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Research and Development of Biosensors in China

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Biosensors will soon play an important role in people's daily lives since they meet real needs in health care, environmental monitoring and control, agriculture, marine and chemical industries. Some universities, research institutes and companies in Mainland China have focused on fundamental and applied biosensor development, out of which some biosensor products have been commercialized with total sales of about one million dollars per annum.

1. Introduction

Calorimetric, electrochemical, optoelectronic, piezoelectric and thermistoric routes linking biology to electronics have demonstrated the basis for a range of biosensors.[1] Therefore a biosensor is defined as an analytical tool or system consisting of a biological or biologically devised sensing element in contact with a suitable physiochemical transducer that converts a biochemical signal into a quantifiable electrical signal.

Back in the 1960s, the first biosensor was established through the pioneering work of Clark and Lyons[2] as the basis for the fabrication of enzyme electrodes. Potential applications of biosensors are widespread in health care, veterinary medicine, the food industry, fermentation industry, environmental monitoring, and the defense and security industries.

The Chinese literature on biosensors has been recently reviewed, and the reader is referred to these publications for a historical appreciation of the field. Since the 1970s Chinese scientists have been working on immobilization of enzymes and viable micro-

organisms. Electrodes with immobilized enzymes have been developed into commercial products in some local market. Several biosensors have been developed and applied to clinical diagnosis, the food industry, environmental monitoring and fermentation process control.

The biosensor research groups in China belong to different universities, research institutes and companies. Generally, research activities deal mainly with enzyme-based electrodes, microbial sensors, immunosensors, tissue-based sensors and ion-selective field-effect transistor (ISFET)-based sensors.

The purpose of this review is to provide an overview of biosensor research in China from both fundamental and application viewpoints.

2. Development of Enzyme-Based Electrodes

2.1 *Oxygen and hydrogen peroxide electrodes*

Research has been most actively conducted on enzyme-based electrodes because it appears that an enzyme electrode can be constructed for any analyte, provided that a suitable oxidase is available. The early choice for a base electrode was a Clark-type oxygen electrode incorporating a biocatalyst which utilized oxygen in the presence of a specific substrate. The presence of the analyte causes a decrease in the current obtained from the enzyme electrode. A classic example is the reaction catalyzed by glucose oxidase:

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic acid} + H_2O_2. \quad (1)$$

For O_2 measurement, a platinum cathode is protected by an O_2 permeable membrane and is poised at –600 mV to –700 mV, relative to an Ag/AgCl reference electrode.

The report on the use of polyacrylamide-immobilized glucose oxidase with oxygen electrode to assay blood glucose level in serum was an early success. The linear response range to blood glucose was around 0 – 300 mg/dl, CV% was 1.03 – 2.5, and one piece of enzyme membrane could be used for 1,000 determinations. Recently a glucose sensor[3] was developed by modifying an immobilized glucose oxidase membrane and coating a Nafion diffusion-limiting membrane over an amperometric H_2O_2 microarray electrode which was composed of an Ag/AgCl reference electrode, a Pt counter electrode and two sets of Pt working electrodes, each set consisting of five bands. The small size of the microelectrodes gives them a number of advantages over electrodes of macroscopic dimensions such as high current densities from nonplanar diffusional contributions to the net Faradaic current; and low ohmic potential drop which allows the use of highly resistive solutions. Table 1 summarizes some of the developments in GOD-based electrodes.

2.2 *Mediated amperometric enzyme electrodes*

Mediated and chemically modified amperometric sensors have received much attention in the last decade and are beginning to supersede oxygen/hydrogen peroxide-based sensors as the main thrust of enzyme electrode research.

The major advantage of the mediated amperometric biosensors is their lack of dependence on oxygen since the mediator acts in place of oxygen as the electron acceptor.

In industry and medicine, the low cost and reliability of enzyme electrodes have spurred

Table 1
Developments in GOD-based electrodes.

Basc clcctrodc	Immobilizcd support	Linear response range
O_2	Polyacylamine	0–300 mg/dl
O_2	Gelatin	18–400 mg/dl
O_2	Alkyamine porous glass	0–500 mg/dl
O_2	Bovine serum albumin	50–500 mg/dl
H_2O_2	Acetate cellulose	30–350 mg/dl
H_2O_2	Nafion membrane	2–40 mM

on greater efforts, which are of great interest in this country, with its large population and huge potential market.

Yang[4] used thionine as a redox mediator and fabricated a microbial sensor to study the growth kinetics of yeast in fermentation.

Hu did additional research in mediated carbon-based enzyme sensors. Using benzoquinone[5] and glucose oxidase enzyme-modified graphite electrodes, a device for glucose assay has been fabricated and used in several local fermentation factories and hospitals. Because of a large number of cases of diabetes as revealed by recent medical survey,[6] much attention has been focused on the self-monitoring of patients not only in clinics but at home as well. A disposable screen-printed carbon strip electrode technology has been developed, which led to the fabrication of a self-monitoring blood glucose assay device.[7] Figures 1 and 2 show, respectively, a screen printer and a model of screen-printed enzyme strip electrodes.

The carbon strip electrodes shown in Fig. 2 are comprised of two conductive carbon tracks made of unmodified graphite powder mixed with conducting mediators to yield an

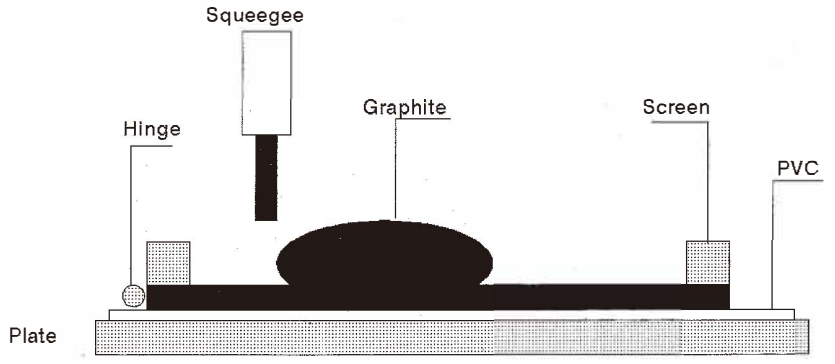

Fig. 1. Screen printer.

Fig. 2. Printed strip.

even suspension which can then be printed through a screen onto an inert plastic support. The reference electrode was prepared by Ag/AgCl ink. An insulating layer with openings allowing external electrical contact with one end of the strip and access of analyte to the working and reference sensor area at the other end, was printed over the whole strip.

A clinical study comparing the COBAS MIRA Auto-Biochemical Instrument revealed a good agreement between the two methods. The following linear regression was calculated from a study of 153 patients: $y = 18.01 + 0.992x$, with correlation coefficient $\gamma = 0.9746$.[8]

Some other enzyme electrodes were also developed recently. Feng described the use of an oxygen electrode[9] for the determination of ascorbic acid level in serum. Feng also described some novel adaptations of a technology using O_2-liberating enzyme at an electrode for the determination of fish freshness to a microprocessor-based system.[10]

These oxygen-based electrodes with several enzymes immobilized on them are used in conjunction with a compensation for the changes in the oxygen concentration in the sample. The enzyme electrodes responded to inosine monophosphate, inosine and hypoxanthine, respectively, and assay results were obtained within 20 min.

Table 2 summarizes some important biological substances that can be detected by an oxygen electrode.

3. ISFET-Based Enzyme Sensors

Some ISFET sensors have been developed. Li[11] constructed a single pH-FET by combination of SOS-pH-FET with an immobilized β-lactamase membrane for penicillin

Table 2
Biosensors for some important biological substances.

Analyte	Base electrode	Biological element	Linear response range
Ascorbic acid	O_2	Ascorbic acid oxidase	10–1000 mg/dl
Ethanol	O_2	Alcohol oxidase	0.02–60 mM
Lysine	O_2	Lysine oxidase	10–100 mg/dl
Lactate	O_2	Lactate oxidase	0–5.0 mM
Glutamate	O_2	Glutamate oxidase	0–100 mg/dl
Hypoxanthine	O_2	Xanthine oxidase	0–10 mM

determination. The linear response range to penicillin was 0.5–25 mM, and the correlation coefficient was 0.9976. The SD and CV% for 8 determinations were 1.67 mV and 2.1, respectively.

A glucose oxidase H^+ -ISFET sensor for glucose detection has been investigated. The linear response range of the sensor to glucose is 0.5–20 mg/dl, and the response time is about 25 s. Table 3 describes three ISFET-based enzyme sensors.

4. Biosensors Based on Tissues

Plant and animal tissues have been successfully used for fabrication of biosensors. Dai[12] immobilized banana pulp on the surface of an oxygen electrode forming a thin layer for dopamine detection. The sensor response time is 10 min, and the lenear response range to dopamine is 0.08–1.6 mM.

Xie et al.[13] fabricated a pig liver sensor with an oxygen-sensitive probe for determination of hydrogen peroxide. Good correlation, precision and recovery were obtained. They also used a layer of jack bean meal-containing urease with a pH-sensitive electrode to detect urea. By using a pumpkin tissue slice containing glutamate decarboxylase, coupled with a carbon dioxide gas-sensing electrode, a system for determination of glutamic acid was developed. The linear response range of the pumpkin tissue electrode to glutamic acid is 0.5–1.0 mM. The response time in a steady state is 10 min. The lifetime is over 7 days.[14] Table 4 summarizes some tissue-based biosensors.

Table 3
ISFET-based enzyme sensors.

Analyte	Immobilized support	Linear response range
Glucose	Polyacrylamide	$5 \times 10^{-6} - 2 \times 10^{-4}$ g/ml
Penicillin	BSA	5 – 100 mg/ml
Glucose	BSA	0.5 – 25 mM

Table 4
Tissue-based biosensors.

Analyte	Base electrode	Bioelement	Linear response range
Dopamine	O_2	Banana pulp	$8 \times 10^{-6} - 6 \times 10^{-3}$ M
H_2O_2	O_2	Pig liver slice	$6.4 \times 10^{-5} - 1.2 \times 10^{-3}$ M
Urea	NH_3	Jack bean slice	$4.6 \times 10^{-5} - 1.0 \times 10^{-2}$ M
Glutamate	CO_2	Pumpkin tissue	$1 \times 10^{-2} - 5 \times 10^{-4}$ M

5. Immunosensor

Li and Wang[15] prepared a new anti-hepatitis B surface (anti-HBs) membrane disc on silk by chemical modification with cyanogen bromide. The disc was immersed in HBsAg(+)serum solution or assayed serum sample and then held at 37°C for 1 h, forming an HBsAG-anti-HBs complex membrane disc. The disc was used to fabricate a working immunoelectrode with Ag/AgCl as an internal reference. The immunoelectrode and saturated calomel electrode were dipped simultaneously into a working buffer for the determination of the concentration of the target material.

The result of determination using 105 samples (61 positive and 44 negative) showed more than 85% agreement with the data obtained from ELISA. It was linear over the range of 20–320 ng/mg with a standard deviation of 10.78% ($n = 4$, $m = 5$).

6. Microbial Sensors

Various microbial sensors based on amperometric determination have been developed in China. A microbial sensor for glutamic acid incorporates immobilized *E. coli* cells in a CO_2 sensing electrode.[16] The linear response range to glutamic acid was 60 –1200 mg/dl. The CV% was between 1.59 and 2.5.

The biochemical oxygen demand (BOD) test[17–19] is one of the most widely used tests in the measurement of organic pollution. Some bacteria and yeast capable of wastewater treatment were used to assay the effluent from fermentation factories (normally glucose or glutamate solution) with concentrations up to 30–40 mg/l. The minimum measurable BOD was 1 mg/l. These BOD sensor systems are stable from 25 to 180 days and are available commercially.

Yeast cells immobilized on an oxygen electrode were used for the determination of sugar.[20] In fermentation broth, the relationship between sucrose concentration and sensor response was linear up to 100 mg/l. The response time was less than 1 min.

Immobilized *E. coli* on acetate cellulose membrane and oxygen electrode was used for vitamin B12 bioassay.[21] The lenear responce range to vitamin B12 was $5 \times 10^{-6} - 2.5 \times 10^{-5}$ mg/ml. The response of the sensor was reproducible within ±3% error. The assay could be completed in 2 h for each sample.

Table 5 listed some example of microbial sensors.

7. Conclusions

In conclusion, it is now universally recognized that biosensors play an increasingly vital role in the pursuit of the most rapid and simple analytical methodologies that are required, not only by skilled analysts or technicians, but also by untrained personnel, who might want to use them in hospitals, doctors' clinics, and other workplaces in remote areas (which is a matter of great concern in this country), and also for self-monitoring of

Table 5
Microbial sensors.

Analyte	Base electrode	Microorganism	Linear response range
Sugar	O_2	*Saccharomyces* sp.	0 – 100 mg/l
Glutamate	CO_2	*E. coli*	8.3 – 630 mg/l
Lysine	CO_2	*Bacillus* sp.	$5 \times 10^{-4} - 5 \times 10^{-2}$ M
BOD	O_2	*Pseudomonas* sp.	1 – 30 mg/l
BOD	O_2	Yeast	1 – 45 mg/l
B12	O_2	*E. coli*	$5 \times 10^{-3} - 2.5 \times 10^{-2}$ mg/l
Fuel-cell	Carbon	*E. coli*	

conditions such as diabetes.

This paper has attempted to present an overview of the development of biosensors in China. Some of the research projects merely followed pioneering studies conducted by international researchers; however, some of them are very reliable and have practical applications. Their utility will depend on the local research environment and economic situation.

Undoubtedly the future development of increasingly selective, sensitive and more stable sensors will present many challenges. It is hoped that the information given in this paper will provide an opportunity for researchers working in this important and exciting area to become familiar with and understand the efforts that are being undertaken by the Chinese.

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