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An Air-Gap Enzyme Electrode: Development and Applications

Ravi M. Shanker,[1,2] Nashed E. Nashed,[1,3] and Siegfried Lindenbaum[1,4]

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A novel air-gap enzyme electrode was developed by amalgamating the principles controlling ion-selective electrodes, enzyme kinetics, and diffusion of molecules across membranes. Commercially available ammonia and carbon dioxide gas-sensing electrodes were modified to measure the respective gases over a head-space. A plexiglass cell was designed and fabricated to house the modified electrodes. The air-gap electrodes exhibited superior sensitivity and response characteristics compared to the original membrane electrodes. The plexiglass cell was divided into two compartments by virtue of a selectively permeable membrane. Enzymatic reactions were conducted in the cell to determine quantitatively the concentration of the substrate. L-Phenylalanine ammonia lyase was used to determine L-phenylalanine with the ammonia electrode and L-glutamate decarboxylase was utilized for the determination of L-glutamic acid by the carbon dioxide electrode. Near-Nernstian slopes were obtained for the response of the enzyme electrodes. In addition to the good reproducibility, the method provided unique ability to reuse the same enzyme solution for several determinations of various concentrations of the analyte. The performance of the two compartment cell and air-gap enzyme electrode was found to be superior in comparison to conventional enzyme electrodes.

KEY WORDS: enzyme electrode; air-gap electrode; L-phenylalanine; L-glutamic acid; ammonia electrode; carbon dioxide electrode.

INTRODUCTION

In the ever-expanding field of potentiometric sensors, gas-sensing membrane electrodes have come to be recognized as an important class of ion-selective electrodes. The gas-sensing probes consist of a sensing electrode, a reference electrode, and a gas-permeable hydrophobic membrane. The membrane separates the test solution from the internal sensing electrolyte and the reference and sensing electrodes. Gas-sensing probes without membranes, or "air-gap electrodes," were first reported by Ruzicka and Hansen (1). These researchers also reported the development of the first air-gap electrode for the enzymatic determination of urea in blood (2). The adaptation of this type of electrode for enzymatic assay of urea by Guilbault and co-workers (3,4), as well as the work of Hsiung *et al.* (5), showed the practical utility of this method. They performed the assay of urea using the air-gap electrode in two separate steps: in the first step, the enzymatic conversion of urea was carried out with the soluble enzyme urease; this was followed by the addition of a strong base to convert the ammonium ions to free ammonia. These workers also reported their evaluations of using a macrochamber and a microchamber; the response time with the latter was smaller and it gave better reproducibility. Guilbault and Tarp (3) improved the above system and developed a direct one-step assay for urea by means of chemically immobilizing the enzyme, urease. The enzyme, immobilized either as powder or as gel, was placed at the bottom of a chamber and then covered by a piece of nylon net. The net was kept in position by means of an O-ring which fitted exactly the inside diameter of the cavity of the sample chamber. The air-gap enzyme electrode exhibited linearity in the range of 2×10^{-2} to 4×10^{-4} *M* urea with near-Nernstian slopes. Guilbault and Stokbro (4) reported the development of an electrode specific for urea by using an immobilized enzyme stirrer. In this study, they used the same design for the air-gap electrode as in the previous study, however, the immobilized enzyme was placed on the surface of a magnetic stirrer and covered by a nylon net, and then the enzyme coated stirring bar was placed in the sample chamber for the assays. The solution was stirred and the equilibrium pH was recorded. With this electrode, linearity was obtained from 5×10^{-2} to 4×10^{-4} *M* urea with slopes close to Nernstian. Urea in blood was assayed by the authors with an accuracy of 1.8% and a precision of 2.0%. Hsiung *et al.* (5) reported an air-gap enzyme electrode for the determination of L-phenylalanine with the detection limit of 6.4×10^{-5} *M* of phenylalanine. However, in this assay the enzyme could not be reused, as it was not immobilized.

The various advantages of the air-gap electrode, such as faster response, rapid baseline recovery, ruggedness of the electrode, being unaffected by the components in the solution, or being minimally affected by the components in solution, have been overshadowed by the complex and inconvenient design of these systems. Riley (6) states that gas-sensing membrane probes are, in general, more useful because they are easier to use but air-gap probes are preferable for the analysis of samples which wet or otherwise impair the performance of the membrane. Therefore, the purpose of this research was to develop a simple and convenient system that would overcome the drawbacks of the previous air-gap enzyme electrodes. To enable the achievement of the above objective, the investigations involved (a) modifying commercially available gas-sensing ion-selective membrane probes to air-gap electrodes, (b) designing a cell that would be convenient to use, be able to house the modified electrode, provide an air-gap between the sample solution and the electrode, and also provide an air-tight compartment, and (c) evaluating the feasibility of developing an air-gap enzyme electrode by combining the modified electrode and the new cell. Further, the following two objectives were also borne in mind to make this combination convenient (i) to reuse the enzyme for several determinations of the analyte and (ii) to conduct the enzymatic reaction at either the pH of optimal activity of the enzyme or at any other desired pH.

MATERIALS AND METHODS

Chemicals

The following chemicals were used without further purification: ammonium chloride and lactic acid (J. T. Baker

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[1] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045.

[2] Present address: Pfizer Central Research, Groton, Connecticut 06340.

[3] Present address: Food & Drug Administration.

[4] To whom correspondence should be addressed.

Chemical Co., Philipsburg, NJ); sodium hydroxide (Mallinckrodt Chemical Works, St. Louis, MO); Victawet 12 (Stauffer Chemical Co., Westport, CT); sodium bicarbonate, trishydroxymethylaminomethane (Tris), methanol (HPLC grade); and ortho-phosphoric acid (Fischer Scientific Co., Fairlawn, NJ); and L-phenylalanine, L-glutamic acid, L-glutamic acid decarboxylase, *trans*-cinnamic acid, octane sulfonic acid (sodium salt), and pyridoxal phosphate (Sigma Chemical Co., St. Louis, MO). L-Phenylalanine ammonia lyase, obtained from Sigma Chemical Co., was purified by size exclusion gel chromatography before use. All the solutions in this study were prepared using freshly collected deionized, double-distilled water.

Instruments

A Mettler H51AR or PL300 balance was used to weigh most substances. For weighing small quantities (<50 mg) a Cahn 21 automatic electrobalance was used. The pH of the buffers was measured with a Corning 112 pH meter equipped with a glass electrode with a silver/silver chloride reference electrode (Cole Parmer). The ammonia electrode (Model 95-12) and carbon dioxide electrode (Model 95-01) were obtained from Orion Research Inc. (Cambridge, MA). The potential detected by the electrode was measured using the Orion Research Microprocessor Ionalyzer (Model 910). The Ionalyzer was connected to a Heath Schlumberger (Model EU-200-01) potentiometric amplifier. The output from the amplifier was recorded using a Fischer Recordall (Series 5000) chart recorder. A Corning PC251 magnetic stirrer was used during the entire study for stirring the solutions in the two compartments of the cell. To avoid interferences from static electricity the electrode was placed in a grounded Faraday cage made from perforated aluminum sheets.

A Perkin-Elmer (Model 555) UV-visible spectrophotometer was used for scanning the absorbance spectrum of the compounds as well as measuring the absorbance of solutions for conducting the protein assay for the purification of L-phenylalanine ammonia lyase. The chromatographic method described in this paper was conducted isocratically using a modular HPLC system that included a Waters Associates Model U6K universal injector, Model 6000A pump, and Model 440 absorbance detector with a 254-nm filter. The chromatographic separations were accomplished using an ODS Hypersil (C18) guard column (50 × 4.6 mm, 5 μm) and an ODS Hypersil analytical column (150 × 4.6 mm, 5 μm). The chromatograms were recorded by a Fischer Recordall (Series 5000) chart recorder.

Modifications of the Electrodes

The gas-sensing membrane probes for ammonia (Model 95-12) and carbon dioxide (Model 95-02) were converted into air-gap electrodes by making certain modifications. The major modification was the removal of the hydrophobic gas-permeable membrane from the body of the electrode. In the original electrode, this membrane also served as a seal for the internal electrolyte solution. Therefore, it was necessary to provide an O-ring seal to retain the solution. As a consequence of this modification the sensing tip of the electrode was now exposed to air without the essential layer of electrolyte. A thin layer of the electrolyte solution was adsorbed on the sensing tip by dipping the modified electrode in an appropriate electrolyte (ammonium chloride or sodium bicarbonate) solution containing the nonionic surfactant, Victawet 12 (14).

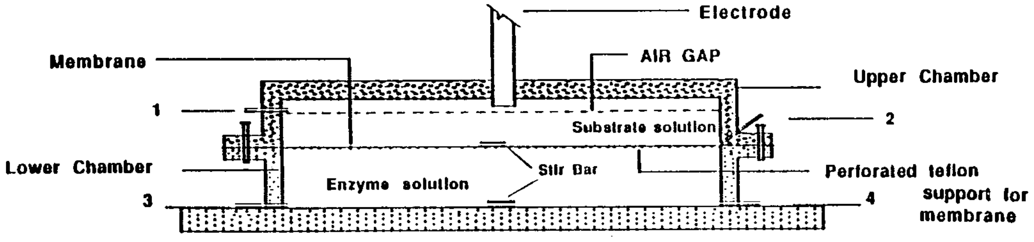
Design of the Cell

A cell was designed and built such that an air gap would separate the thin film of electrolyte on the electrode from the the test solution. The cell, made from plexiglass, consisted of two parts that were held together by four fastening screws. Both the upper and the lower parts of the cell had two ports each for injecting and withdrawing solutions. The upper part of the cell was drilled and tapped for easy mounting of the modified electrode. After mounting the electrode, the cell was rendered airtight by clamping the silicone tubes attached to the four ports. Figure 1 is a schematic diagram of the assembled cell with the modified electrode mounted on it. The cell was divided into two compartments separated by a membrane. Stirring of the solutions in the two compartments was accomplished by placing a magnetic stir bar in each compartment. The assembled cell was placed on a magnetic stirrer, the electrode was connected to the Ionalyzer, and finally, the output from the Ionalyzer was connected to the chart recorder via the amplifier.

Calibration of Electrodes

Membrane Electrodes

Both ammonia and carbon dioxide membrane electrodes were calibrated according to the procedures described in the instruction manual (7,8).



1, 2, 3 and 4 - Ports for injecting / withdrawing solutions

Fig. 1. Schematic diagram of the assembled cell and air-gap electrode.

Air-Gap Electrodes

Ammonia Electrode. Between sample measurements and for overnight storage the modified ammonia electrode was placed in an electrolyte solution. The electrolyte solution consisted of 5×10^{-4} *M* ammonium chloride with 1×10^{-4} *M* sodium chloride. This solution was saturated with the nonionic surfactant, Victawet 12. Four milliliters of the sample solution was placed in the cell, the silicone tubes were closed with pinch clamps, and the electrode was mounted on the cell. The solution was stirred with a magnetic stir bar. The electrode reading was recorded until a stable reading was obtained. Then 500 μl of sodium hydroxide (5 *M*) solution was injected into the cell via one of the ports. The final electrode response was recorded after a new stable reading was obtained. The electrode was removed, and the tip rinsed with water and then placed in the electrolyte solution, rendered ready for the next sample. The cell was rinsed with water, followed by 1 *N* hydrochloric acid solution, and finally, rinsed with water.

Carbon Dioxide Electrode. Procedures very similar to that of the ammonia electrode were used for the carbon dioxide electrode. However, the electrolyte consisted of 2×10^{-3} *M* sodium bicarbonate solution saturated with Victawet 12. Here 500 μl of lactic acid (10%) was used to bring about the change in pH of the sample solution instead of sodium hydroxide. Also, the cell was rinsed with water, followed by 1 *N* sodium hydroxide solution (instead of hydrochloric acid), and again the final rinse was with water.

Enzymatic Determination of Amino Acids

The enzymatic reactions investigated in this study were as follows.

$$\text{L-Phenylalanine} \xrightarrow{\text{L-phenylalanine ammonia lyase}} \text{Ammonia} + \textit{trans}\text{-Cinnamic acid}$$

$$\text{L-Glutamate} \xrightarrow[\text{Pyridoxal phosphate (cofactor)}]{\text{L-Glutamate decarboxylase}} \text{Carbon dioxide} + \text{4-amino butyrate}$$

Conventional Techniques

The enzymatic determination of L-phenylalanine using the gas-sensing ammonia electrode was conducted using previously reported immobilization techniques (3,4). In the first technique the enzyme was immobilized on the electrode by using a cellophane membrane. The second technique used a magnetic stir bar to immobilize the enzyme.

Air-Gap Electrode and Cell

Ammonia Electrode. The cell was assembled as described previously but with a dialysis membrane between the two parts of the cell. The membrane had a molecular weight cutoff in the range of 12,000–14,000. Purified L-phenylalanine ammonia lyase (about 5 units) was injected into the lower (reaction) compartment. Four milliliters of the test solution (standard or sample) of L-phenylalanine was injected into the upper (detection) compartment. The solutions were allowed to incubate for 45 min with constant stirring. Then the enzyme solution was removed from the reaction compartment and replaced by a buffer solution. The electrode was mounted on the cell and the stable baseline reading was recorded. Next 500 μl of sodium hydroxide was added and the final, steady, stable reading was recorded. The subsequent steps were similar to those described for the calibration of the air-gap electrodes. Between samples, the enzyme solution was dialyzed in the reaction compartment of the cell using the buffer solution.

Carbon Dioxide Electrode. Procedures were almost identical to those adopted for the ammonia electrode. In this case, the enzyme L-glutamate decarboxylase (about 5 units) was injected into the reaction compartment together with the cofactor, pyridoxal phosphate (5 m*M*). The enzyme and substrate solutions were allowed to incubate for 45 min and then the enzyme solution was replaced by a buffer solution. All of the other steps were as described for the calibration of the air-gap carbon dioxide electrode.

HPLC Assay

Reverse phase, ion-pair chromatographic assay was developed to separate and quantitate L-phenylalanine and *trans*-cinnamic acid. The mobile phase consisted of 45% 10 m*M* phosphoric acid (pH 2.4), to which 0.1% octane sulfonic acid was added as the ion-pairing agent, and 55% methanol. Twenty microliters of the test solution was injected using Precision Scientific syringes. All analyses were done at ambient temperature. Calibration plots for both compounds were generated by making serial dilutions of the stock solutions of L-phenylalanine and *trans*-cinnamic acid and plotting the recorded peak heights against the respective concentration of the analyte.

RESULTS AND DISCUSSION

Membrane Electrodes

Gas sensing electrodes have evolved from the combination of ion-selective electrodes and the availability of synthetic microporous gas permeable membranes. The construction of various gas-sensing probes has been described in several papers (9–11). The usual electrode assembly consists of a glass electrode as the internal ion-selective electrode and a reference electrode that is joined by an internal electrolyte layer. The two electrodes and the electrolyte are separated from the sample solution by a hydrophobic membrane that is permeable to the gas but impermeable to the aqueous solution. The gas diffuses into the film of the electrolyte at the tip of the electrode from the sample through the membrane. The equilibrium amount of the analyte in the film gives rise to the characteristic pH that is measured by the electrode.

Calibration plots for the response of the membrane-based ammonia and carbon dioxide commercial electrodes was obtained as a function of the concentration of ammonium chloride and sodium bicarbonate, respectively. The plot for the ammonia electrode showed linearity in the range of 1×10^{-5} to 1×10^{-2} M with a slope of -56.4 ± 1.2 mV ($r^2 = 0.996$). Similarly, for the carbon dioxide electrode the concentration range for linearity was 1×10^{-4} to 1×10^{-1} M with a slope of $+58.1 \pm 1.8$ mV ($r^2 = 0.985$). It is observed that the slopes of the two calibration plots are opposite in their sign: negative for the ammonia electrode and positive for the carbon dioxide electrode. The reason for this difference in sign is due to the direction of change in potential as a result of the change in the hydrogen or hydroxyl ion level in the internal electrolyte of the electrode. The Nernst relationship can be expressed as

$$E = E_o - S \log [\mathrm{OH}^-] \qquad (1)$$

or

$$E = E_o + S \log [\mathrm{H}^+] \qquad (2)$$

where E is the measured electrode response in millivolts, E_o is the reference potential (a constant), $[\mathrm{OH}^-]$ and $[\mathrm{H}^+]$ are the hydroxyl and hydrogen ion concentrations respectively, and S is the electrode slope. The equilibrium relationships for the ammonia and carbon dioxide electrodes may be represented as

$$\mathrm{NH_3 + H_2O = NH_4^+ + OH^-}; \quad \frac{[\mathrm{OH^-}][\mathrm{NH_4^+}]}{[\mathrm{NH_3}]} = \text{constant} \qquad (3)$$

$$\mathrm{CO_2 + H_2O = HCO_3^- + H^+}; \quad \frac{[\mathrm{H^+}][\mathrm{HCO_3^-}]}{[\mathrm{CO_2}]} = \text{constant} \qquad (4)$$

Considering that the internal electrolyte contains a constant concentration of the ion of interest (ammonium or bicarbonate), the above equilibrium expressions may be written as

$$[\mathrm{OH^-}] = [\mathrm{NH_3}] \cdot \text{constant}^* \qquad (5)$$

and

$$[\mathrm{H^+}] = [\mathrm{CO_2}] \cdot \text{constant}^\wedge \qquad (6)$$

Since the hydroxide ion concentration is proportional to the concentration of ammonia and the hydrogen ion concentration is proportional to the concentration of carbon dioxide,

$$E = E'_O - S \log [\mathrm{NH_3}] \qquad (7)$$

and

$$E = E^*_O + S \log [\mathrm{CO_2}] \qquad (8)$$

where E'_O and E^*_O are partly determined by the internal reference element, which responds to a fixed composition of the internal filling solution.

Air-Gap Electrodes

The preceeding equilibrium relationships are also applicable in the case of the air-gap electrodes. In this case, instead of the entire solution of the internal electrolyte being subjected to the change in equilibrium, only a thin film of electrolyte present on the tip of the electrode interacts with the gaseous analyte and, therefore, causes the change in the electrode potential. However, the internal reference element still remains the same as in the case of the membrane electrode. Consequently, both E'_O and E^*_O [Eqs. (7) and (8)] are the same for the membrane and air-gap electrodes. The current design of the cell made the chamber airtight, therefore, the activity of the gas (ammonia or carbon dioxide) in the solution, the air gap, and the thin film of the electrolyte was the same. Thus, the air-gap electrode provided a more sensitive measurement of the analyte gas. This was exhibited by the extension in the range of linearity by almost an order of magnitude when compared to the range obtained with the membrane electrodes. In the case of the ammonia electrode the linearity range was found to be 1×10^{-6} to 1×10^{-1} M with a slope of -55.6 ± 1.7 mV ($r^2 = 0.992$). Similarly, for the carbon dioxide air-gap electrode the range was determined to be 1×10^{-5} to 1×10^{-1} M with a slope of $+57.3 \pm 1.9$ mV ($r^2 = 0.993$). Results from these studies are shown in Figs. 2 and 3; these are average values of at least five

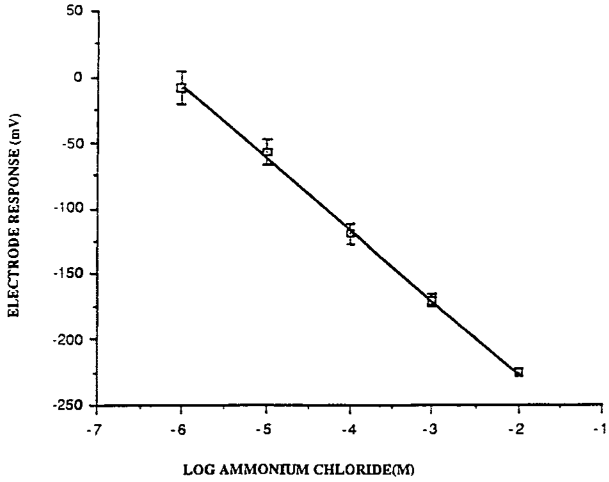


Fig. 2. Performance data for the air-gap ammonia electrode. The plot exhibits a linear response from 1×10^{-6} to 1×10^{-1} M with a slope of -55.6 ± 1.7 mV ($r^2 = 0.992$).

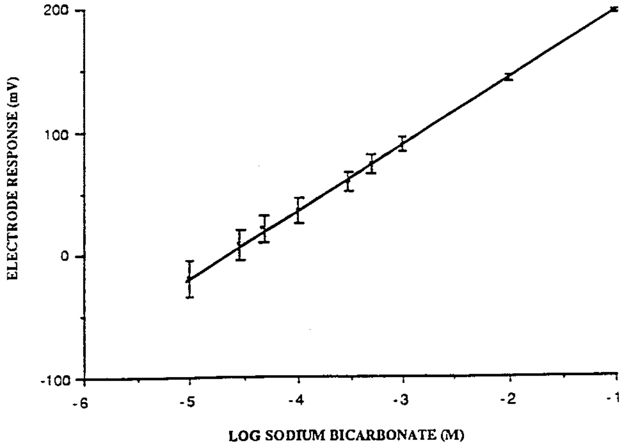


Fig. 3. Performance data for the air-gap carbon dioxide electrode. The plot exhibits a linear response from 1 × 10^{-5} to 1 × 10^{-1} *M* with a slope of 57.3 ± 1.9 mV (r^2 = 0.993).

determinations for each concentration and the vertical error bars represent the standard deviations for the average values. The time required by the electrode to reach the maximal response was significantly shorter with the air-gap electrode compared with the response time for the membrane electrodes (Fig. 4). Although Fig. 4 does not depict the time for recovery to the baseline, it was found that in the case of air-gap electrodes the recovery was instantaneous in comparison to the membrane electrodes, which required more than 25 min to recover to the baseline.

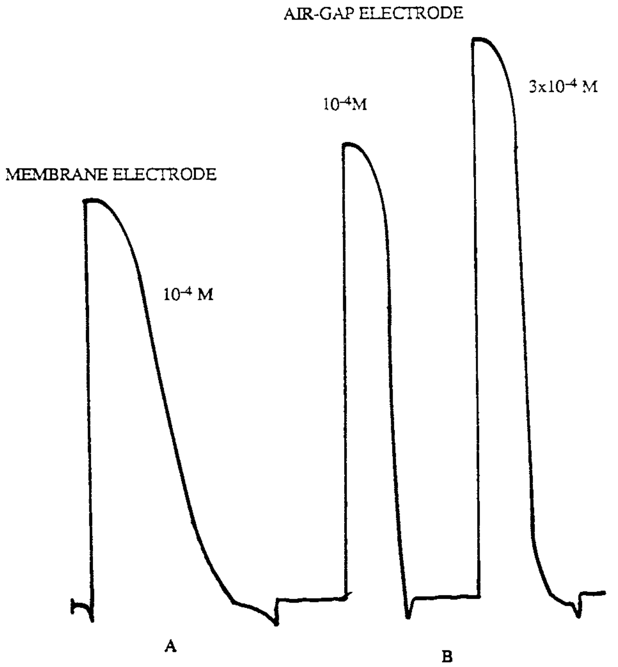


Fig. 4. Recordings of the typical response times of (A) the ammonia gas sensing membrane electrode and (B) the air-gap ammonia electrode using ammonium chloride as the analyte. Chart speed: 0.25 cm/min.

Enzyme Electrodes

Conventional Techniques

Enzyme electrodes have been used as analytical tools because of their selectivity, sensitivity, and convenience. Several different techniques are used to immobilize enzymes at the electrode surface and excellent reviews have been published on these techniques. Use of gas-diffusion membranes together with immobilization techniques has rendered better selectivity to such electrodes. However, the response characteristics of enzyme electrodes are subject to many variables, particularly with regard to their design and construction. Nevertheless, the basic principle remains the same: the enzyme is immobilized on the ion-selective electrode; the substance to be assayed diffuses into the enzyme layer and either produces products or consumes a reactant which is measured by the ion-selective electrode. The measurement may be either potentiometric or amperometric. In the case of potentiometric measurements, the electrode response is related logarithmically to the concentration of the substrate.

Two conventional techniques were investigated in this study to provide a better comparison with the new technique reported. For both of these studies the enzyme L-phenylalanine ammonia lyase was used to assay L-phenylalanine. When the enzyme was immobilized on the tip of the ammonia electrode with a dialysis membrane, the range of linearity for L-phenylalanine was determined to be 5 × 10^{-4} to 1 × 10^{-2} *M*. The slope for this range was −57.2 ± 1.8 mV (r^2 = 0.991). However, the response of the electrode was extremely slow and the recovery to the baseline was even slower. The problem of slow response and very slow recovery was partially overcome by immobilizing the enzyme on a magnetic stir bar with a dialysis tubing. This method exhibited linearity over the range of 1 × 10^{-4} to 1 × 10^{-2} *M* with a slope of −56.5 ± 1.3 mV. Although this method was better than the previous one, it still possessed the disadvantages of the membrane electrode and the stir bar did not have a large surface area to render the reaction more efficient. Previous researchers have also reported similar sensitivity and poor response and recovery times using different substrates and enzymes (4,12). To overcome some of these problems, Guilbault *et al.* (13) designed a new electrode-sample cell that required the internal electrolyte to be completely replaced after assay of each sample. However, none of these systems are capable of providing features for convenience and applicability for the assay of several samples without compromising on either the sensitivity or the reusability of the enzyme.

Air-Gap Electrodes

The higher sensitivity along with the faster response and recovery times of the air-gap electrodes reported in this study was also used to study the two enzymatic reactions. Combination of the air-gap electrode with the two-compartment cell provided a very efficient, convenient, and reproducible system for conducting electrochemical enzymatic assay of the substrates. The plot for the determination of L-phenylalanine by the air-gap ammonia electrode is shown in Fig. 5. The electrode response showed linearity

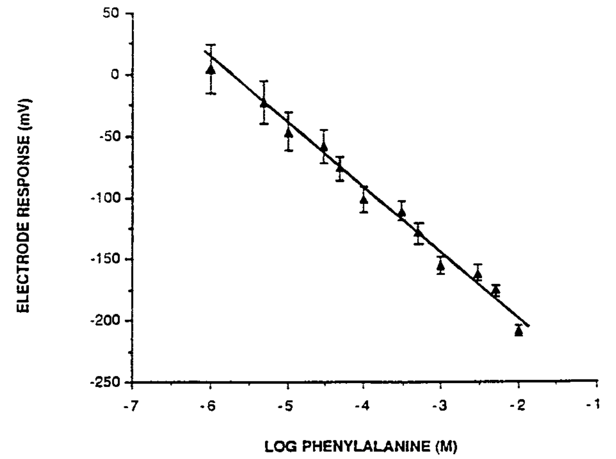


Fig. 5. Performance data for the air-gap L-phenylalanine electrode. The plot exhibits a linear response from 1 × 10^{-6} to 1 × 10^{-2} *M* with a slope of −56.9 ± 1.2 mV (r^2 = 0.977).

over the concentration range of 1 × 10^{-6} to 1 × 10^{-2} *M* L-phenylalanine with a slope of −56.9 ± 1.2 mV (r^2 = 0.987). The enzymatic reaction was conducted at pH 7.5, the optimal pH for the activity of the enzyme. At this pH the product of the enzymatic reaction, ammonia, existed mostly as ammonium ions and, therefore, was contained in the solution. Upon subjecting the solution to a pH change by the addition of sodium hydroxide, the ammonium ions were converted to ammonia and the latter was detected by the electrode. In comparison to either the magnetic stir bar or when the enzyme was immobilized on the electrode, the two-compartment cell had a larger capacity to hold the enzyme and provided more surface area for the substrate to diffuse through the membrane. Therefore, the two-compartment system was more efficient in conducting the enzymatic reaction. Further, the versatility of the system is indicated by the fact that the entire set of data points depicted in Fig. 6 was obtained by using the same enzyme solution over a period of several days. The activity of the enzyme solution was monitored each day and a comparison was done for the electrode response obtained by assaying a sample of 1 × 10^{-4} *M* solution of L-phenylalanine. If the response was less than 70% of the initial response, then the enzyme solution was discarded.

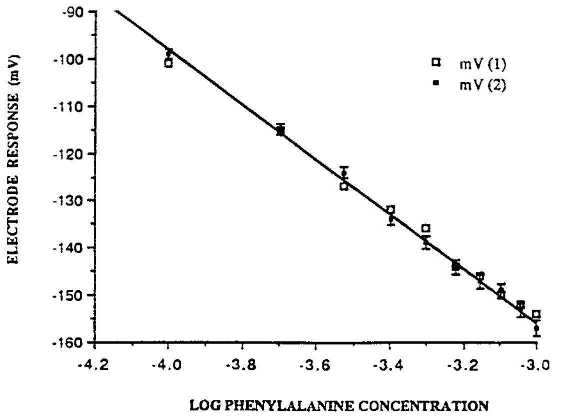


Fig. 6. Performance data for the air-gap L-phenylalanine electrode over one decade of concentration of the analyte. The graph represents two sets of data obtained from two samples of the enzyme, L-phenylalanine ammonia lyase. Slope of the line is −57.8 ± 1.2 mV (r^2 = 0.998).

Although the data in Fig. 6 show the applicability of the system for the determination of L-phenylalanine over a wide range of concentration, the reproducibility of the method within a narrow range of concentration of the substrate needed further investigation. Figure 6 shows two sets of data for the enzymatic determination of L-phenylalanine by the air-gap enzyme electrode. Each of the two sets of data was obtained from a fresh solution of the enzyme but the data within a set were obtained from the same enzyme solution. Each data point represents the average and standard deviation of four determinations. The two sets of data are not statistically different from each other and exhibit excellent linearity over the range of 1 × 10^{-4} to 1 × 10^{-3} *M* concentration of L-phenylalanine. The slope of the regression line was −57.8 ± 1.2 mV and is not statistically different from the slope of the regression line obtained for the data represented in Fig. 6. Thus, this method proved to be convenient, sensitive, and reproducible. To confirm and corroborate the electrochemical assay a HPLC method was developed to separate and quantitate L-phenylalanine and *trans*-cinnamic acid. HPLC analysis was conducted on the same samples that were subjected to electrochemical assay. Excellent agreement was found between the electrochemical and the HPLC data for the enzymatic assay of L-phenylalanine (14).

Studies were conducted with a carbon dioxide electrode to demonstrate the applicability of the system with other gas sensing electrodes. Carbon dioxide produced as a result of the enzymatic conversion of L-glutamic acid by L-glutamate decarboxylase was monitored utilizing the newly developed technique. Linearity was obtained over the concentration range of 5 × 10^{-5} to 1 × 10^{-1} *M* L-glutamic acid, with a slope of 55.1 ± 1.4 mV. Although the sensitivity of the carbon dioxide electrode was not comparable to the sensitivity of the ammonia electrode, it was better than the sensitivity for carbon dioxide enzyme electrodes obtained by conventional techniques. Figure 7 shows the electrode response

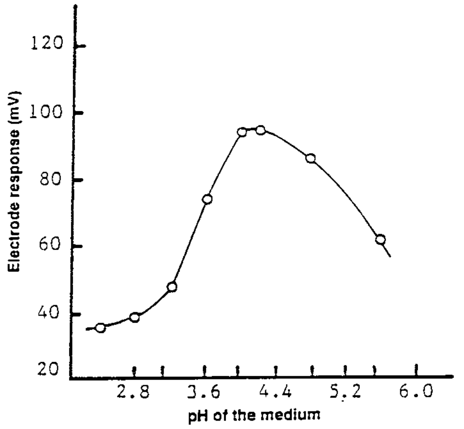


Fig. 7. pH–response profile for the catalytic activity of glutamate decarboxylase determined by the air-gap carbon dioxide electrode.

plotted against the pH of the medium for the determination of L-glutamic acid. The enzyme was found to have optimal activity at pH 4.4; this was in close agreement with report of Shukuya and Schwert (15), who conducted the study in a homogeneous system, when the substrate solution was mixed with the enzyme solution and the product was monitored by measuring the absorbance. These data indicate the flexibility provided by the system to conduct enzymatic reaction at any desired pH.

CONCLUSIONS

This report describes successful modification of commercially available gas-sensing membrane electrodes together with the design of a new cell to house the modified electrodes. Through the application of the principles of ion-selective electrodes, enzyme kinetics, and diffusion, a system was developed to provide a method for efficiently conducting potentiometric enzymatic assays. Modifications on the electrodes resulted in improving the sensitivity and response characteristics of the electrodes. The system also made it possible to reuse the enzyme solution for several determinations of the analyte without chemically or physically modifying the enzyme.

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