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综述

DEVELOPMENT AND FUTURE PROSPECTS OF BIOSENSOR MEMBRANES FOR ANALYSIS

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(一) INTRODUCTION

In recent years, the development of a sensor capable of detecting and analyzing a variety of components with little effort is being watched with interest.[1] The function of the chemical sensor, that of specifically identifying chemical substances is particularly significant. Because of this, new bio-functional polymer membranes are being designed, in which living organism substances of higher specific properties have been immobilized in/on the polymer membranes. These are called biosensor membranes.

This paper deals with the current situation in the development of biosensor membranes for analysis during the past 2—3 years, as well as its future prospects, focussing on the biosensor membranes.

(二) TYPE OF BIOSENSOR MEMBRANES (BM)

BM is a product made through the process in which a molecule identification element such as an enzyme etc. has been immobilized in/on natural or synthetic polymer membranes. Chemical substances including the substrate etc. will enzymatically react with the biosensor membranes. Electrode-sensitive substances produced or consumed through as a result of the reaction will diffuse in the BM and reach to the electrochemical device (transducer). In the electrochemical device, the output signal corresponding to the concentration of this substance is obtained. Therefore, the concentration of the chemical substance can be detected.

As shown on Fig.1 a variety of BM have been designed by using enzymes, bacteria, organella, antibodies, antigens, receptors, tissues of animals and plants etc. Recently, these molecule identification elements-combined multi-enzyme biosensor[2], hybrid biosensor[3-5] and high sensitivity-

aimed chemically amplifying biosensor[6,7] have been designed. In addition, the semiconductor biosensor piezobiosensor[8] and optobiosensor[9] are being developed. Here, we will comment on typical BM and discuss the case studies we have made recently.

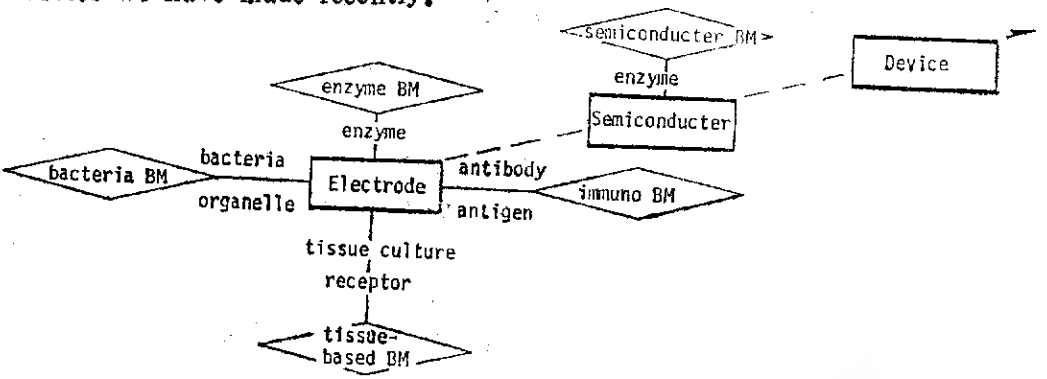


Fig. 1 Type of biosensor membranes

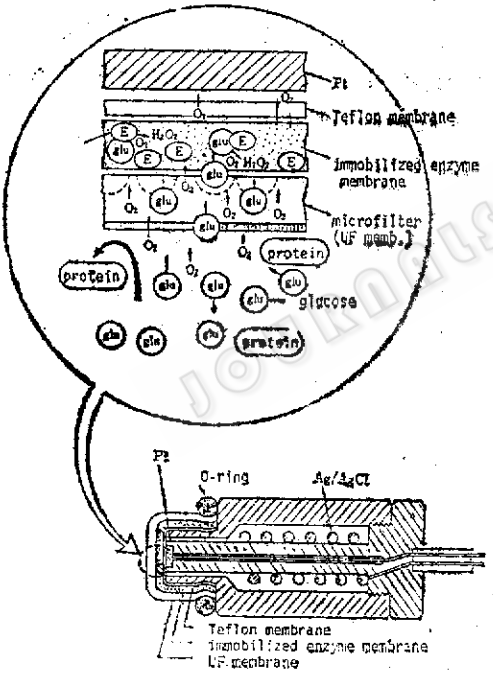


Fig. 2 Example of an enzyme biosensor (glucose biosensor)
UF, Ultrafiltration membrane

1. Enzyme-biosensor membranes (EBM)

The enzyme-biosensor (Enzyme electrode) is the prototype[10] of the biosensor proposed by Updike and Hicks. Fig. 2 illustrates one example of an enzyme-biosensor, (glucose sensor). The UF membranes (membranes excluding protein) remove the macromolecular substances (proteins etc.) present in the solution and permeate the glucose substrate (glu), and oxygen. In the EBM, the enzyme reaction takes place as shown in the formula below:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconolactone} + H_2O_2$$

Consumed oxygen diffuses through the gas-permeable membranes and is converted into an electrical signal by the oxygen electrode. The substances (e.g. O_2, H_2O_2, CO_2, NH_3 etc.) consumed or produced by EBM are measured by the variation in current value affected by the oxygen electrode etc., or the variation in potential difference affected by the ammonia gas electrode etc.

The recent study for EBM represents; firstly, the development of new

EBM through combination with new devices; secondly, the design of multi-EBM and its new measuring method; and thirdly, the fundamental analysis of BM on the basis of EBM as a model, etc.

2. Bacterial biosensor membranes (BBM) [11]

Bacterial biosensors are those sensors which directly utilize the complicated bacteria-enzyme reactions. Fig. 3 illustrates the example of the BBM preparing method, using agar-gel. These type membranes have been manufactured for their practical use in the fermentation field in order to measure the amino acid present in the fermentation tank, the organic acids alcohol etc. Also, the BOD sensor which utilizes the characteristics of bacteria has appeared on the market recently.

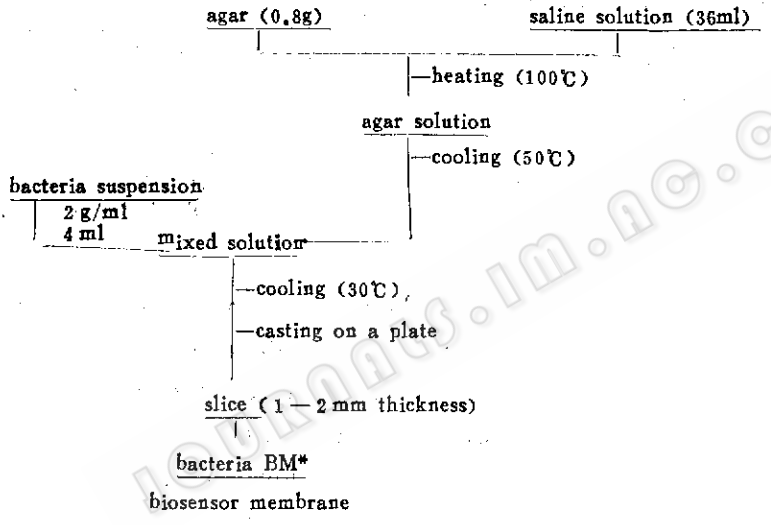


Fig.3 Preparation of an agar-bacteria biosensor membrane

3. Immuno biosensor membranes (IBM) [12-14]

There is an immuno assay available in the clinical analysis field known as the micro quantitative method of ingredient such as peptide and steroid hormons in body fluid. The immuno biosensor is made of an immobilized antibody, a serum containing antigen and a labelled antigen will come into contact with the IBM competitively. As a result, the membrane electric potential which depends on the quantity of antigen present in the serum can be detected. Fig. 4 illustrates the membrane formula of IBM, Fig. 5 shows the example of measuring insulin with IBM immobilizing anti-insulin antiserum. At present, the enzyme immuno assay(EIAi)s taking the place of the radio immuno assay (RIA) which requires a specific installation. However, the sensitivity of EIA is far inferior to that of RIA. Further development of EIA as a new measuring system to take the place of RIA through impro-

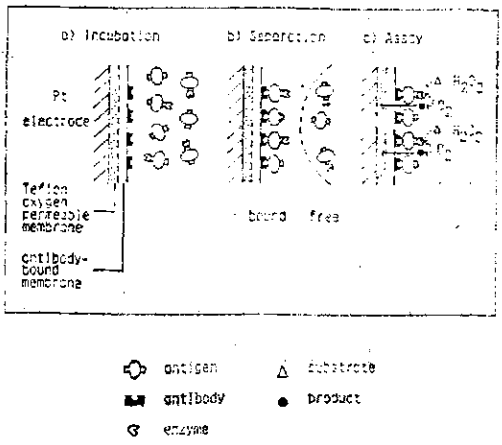


Fig. 4 Example of an immuno biosensor membrane

vements in the selectivity of antibodies and the higher sensitivity of IBM is anticipated in the future.

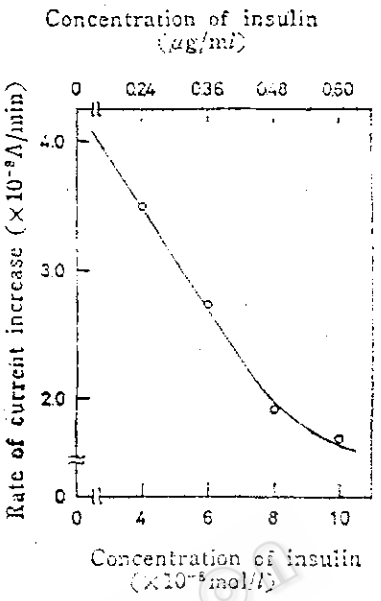


Fig. 5 Calibration curve for an insulin biosensor
Incubation: pH 8.0, 30℃, 10 min
Assay: in 10 mmol/l H_2O_2 at pH 7.0

4. Tissue-based biosensor membranes (TBM) and receptor biosensor membranes (RBM)

Rechnitz et al. have designed a TBM made of immobilized mammalian tissue and have demonstrated that adenosine[16], adenosine-5′-l phosphoric acid[18], guanine[17] and glutamine[18] can be measured by it. As well, they are proposing the TBM made of an immobilized plant tissue slice of corn[19] or yellow squash[20] (Fig. 6), the reaction example[20] is shown by the formula below:

$$\text{L-glutamic acid} \xrightarrow[-CO_2]{\text{glutamate decarboxylase}} \gamma\text{-aminobutyric acid}$$

One of us is formulating RBM through the isolation and reconstitution of acetylcholine receptor from the electro-organ of Torpedo fish[21]. Fig. 7 shows the contrast of the sectional structure of RBM with a synaptic membrane. Schubert[22] et al. have formulated the BM made of entrapped liver cell of rat with gelatine and have demonstrated that NADH is measurable with this and the oxygen electrode. TBM and RBM are in early stages of study. However, in line with the progress in biotechnology, the further possibility of the production of a tissue-oriented sensor would increase once the genuineness and function of animal and plant tissues have been clarified. In addition, a peptide hormone receptor has been isolated as cell membranes fragment and the structure of one of them is being determined through the analysis of genes.

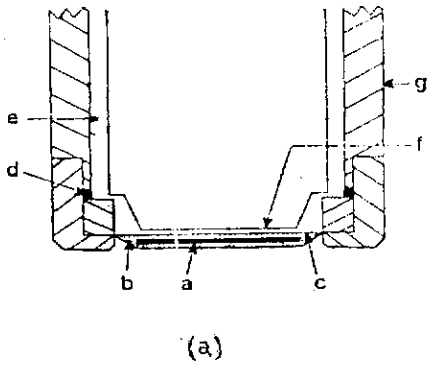


Fig.6a Schematic diagram of the squash tissue-based biosensor membrane, (a) slice of yellow squash tissue; (b) BSA conjugate layer; (c) carbon dioxide gas-permeable membrane; (d) O-ring; (e) internal electrolyte solution; (f) pH-sensing glass membrane; (g) plastic electrode body

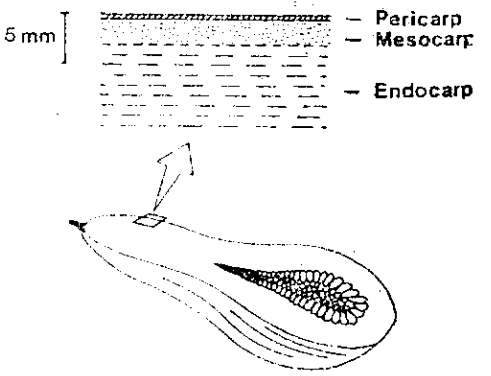


Fig.6b Cross-section of yellow squash showing origin of mesocarp biocatalytic layer

5. Semiconductor biosensor membranes (SBM) [23,24]

The BM discussed so far cover the cases which use the electrode as an electrochemical device. Recently, SBM consisting of ISFET (ion selective field effect transistor) and EBM have been developed to take the place of electrodes, for the sake of high sensitivity and the compactness of the biosensor. ISFET, in which an insulated gate-type field-effect transistor and ion sensor have been integrated, is compact (3 mm max.) and is an ion sensor having a lower output impedance. From Fig. 8 SBM is casted on the gate insulation membranes (Si_3N_4/SiO_2) of ISFET. Because of the enzyme

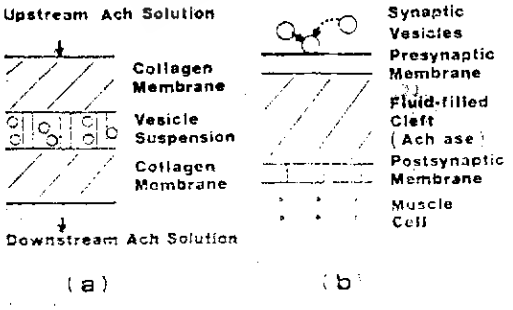


Fig. 7 Schematic diagram of the receptor biosensor membrane (AchI receptor) (a) and postsynaptic membrane (b)

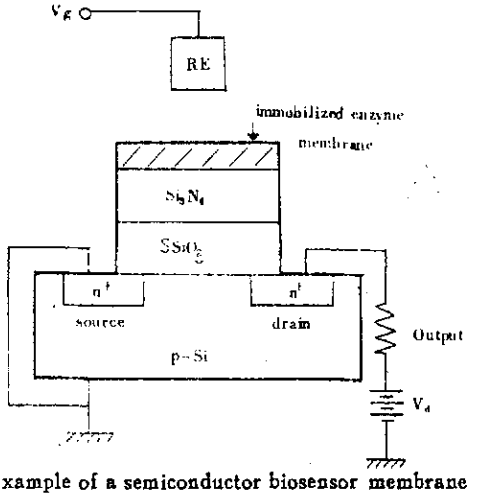


Fig.8 Example of a semiconductor biosensor membrane

reaction, the ion concentration near to SBM varies and the drain current is modulated by the change in the interfacial electric potential of Si_3N_4. In this way the substrate corresponding to the modulation quantity or the

concentration of product can be detected.

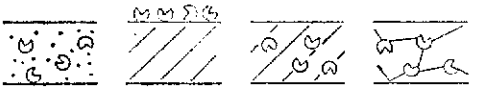
Since the semiconductor is highly sensitive and compact, the method of manufacturing SBM requires a more refined membrane manufacturing technology such as ultra-thin membrane manufacturing technology, membrane thickness homogenizing (identical) technology, semiconductor monolithic molding technology, integration technology, and immobilizing technology etc. Furthermore, there is a need for the development of ultra-high sensitive and ultra-compact (10μm max.) microsensors[25-27]. Microsensors will make it possible to elucidate the brain physiology, directly detect the nerve transmitter, micro"in vitro" assay with only one cell etc., and this new sensor technology will be developed. For this reason, an ultra-fine immobilizing technology would be indispensable.

(三) THE STRUCTURE OF BIOSENSOR AND ITS IMMOBILIZATION BM IS MADE AVAILABLE BY IMMOBILIZING A MOLECULE-IDENTIFICATION ELEMENT ONTO SYNTHETIC OR NATURAL POLYMERS

The difference in the structure of BM under the various kinds of immobilizing methods is illustrated on Fig. 9 typically. We shall take this opportunity of stating the cases of our study made recently regarding the application of the immobilizing method to the BM.

1. Insulation-BM

The suspension of enzymes, bacteria, and mammal and plant tissue is sealed between the gas-permeable membranes and UF membranes to form BM membranes. There are two cases available for Insulation-BM; in one case the supporters are not used and in the other, the supporters of porous polymer membranes etc. are used to adjust the thickness of membranes and the quantity to be immobilized. The latter is called an impregnated BM.



(a) Insulation (b) Adsorption (c) Entrapment (d) Binding

Fig.9 Typical structures of biosensor membranes

It is easy to prepare and particularly effective with bacteria, mammal and plant tissues and recepters etc. And some of them now has been commercialized such as BOD sensor.

2. Adsorption-BM

Enzyme and antibody are adsorbed onto the polymer membranes hydrophobically to form adsorption-BM. The adsorption function of the protein and cell to the polymer matrices varies substantially depending upon the polymer materials and their surface conditions. Polypropylene[28], onto which the protein adsorbs less readily than it does onto glass, has been used in making microtubes for biochemical experiments. Since nylon ad-

sorbs protein well, porous nylon membranes have been developed as a blotting material of cell translation in genetic engineering. Since polycarbonate adsorbs the cell, it has been used as a cell selecting plate of monoclonal antibodies. Recently, enzyme-immobilizing carriers (membranes) have been developed because of the special process of polyvinyl chloride (PVC) with added adsorption function[29] (Table 1). The characteristics of these"wet" PVC membranes is that the several kinds of enzymes can be immobilized simultaneously under normal conditions, such as oxalacetate decarboxylase and pyruvate oxidase have been immobilized simultaneously and that GOT (glutamate oxalacetate transaminase) /GPT (glutamate pyruvate transaminase) can be measured consecutively for the diagnosis of liver fuction[30].

Table 1 Adsorption of various substrates into a wet PVC membrane

Substrates	M	Solvent	pH (solvent)	N-content (%)	Amount sorbed (μg/cm²)
Urea	60	H_2O	7.0	46.7	0
Glycine	75	H_2O	7.0	18.7	0
Uric acid	168	H_2O	7.0	33.3	0
Glucose	180	H_2O	7.0	—	0
Gly-gly-glycine	189	H_2O	7.0	21.7	0
Acetylcholine iodide	273	H_2O	7.0	—	0
Tetrahydrocortisone	364	H_2O	7.0	—	0
Albumin	45000	H_2O	7.0	15.6	64.5
Hemoglobin	68000	H_2O	7.0	14.6	101
γ-globulin	156000	H_2O	7.0	14.7	122
Fibrinogen	400000	H_2O	7.0	10.6	198
Uricase	120000	borate	8.5	14.6	75.7
Glucose oxidase	186000	phosphate	5.6	13.0	69.9
β-glucuronidase	280000	acetate	7.0	14.4	80.9
Urease	480000	phosphate	7.0	12.7	88.6

a. Micro-Kjeldahl method.
b. Ion-electrode method described in this paper.
c. Refer to a paper (S.Hirose et al., *J.MOl.Catal.*,6:251 (1979)
d. Refer to a paper (S.Hirose et al.,*J.Appl.Biochem.*,2:45 (1980)

3. Entrapment-BM

Enzyme and bacteria are entrapped by and fixed onto polymer matrices to form entrapment-BM. Enzymes and bacteria are entrapped onto a three-dimensional macromolecular structure, while substrates and products permeate and diffuse freely in the matrix.However, if the substrate is that of having high molecular weight, entrapment-BM is unsuitable. The typical example of entrapment-BM is the polyacrylamide gel and acrylic acid derivative,

4. Binding-BM[31]

Through the utilization of ions or functional groups present in the polymer matrix, the enzyme is ion bonded, covalent bonded, and crosslink bonded to form binding-BM. An example of this is where lactase has been immobilized on DEAE cellulose membranes to form ion-binding BM, however, no report of this kind has been made available recently. The enzyme is covalent bonded to matrices by the peptide method, alkylation method, or diazo method etc. to form covalent-binding BM. Covalent-binding BM gives no elution of enzyme and is more stable. BM with better response properties has been obtained by formulating thin polymer membranes. On the other hand, the formulation method is complicated and the enzyme tends to be deactivated chemically. Miyahara[23] et al. utilized covalent-binding BM for SBM. Crosslinking-BM is obtainable through the mutual cross linking of enzyme-protein (so that they form on the thin membranes), using a multifunctional reagent such as glutaraldehyde. While the membranes formation is simple, it has the same weakness as that for the covalent-binding BM. Janata[24] et al. have designed penicillinase-SBM.

(四) PHYSICAL PROPERTIES OF BIOSENSOR MEMBRANES (BM)

The physical properties of BM can be briefly divided into two categories, one for the fundamental properties of BM using a model solution and the other for the practical properties using an actual solution. The properties of both are collated on Table 2.

1. Response properties[32-34]

The response properties of BM represent the condition (properties) of change in output during the period from the time (t_0) when the specimen was added until the time (t_5) when the output reverted to the original condition. During a series of these processes, the response properties of BM correlate mutually with a variety of properties shown on Table 2.

First of all, it is thought about the material diffusion of BM. In general, diffusion coefficient (D'_{eff}) is defined in as the following formula by Fick's law.

$$\frac{dc}{dt} = D'_{eff}\frac{d^2c}{dx^2} \qquad (1)$$

where c is the concentration, t is the time, and x is the displacement in the direction where diffusion generates.

Now, in order to derive the diffusion coefficient of BM experimentally, the relationship between the entire diffusion quantity (Qt) and the time (t) is derived from the following formula.

Table 2 Performances of biosensor membranes

fundamental properties model solution(reagents, control serum,control culture sup)	practical properties actual solution(blood of disease,swage water,culture sup)
1. strength of biosensor membrane 2. membrane formation (immobilization,reproducibility) 3.yield of immobilized enzyme(amount) 4. immobilized enzyme activity 5. diffusion coefficient 6. response time 7. range and limit of detected concern tration 8. activity-profile of various properties (pH,ionic strength,temp., buffers) 9. activity-stability of various properties 10. kinetic properties (Km, Vmax) 11. specificity of substrate 12. effects of interfering substances 13. storage stability 14. life time(half life of activity)	1. Confirmation of fundamental properties using actual solutions 2. comparison between batch system and continuous one 3. comparison between rate assay and steady-state assay 4. adsorption of interfering substances on biosensor membranes 5. injection of samples and buffers 6. washing of biosensor membranes 7. stirring in a reaction vessel 8. control of temperature 9. automation of apparatus 10. standarized biosensor membranes (wet/dry, kit/cartridge, packing, guarantee)

$$Qt = \int_0^t JAdt = \int_0^t \left(V\frac{dc}{dt} + qc\right)dt \qquad (2)$$

where J is the diffusion quantity per unit time and unit area, A is the area of BM, V is the cell volume, and q is the flow rate. From the relationship of equation(2), time-lag(θ) is derived on the graph, and the diffusion coefficeint is derived from the following equation

$$D'_{eff} = \frac{L^2}{6\theta} \qquad (3)$$

where L is the membrane thickness of BM.

An diagram of the apparatus for deriving the diffusion coefficient of BM is shown in Fig.10, while an example of the relationship between the entire diffusion quantity of acetyl choline iodide (AchI) and the time,using wet PVC-BM is shown in Fig.11.

D'_{eff} relies on the material properties of BM and occasionally on the concentration of substrate, but not on the membrane thickness in homogeneous BM.If D'_{eff} relies on the concentration of substrate,it is not desirable as BM since the linear relationship between the output of sensor and the concentration of substrate does not bring into existence even in the range of relatively low concentration of it.

BM usually consists of de-protein membranes (UF membranes), which remove impurities present in the solution,and gas-permeable membranes,in addition to what are called "biosensor membranes" made of fixed enzyme. One of us[21] are elucidating experimentally for D'_{eff} in triple layer membranes in which RBM has been pregnated with collagen membranes. The concentration of the substrate is one of the elements which influence the res-

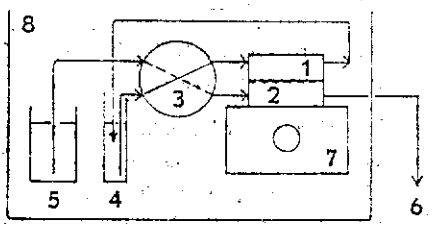


Fig.10 Apparatus for measuring diffusivities of a biosensor membrane: (1) biosensor membrane, (2) diffusion cell, (3) Masterflex pump (1.55ml/min), (4) recycled radio active Ach solution (10ml) (upstream), (5) flux buffer (pH 7.5), (6) microfuges (1.5ml) for sampling, (7) stirrer, and (8) water bath (3℃)

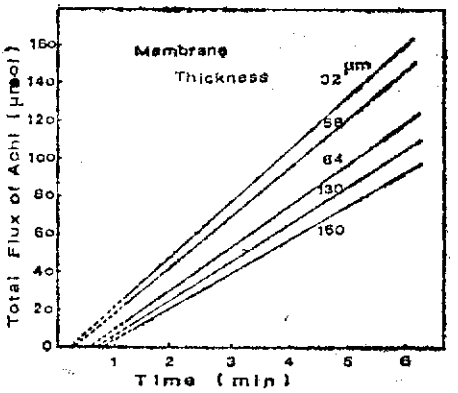


Fig.11 Plot of total flux of AchI against time under the conditions of various kinds of wet PVC membrane (for biosensor membranes) thickness.

ponse properties. In the case of a higher concentration of substrate, the enzyme reaction and the diffusion of substrate into BM are accelerated, and the output of the sensor becomes constant against the concentration of substrate. Therefore, the range of concentration to bedetected, the detection limit (upper and lower limit), and the dilution method of specimen solution etc. are in the process of being evaluated.

The enzyme reaction condition is also one of the important elements which influence the response properties. The suitable condition of BM often differs from that of the liquefied enzyme since the enzyme has been immobilized. Besides this, the elimination and deactivation of enzyme present in BM, washing method of BM and adsorption of interfering substances to BM substantially influences the response properties.

As can be seen from the above, there are many points to be taken into consideration when selecting BM material having a larger D'_{eff} changing triple layer membranes to single layer membranes (thinning of membranes), selecting an efficient immobilizing method and so on.

2. The life of biosensor membranes (BM)

Enzymes will be deactivated by heat and a change in pH. The enzyme present in the solution will usually deactivate within 2—3 days. Thus, it is known that the immobilization of an enzyme onto polymer matrix will make the activity of the enzyme durable. The commercialized glucose biosensor membrane onto which glucose oxidase has been immobilized has maintained its life for more than one month.

The measurement of BM life is conducted over a long life test period of more than one month, and it is consecutively tested several thousand

times etc. In cases where it is difficult to determine the life of BM under the same conditions over a long period of time, BM life can be estimated by calculating the half-life period of the enzyme activity. When it is desirable to determine the BM life in a short period of time, the time-course of BM activity with over a period of time is measured uner several temperature conditions (25—60℃). The BM life can be estimated under normal temperature by this accelerated test. It is often observed that BM life where the model solution is used will substentially differ from the case with the practical solution. It is necessary to make a further detailed evaluation of the influence of an inhibitor on the BM, the difference in the washing conditions of BM etc.

To retain the BM life, it is desirable to measure it under most suitable conditions for enzyme reaction. Recently, multi-BM made of immobilizing more than two kinds of enzymes is being used. Since the suitable conditions for each enzyme vary, the optimum condition of the whole measuring system must be established. Rapid improvement in the length of BM life can be expected if BM can be preserved in a dry condition rather than a wet condition. The dry treatment technology together with the surface treatment technology of BM for the prevention of contamination should become one of the important know-how technologies in the development of functional polymer materials.

(五) CHARACTERISTICS OF BIOSENSOR MEMBRANES (BM)

The attributes of BM and biosensors in which BM are used are as follows:

1. Unstable molecule identification elements can be used repeatedly.
2. Specimens can be analyzed directly.
3. The analytical operation is simple.
4. It only requires a trace amount of the specimen.
5. It requires no reagent other than a buffer solution.
6. It requires no clarity in the specimen.
7. It enables automatic measurement.

The characteristics of BM by using each immobilizing method are collated on table 3.

Insulation-BM and entrapment-BM are superior as regards the immobilization quantity of the molecule identification element, while insulation-BM and adsorption-BM are superior in response properties, and entrapment-BM and biding-BM are superior in stability (life). there are both BM systems and column systems available for biosensors depending upon the sha-

Table 3 Characteristics of biosensor membranes by using each immobilizing method

Immobilized method	Elements for biosensor							Amount of imm. enzyme	Responsibility	Stability	Reproducibility
	Enzyme	Bacteria	Organelle	Antibody	Tissue culture	Multi-enzyme					
Insulation	○	○	○	○	○	○		○	○	×	△
Adsorption	○	×	×	○	×	○		△	○	△	△
Entrapment	○	○	○	○	○	○		○	×	○	×
Binding	○	×	×	○	×	○		×	△	○	○

pe of a immobilized method. The former is the system in which an enzyme reaction, portion and device (mainly electrode) have been consolidated in one unit, while the latter is the isolated system. Compared to the column system, the BM system has the following merits: a compact unit; good response properties; the less influences from the flow rate of the buffer solution, stirring condition etc. and the better reproducibility of output. While its demerits are: firstly, as a device, its systemization with devices other than an electrode (semiconductor inclusive) is difficult; and secondly, in the case of a shorter life of BM resulting in frequent replacement of BM, it is difficult to keep the system condition constant.

(六) EXAMPLES OF BM IN PRACTICAL USE AND ITS DEVELOPMENT

Until now glucose-BM is the most frequently used for clinical examination, fermentation, food and medical treatment. In addition, there are alcohol-BM, lactate-BM, BOD-BM etc. available (table 4).

1. Glucose biosensor membranes (glucose-BM) for clinical examination

Table 4 Commercialized biosensor with use of biosensor membranes for analysis [*]

Biosensor membrane (BM)	Manufacture	Need	Assay item	Reference
Enzyme-BM	TOA Electric Co. Analytical Instr. Co. Fuji Electric Co. Yellow Spring Ins. Toyojyozo Co.	Clinical Analysis Fermentation	Glucose Ethanol Lactic acid	
	Ishikawa Co. Oriental Electric	Fermentation Food	Glucose	
	Technicon Co. (U.S.A) Kyoto Daiichi Elec. Co. Mitsubishi Chemical Co.	Clinical Analysis	Glucose	Immobilized column
	Tateishi Electric Co. Nikkiso Fujisawa pharmaceutical	Medical	Glucose Lactic acid	
Bacteria BM	Denki Kagaku Keiki Nisshin Electric	Fermentation Environment	Ethanol Acetic acid BOD	

* Nikkei Biotech, Jan. 28, 1985

In the second half of 1970, an immobilized enzyme type glucose-BM made by the YSI Company of the U.S.A. has been commercialized. since then, glucose-BM has been developed by several Japanese companies. Glucose is one of the important items in screening examinations as a basic metabolism product in human body. Since glucose examination requries the pre-treatment of blood, it does not allow measurement simultaneously with the other measuring items. The glucose biosensor method has the following merits, when compared to the colorimetric measurement method (HK-G6PDH Method) ; firstly, the unit is compact and measurement is simple, secondly, BM life is long and almost maintenance-free, and thirdly, a reagent is not required.

Typical examples of glucose-BM are illustrated on Table 5. Recently, BM with 3 kinds of BM functions in one layer has been developed.It has made it possible to measure one test specimen within 10 sec. and 1500 test specimen/membrane.

In the future, BM of urea, creatine, CPK, amylase etc., which are urgent examination items, are expected to be developed.

2. Glucose biosensor membranes (Glucose-BM) for fermentation

Recently glucose-BM for fermentation that is capable of solving these problems has been developed. The utilization of glucose-BM for the automatic control fermentation system of glucose, together with soluble oxygen,

Table 5 Examples of commercialized glucose biosensor membranes

Function of biosensor membrane	Polymer materials			Ref.
	A Co.	B Co.	C Co.	
Gas-selective membrane	Cellulose acetate	GOD -cellulose	wet-PVC[2]	Permeated H_2O_2 high activity of sensor membrane, rejection of protein, blood corpuscles, etc.
Immobilized enzyme membrane	GOD[1] -cellulose			
Microfilter	Polycarbonate	Cellulose acetate		
A number of membranes	3 layers	2 layers	1 layer	

1) GOD: glucose oxidase
2) PVC: polyvinylchloride

pH, and alcohol concentration etc. are expected to be developed.

3. BOD biosensor membranes(BOD-BM) for environmental measurement

The BOD biosensor is characterized by its ability to measure BOD in a short period of time, being compact and requireing no special unit. BOD biosensor membranes available on the market are prepared by immobilizing the bacteria present in the sludge by the insulation method. Materials for

manufacturing membranes are available in kit-form and can be used as teaching materials to study a biosensor.

4. Other biosensor membranes

There are some biosensor membranes available as a measuring system for personal use in a factory, though they are not available on the market. For example, they are amino acids, organic acids etc. They are being utilized as part of a system such as the monitoring of artificial pancreas, or as unit monitors at the Aqua-Renaissance Project (Japan government).

5. Development

Biosensor membranes started from EBM owe very much to the immobilizing technology which permits the utilization of a variety of molecule identification elements. Today, the development of electrochemical devices presented new opportunity to develop the SBM. The compactness of the wire electrode and solid state electrode etc. is progressing in the electrochemical device and it will not be long before micro-BM wields its power finer materials and micro materials (cells etc.). On the other hand, in the molecule identification element field, an element with superior specific properties such as monoclonal antibody is being developed, and the development of BM which utilizes this may take place. In line with the progress of electronics, biosensor is systemized, and a "feeling" biosensor and an image biosensor may be utilized. Advanced BM may be multifunctioned and integrated as biochip [35] and a biocomputer element [86]. As we have seen with regard to the development of BM, the significance of the development of functional polymer materials in biochemistry and electrochemistry is growing everyday.

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