spectrally analyzed and PCA processed. Their distinctive nephelometric coordinates were inserted in a wider nephelometric 3D map including extra virgin olive oils coming from elsewhere, as well as non-extra virgin oils. The Figure above shows fue nephelometric 3D maps in fue PCI-PC2-PC3 subspace, exhibiting sharply defined clouds that are capable of clustering fue oils from Tuscany with respect to fue other groups. Similar maps can be obtained by considering fue oils from Calabria.

Acknowledgements. The financial support of EC project 'OPTIMO' (contract #SMT4-CT97-2157), ofRegione Toscana project 'CARABIOTEC', and of A.R.S.I.A. is acknowledged.

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DISSOLVED OXYGEN SENSERS FOR CONTAMINATED AND AGGRESSIVE AQUEOUS ENVIRONMENTS

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Dissolved oxygen (DO) sensors which will operate for prolonged periods in hostile, contaminated and corrosive environments are in considerable demand for industrial and environmental applications. Conventional electrochemical (Clark electrode) detectors are not suitable for such environments since they are easily poisoned by sample constituents such as metal ions, proteins, oxidants and reductants. Consequently, considerable effort has been expended in the development of robust optical sensors based on oxygen sensitive luminescent dyes encapsulated in protective chemically resistant sol-gels or polymers. Besides being inert, suitable matrices must be able to dissolve the dye, be permeable to oxygen and impermeable to liquid water, and dissolved contaminating species which interfere with the operation of the sensor.

Luminescent quenching of organic fluorophores isolated in chemically inert matrices can form the basis of DO detectors due to their fast response, high sensitivity and specificity.[1] Unlike electrochemical detectors, they are less easily poisoned. Most of these sensors have been based on transition-metal complexes trapped in polymer or sol-gel matrices.[2]

In many respects, highly fluorinated polymers are ideal matrices for organic fluorophores. They are chemically inert, permeable to oxygen and since they are highly hydrophobic offer protection from many charged species which may poison the dye. Unfortunately, many highly fluorinated organic polymers are insoluble in common solvents rendering them unsuitable for fabricating thin film sensors. Some fluorinated polymers, however, such as Nafion and copolymers of terafluoroethylene, vinylidine fluoride and propylene, are soluble in suitable solvents. These materials were utilised in this work.

Some fluorinated polymers however, although inert are relatively soft and cannot withstand abrasive aqueous environments for extended periods. Also, due to low glass transition temperatures, the dye molecules tend to form aggregates at elevated temperatures and during the film deposition process in some systems. To address these problems silica sol-gel / fluoropolymer composite films were investigated as possible dye hosts. It was also hoped that these composites will reduce or eliminate some of the problems frequently encountered with pure silica sol-gels such as cracking and loss of transparency, at the same time retaining some of the key advantages of the fluoropolymer matrix. A series of composites were prepared and evaluated as dissolved oxygen sensors with several oxygen sensitive phosphorescent dyes. The contribution of dynamic and static triplet-state quenching to the Stern-Volmer curve was also studied.[3] Finally, the suitability of these composite systems for constructing thin film optical sensors for operation in contaminated and corrosive aqueous environments was evaluated.

Acknowledgments. The authors are pleased to acknowledge a grant to RNG from Bell College Research Grants Committee in support of the Joint Optical Sensor Programme.

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OPTICAL CHEMICAL HEAVY METAL ION SENSING VIA SOL-GEL DOPED INDICATOR CALCEIN

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The importance of continuous monitoring and controlling of heavy metals in aquatic samples is unquestionable. It is already clear which harmful effects have these metals on the environment and living organisms when present in high concentrations.

The growing activity in the field of ion-sensitive optical devices and related optical methods for trace analysis of heavy metal ions has resulted in numerous sensing schemes, new indicator dyes, and in highly diversified methods of immobilization. Both, the method of immobilization and the type of polymer matrix exert a significant effect on the performance of ion-sensitive layers. The proper choice of a polymer matrix for an ion-sensitive membrane layer is governed by parameters like permeability for the analyte, mechanical stability, and suitability for dye immobilization.[1]

The sol-gel technique offers a low-temperature method for synthesizing amorphous materials which are either totally inorganic in nature or composed of inorganic and organic. The process is based on hydrolysis and condensation reactions of organometallic compounds in alcoholic solutions. The most widely investigated system involves silica-based glasses which are prepared by polymerization of a silicon alkoxide, $Si(OR)_4$.

The ability to synthesize inorganic polymers using sol-gel processing with little or no heating makes it possible to dope these gels with a variety of organic and organometallic molecules especially dyes having desired luminescent properties. More recently, sol-gel glasses have gained interest as a matrix for chemically sensitive optical materials because of their optical transparency, mechanical stability, chemical inertness, and flexibility in terms of shaping sensor configurations. Hence, sol-gels have been extensively studied with respect to their application to chemical sensing of analytes such as pH, metal ions, inorganic anions, glucose, oxygen and ammonia.[2] The sol-gel can be cast as monoliths, coated as thin films on slides and fibres, or ground into powder. This allows various configurations of sensing elements.[3]

Up to now, several sensing schemes for heavy metals have been presented that use fluorescence measurements. Among different fluorophors used in sensors systems also calcein, a fluorescein derivative, is well known indicator. Its fluorescence is usually quenched by complexation with various metal ions and at various pH values. Here we present a calcein doped sol-gel sensing scheme for heavy metals like Cu^{2+}, Co^{2+} and Ni^{2+}. Acid catalysed sol-gel process and tetramethoxysilane precusor (TMOS) were used. Sensing layers were prepared using very fine-triturated sol-gel monoliths. Calcein in solution shows excitation/emission maxima at 480/507 nm, whereas calcein immobilized in sol-gel matrix shows excitation/emission maxima at longer wavelegths (494/515 nm). Sensing system shows high selectivity and reversible response for all tested ions.

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DOUBLE GRATING WAVEGUIDE STRUCTURES: 350-FOLD ENHANCEMENT OF TWO-PHOTON FLUORESCENCE APPLYING ULTRASHORT PULSES

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Double grating waveguide structures (DGWS) have a planar multilayer configuration comprising a substrate and a thin waveguide layer with one grating under and one on top of the waveguide. Since new industrial fabrication strategies take advantage of writing the grating into the substrate material and preserving the structured surface during the coating process of the waveguide layer, these devices have recently become more and more important as sensor chips in biotechnology applications such as fluorescence-based microarray platforms. For resonance conditions, i.e. specific wavelength, polarisation and angular orientation of the incident beam, DGWS show destructive interference of the subsequently diffracted, guided and rediffracted incident beam with the directly transmitted beam, while most of the incident light is reflected. This guided mode phenomenon is characterised by a large field enhancement at the waveguide surface inducing large fluorescent signals of, for example, biomolecule markers or proteins.[1]

The spectral as well as the angular bandwidth of the resonances strongly depend on the gratings, leading to broader resonances with increasing grating depth. Specifically, the resonance bandwith of a DGWS can be assumed as the sum of the resonance bandwidths that are caused by two single gratings, one on top and one under the same waveguide layer.[2] As a consequence, resonant DGWS have broader spectral bandwidths and, therefore, are perfectly qualified for the excitation of two-photon flourescence (TPF) applying broadband ultrashort pulses for which high coupling efficiencies can only be achieved by comparably broad resonance bandwidths.

We report a 350-fold enhancement of TPF obtained with a DGWS on which Rhodamine B (5 µl of a 10^{-6} M solution) was deposited. After evaporation of the solvent ethanol (providing a monolayer of immobilised dye molecules at the surface), the DGWS was illuminated by a mode-locked Ti:sapphire laser with 800 nm pulses of around 100 fs. The enhanced TPF signal was recorded under the resonance angle for TE polarisation by a CCD camera, equipped with IR blocking filters and an objective. These results are documented in Figure 1 as a function of incident laser power and compared to measurements under the same experimental conditions for TM polariston, *i.e.* out of resonance. The logarithmic representation in Figure 1 demonstrates the enormous enhancement of more than two orders of magnitude of the nonlinear TPF signal induced by the evanescent field of the resonant DGWS.

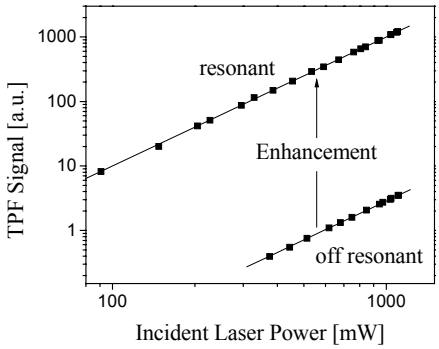

Figure 1. Two photon fluorescence signal of Rhodamine B deposed at a DGWS under resonance (TE polarisation) and out of resonance conditions (TM polarisation) for the incident 800 nm fs-pulses.

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FLUORESCENCE LIFETIME-BASED SENSOR DEVICE FOR MEASURING OXYGEN CONCENTRATION IN HOT FLUE GAS

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Heating systems for private houses and larger facilities contribute considerably to air pollution. Many countries therefore apply strict regulations to flue gases emitted from heating systems. So far compliance with these regulations is monitored in periodic intervals, but it is more and more accepted that continuous control is necessary. As a key parameter for the combustion process serves the excess air ratio (air flow/stochiometric air flow), that can be determined from the oxygen concentration in the flue gas.

Flue gas temperatures are between 50 °C and 90 °C for condensing boilers, and for non condensing boilers between 170 °C and 250°C. In the flue gas we have the presence of CO < 60 mg/kWh (nearly 50 ppm at 3 % O_2), NO_x < 80 mg/kWh (nearly 40 ppm at 3 % O_2), HC < 2 ppm.

The burners have to meet European standards EN676 for gas and EN267 for oil. For methane (EN676), maximum permitted excess air ratio is between 1.2 and 1.5. An excess air ratio of 1.2, correspond to 3.8% O_2 in the flue gas. An efficient combustion process has normally a CO_2 value of 10.5% (equivalent to an O_2 value of 2.1%). Higher CO_2 values are dangerous due to a possible strong CO emission increase. When it is difficult to control the air flow, it is generally preferred to work at a CO_2 value of 9.5% (equivalent to an O_2 value of 4%). The presence of an oxygen sensor permits to work at higher CO_2 values (higher efficiencies). Therefore, the oxygen sensor must have the following characteristics: oxygen range from 0% to 5% O_2 in the flue gas, accuracy ± 0.5% O_2.

The sensor may be serviced or exchanged only at the regular service intervals (typically once per year), and no calibration or other kind of maintenance is allowed in between. That means that the sensor must fulfill the accuracy specifications under the above-mentioned special conditions for more than twelve months.

For a commercial application, an especially stringent condition is the price: The retail price of the sensor element should not exceed € 20, the complete sensor device (including electronics and display) should cost about € 100.

A sensor device meeting the above requirements has been developed. To achieve the necessary long-term stability, a fluorescence lifetime-based sensing scheme was applied. With a ruthenium complex as the indicator and a carefully selected polymer matrix a sensor for the range up to 90 °C could be implemented. At a temperature of 80 °C the fluorescence decay time of our sensor dye changed by about 50 ns per % oxygen. The noise level was ± 8 ns corresponding to ± 0.2 % oxygen. No cross sensitivity to CO, NO_x, or HC within the specified ranges was present. A long-term study is under way.

High-temperature investigations proved that Ru complexes may be used even up to 250 °C, but the decrease in fluorescence lifetime with temperature presents some problem. More difficulties, however, result from the changing properties of the matrix that not only concern oxygen permeability and solubility, but also quantum efficiency and fluorescence lifetime of the indicator.

FIBER OPTIC SENSOR WITH LIQUID CORE FOR CHEMICAL TRACE ANALYSIS

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Liquid core optical fiber wave-guides are capillaries that contain a liquid core – liquid sample for spectroscopic analysis. The technique presented in this work can be used to markedly extend the detection capabilities of many existing solution. Based measurements obtained via absorbance spectroscopy. The sensitivity of absorbance spectroscopy can be improved by extending optical path length. Liquid core wave-guides provide for long optical path lengths by constraining light propagation within a liquid medium, which has a higher refractive index than the surrounding solid tubing.[1] Since total internal reflection occurs only if the refractive index of the core is greater than that of the capillary tubing, conventional glass and fused silica wave-guides are limited to use with high refractive index liquids such as aromatics, carbon disulfide, and various halogenated compounds.[2] We present a simple optic method that allows extending the sensitivity of conventional spectroscopic measurement. The analytical apparatus required for this analysis is very simple and robust. Benefit of this solution is possibility to use of small volume samples, long path lengths by constraining light propagation within a liquid medium which has a higher refractive index than the Teflon AF tubing, sensitivity increase of conventional absorbance spectroscopy by two or more orders of magnitude. The refractive index of Teflon AF 2400 is lower than that of virtually all standard temperature and pressure liquids, and it is near theoretical minimum for organic polymer predicted by Groh and Zimmerman.[3] No preconcentration is required in chemical analysis. The analytical procedures employed in long path length absorbance spectroscopy are amenable to miniaturization and autonomous operation. In the present paper we describe a liquid core optical fiber based on tubing made entirely of Teflon AF 2400. We show that this device can transmit visible light with low loss when filled with low refractive index liquid. Attenuation at 632.8 nm versus effective liquid core fiber length was used for optical characterization of this type of fiber wave-guides. In absorbation spectroscopy, loss is equivalent to baseline attenuation and therefore governs the maximum length of fiber that can be used. Inherent wave-guide loss is equal to approximately 6 dB/m for 288/481 μm capillary filled with water, and 14 dB/m for 478/674 μm Teflon AF capillary. We believe that liquid core Teflon AF fibers offer significant benefit in diverse spectroscopic applications.

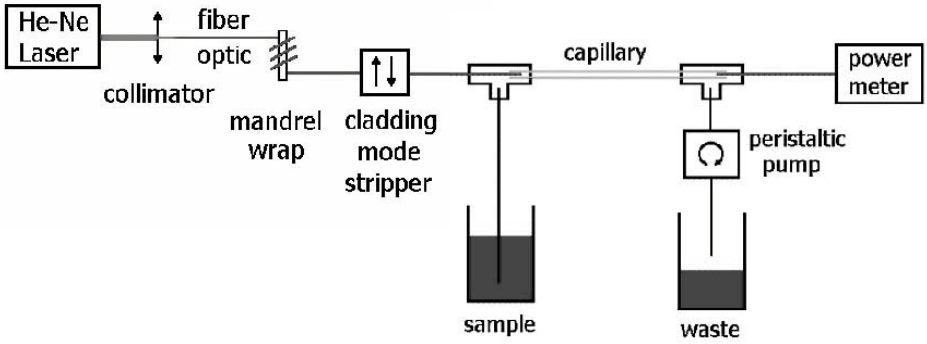

Figure 1. Overview of experimental setup

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USING OPTICAL SENSOR NIR FOR ON-LINE VIRGIN OLIVE OILS CHARACTERIZATION

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Near infrared transmittance spectroscopy was applied to on-line control quality and characterization of virgin olive oils.

The transmittance spectrum (log 1/T) were obtained through a dispersal equipment, scanning the samples between 750-2500. A 1mm optical pass flux tray and holding 120 ul was employed.

The samples are set in the tray with the help of a peristaltic pump. This system allows to the analysing equipment to be adapted to the virgin olive oil process line easily, since with the help of the pump a by-pass is created, which pumps the oil sample up from the main oil current and gives it back once it has been scanned.

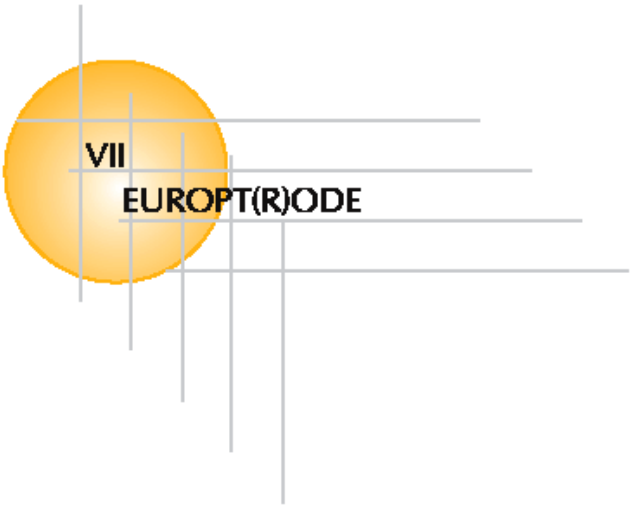
Calibrations models for: acidity value (AV), bitter taste (K225) and fatty acid composition (FAME), were previously developed in the laboratory using partial least squares (PLS) regression. A total of 190 virgin olive oil samples, gathered during the usual harvesting time of three olive crop seasons, were used. A initial smoothing followed by a first derivative treatment was the best signal correction, The validation set gave a correlation coefficients and standard error of prediction of 0.999 and 0.35 %, 0.936 and 0.058, 0.998 and 0.604%, 0.992 and 0.674% for AV, K225, fatty acid oleic and fatty acid linoleic, respectively.

These PLS models, previous a slope/bias correction, were used to monitor on-line values of these parameters during virgin olive oils processing in real olive mills. Sample of oils were obtained for chemical analysis.

The results indicates similarity between information both NIR and reference laboratory methods.

Application of NIR transmittance spectroscopy, on-line to virgin olive oil production line, has allowed to detect, at real-time, the changes produced in the characteristics of the oils during his production.

Keywords.- Near infrared transmittance spectroscopy; on-line analysis; virgin olive oil; control quality.



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