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Immobilized Enzymes as Analytical Reagents

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Abstract

Immobilized enzymes are becoming increasingly popular as analytical reagents because of their reusability, stability, and sensitivity to many inhibitors that would seriously interfere in assays using soluble enzymes. In this article, some of the kinetic and catalytic effects of immobilized enzymes in analysis will be discussed. The shift of the activity–pH profile curves on immobilization, the changes in temperature dependence, the inhibitor constants (K_I), Michaelis constants (K_m), and the maximum velocity (V_{max}), plus others, will be discussed. Finally, the use of these immobilized enzymes in fluorometric and electrochemical monitoring systems will be shown, and the future of these reagents in various areas will be discussed. A survey of enzyme electrodes will be presented as an example of the use of immobilized enzymes. Application of immobilized enzyme technology to the assay of BUN, glucose, uric acid, amino acids, ethanol, and other metabolites will be discussed.

Index Entries: Immobilized enzymes, as analytical reagents; enzymes, immobilized, as analytical reagents; analytical reagents, immobilized enzymes as; reagents, immobilized enzymes as analytical; enzyme electrode, in analysis; glucose oxidase; cholinesterase; peroxidase; antigen; antibody; immobilized cells.

Introduction

Excellent chemical analysis can be performed with enzymes, which are biological catalysts; the real advantages of immobilized enzymes are many in analyses using electrochemical probes or other methods of analysis. One advantage of the immobilized enzyme is a pH shift; i.e., the pH optimum can be shifted to that region at which one wants to make a measurement, by choosing the right support for immobilization. Take an enzyme with a narrow pH range of, say, 6–8; this can be shifted

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on immobilization down to the acidic side or, conversely, up to the basic side. Futhermore, the enzymes are much more stable. In some work at Edgewood Arsenal, Maryland, we actually heated our enzymes to 150°F and brought them back down to room temperature, with very little loss of activity. No soluble enzyme could be treated in this fashion.

One advantage often overlooked is that better selectivity can be realized with the enzyme when immobilized; this insolubilized reagent becomes much more selective for an inhibitor, and only the most powerful inhibitor can actually attack the enzyme. We demonstrated this several years ago in an immobilized cholinesterase alarm for the assay of organophosphorus compounds in air and water. No other common interferants disturbed the alarm—it responded only to organophosphorus compounds.

In 1961 at Edgewood Arsenal, I first experimented with some soluble enzymes, such as glucose oxidase, and developed an electrochemical assay for glucose. This led to the use of immobilized enzymes with a commercially available ion-selective electrode sensor to form one self-contained sensor that could be used to measure either organic or inorganic compounds, which are primary or secondary substrates for the immobilized enzyme. The base sensor can be glass; i.e., the cation response can be measured (the ammonium ion, for example), or the pH change in a penicillin electrode can be measured, as done by Mosbach and Papareillo and others. Or a gas membrane can be used as a base sensor, such as the ammonia or the CO_2 membrane. Next are the polarographic sensors that measure peroxide or oxygen, or any of the solid membrane electrodes, i.e., the cyanide electrode. For example, the enzyme can be placed on top of a flat glass electrode sensor; a membrane is then put over the outside of this sensor to hold the enzyme in and keep things like catalase and bacteria out. This protects the enzyme from bacterial spoilage, which is one of the primary reasons for loss of enzyme activity.

With potentiometric devices, we can measure the response either by a steady-state (i.e., equilibrium) method measuring millivolts or microamperes, or by a rate method that senses the change in millivolts or microamperes per minutes. Measurements of substrate can be performed by either a steady-state or a rate method. But measurements of enzyme activity must be done by a rate method. This is a point often hazy in the literature—one can find many claiming that they are measuring enzymes by steady-state methods, though this is impossible under any standard definition of enzyme activity. Enzymes are catalysts and have to be measured by a rate method, but this may be either an interrupted or a continuous measurement of rate.

Enzyme Electrode Probes

An enzyme electrode probe is a union of an enzyme (that highly selective, ultrasensitive catalytic reagent) and an electrode sensor. The result is a device that can measure either organic or inorganic substances in solution directly, usually with no pre-treatment or separation of the reaction mixture, at concentrations of from 10^{-1} to $10^{-5}M$. The principle is quite simple: an enzyme is chosen for use

that reacts, either totally specifically or highly selectively, with the substance to be determined. This enzyme is mounted, either in a soluble or immobilized (insolubilized) form, onto a conventional ion selective electrode that measures either a product of the reaction or one of the original reactants. For example, a glucose electrode can be constructed by mounting glucose oxidase, an enzyme that reacts quite selectively with β-D-glucose, onto either a pH electrode

$$\beta\text{-D-Glucose} + O_2 \underset{\text{oxidase}}{\overset{\text{Glucose}}{\rightleftharpoons}} H_2O_2 + \text{Gluconic acid} \quad (1)$$

$$H_2O_2 + I^- \xrightarrow{\text{Peroxidase}} I_2 + H_2O \quad (2)$$

[which senses the gluconic acid liberated in Eq. (1), a Pt electrode (which can measure the H_2O_2 liberated at + 0.6V vs SCE or the O_2 uptake at − 0.6V vs SCE), an iodide membrane electrode (to sense the I^- taken-up in the indicator reaction (2)] or a gas membrane, Clark-type O_2 electrode, which will measure the O_2 uptake in reaction (1). Of all of these possibilities, the best approach is to measure either the O_2 uptake or the H_2O_2 liberated.

Similarly, an urea electrode can be constructed by placing a layer of urease onto either a glass NH_4^+ ion selective electrode, an antibiotic nonactin NH_4^+ ion electrode or onto a gas membrane (NH_3 or CO_2) electrode. The gas NH_3 electrode is preferred, since it is specific

$$\text{Urea} \xrightarrow{\text{Urease}} NH_4^+ + HCO_3^- \quad (3)$$

$$NH_4^- \xrightarrow{OH^-} NH_3 \quad (4)$$

for NH_3, in contrast to the NH_4^+ electrodes, which sense K^+ and Na^+ as well. The CO_2 gas membrane electrode is also specific, but is slower in response and cannot sense as low concentrations of CO_2, as can the NH_3 electrode measure NH_3.

A list of the enzyme electrode probes described in the literature for the analysis of substrates, is given in Table 1. Over 40 substrate electrodes have been described, many totally specific for the analysis of their substrates. In this table is given also the enzyme used for each electrode, the type of base ion sensor used and the reference. In some cases, i.e., glucose or urea to mention but two, several different types of electrode have been proposed for each substrate, so that in all, 63 different electrodes have been proposed.

Some typical response characteristics of the electrodes are listed in Table 2; the type of immobilization used in construction of the probe, the stability of the electrode (the useful life time of a probe assuming 10 assays per day), the response time in minutes, and the linear range in moles per liter.

The response of any enzyme electrode owes to the diffusion of the substrate into the enzyme layer, where the substrate reacts with the enzyme to yield products:

$$\text{Substrate} \xrightarrow{\text{Enzyme}} \text{Products} \quad (5)$$

The products must then diffuse through the enzyme layer to the electrode surface, where they are sensed by the base probe. A potential is produced that is a log function of the concentration of substrate if a potentiometric sensor (e.g. NH_4^+, I^-,

TABLE 1
Enzyme Electrode Probes for Substrates

Type	Enzyme	Sensor	Reference
Acetic, formic acids	Alcohol oxidase	Pt	*1*
Acetylcholine	Acetylcholinesterase	Choline	*2,3*
Acetyl-β-methylcholine	Acetylcholinesterase	Acetylcholine	*4–6*
Adenosine monophophate (AMP)	5-Adenylate deaminase	NH_4^+	*7,8*
Adenosine triphosphate (ATP)	Glucose oxidase–hexokinase	O_2	*115*
Alcohols	Alcohol dehydrogenase	Pt	*9*
	Alcohol dehydrogenase/diaphorase	Pt	*10*
	Alcohol oxidase	Pt	*11*
D-Amino acids[b]	D-Amino acid oxidase	NH_4^+	*12,13*
L-Amino acids[c] (general)	L-Amino acid oxidase	Gas (NH_3)	*14*
		NH_4^+	*15–19*
		Pt	*20*
	Decarboxylases	CO_2	*21*
L-Arginine	Arginase	NH_4^+	*22*
L-Asparagine	Asparaginase	NH_4^+	*17–23*
L-Cysteine	*Proteus morganii*	H_2S	*24*
L-Glutamine	Glutaminase	NH_4^+	*25*
L-Glutamic acid	Glutamate dehydrogenase	NH_4^+	*26*
	Glutamate decarboxylase	CO_2	*25,27*
L-Histidine	Histidinase	NH_4^+	*28*
L-Lysine	Lysine decarboxylase	CO_2	*29,30*
L-Methionine	Methionine ammonia lyase	NH_3	*31*
L-Phenylalanine	Phenylalanine ammonia lipase	NH_3	*82*
L-Tyrosine	Tyrosine decarboxylase	CO_2	*32,144*
	Tyrosinase	Gas (O_2)	*33*
Amygdalin	β-Glucosidase	CN^-	*34,35*
Butyryl thiocholine	Cholinesterase	Pt(SCh)	*36*
Catechol	Catechol 1,2-oxygenase	O_2	*116,117*
Cholesterol	Cholesterol esterase/cholesterol oxidase	Pt(H_2O)	*37*
		Pt(O_2)	*38–41*
	Cholesterol oxidase	Pt(O_2)	*42*
Creatinine	Creatininase	NH_4^+	*43*
	Creatininase (purified)	NH_3	*44*
Glucose	Glucose oxidase	pH	*45*
		Pt(H_2O_2)	*46–50*
		Pt(Quinone)	*51*
		Pt(DCIP)	*52,53*

(continued)

TABLE 1 (*continued*)

Type	Enzyme	Sensor	Reference
		$Pt(O_2)$	*1,54*
		I	*55–57*
		$Gas(O_2)$	*58–63*
	Glucose oxidase peroxidase	Pt	*64*
Lactic acid	Lactate dehydrogenase	$Pt\|Fe(CN)_6^4$ | C(NADH)	*51* *65*
	Cyt-b_2	Pt	*52,53,66–70*
Lactose	β-Galactosidase/glucose oxidase	$Gas(O_2)$	*71,72*
Lectin	Glucose oxidase	$Gas(O_2)$	*118*
Maltose	Maltase/glucose/oxidase	$Gas(O_2)$	*71,72*
NADH	Alcohol dehydrogenase	Pt	*74*
	Mitochondria	$Gas(O_2)$	*73*
Nitrate	Nitrate reductase/ nitrite reductase	NH_4^+	*75,96*
Nitrite	Nitrite reductase	$Gas(NH_3)$	*76*
Oxalic acid	Oxalate decarboxylase	$Gas(CO_2)$	*77*
Penicillin	Penicillinase	pH	*45,78–81*
Peroxide	Catalase	$Pt(O_2)$	*83*
Phenol	*Trichosporon cutaneum*	$Gas(O_2)$	*119*
Phosphate	Phosphatase/glucose oxidase	$Pt(O_2)$	*84*
Saccharose	Invertase/mutarotase/ glucose oxidase	$Pt(O_2)$	*85*
Succinic acid	Succinate dehydrogenase	$Pt(O_2)$	*86*
Sucrose	Sucrase/glucose/oxidase	$Pt(H_2O_2)$	*87*
Sulfate	Aryl sulfatase	Pt	*88*
Thiosulfate	Rhodanase	CN	*89*
Urea	Urease	NH_4^+	*90–93,129*
		pH	*45*
		$Gas(NH_3)$	*22,94–95*
		$Gas(CO_2)$	*97,144*
Uric Acid	Uricase	$Pt(O_2)$	*98*

[a]Responds to methanol, ethanol, and allyl alcohol.

[b]Responds to D-phenylalanine, D-alanine, D-valine, D-methionine, D-leucine, D-norleucine, and D-isoleucine.

[c]Responds to L-leucine, L-tyrosine, L-phenylalanine, L-tryptophan, and L-methionine.

pH) is used, or a current is produced that is a linear function of the substrate concentration if an amperometric sensor (e.g., Pt) is used. If a highly active ($>$ 10 units) layer of enzyme is used, and if the reaction solution is well stirred, the response time of the enzyme electrode approaches that of the base sensor itself (about 1–2 min for most electrodes).

Also, no longer is it necessary to prepare reagents, make a standard curve, effect

TABLE 2
Typical Response Characteristics of Electrodes

Type	Immobilization[a]	Stability	Response time[b]	Range, m[c]
L-Amino acids	Chemical	4–6 months	2 min	10^{-2}–10^{-4}
(general)	Physical	2 weeks	1–2 min	10^{-2}–10^{-4}
L-Tyrosine	Physical	3 weeks	1–2 min	10^{-1}–10^{-4}
L-Glutamine	Soluble	2 days[d]	1 min	10^{-1}–10^{-4}
L-Asparagine	Physical	1 month	1 min	10^{-2}–5×10^{-5}
L-Lysine	Chemical	6 months	5 min	10^{-2}–5×10^{-5}
L-Methionine	Chemical	6 months	2 min	10^{-2}–10^{-5}
D-Amino acids	Physical	1 month	1 min	10^{-2}–5×10^{-5}
Alcohols	Soluble	1 week	1 min	10^{-2}–5×10^{-5}
	Chemical	>4 months	30 s	10^{-2}–5×10^{-5}
Amygdalin	Physical	3 days	2 min	10^{-2}–10^{-4}
Cholesterol	Chemical	2 months	5 min	10^{-2}–10^{-4}
Glucose	Soluble	1 week	5–10 min	10^{-1}–10^{-3}
	Physical	3 weeks	2–5 min	10^{-2}–10^{-4}
	Chemical	>14 months	1 min	2×10^{-2}–10^{-4}
Penicillin	Soluble	3 weeks	2 min	10^{-2}–10^{-4}
	Physical	2–3 weeks	0.5–2 min	10^{-2}–10^{-4}
Phosphate	Chemical	4 months	1 min	10^{-2}–10^{-4}
Sulfate	Chemical	1 month	1 min	10^{-1}–10^{-4}
Urea	Physical	2–3 weeks	1–2 min	10^{-2}–10^{-4}
	Chemical	>4 months	1–2 min	10^{-2}–10^{-4}
Uric acid	Chemical	>4 months	30 s	10^{-2}–10^{-4}

[a]Physical refers to polyacrylamide gel entrapment; chemical is attachment to glutaraldehyde with albumin, polyacrylic acid, or glass beads; soluble is nonimmobilized.
[b]Time required to reach 95% of total response.
[c]Analytically useful range, either linear or with reasonable change if curvature is observed.
[d]Preparation lacks stability as evidenced by constant decrease in signal each day.

a reaction and measure. A single self-contained electrode is placed into solution, and the concentration is read directly, without any difficulty; only a buffer is required as the reagent. Furthermore, if the enzyme is chemically immobilized, the same electrode can be used for up to several thousand assays, decreasing the cost per test to fractions of a cent.

The linear range of most electrodes is at least 10^{-2}–$10^{-4}M$, with some electrodes sensitive to $10^{-5}M$, and responsive to as high as $10^{-1}M$ concentrations.

The stability of the electrode depends on the type of entrapment. Here again there is much ambiguous reporting of immobilization data in the literature. Some individuals use dry storage for a long period of time and then report a fantastically long lifetime. Realistically, the immobilization characteristics and the stability of the enzyme should be defined in terms of dry storage and use storage. The lifetime of most soluble enzymes, except perhaps in the case of some types of glucose oxidase, which are quite stable in the crude form, is generally about 1 week, or 25–50 assays. However, one must realize that there are potential interferences that arise in

the use of soluble enzymes that are not found in the use of an entrapped enzyme. The physically entrapped enzyme lasts about 3–4 weeks or 50–200 assays. For the chemically bound enzyme, 200–1000 assays is a good range. In many cases, we and others have achieved at least this number, and furthermore there are many enzymes available—bound onto nylon tubes, for example, such as those Technicon is producing for use on SMAC or the Auto-Analyzer, those Boehringer has been experimenting with, and those Miles is selling under the trade name Catalink—that are very stable. These tubes have been demonstrated to last for 10,000 assays.

The stability is dependent also on the content of enzyme in the gel, on the optimum conditions, as was mentioned, and finally on the stability of the base sensor itself. The type of chemical bonding serves two purposes: (a) it selects the pH range and (b) it provides the best immobilization method for each enzyme.

Another factor that will affect the stability of an electrode is the choice of operating conditions. Consider, for example, the amygdalin electrode—amygdalin (a derivative of the drug laetrile) is cleaved by β-glucosidase to CN^- ions that are sensed by a CN^- ion selective electrode. Since the CN^- electrode reponds best to free CN^- ions, obtainable only at pH values >10, Rechnitz and Llenado (*34*) used this pH of 10 for the operation of their electrode. Even though the electrode was physically bound, it lost activity continually and had a life-time of only a few days. It is known that almost all enzymes will lose activity at pH values < 3 and >9, and undoubtedly this was a key contributory factor to the poor stability. Mascini and Liberti (*35*) on the other hand, used a pH of 7, the optimum pH of the amydgalin–β-glucosidase reaction. Their electrode not only had excellent stability, but responded faster and better than that of Llenado and Rechnitz. Thus, the reader would be well-advised always to use the optimum pH of the enzyme system employed, and to force the electrode to conform. Usually it will, as was also demonstrated by Anfalt et al. (*22*) who showed that a urea electrode, constructed with a layer of urease on a gas membrane–NH_3 electrode, responds better at pH 7 (the pH optimum for the urea–urease reaction) than at pH 9 [where the NH_3 electrode can best sense the free NH_3 liberated from NH_4^+ ion; Eqs. (3) and (4)]

Still another factor affecting the stability of some enzyme electrodes is the leaching out of a loosely bound cofactor from the active site, a factor that is essential for enzymatic activity. For example, Guilbault and Hrabankova (*12*) found that D-amino oxidase rapidly loses activity owing to the lability of the bond between protein and coenzyme (flavine adenine dinucleotide, FAD). If the enzyme is stored in a solution of FAD, little activity is lost in three weeks.

Interferences can be in the sensor itself or from other substrates for the enzyme. For example, alcohol oxidase can be used for an excellent acetic acid electrode; an interference would be the native substrate, ethanol. Inhibitors of the enzyme are also interferences, and here the immobilized enzyme makes the enzyme much less susceptible to environmental factors.

Anfalt et al. (*22*) used an NH_3 electrode in combination with immobilized urease and presented a probe that completely eliminates the interference of K^+ and Na^+. Using this probe, Guilbault and Mascini (*95*) developed a specific, fast responding, sensitive system for assay of urea in blood and urine. The use of an NH_3 probe is recommended for all assays of urea in blood or urine.

In the Pt electrode devices proposed for use in assaying for oxidative enzymes, two different approaches have been proposed. One is to monitor the peroxide produced either by measuring the rate of change in the current (*48, 100*) or the total current change at +0.6V vs SCE (*48, 100, 101*). The second is to measure the uptake of O_2 in the enzymatic reaction by noting the reduction of O_2 at −0.6V vs SCE (*1, 98*). Any oxidizable or reducable substances present would interfer in either system. This problem was minimized by Clark (*101*) who used a second uncoated Pt electrode held at the same potential as the enzyme electrode to measure any interferences present in blood. This system, as modified, forms the basis for a Yellowsprings Instrument Co. electrode for glucose. Another method of eliminating this effect is to monitor the rate of change of current very quickly (20 s). This method was proposed by Guilbault and Lubrano (*48*), who noted that more than 200 different blood samples could be assayed with errors of less than 2%. Finally, since a blank value, arising from all the other compounds present in blood that consume O_2, can be subtracted before measurement with the enzyme sensor (*1*) still better selectivity can be obtained using the measurement of O_2 at −0.6V.

Solid precipitate electrodes, such as the CN^- probe used for amygdalin, suffer interferences from any ions capable of forming insoluble precipitates with silver salts. Other interferences include substances capable of reducing silver ion, heavy metal ions, and transition metal ions capable of forming cyanide complexes.

In the glass electrode systems for pH measurement, such as the pencillin electrode (*45, 78–81*), any acidic or basic components present would interfere in the measurement. However, by adjusting the solution to a definite pH before starting the enzyme reaction, and assuming that only reaction will give use to a pH change, these effects can be minimized or eliminated.

Interferences with the enzyme reaction fall into two classes—substrate interferences (other than the analyte) that can be reacted upon by the enyzme, and substances that can either activate or inhibit the enzyme.

Some enzymes, such as urease or uricase, are specific for their substrates, urea and uric acid—and there are no interferences. Others, such as penicillinase (*45,78–81*), react with a number of substrates: ampicillin, naficillin, penicillin G, penicillin V, cyclibillin, and dicloxacillin, all of which can be determined with the penicillin electrode.

Similarly D- and L-amino acid oxidases are less selective in their responses, as is alcohol oxidase (see footnotes to Table 1 for the substrate selectivities). Hence, in using electrodes of these enzymes, a separation must be effected if two or more substrates are present, or else the total is determined. However, totally specific electrodes for the assay of L-amino acids, such as L-methionine (*31*), L-lysine (*29, 30*), L-tyrosine (*32*), or L-phenylalanine (*82*), can be developed by inducing, isolation, separating, and purifying decarboxylative or deaminative enzymes that react totally specifically with one L-amino acid. This same principle can be applied to many enzyme systems that have previously been inaccurately reported not to be specific, yet that, after purification, react with only one substrate. An example of this is creatininase, reported to react with several substrates (*43*), yet after purification, the only substrate is creatinine (*44*).

The activity of the enzyme can be adversely affected by the presence of certain

compounds, called inhibitors (*104*). These are generally heavy metal ions such as Cu^{2+}, Ag^{+}, or Hg^{2+}, but can also be sulfhydryl-reacting compounds, such as *p*-chloromercuribenzoate or phenylmercury (II) acetate, which can react with the free S—H groups present at the active site of many enzymes, especially oxidases.

The key point to emphasize, however, is that the immobilized enzyme is much less susceptible to inhibitors, especially weak or reversible inhibitors, owing to the protection of the immobilization matrix. Thus, by using an enzyme in an immobilized form, most of the interferences arising from inhibitors or activators are eliminated. The user should be aware of the potential dangers of activators or inhibitors, especially in assaying solutions containing metal ions and pesticides.

Construction of an Enzyme Electrode

I would like next to describe the basic procedures in the construction of an enzyme electrode. To do this one needs only (a) to pick an enzyme that reacts with the substances to be determined; (b) to obtain the enzyme, either from a commercial source or by direct isolation; (c) to immobilize the enzyme by standard procedures (chemical attachment is recommended) or buy it already immobilized; and (d) to place the immobilized enzyme around the appropriate electrode to monitor the reaction that occurs [this last step will probably be the limiting factor, since steps (a)–(c) are always possible]. To be useful in a probe, the enzyme must be highly active ($>$ 10 units/probe) and must be in a properly immobilized form.

For details on construction of an enzyme electrode probe, the reader is invited to peruse refs. *102* and *103*.

Antigen–Antibody Probes

Another possible application of biological probes would be the construction of sensor probes utilizing bound antibodies or antigens.

We have, for example, successfully immobilized creatine kinase M antibody as a pretreatment for the detection of cardiospecific CK-MB isoenzyme. Goat anti-human CK-M IgG was immobilized on a carrier (glass beads) through glutaraldehyde coupling, and the immobilized carrier was packed into a magnetic stirring device, which is a rotating porous cell with a removable lid (*120*). The bound antibody could be used for several hundred assays, is regenerable, and excellent results in use of this "immuno-stirrer probe" were obtained in the assay of CPK-MB specifically.

An alternative approach was presented by Suzuki (*121*) who bound an antigen, and developed an assay for syphilis in blood. The contact potential was measured, with very low ΔmV changes (e.g., 1–3 mV).

Probes Using Immobilized Whole Cells

Still another possibility is to use the whole immobilized cell or tissues for electrodes. This field has been reviewed recently by Mattiasson (*122*).

This technique has the advantages: (1) there is no need to purify an enzyme; (2)

the system can catalyze a series of reactions; and (3) the whole cells can be even kept alive for long periods of time. Disadvantages are: (1) generally the response is slow, unless the whole cells contain very high enzyme activity, which is not common; and (2) the response of the probe is not as selective as that obtained with a highly purified enzyme. Some examples of analytical uses are: sliced porcine kidney (glutaminase) for assay of glutamine (*123*), whole living cells of *Sarcina flava* (glutaminase) for glutamine (*124*), intact living cells (*Streptococcus faecalis:* L-arginase) for arginine (*125*), *Azotobacter vinelandii* (nitrate reductase) for nitrate (*126*), and immobilized nitrifying bacteria for ammonia (*127*).

Future Applications

The immobilized enzymes are likely to bring a new future to enzymic analysis and to biochemistry and medicine in general. Enzyme electrodes (transducers with immobilized enzymes), would allow direct, simple, continuous in vivo analysis of important body chemicals. A glucose electrode, for example, would permit a continuous analysis of blood glucose levels in patients, or the analysis of glucose in urine or blood samples in a hospital or clinical laboratory in as simple a manner as a pH measurement. Similarily, implanted transducers using immobilized enzymes could be used for patient therapy. The use of immobilized enzymes in synthesis and therapy and their application in automated analysis, using the enzyme tubes of Technicon, Miles, and Carla, will open new horizons for this very exciting area.

Several recent attempts have been devoted to the design of stable, self-contained enzyme electrode probes that can be easily fabricated in large scale. Guilbault and Lubrano (*105*) described the production of such electrodes for glucose and L-amino acids using various membrane films. A generally useful mild coupling method for enzyme or collagen membranes, using acyl azide activation, has been reported by Coulet et al. (*106–110*). Stable, very sensitive glucose sensors have been described (*107–111*) that can measure as little as 1×10^{-8} mol/L concentrations (*111*).

Finally, it should be mentioned that immobilized enzymes, together with electrochemical sensors, are used in several instruments available commercially. Owens-Illinois (Kimble) has designed a urea instrument using immobilized urease and an ammonia electrode probe, and a glucose instrument using insolubilized glucose oxidase and a Pt electrode (*112, 113*). Patent rights to this system have been purchased by Technicon Inc. Yellowsprings Instrument Company (Ohio) markets a glucose instrument with an immobilized glucose oxidase pad placed on a Pt electrode, and has other instruments available for triglycerides, lipase, and amylase. Universal Sensors (2110 Leroy Johnson Dr., New Orleans) offers enzyme electrode probes for glucose, urea, creatinine, lactate and amino acids that have excellent stability and reproducibility.

Undoubtedly, the future will see many self-contained enzyme electrode probes offered commercially, in addition to the "systems" now sold.

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