RECENT DEVELOPMENT OF ENVIRONMENTAL SENSORS


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ABSTRACT: The most recent developments in the electrochemical sensor and biosensor research and their applications to the environmental field performed in our laboratory are described. Particular attention is paid to the total toxicity determinations to the pesticide analysis and to the monitoring of inorganic ionic pollutants.

Keywords: sensors, biosensors, environment.

INTRODUCTION

Environment is always more the preferred subject of study and control; so new approaches, new methods and new devices are everywhere in congresses and scientific journals presented so stimulating other research in a self feeding process.

We too since many years are dealing with environmental sensors and biosensors always trying to contribute to the needs of the monitoring activity that are accuracy, sensitivity, easy handiness, possibly low costs. Here we describe some our recent successes in producing some environmental probes finalised to determine some common pollutants or risk situations [1-16].
RESULTS

One of the most interesting results of modern biosensor research is certainly represented by the development of toxicity biosensors. For several years our laboratory has been engaged in this type of research. As a result, a number of integral toxicity biosensors have been developed using immobilised Saccharomyces cerevisiae yeast cells and different types of electrochemical transducers [1]. One of the crucial aspects of this research was the development of a suitable method for immobilising the yeast cells. For this purpose, two classical immobilisation methods were adopted. The first method involved the immobilisation of yeast cells in a dialysis membrane, and the second the entrapment in a membrane of cellulose triacetate. Unfortunately, the results obtained using such methods were not encouraging. It was consequently tempted to develop a new immobilisation method involving the entrapment of Saccharomyces cerevisiae cells in agar gel containing culture medium [2,3].

The results obtained using this method were immediately seen to be very interesting: the agarized medium layer on a Petri dish, containing the entrapped yeast cells, can be stored at +5 °C for more than one month. During this period, whenever the toxicity biosensor has to be used to make a measurement, all that needs to be done is to cut a 0.5 cm diameter disk out of the agarized layer and place it on the selected indicating electrode using a suitable net or dialysis membrane. At this aim, the following indicator electrodes have been tested: gas diffusion amperometric electrode for oxygen, gas diffusion potentiometric electrode for carbon dioxide, glass electrode for measuring pH and an H_3O^+ sensitive FET.
The toxicity biosensors thus obtained were used to measure integral toxicity values in solutions containing several different pollutants such as metal ions, phenols, and anionic or cationic surfactants, as well as to evaluate the toxicity of chemotherapeutic agents such as cholic acids or cisplatinum.

In all these applications our method of immobilising Saccharomyces cerevisiae cells proved to be completely valid and fully satisfied the purposes for which it had been developed. Indeed, from the applications point of view, the cellular stability in the used immobilisation medium not only allowed a large number of toxicity biosensors to be designed and built by coupling the selected cells to different types of electrochemical transducers, but also made possible to extend the range of the assayable analytes by exploiting the possibility of “incubating” in presence of the toxic compound the immobilised yeast cells both for relatively short periods (about 10-15 min) and for longer ones (up to one week) before performing the toxicity evaluation.

An other class of pollutants about which there are always great movements of ideas and innovation rates are pesticides [4,5].

We studied the possibility of fabricating a new OPEE (organic phase enzyme electrode) i.e. a bienzymatic inhibition biosensor, for the analysis of organophosphorus and carbamate pesticides, able to operate also when dipped directly in non aqueous medium [6].

The biosensor was constructed by modifying the response of a gaseous diffusion amperometric oxygen electrode (with the external body made entirely of teflon) sandwiched between two kappa-Carrageenan membranes entrapping the two enzymes butyrylcholinesterase and
choline oxidase. After exploring the possibility of operating in single organic solvents, even with widely differing polarities, such as n-hexane or chloroform, the best results so far were obtained using a 50% (v/v) water saturated chloroform-exane mixture. The measurement procedure providing the best results was that involving the recording of biosensor response to the addition of a fixed concentration of butyrylcholine both before and after contact for a fixed period of time with a solution of the solvent containing the pesticide to be determined (incubation time 20 min). The results show that the limit of detectability of the method for pesticides such as Aldicarb or Paraoxon is about 4.5 ppb, the linearity range about half a decade and the response time 6-10 min, according to the pesticide analysed.

We were also engaged on the development of pesticide sensors based on solid state and biosensor technology. In this case the biosensor is obtained by coupling pH solid-state electrode with butyrylcholinesterase immobilised in a functionalised nylon membrane. The solid state pH electrode is obtained by a polymeric membrane coated on a small graphite electrode. The graphite electrode is embedded in a teflon body and the polymeric membrane contains PVC-PVA-PVAc as base polymer and tridodecylamine as ionophore [7]. The enzyme butyrylcholinesterase is immobilised in a nylon functionalised membrane using carbodiimide and fixed on the head of the pH electrode by means of a rubber O-ring. The linearity range extends from 2.5 to 20 ?g/L and the minimum detection limit may reach about 1 ?g/L of Paraoxon with a response time ? 5 min.
Lastly a combination of the sensitivity of the Ion Selective Field Effect Transistor (ISFET) with the specificity of the enzyme system providing a simple procedure for pesticides determination, was carried out in our laboratory [7], using integrated chips supplied by HEDCO laboratory of Utah University and polyazetidine for butyrylcholinesterase enzyme immobilisation, so obtaining encouraging results. Increasing attention is paid to radicalic species as on one side are present in the environment and on the other are responsible for damages to humans being's health and safety and for the occurring of many degeneration phenomena in foods and organisms.

We recently developed other suitable ISFET devices for detecting several compounds of environmental interest [8]; particularly new polymeric membrane ISFETs respectively for nitrate analysis [9], using tetradodecylammonium nitrate as ion exchanger, for ammonia surfactant analysis [10], using benzylidimethylhexadecylammonium cholate, or trioctyldodecylammonium cholate as exchangers and for cationic surfactant analysis [11], using benzylidimethylhexadecylammonium reineckate, or exadecylpiridinium reineckate as exchangers, were fabricated.

In all cases the exchanger was dispersed in a polymeric membrane, prepared using polyvinilchloride as base polymer and sebacate or dibutylphtalate as plasticizer. Measurements were performed in feedback mode by recording the gate output voltage variation for the ISFET, on increasing the analyte concentration in solution. After a full electrochemical characterisation, the new ISFETs, were applied to the analysis of authentic aqueous matrices of
environmental interest, (river, lake, sea water, aqueous soil extracts and so on). The analytical results were generally satisfactory, especially taking into account that several times it was easily possible also to use the sensor directly dipped in the environmental matrix.

Recently we also studied two different kinds of biosensors to determine superoxide radicals [12] obtained by coupling a transducer consisting of an amperometric gaseous diffusion amperometric electrode for oxygen, or other amperometric electrode for hydrogen peroxide, with superoxide dismutase enzyme immobilised in Kappa-Carrageenan gel. Both the sensors showed a good response to the superoxide radical. Nevertheless we consider that the SOD/H$_2$O$_2$ biosensor is now mature enough both from an engineering and an operative point of view. An application of this biosensor was implemented by us to study in vitro the effects on the superoxide radical of molecules considered as radical scavengers and having substantial biomedical or pharmaceutical interest. Initially the response of biosensor to the superoxide radical in the presence of three very common scavengers (ascorbate, glutathione, cysteine) was investigated. Currently we are studying other important molecules such as melatonine, b-carotene and tocopherol as scavanger molecules of great interest.

Benzene is the new emergence of the environment due to its increased spreading in connection with green fields use. A biosensor based on Pseudomonas putida cells immobilised in an agarized culture medium was constructed for the purpose of determining traces of benzene in aqueous solutions using a classical gaseous diffusion amperometric oxygen electrode as transducer [13]. By the performing a complete
electroanalytical characterisation of the biosensor, it was observed that the response to several benzene derivates (toluene, chlorobenzene, nitrobenzene, ethylbenzene) can justify an use of the biosensor also for other hydrocarbons than benzene, for this aspect our biosensor being better than the previously proposed one.

The characteristics of selectivity and response time result to depend on the procedure used during the cells growth phase, especially for what concerns the molecular configuration of the benzene dioxygenase enzyme contained in the cells of the Pseudomonas putida used.

Sulfite assay in environmental matrices such as sea or river water is of considerable interest in the filed of environmental protection. For instance, when it is oxidised, sulfite reduces the oxygen content in natural water, which has a detrimental effect on aquatic life.

As an alternative to classical Monier-Williams idiometric or pararosaniline methods, several up-to-date methods have been proposed by different authors, e.g. ion chromatography and enzymatic-spectrophotometric methods. Also enzyme sensors have been proposed for sulfite determination.

A new sulfite oxidase enzyme electrode for sulfite analysis in sea and river water samples was developed by us [14]. The biosensor was constructed by coupling an oxygen sensor to a nylon membrane with the enzyme chemically immobilised on it. Measurement on standard solutions and in matrices of environmental interest were carried out both under steady-state and flow conditions with positive results. Fructose was profitably used as stabilising agent for sulfite both for standard solutions and aqueous environmental samples.
In previous papers [15,16] we described the results obtained in the case of semiconductor assisted photodegradation chlorophenols, pesticides and surfactants. In the first researches, generally inorganic semiconductors (TiO$_2$, ZnO, CdS), directly dispersed in the aqueous matrix, were used as catalysts for the photodegradation (by UV light) of organic pollutants and TiO$_2$ resulted the best; this procedure is time consuming because at the end it needs a recovery of the catalyst by filtration. One of our goals in this field was the UV photodegradation of several kinds of organic pollutants (chlorophenols, pesticides, surfactants) by using a catalytic membrane consisting of TiO$_2$ and polyaniline (a typical conducting polymer) as mixed catalyst, polyvinylchloride (PVC) as support and dibutylphthalate (DBP) as plasticiser.

Owing to the different structures of the compounds to be degraded, it was not possible to use a single method to follow the process. Consequently a chromatographic method using high performance liquid chromatography (HPLC) was used for the chlorophenols, an amperometric biosensor with oxygen indicator for the pesticide and a CO$_2$ electrode and surface tension measuring device for the surfactant.

The CO$_2$ electrode was also used to control if the degradation was quantitatively complete; this let us to consider a new research project: the CO$_2$ electrode can be modified by coupling with a catalyst so obtaining a sensor that, when in contact with a polluted matrix irradiated by UV light, allows to perform the Total Organic Carbon (TOC) analysis.

At this aim a suitable flow cell was constructed on the top of which the modified electrode is dipped while an optic fibre, with high transmission in
the used UV range, is inserted from the bottom. In the first tests, TiO$_2$ was used as catalyst; it was directly formed (chemically or physically) on the surface of a titanium foil encircling the head of the electrode. In next steps polyaniline could be added to test eventual improvements and finally a biosensor could be constructed by adding, to one or both the above indicated catalysts, enzymes of the oxidase or peroxidase type.

CONCLUSIONS
The described sensors and biosensors represent the most recent developments of our research group so without considering older studies concerning polymeric membrane ISEs to determine anions and cations of environmental interest, biosensor for phenols in wastewater and inhibition biosensors to determine heavy metals, phosphate and pesticides. In any case it emerges that the fields deserves particular attention considering the wide unexplored opportunities.

REFERENCES


