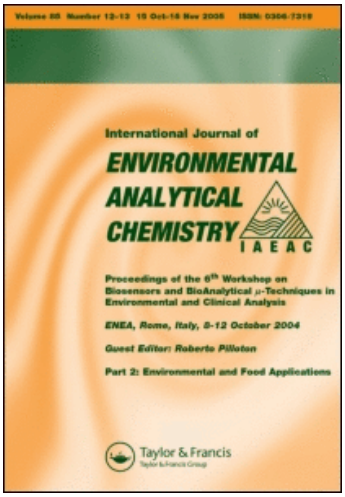

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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G. G. Guilbault[a]; T. J. Rohm[ab]
[a] Department of Chemistry, Louisiana State University in New Orleans, New Orleans, Louisiana, U.S.A. [b] Proctor and Gamble, Cincinnati, Ohio

To cite this Article Guilbault, G. G. and Rohm, T. J.(1975) 'Ion-selective Electrodes and Enzyme Electrodes in Environmental and Clinical Studies', International Journal of Environmental Analytical Chemistry, 4: 1, 51 — 64
To link to this Article: DOI: 10.1080/03067317508071101
URL: http://dx.doi.org/10.1080/03067317508071101

Intern. J. Environ. Anal. Chem., 1975, Vol. 4, pp. 51–64

Printed in Great Britain

Ion-selective Electrodes and Enzyme Electrodes in Environmental and Clinical Studies

G. G. GUILBAULT and T. J. ROHM†

Department of Chemistry, Louisiana State University in New Orleans, New Orleans, Louisiana 70122, U.S.A.

(*Received June 25, 1973*)

Electrodes have been developed for the assay of glucose, urea, amino acids, uric acid, phosphate, nitrate and perchlorate. The electrodes for the organic compounds are enzyme electrodes which are prepared by chemically immobilizing an enzyme over the outside of a conventional ion-selective electrode. These electrodes will be discussed in depth.

The progress and the development of the electrodes that show sensitivity and selectivity for phosphate, nitrate and perchlorate will be outlined. The basis of these sensors is a complex of a transition metal of either an analog of thiourea or an organic chelator, such as 1,10-phenanthraline. Such electrodes respond linearly to phosphate, nitrate or perchlorate, and show selectivity over sulphate, halides and acetate.

The linear range of all these electrodes is approx. 10^{-1}–10^{-5} M with a near Nernstian slope and a reproducibility of 1%. The electrodes are stable and can be used continuously.

KEY WORDS: Electrodes, ion-selective, enzymatic, clinical.

INTRODUCTION

One of the most commonly made measurements in the chemical laboratory is that of pH. The glass electrode, selective for hydrogen ion, a reference electrode and a pH meter combine to form an extremely useful analytical tool. The advantages of this measuring system, often taken for granted, are speed, sensitivity, cost, reliability and the sample is not destroyed or consumed in the process. The same advantages apply to other ion-selective electrodes which have become available in the past few years. At this time,

† Present Address: Proctor and Gamble, Cincinnati, Ohio.

ion-selective electrodes which are sensitive to particular cations and anions can be purchased or constructed at moderate cost. The analytically useful range of these sensors is generally from 10^{-1} *M* to 10^{-4} *M* although there are several sensors which are useful at much lower concentrations. Since the response of ion-selective electrodes is logarithmic, the precision of measurements is constant over their dynamic range.

Potentiometry with ion-selective electrodes is finding wide application in chemical studies. Particularly useful applications are in the fields of clinical and environmental chemistry where a large number of samples, and the need for a rapid method of analysis rule out many slower, more involved methods.

Enzyme electrodes represent the most recent advance in analytical chemistry. These devices combine the selectivity and sensitivity of enzymatic methods of analysis with the speed and simplicity of ion-selective electrode measurements. The results is a device that can be used to determine the concentration of a given compound in solution quickly and a method that requires a minimum of sample preparation. Enzyme electrodes for the determination of glucose, urea, l-amino acids, l-phenylalanine and other substances of clinical importance have been developed and employed to measure the concentration of these substances.

ION-SELECTIVE ELECTRODES

The hydrogen-selective or pH electrode is the best-known ion-selective electrode. Its discovery is credited to Cremer[1] and Haber and Klemensiewicz[2] who found that certain glasses respond to hydrogen ion activity. The response of the glass electrode was commonly believed to be a result of migration of hydrogen ions through the thin glass membrane. The studies carried out by Karreman and Eisenman[3] and the work of Stephanova *et al.*[4] provided the insight necessary for the development of new ion-selective electrodes. At present, ion-selective electrodes for Na^+, K^+, Mg^{++}, Ca^{++}, Cd^{++}, Cu^{++}, Ag^+, NH_4^+, $S^=$, I^-, Br^-, Cl^-, CN^-, SCN^-, F^-, NO_3^-, ClO^-, BF^-, as well as H^+, are available. The electrodes are available from several manufacturers and as newer methods of preparation of I-SE have been developed several kits for the preparation of different electrodes using a common body or housing have been introduced.

The field of ion-selective electrodes was reviewed by Pungor[5] and by Rechnitz.[6] More recent developments and applications of ion-selective electrodes may be found in the report of the Symposium held at the National Bureau of Standards, Gaithersburg, Maryland[7] or the article by Moody and Thomas.[8]

An ion-selective electrode may be defined as a device that develops an electrical potential proportional to the logarithm of the activity of an ion in solution. The term "specific" is sometimes used to describe an electrode. This term indicates that the electrode responds to only one particular ion. Since no electrode is truly specific for one ion, the term ion-selective is recommended as more appropriate.

The response of an ion-selective electrode to an ion, i, of activity $\mathscr{A}_i$ and charge z, is given by the Nernst equation:

$$E = \text{constant} + \frac{2.303\ RT}{zF} \log [\mathscr{A}_i + K_{ij}(\mathscr{A}_j)^{z/y}] \tag{1}$$

in which E is the measured potential, R is the gas constant and is equal to 8.314 joules deg^{-1} $mole^{-1}$, T is the absolute temperature in °Kelvin, F is the Faraday constant equal to 96,487 coulombs $equiv^{-1}$, K_{ij} is the selectivity coefficient, j is any interfering ion of charge y and activity $\mathscr{A}_j$. The sign of the second term on the right-hand side of equation (1) is positive for cations and negative for anions.

The selectivity coefficient is a numerical description of the preferential response of an ion-selective electrode to the major ion, i, in the presence of the interfering ion j. The lower the numerical value of K_{ij} for a particular ion-selective electrode the greater concentration of j can be tolerated before causing errors in the measurement. Values of K_{ij} can be calculated from:

$$\pm \frac{E_2 - E_1}{2.303\ RT/zF} = \log K_{ij} + \left(\frac{z}{y} - 1\right) \log \mathscr{A}_i \tag{2}$$

in which E_1 and E_2 are the measurements of separated solutions of the principle ion and interfering ion, respectively, at the same activity. Or, more realistically, K_{ij} may be obtained by making measurements of the potential of an ion-selective electrode in solutions of constant interferent activity, $\mathscr{A}_j$, and changing primary ion activity, $\mathscr{A}_i$. Then, K_{ij} may be determined by

$$\mathscr{A}_i = K_{ij}(\mathscr{A}_j)^{z/y} \tag{3}$$

The value of $\mathscr{A}_i$ is taken at the point where serious deviation from Nernstian response is noted.[8]

At present, ion-selective electrodes can be divided into several categories according to the composition of their sensor membranes.

1.) Glass electrodes are ion-selective electrodes in which the sensing membrane is a very thin membrane of glass usually in the shape of a bulb. The composition of the glass determines the selectivity of the membrane. Glass electrodes are available which are sensitive to H^+ (pH electrodes) and to cations in the order $Ag^+ > H^+ > K^+ > NH_4^+ > Na^+ \gg Li^+$, Ca^{++}, Mg^{++} over a concentration range 10^{-1} to $10^{-5}M$.

2.) Solid-state electrodes are ion-selective electrodes in which the sensor is a thin layer of a single or mixed crystal or precipitate which is an ion conductor. Two classes of these electrodes are distinguished: homogeneous and heterogeneous. Homogeneous electrodes refer to those electrodes in which the membrane is a pellet prepared from a precipitate, mixture of precipitates, or a single crystal. In the heterogeneous electrodes a precipitate or mixture of precipitates is dispersed in an inert supporting matrix such as silicone rubber or poly(vinyl chloride) (PVC).

3.) Liquid ion-exchange electrodes are prepared by dissolving an organic ion exchanger in an appropriate solvent. The solution is held in an inert matrix. Ion exchangers at present used in the preparation of these electrodes may be a ligand association complex such as those formed by the transition metals with derivatives of 1,10-phenanthroline, quarternary ammonium salts, organic-phosphate complexes and antibiotics. In some cases the exchanger and solvent are entrapped in an inert polymer matrix such as PVC or poly(methyl methacrylate) and coated on a platinum wire or graphite rod.

4.) There are special electrodes which employ a coating over the membrane of an ion-selective electrode. The coating may be a gas-permeable membrane in which case electrodes sensitive to CO_2 or NH_3 are the result. The gas diffuses through the membrane and alters the pH of an internal filling solution. The pH change is measured with a glass electrode and is proportional to the concentration of gas which enters the membrane. Another coating, which has been used successfully, contains an enzyme which converts a substrate to an ion which is detected by an ion-selective electrode. These electrodes will be discussed in some detail in the second part of this paper.

When using an ion-selective electrode to make potentiometric measurements of the activity of a given ion in solution, it is important to remember that the device is affected by the activity of the ion. Therefore, species which may complex the ion of interest and lower its activity must either be removed or masked. It is often necessary to use a buffer solution to control ionic strength, pH and to prevent changes in the activity of the ion being measured by oxidation, reduction or complexation.

There have been a large number of reports of applications and progress in the design and manufacture of ion-selective electrodes in the literature. An important development has been the use of PVC in the preparation of ion-selective electrodes. Electrodes manufactured with PVC are much lower in cost, they have essentially the same response characteristics and usually can be used for longer periods of time than previous electrode assemblies. Moody *et al.*[9] constructed a calcium-selective electrode using a liquid ion exchanger incorporated in a PVC matrix. The optimum concentration of calcium exchanger used in the preparation was described by Griffiths *et al.*[10]

The electrode constructed in this manner gave a near-Nernstian response (30 mV per pC a unit) over the range 2.6×10^{-2} to 6.0×10^{-5} M in $CaCl_2$ solution. Davies *et al.*[11] prepared nitrate ion-selective electrodes by incorporating commercially available liquid ion exchangers in PVC. These electrodes overcome the problem of leakage that is associated with other liquid ion exchange assemblies.

Pick *et al.*[12] have used valinomycin in a variety of neutral carriers to prepare ion-selective electrodes for potassium. The response time of this type of electrode is usually less than 3 sec and the useful range of the electrodes is 10^{-1} to 10^{-5} M. This electrode prepared from this material is exceptional in that there is very little or no drift in potential over a three-day period. As the working characteristics of electrodes are improved and new electrodes are introduced many new applications of these devices can be expected.

With the exception of the glass electrode, the ion-selective electrode that has found the most use is the fluoride electrode. Commercially available fluoride electrodes have a working range of 10° to 10^{-6} M fluoride. The electrode has been used extensively to determine the concentration of fluoride in drinking waters and natural waters. In most cases, the process consists of mixing the samples with a total ionic strength adjustment, TISAB, buffer prior to potential measurement with the fluoride electrode. The concentration is found by comparing the measured potential to a series of potential measurements of fluoride solutions of known concentration.[13,14] Sea water fluoride concentration can be measured in a similar manner, but the standard solutions must contain the electrolytes found in sea water of the type analyzed.[15,16] The fluoride content of a great number of other substances and solutions has been determined using the fluoride ion-selective electrode. Among the materials examined are bone,[17] soils,[18] urine,[19] and serum.[20]

The use of ion-selective electrodes for the determination of the calcium content of biological materials has been investigated vigorously since calcium is one of the most important electrolytes in human physiology.[21] The electrode most commonly used in these studies has been a liquid ion-exchanger of the calcium salt of didecyl phosphoric acid in didecyl-phenyl phosphonate. Such an electrode has a working range of 10^{0} to 10^{-5} M in calcium. The electrode responds to ionized calcium and if the total calcium content of a sample is to be measured, calcium must be freed from ligands or chelates prior to measurement.

The normal values of calcium in serum range from 8.5 to 10.5 mg/100 ml in elders to 9.0 to 11.0 mg/100 ml in children.[22] Of this, 30 to 55% is present as protein-bound calcium, 5 to 10% is present in the form of complexes and chelates and the remainder is ionized calcium.[23] Studies of apparent

ionized serum calcium have been carried out by Hattner *et al.*[24] and by Li and Piechoki.[25]

The calcium content of soils was determined using a calcium ion-selective electrode by Woolson *et al.*[26] and that in water by Hadjiioannou and Papastathopoulous[27] and by Tackett[28] using titration with EDTA with a potentiometric indicating system.

One problem associated with the use of commercially available calcium electrodes is the relatively poor selectivity coefficients of the sensors. Moody and Thomas[29] report the following selectivity coefficients for the Orion Calcium Electrode 92–20:[30]

$$K_{CaH} = 10^5,\ K_{CaZn} = 3.2,\ K_{CaFe} = 0.80,\ K_{CaMg} = 0.014,$$

$$\text{and } K_{CaNa} = 10^{-4}.$$

The same authors report the following for the Corning Calcium Electrode 476041:[31]

$$K_{CaBa} = 10^{-2},\ K_{CaSr} = 10^{-2},\ K_{CaNi} = 10^{-2},\ K_{CaMg} = 10^{-2},$$

$$K_{CaNa} = = 10^{-3}, \text{ and } K_{CaK} = 10^{-3}.$$

It is therefore very important to either mask or remove these interfering ions, particularly iron and zinc before performing direct measurements with a calacium-selective electrode. Alternately, as is the case in serum measurements, standards are prepared using solutions with sodium content (150 mM) that approximates the sodium concentration of serum samples. A new calcium-selective electrode based on a neutral carrier has been developed by Ammann *et al.* [32] This electrode is reported to have superior selectivity for calcium over sodium and magnesium.

The family of electrodes prepared from silver sulfide alone or mixed with halogen salts of silver are well characterized.[5,6] The precipitates are used either in the form of a pellet or mixed in an inert supporting matrix such as silicone rubber. When silver sulfide is used alone, the electrode responds to sulfide and silver ions over the concentration range 10^0 to $10^{-7}M$. The sulfide content of natural waters[33] and low levels of silver[34] have been determined with this electrode.

When a particular silver halide is mixed with silver sulfide to form a sensor membrane, the membrane behaves as though it was composed of the halide salt alone. Electrodes for iodide, bromide and chloride have been prepared in this manner. The iodide electrode also responds to cyanide. An electrode for thiocyanate can be prepared by mixing silver thiocyanate with silver sulfide to form a sensor membrane. The selectivity coefficients of the halide and pseudo-halide electrodes can be estimated by

$$K_{ij} = \frac{\text{Solubility product of Agi}}{\text{Solubility product of Agj}} \quad (4)$$

The chloride electrode has been used to determine the chloride content of sea water[35] and the sulfide electrode has been used to determine the chloride content of biological fluids[36] The bromide present in waters has been determined using a bromide ion-selective electrode.[37] Low levels of cyanide (0.05 to 0.4 ppm) can be determined in a variety of materials with a silver sulfide electrode and suitable masking agents to complex metals, such as nickel, which forms stable cyanide complexes.[38]

Another group of electrodes is prepared by mixing copper, cadmium or lead sulfide with silver sulfide. The electrodes constructed from these mixed sulfides are sensitive to Cu^{++}, Cd^{++} and Pb^{++}, respectively, over the concentration range 10^{0} to 10^{-7} *M*. Silver and mercury(II) are serious interferences and high levels of ferric ion also interfere, but this interference can be overcome by the addition of fluoride to complex the Fe(III). These electrodes have not been extensively applied in the fields of environmental and clinical chemistry.

Liquid ion-exchange electrodes which are sensitive to nitrate, perchlorate, fluoroborate and chloride generally have a useful working range of 10^{-1} to 10^{-5} *M*. The liquid ion-exchange electrode for chloride is not as seriously affected by the presence of sulfide and the halogens as the solid-state chloride electrode. Therefore, it can be used for many assays in which the interference caused by sulfide or the halogens is not negligible. The perchlorate and fluoroborate electrodes have limited applications in analysis. However, the nitrate electrode has been used to directly determine nitrate in many types of samples. Bremner *et al.*[39] determined the nitrate in soils, and nitrate in water was determined by Shaw and Wiley[40] and by Langmuir and Jacobson.[41]

The glass electrodes for determination of monovalent and divalent cations have been used in a variety of clinical and environmental studies. Garrels used a potassium/sodium glass electrode for measuring these cations in sea water.[42] Parts-per-billion quantities of sodium in water were measured by Budd and Jones[43] and Annino[44] used a sodium ion-selective electrode to measure the sodium content of urine. There are many other applications of glass electrodes in the areas of environmental and clinical chemistry. The text edited by Eisenman[42] and the chapter of the N.B.S. publication written by Khuri[45] are recommended to those who are interested in these areas.

Antibiotics and similar compounds have been used successfully to prepare cation-selective electrodes. The calcium ion-selective electrode developed by Ammann *et al.*[32] has been mentioned earlier. Pioda *et al.*[46] have studied

the properties of the antibiotic nonactin for use as a sensor membrane. An electrode prepared from this material in an inert matrix is sensitive to NH_4^+ with linear near-Nernstian response of 51 mV per decade change over the concentration range 10^{-1} to 10^{-4} M NH_4^+.[47] This electrode responds poorly to Li^+ ion activity and the selectivity for NH_4^+ over K^+ and Na^+ was reported to be superior to glass electrodes sensitive to ammonium ion. The nonactin electrode has been used in an enzyme system and will be discussed in the second part of this review. Valinomycin-based potassium ion-selective electrodes have been studied.[48] The antibiotic may be used in a suitable organic solvent or an inert matrix[12] and electrodes prepared in this manner have been used to measure the potassium content of serum.[49] Greater concentrations of sodium can be tolerated with the antibiotic electrode than with the sodium glass electrode. For a more detailed account of the neutral carrier ion-selective electrodes, the chapter by Eisenman[50] in the N.B.S. proceedings is recommended.

The final electrodes to be considered are the gas sensing type prepared by placing a gas-permeable membrane over a housing which contains a pH electrode and internal filling solution. Electrodes of this type are available for the ammonia, carbon dioxide and hydrogen sulfide. The CO_2 electrodes have found most use in the determination of blood P_{CO_2}.[51,52] Ammonia in natural waters and solids[53] has been determined directly using an ammonia electrode and the measurement of the ammonia or ammonium content of a wide variety of samples is feasible since only gases which diffuse through the membrane would interfere with the operation of the device.

ENZYME ELECTRODES

The role of enzymes in biological systems is one of the most interesting and promising areas of science at present under investigation. Enzymes are the regulators of many biological systems upon which depend the very existence of life as we know it. It is not surprising then that there is a need for methods to determine the activity of these extremely important agents. Enzymes are proteins which catalyze a given chemical conversion. As catalysts, they are effective at very low concentrations, they enhance the rate of a reversible reaction without affecting the equilibrium of that reaction and are unchanged in the reaction. The term "unchanged" is naive. Since enzymes bind the substrate which is undergoing a chemical change they are altered. The enzymes, however, subsequently release the modified substrate and return to their original form. As proteins, the activity of enzymes is influenced by the factors which are critical in biological systems. Temperature, pH, the presence or absence of inhibitors, ionic strength and the concentration of substrate all are factors which govern the activity of an enzyme.

The determination of enzyme activity is of great importance in clinical studies and in the area of biochemistry. Electrochemical methods have been applied for these assays. Probably the most common electrochemical method for the assay of enzymes which produce or consume an acid is to follow the pH change of the reaction mixture as a measure of the activity of the enzyme. This method is not generally employed directly since the activity of a given enzyme is affected by changes in pH. Instead a "pH stat" method is used in which the pH of the assay mixture is maintained by the addition of an acid or base. The rate of the addition of reagent gives the reaction velocity.

The activity of an enzyme in a system in which oxygen is consumed can be determined using an oxygen electrode. The electrode is a gold cathode separated by an epoxy casting from a silver anode. The inner sensor body is housed in a plastic casing and comes in contact with the assay solution only through the membrane. When oxygen diffuses through the membrane it is electrically reduced at the cathode by an applied potential of 0.8 V. This reaction causes a current to flow between the anode and cathode which is proportional to the partial pressure of oxygen in the sample. The rate of uptake of oxygen can be related to the activity of the enzyme or the concentration of substrate in the assay mixture. Good correlation between glucose values determined in blood by a measurement of oxygen uptake with those obtained by standard chemical tests was found by Kadish and Hall[54] and by Makino and Koono.[55]

Ion-selective electrodes have been used to determine the activity of rhodanase and cholinesterase. In the rhodanase system, a cyanide-selective electrode followed the decrease of cyanide ion during the reaction,

$$CN^- + S_2O_3^{2-} \xrightarrow{\text{rhodanase}} SCN^- + SO_3^{2-} \qquad (5)$$

which is catalyzed by rhodanase.[56] Results obtained by this method are comparable to those obtained by spectrophotometric procedures. The method is easily adapted to automated systems. The cholinesterase assay was performed using a sulfide-selective electrode to monitor the amount of thiocholine released under the influence of cholinesterase,

$$\text{Acetylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{thiocholine} + CH_3COOH \qquad (6)$$

The amount of thiocholine released is proportional to the activity of cholinesterase.[57] Llenado and Rechnitz[58] used systems very similar to those described above for the assay of β-glucosidase, rhodanase and glucose oxidase. An ion-selective electrode for cyanide was used to follow the production of cyanide ion in the assay of β-glucosidase,

$$\text{Amygdalin} + H_2O \xrightarrow{\beta\text{-glucosidase}} \text{benzaldehyde} + \text{glucoside} + HCN \quad (7)$$

and the consumption of cyanide in the rhodanase system as was described previously.[56] An iodide-selective electrode was used with the glucose oxidase assay,

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{D-gluconic acid} + H_2O_2 \quad (8)$$

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{Mo(VI)} 2H_2O + I_2 \quad (9)$$

to measure the decrease in iodide concentration resulting from oxidation of iodide to iodine by hydrogen peroxide.

In addition to determinations of enzyme activity electrochemical methods have been combined with enzymatic systems to provide highly selective and sensitive procedures for the determination of the concentration of a given substrate. This is possible because under controlled conditions, the rate of an enzyme catalyzed reaction is proportional to the concentration of substrate. The concept of using an enzyme as a reagent in conjunction with an electrode was introduced by Clark and Lyons[59] and the first working enzyme electrode was reported by Updike and Hicks[60] using glucose oxidase immobilized in a gel over a polarographic oxygen electrode to measure the concentration of glucose in biological solutions and in tissues. The use of immobilized enzymes is a more recent development and it overcomes several of the objections which were associated with the use of enzymes as reagents. The high cost of these materials makes routine use of enzymes impractical and the activity of a particular enzyme preparation often varies from manufacturer to manufacturer and from batch to batch. However, by immobilizing the enzyme, the amount of the material required to perform routine analysis is greatly reduced and the need for frequent assay of the enzyme preparation is not necessary. Furthermore, the stability of the enzyme is often improved when it is incorporated in a suitable gel matrix. An electrode for the determination of glucose prepared by covering a platinum electrode with glucose oxidase in a polyacrylamide gel has been used for over 300 days.[61]

Two methods are used to immobilize an enzyme: (a) the chemical modification of the molecules by the introduction of insolubilizing groups. This technique, which results in a chemical bonding of the enzyme, is in practice sometimes difficult to achieve because the insolubilizing groups can attack across the active site destroying the activity of the enzyme; (b) the physical entrapment of the enzyme in an inert matrix such as starch or polyacrylamide gels. The latter procedure is often faster and simpler than chemical immobilization. The immobilized enzyme is then placed over the sensor of an

electrode which is sensitive to the product of the enzyme-substrate reaction. When the enzyme electrode is placed in a solution which contains the substrate for which the electrode is designed, the substrate diffuses into the enzyme layer where the enzyme-catalyzed reaction takes place producing an ion which is detected by the electrode.

Guilbault and Montalvo[62] prepared an enzyme electrode for the substrate urea by immobilizing urease in polyacrylamide and placing this gel over the surface of a cation electrode (Beckman 39137). The ammonium ion produced in the reaction of urea and urease is sensed by the cation electrode; the steady-state potential developed is proportional to the logarithm of the urea concentration. An improved urea electrode was reported by Guilbault and Nagy[63] using an ammonium ion-selective electrode in place of the cation electrode.

Enzyme electrodes for the determination of l-amino acids were developed by Guilbault and Hrabankova[64] who placed an immobilized layer of l-amino acid oxidase over a monovalent cation electrode to detect the ammonium ion formed in the enzyme catalyzed oxidation of the amino acid. Two different kinds of enzyme electrodes were prepared by Guilbault and Nagy for the determination of l-phenylalanine.[65] One of the electrodes used a dual enzyme reaction layer—l-amino acid oxidase with horseradish peroxidase—in a polyacrylamide gel over an iodide-selective electrode. The electrode responds to a decrease in the activity of iodide at the electrode surface due to the enzymatic reaction and subsequent oxidation of iodide.

$$\text{l-Phenylalanine} \xrightarrow{\text{l-amino acid oxidase}} H_2O_2 \qquad (10)$$

$$H_2O_2 + 2H^+ + I^- \xrightarrow[\text{peroxidase}]{\text{horseradish}} I_2 + H_2O \qquad (11)$$

The other electrode was prepared using a silicone rubber based nonactin type ammonium ion-selective electrode covered with l-amino oxidase in a polyacrylic gel. The same principle of diffusion of substrate into the gel layer, enzymatic reaction and detection of the released ammonium ion applies to this system.

Another type of enzyme electrode makes use of a platinum electrode to detect the hydrogen peroxide produced in an enzymatic reaction. The enzyme electrodes for glucose, one of which was mentioned previously,[61] used preparations of glucose oxidase which were non-bonded, physically bonded or chemically immobilized as chemical transducers over a platinum electrode. The electrode was poised at a potential of +0.6 V versus S.C.E. Determinations of the glucose concentration of serum samples made using these electrodes required less than 12 sec and the only reagent required was a phosphate buffer. Of the enzyme preparations studied, the chemically immobilized gel was the most stable.

Alcohol oxidase catalyzes the oxidation of lower primary aliphatic alcohols.

$$RCH_2OH + O_2 \xrightarrow[\text{oxidase}]{\text{alcohol}} RCHO + H_2O_2 \qquad (12)$$

The hydrogen peroxide produced in these reactions may be determined amperometrically with a platinum electrode, as in the determination of glucose above. Guilbault and Lubrano[66] used the alcohol oxidase obtained from Basidiomycete to determine the ethanol concentration of 1-ml samples over the range 0 to 10 mg/100 ml with an average relative error of 3.2% in the 0.5 to 7.5 mg/100 ml range. This procedure should be adequate for clinical determinations of blood ethanol since normal blood from individuals who have not ingested ethanol ranges from 10 50 mg/100 ml. Methanol is a serious interference in the procedure since the alcohol oxidase is more active for methanol than ethanol. However, the concentration of methanol in blood is negligible compared to that of ethanol.

Papariello *et al.*[67] used an immobilized preparation of penicillinase and a pH electrode to determine penicillin concentrations over the rage 10^{-1} to 10^{-4} *M*. The reproducibility of this work is poor in comparison to the procedure described above. A better electrode was described by Mosbach *et al.*[68]

The combination of the sensitivity and selectivity of enzymatic methods of analysis and advances in the development of ion-selective electrodes will provide the analyst with an extremely useful tool for the analysis of a wide variety of samples.

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