Electrochimica Acta, Vol. 31, No. 5, pp. 515–520, 1986.
Printed in Great Britain.

0013–4686/86 $3.00 + 0.00

REVIEW ARTICLE

ELECTROCHEMICAL SENSORS BASED ON BIOLOGICAL PRINCIPLES*

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(*Received* 30 *September* 1985)

Abstract—A number of electrochemical sensors are based on biological principles such as membrane ion transport mediated by ionophores, enzymatic reactions resulting in formation of electrode or membrane active products or in consumption of similar substances, and immunochemical reactions by effect of which concentrations of electrode active substance are changed. These sensors include a certain type of ion-selective electrodes, further enzyme, tissue and bacterial electrodes and immunoelectrodes. The present situations of these sensors and their prospects are critically evaluated.

INTRODUCTION

Application of principles taken from biology to electroanalytical chemistry dates from the beginning of this century. In the search for a suitable biological membrane model, Cremer[1] used thin glass membranes and found that the membrane potential depends on hydrogen ion concentration in the bathing solution. This finding led Haber and Klemensiewicz[2] to the construction of the glass electrode. Although in this way the subject of the present paper could be extended to the whole field of ion-selective electrodes (for review see ref.[3]) a restriction to more specific biophysical or biochemical processes as the basis for construction of electrochemical biosensors is envisaged as the theme of the present paper.

The processes from which electrochemical biosensors originated include:

i. Membrane ion transport mediated by ionophores;
ii. Enzymatic reactions resulting in formation of electrode or membrane active products or in consumption of similar substances;
iii. Immunochemical reactions by effect of which concentrations of electrode active substance are changed.

IONOPHORE MEDIATED ION TRANSPORT[4]

Since 1951 when Berger *et al.*[5] described solubilization of sodium and barium ions in low permittivity solvents by a metabolite later identified as the antibiotic, nigericin[6], a number of analogous natural and synthetic substances were described. They are either macrocyclic or acyclic but form cyclic structures when entering into complexes and possess the following common properties (ref.[3], p. 175):

i. They form relatively stable complexes with alkali metal (in some cases with alkaline earth metal) ions while the stability of the complexes follows ion-selective series.
ii. They enable transfer of hydrophilic ions across the lipid membranes of the cells and cell membranes, across artificial lipid bilayer membranes (BLM) and across relatively thick membranes of organic solvents.
iii. They ion-selectively uncouple oxidative phosphorylation in mitochondria.
iv. Their action results in formation of a defined membrane potential at BLM and thick membranes forming a basis for their use in ion-selective electrodes, as first demonstrated by Stefanac and Simon[7].

These substances were called ionophores by Pressman[8] while the term 'ion-carrier' is also used. Their basic structure can be exemplified by the typical member of this group, valinomycin[9] (Fig. 1). The polar carbonyl groups of the carboxyls stretch into the internal cavity of the molecule while the external 'envelope' of the structure is made hydrophobic by the isopropyl groups. This enables complex formation with alkali metal ions, particularly with potassium ion, which fits well into the internal cavity while the hydrophobic outer surface causes dissolution of the complex in solvents of low polarity as well as in the interior of BLM and biological membranes.

ENZYMATIC REACTIONS[10–12]

The vast majority of biochemical pathways are catalysed by specific proteins, the enzymes. They act according to the scheme

$$\text{substrate} + \text{cofactor} \xrightarrow{\text{enzyme}} \text{products}.$$

The utilization of enzyme catalysed reactions for electrochemical sensors requires that either the cofactor or the products be electrode or membrane active. An example for the first group of sensors is the

*This paper is based on the lecture given at the 36th Meeting of ISE, Salamanca, 24 September 1985.

oxidation of β-glucose by oxygen which is catalysed by β-glucose oxidase:

$$\beta\text{-glucose} + O_2 \xrightarrow[\text{oxidase}]{\beta\text{-glucose}} \text{gluconic acid} + H_2O_2. \quad (1)$$

Both O_2 and H_2O_2 are electroactive.

The second group is exemplified by the hydrolysis of urea catalysed by urease:

$$CO(NH_2)_2 \xrightarrow{\text{urease}} CO_2 + NH_3\,(NH_4^+). \quad (2)$$

Ammonia or ammonium ion influence membrane potentials of various ion-selective electrodes (ISEs).

IMMUNOCHEMICAL REACTIONS[13–16]

The reaction between the antigen and the antibody would not directly supply effects which could be made use of in electrochemical processes. Thus, this kind of reaction is coupled either with an enzymatic reaction supplying electroactive species[14] or with an immunochemical process resulting in destruction of a membrane[15] or influencing an antigen-labelled ionophore present in the membrane[13].

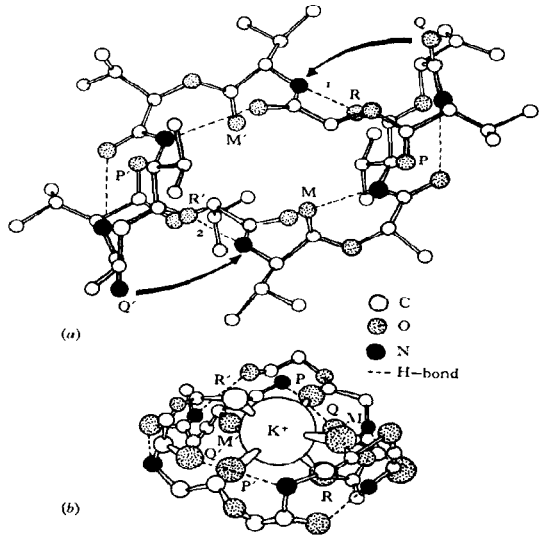


Fig. 1. Conformations of free valinomycin and valinomycin–potassium complex (after Ovchinnikov *et al.* and Duax *et al.*).

PRINCIPLES OF MEASUREMENT

Generation of the electrical signal in the sensor will be dealt with, *ie* the manner by which the extent of biochemical transformation of the determinand is converted to an electrical quantity (in the biochemical way of speaking, the purely electrochemical part of the sensors acts as a transducer of energy).

With sensors based on ionophore-facilitated ion transfer two alternatives will be discussed. In the most frequent version, *ie* in potentiometry with an ionophore based ion-selective electrode[3] the electrical signal is the *emf* of the cell consisting of the inner electrode of the ion-selective electrode, of the membrane and of the reference electrode:

reference electrode	analyte	membrane	inner electrolyte	reference electrode 2

The *emf* E is given by the equation (when the liquid-junction potentials between the reference electrodes and the analyte and the inner electrolyte are neglected):

$$E = E_2 + \Delta\phi_M - E_1$$

where E_2 and E_1 are the potentials of the reference electrodes and $\Delta\phi_M$ is the membrane potential.

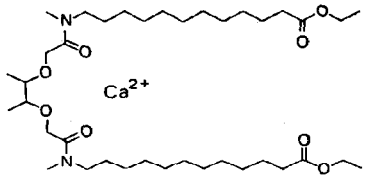
The second alternative is the voltammetric version of potentiometry with ion-selective electrodes, electrolysis at the interface of two immiscible electrolyte solutions (ITIES)[17, 18]. Here, the potential difference at ITIES is varied in a system of two reference and two auxiliary electrodes by means of a four-electrode potentiostat. The interface is formed of an aqueous solution of a hydrophilic electrolyte and of an organic solvent solution of a hydrophobic electrolyte. The resulting E–I–t characteristics are governed by the same principles as in the system of an electrode polarized in an electrolyte solution.

With enzyme electrodes the usual method is to determine the cofactor or the product amperometrically. Very often the Clark oxygen electrode is used. A particular version of the amperometric method is based on a chemically modified electrode with the cofactor bound to the electrode[19]. The second mode of measurement is potentiometry with ion-selective electrodes by which the concentration of the product is measured. Finally, the differential capacity changes due to reversible adsorption of the substrate of an enzymatic reaction is measured[20].

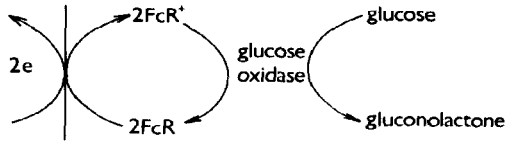
In the case of immunoelectrodes, the Clark oxygen sensor is used in the case of enzyme (usually catalase)-labelled antibodies[14]. Otherwise, the quarternary ammonium ion-selective electrodes function as internal transducers[15].

STRUCTURE AND FUNCTION OF ELECTROCHEMICAL BIOSENSORS

The membranes of ionophore-based ion-selective electrodes are prepared from a poly(vinylchloride) matrix containing an appropriate ion-exchanging solution as plasticizer[21]. This solution contains the salt of the ionophore complex of the determinand cation with strongly hydrophobic anion like tetraphenylborate or *p*-chlorotetraphenylborate[22]. As solvents, *o*-nitrophenyloctylether or phthalic acid esters are used. The ion-selective electrodes of this type include the potassium electrode based on valinomycin[22], the sodium electrode based on monensin[23] and the calcium electrode with the carrier shown in Scheme 1[24].



Scheme 1.



(after Cass et al.)

Fig. 2. A reaction scheme for a glucose enzyme electrode based on a ferrocene modified electrode as internal sensor[19].

Voltammetry at ITIES is particularly suitable for determination of ionophores[25]. In this case a rather high concentration of the salt of the cation which is complexed by the ionophore is used as the electrolyte of the aqueous phase while the organic phase contains a low concentration of the ionophore. With the mode of triangular-sweep voltammetry the current is controlled either by diffusion of the ionophore or of the complex formed to ITIES. This method was applied to determination of the coccidiostat, monensin, in cultures of *Streptomyces cinnamonensis*[26].

Typical enzyme electrodes consist of an immobilized enzyme layer attached to the internal sensor. The immobilization of the enzyme is carried out either by physical adsorption or, preferably, by chemical binding to a polymer layer.

The most important enzyme electrode with amperometric indication is the glucose sensor [27, 28] which is based on reaction (1). Glucose oxidase is immobilized in a gel matrix by reaction with glutaraldehyde and placed on the tip of an oxygen electrode. Glucose and oxygen which diffuse to the enzyme matrix react inside of it. The unreacted oxygen passes through a Teflon membrane and is reduced at a platinum electrode (with a silver/silver chloride electrode as reference). The current is proportional to the oxygen concentration and decreases with increasing glucose concentration. A calibration graph is then used for the determination of glucose. Hydrogen peroxide, which is the product of reaction (1), can also be determined by means of anodic oxidation. However, various interferents prevent proper utilization of this alternative[29].

The main application of the glucose electrode is glucose determination in blood[12]. The necessity of an *in vivo* glucose sensor for artificial pancreas is obvious but in spite of various attempts it has not yet been introduced to clinical practice[30].

An oxygen independent glucose enzyme electrode is based on a ferrocene modified carbon electrode[19]. In this system the immobilized glucose oxidase catalyses the oxidation of glucose to gluconolactone by ferricinium ion. The product, ferrocene linked to the electrode, is then oxidized in an electrode reaction (Fig. 2).

The typical potentiometric enzyme sensor, the urea electrode[31], is based on reaction (2). The product, ammonia or ammonium ion, is then determined either by the ammonia gas probe[32] or by the ammonium ion-selective electrode based on the ionophore, nonactin[33].

In some cases coupled enzyme reactions are used for preconditioning of the determinand by an enzymatic reaction[34], for elimination of the interferents[35] and for recycling the substrate to amplify product formation[36]. For example, a sensor for the determination of sucrose is based on two enzyme layers, one containing invertase which catalyses hydrolysis of sucrose to glucose and fructose. The new substrate, glucose, is then determined by means of reaction (1)[34].

On the whole, the enzyme electrodes with an amperometric indication have definite advantage against the potentiometric sensors, in the first place because of consumption of the product of the enzymatic reaction in the electrode reaction which decreases the response time of the sensor.

The enzymatic processes resulting in concentration change of electroactive cofactors or products can also be substantiated by direct application of biological systems, *ie* by tissues, microorganisms and cell organelles.

In the tissue electrodes[37] a thin layer of a tissue is fixed to the internal indicating system which senses the products of enzymatic reactions of the substrate taking place in the tissue layer. In the glutamine electrode a 0.05 mm thick slice of pig liver is used and the ammonia gas probe functions as internal sensor. An analogy of this system is the adiuretin sensor[38] based on a sodium ion-selective glass electrode covered by toad bladder. In the presence of the antidiuretic hormone the bladder wall becomes permeable for sodium ions which are then sensed by the glass electrode.

Bacterium electrodes[39] usually contain a layer of bacteria fixed to the surface of the internal probe by means of a dialysis membrane. Thus, in the case of the arginine electrode, the enzyme present in *Streptococcus faecalis*, arginine deaminase, catalyses transformation of L-arginine to citraline and ammonia which is then indicated by the internal gas probe.

The substrates, enzymes and inhibitors investigated so far by means of enzyme, tissue and bacterium electrodes are listed in Table 1[12]. The number of measuring systems which have been commercialized is, of course, much smaller (see Table 2). The low stability, particularly of tissue and bacterium electrodes, as well as insufficient sensitivity and accuracy of some determinations, is the main obstacle to wider application.

Finally, two examples of electrochemical immunosensors will be described. They show quite interesting properties in spite of the fact that the methods of radioimmunoassay and of enzyme-immunoassay show considerably superior performance. Aizawa *et al.*[40] described an electrochemical immunosensor where a Clark oxygen electrode is covered with a polymer layer containing the antibody Ab (Fig. 3). This electrode is bathed in a solution containing a mixture of the

Table 1. Substances determined with biospecific electrodes (after Scheller *et al.*)

Amino acids	Amines, amides, heterocycles	
D-alanine	aminopyrine	freshness of meat
L-arginine	aniline	mutagens
L-asparagine	aromatic amines	vitamins
L-aspartic acid	acetylcholine	
L-cysteine	choline	Carbohydrates
L-glutamine	phosphatidylcholine	amygdaline
L-glutamic acid	creatinine	galactose
glutathione	creatine	glucose
L-histidine	guanidine	glucose-6-phosphate
L- and D-leucine	guanosine	lactose
L-lysine	penicilin	maltose
L- and D-methionine	spermine	sucrose
N-acetylmethionine	uric aid	starch
L- and D-phenylalanine	urea	
sarcosine	xanthine	Alcohols, phenols
serine	hypoxanthine	acetaldehyde
L-tyrosine		bilirubin
L-tryptophan	Carboxylic acid	catechol
D-valine	acetic acid	cholesterol
	formic acid	cholesterol esters
Gases	gluconic acid	ethanol
	isocitric acid	glycerol
NH_3	L-ascorbic acid	glycerol esters
H_2	lactic acid	methanol
CH_4	malic acid	phenol
SO_2	nitrilo-triacetic acid	
NO	oxalic acid	Inorganic ions
	pyruvic acid	fluoride
Cofactors	succinic acid	nitrite
		nitrate
AMP	Complex variables	phosphate
ATP		sulphate
NAD(P)H	antibiotics	sulphite
H_2O_2	assimilable carbohydrates	Hg^{2+}
	assimilable substances	Zn^{2+}
	biological oxygen demand	

Table 2. Commercially produced enzyme electrodes (all amperometric)

Determinand	Enzyme
glucose	β-glucose oxidase
sucrose	invertase + β-glucose oxidase
lactose, galactose	galactose oxidase
uric acid	uricase
D-lactate	D-lactate oxidase
L-lactate	L-lactate oxidase cytochrome b_2
choline	choline oxidase
L-lysine	L-lysine oxidase
alcohol	alcohol oxidase
α-amylase	β-glucose oxidase + maltase

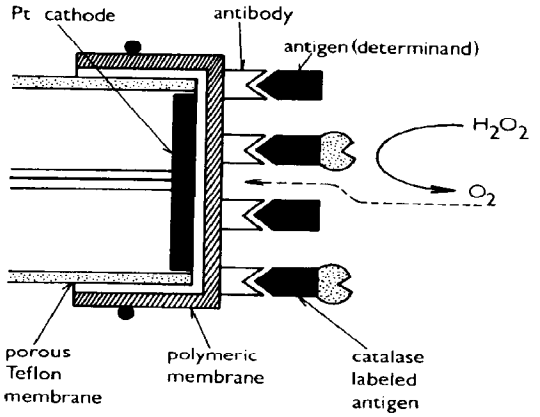


Fig. 3. An immunoelectrode based on catalase-labelled antigen[40].

antigen to be determined, Ag (insulin, choriogonadotropin, etc.), and an analogous catalase labeled antigen EAg which is present at a known concentration. During the bathing period the following antigen–antibody reactions occur:

$$Ag + Ab \rightleftharpoons AgAb$$

$$EAg + Ab \rightleftharpoons EAgAb.$$

By subsequent washing the unspecifically adsorbed antigens are removed from the surface of the sensor which is then put into contact with a solution containing H_2O_2. By effect of catalase H_2O_2 is decomposed and the oxygen formed diffuses to the internal probe.

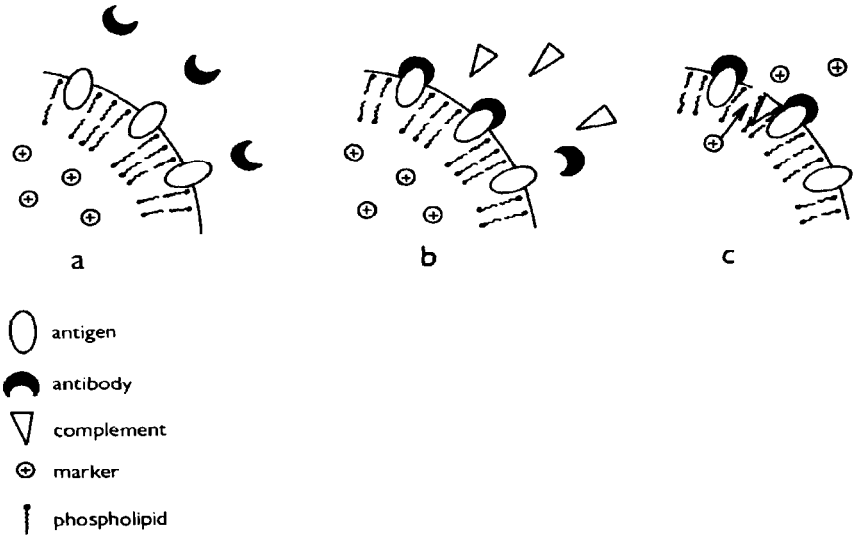


Fig. 4. An immunoelectrode based on lysis of the membrane of a liposome with membrane-bound antigen[41].

Since the amount of the catalase bound to the antibody layer depends on the adsorption equilibrium between the labelled and non-labelled antigen the oxygen current decreases with increasing concentrations of the non-labelled (determinand) antigen in the analyte.

Another approach to immunoanalysis is based on liposomes (vesicles with a bilayer lipid membrane) which contain a liquid antigen in the membrane and a quarternary ammonium ion as a marker inside[41]. The product of the antigen–antibody reaction evokes in the presence of a complement protein a series of reactions resulting in the lysis of the liposome (Fig. 4). In this way the markers are released and then sensed by an ion-selective electrode.

An important step forward would be achieved if some of these biosensors could be miniaturized as it is usual with ion-selective microelectrodes. An ion-selective field-effect transistor (ISFET) could serve as a suitable internal probe[42]. However, a major construction breakthrough will be needed before practical biochips will appear in analytical or clinical laboratories.

The practical application of electrochemical biosensors of the type of enzyme electrodes is still rather small. The most elaborate and mainly used enzyme electrode, the glucose sensor, can only be found in a fraction of clinical laboratories where spectrophotometric methods are mostly used[43]. The situation can be compared with the penetration of the glass electrode into analytical laboratories where it took about 30 years from Cremer's and Haber and Klemensiewicz's discovery to the marketing of the first pH-meter by the Beckman company (see Table 3[43]). A more recent example is the history of sodium and potassium ion-selective electrodes. The sodium glass electrode was known since the 1930s but as there was no suitable electrochemical sensor for potassium the analysis of these ions was made exclusively by means of flame photometry in clinical laboratories. However,

Table 3. ISEs in clinical chemistry (after J. D. Czaban)

1906	H^+ response of glass membrane demonstrated
1924	Glass membranes respond to other ions (Na^+, K^+, Li^+)
1936	Arnold Beckman markets first pH meter
1952–58	Electrodes for P_{CO_2} and P_{O_2} developed
1958	Three channel (pH, P_{CO_2}, P_{O_2}) blood gas analyser developed
1962	Development of non-glass ISEs
1967	First enzyme electrode (glucose)
1969	Valinomycin-based K^+ electrode developed
1971	First Ca^{2+} analyser
1972	Flame photometer replaced by Na^+/K^+ ISEs (in direct potentiometry)
1974	First direct potentiometric analyser for Na^+/K^+ in whole blood
1974	First commercial enzyme electrode
1976	Microprocessor-based Na^+/K^+ blood analyser
1981	Direct potentiometry for Na^+/K^+ becomes more popular

when the valinomycin potassium electrode was discovered by Simon in 1969[44] the automatic potentiometric method for Na^+/K^+ determination found a widespread use so that, after 15 years, more than half of the assays are done by ion-selective electrodes than by flame photometry[43].

Thus, the big effort devoted to the study of biosensors will doubtless not be wasted. The increase of the stability of these sensors as well as an important exemplary success like a glucose sensor for the artificial pancreas will open their way to practical application.

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