Content archived on 2024-05-21

Novel organic-inorganic materials in opto-electronic systems for the monitoring and control of bio-processes

Results

Project Information

MATINOES

Grant agreement ID: G5RD-CT-2002-00752

Project closed

Start date
1 November 2002

End date
31 January 2006

Funded under
Programme for research technological development and demonstration on "Competitive and sustainable growth 1998-2002"

Total cost
€ 1 998 144,00

EU contribution
€ 1 295 007,00

Coordinated by
UNIVERSITY OF MANCHESTER
United Kingdom

This project is featured in...

RESEARCH*EU MAGAZINE

Results Supplement No. 023 - Food for life: safety, services and the food chain

NO. 23, APRIL 2010

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Deliverables

Proof of concept instrument

The University of Manchester's responsibility was to design an overall instrument platform to enable detection of the effects of oxygen quenching on fluorophores and enzymes incorporated into an ORMOCER film.

UoM designed and built a a prototype for use by the other partners to allow them to evaluate the biosensor coatings developed. The prototype was built so that as well as being a development tool, it will form the basis of a field trial unit when the other partners have completed the development of the sensitive ORMOCER films incorporating Ruthenium complex and enzymes.

The detection method was lifetime changes due to oxygen quenching due to immunity to coupling and ageing effects within the optical engineering pathway. A Photomultiplier tube (PMT) is used for detection together with ratiometric photon counting.

Excitation light at 470 nm is delivered to the sensitive film supported on a glass substrate by a fibre bundle. Fluorescent light centred on 590 nm is collected from the film and returned to the instrument housing where it is amplified by the Photomultiplier tube to produce discrete pulses corresponding to collection of individual photons. These pulses are counted electronically and measure the fluorescent intensity decay at precise points in time. The ratiometric method assumes the lifetime decay to be amplitude independent and only depends on exponential decay processes characterised by decay constants (lifetimes). By measuring the light intensity at two contiguous points within a few microsecond after the cessation of the excitation and forming a ratio from these two intensities then a value is produced which is a function of - and is single valued for any value of the decay lifetime. This functional dependance of the ratio is used together with a pre-calibrated lookup table to measure the value of the oxygen at the fluorophore sites.

The above technology has been incorporated into an industrial housing suitable for field trials.

Novel Pre-Immobilised Enzymes for Sensing Applications

The encapsulation of enzymes by trapping during polymerisation using different compounds may be quite complicated when using very reactive or hydrophobic reagents, because they can directly inactivate the enzyme or reduce their stability by generation of non-favourable environments considering enzyme stability. Our group proposes the pre-immobilization of the enzymes on pre-existing porous micro-particles, mainly if the polymer is extremely hydrophobic as in the case of ORMOCER. In this way, the stability of the enzyme will be determined by the properties of the support used in the immobilization and the techniques and know-how from our lab can be directly applied to this new problem, while the enzyme will not be in contact with the polymer, decreasing the requirement imposed to it. Moreover, because these beads present some rigidity, the percentage of biocatalyst percentage weight may be higher that using soluble enzyme. This way, the higher activity recovery, the higher stability and the higher amount of biocatalyst may greatly improve the biosensor performance.

Moreover, new peririfications techniques will be developed to the industrial purification of the target enzymes.

Hybrid Organic-inorganic Polymers

The task of the Fraunhofer institute comprises the development of coating materials to be used on extrinsic or intrinsic optical sensors. Based on the synthesis of novel organo alkoxysilane monomer starting compounds the manufacture of new coating materials was successful. The new monomers can be reacted with water (sol-gel process) thus forming an organically modified siloxane, which is used as coating materials for biosensors. The developed materials exhibit the following properties:

- bearing reactive acrylate groups for fast UV-curing; UV-curing is advantageous to thermal curing, due to heat sensitivity of enzymes to be incorporated into the coating
- bearing hydrophilic groups facilitating the miscibility with water based enzyme solutions for incorporation of enzymes and facilitating the migration of hydrophilic analyses like glucose
- bearing hydrophobic groups in order to facilitate the migration of oxygen, thus increasing oxygen sensitivity
- Ru-complexes can be incorporated into the coating materials, acting as oxygen sensor
- a variable basic structure facilitates the formation of various degrees of cross linking of the sol-gel derived siloxane network as well as of the organic network, obtained via UV polymerisation during the film curing step.

Based on the variety of different starting compounds and different coating materials derived thereof it is possible to pursue two different approaches for an optical sensor.

First approach: sandwich structured biosensor as follows:

1. a primary siloxane film on the glass substrates, with Ru-complex incorporated, used as oxygen sensor
2. a secondary siloxane film on the 1st coating, with enzymes incorporated.

Second approach: a single siloxane layer on the glass substrate containing both the oxygen sensor as well as the enzymes.

The coating materials developed in the project are suitable to be used in a family of novel enzyme based optic sensors allowing real time, continuous, local, in line monitoring of biotechnological processes

Novel Optical Fibre Formulations

Special silica and glass optical with inverted-graded and step-index fibbers are designed and prepared in the Institute which are employed as substrates for the application of detection layers sensitive to oxygen and glucose.
Commercially available plastic optical fibbers are used for the same purpose, too. Detection layers prepared by the sol-gel method are applied onto the substrates by the dip-coating method. The sensitivity of the layers to the analytes is determined by using laboratory set-ups by employing fluorescence changes of Ru transducers due to the analytes, which are measured in the reflection arrangement. Commercially available plastic and silica couplers are used in these set-ups. Fibber-optic elements sensitive to gaseous and dissolved air or to glucose in a concentration range of 0.1-2 mM have been fabricated The technology of the preparation of sensing fibbers and data on their sensitivity create the basis for the development of intrinsic fibber-optic sensors for the oxygen and glucose detection in biotechnological processes.

Laboratory Demonstrator of Novel Sensing Technologies

Optical sensor system for fluorescence lifetime measurement was developed in the project. Sensor uses high sensitive photo multiplier tube (PMT) as detector of fluorescence signal. System is able to use intrinsic and extrinsic sensor probe too. High-speed synchronous principle of detection is able to eliminate optical interference (ambient light). There are measured fluorescence intensities in several times during fluorescence decaying. The unique data processing method was developed to obtain low noise results.
Measurement parameters were optimised in order to get good system response, sensitivity and resolution. It was confirmed that this process was very important to get real fluorescence lifetime

changes measurement. Two systems were developed in the project one channel and multi-channel. One channel system was adapted for industrial use. Extrinsic sensor probe using plastic optical fibres was used. Silica or PMMA lens with prepared sensing layer (ORMOCER) were placed in removable probe cap. Sensor was installed at industrial partner Moorepark during project. The second one multi-channel system is able to detect a few different analytes. This system is tested in laboratory bioreactor (installed at ICPF). It is possible to effective evaluating of new prepared ORMOCER layers.

There are a few responses from industry and research institutions from Czech Republic.
These institutions are interested in testing the system in their processes.

Last update: 13 June 2006

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