

# Preparation and Use of an Enzyme Electrode for Specific Analysis of L-Lysine in Cereal Grains

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## ABSTRACT

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An enzyme electrode selective for L-lysine consisting of L-lysine decarboxylase immobilized at the surface of a CO<sub>2</sub> electrode is described. An electrode responded linearly over a range of 10<sup>-4</sup> M to 10<sup>-2</sup> M L-lysine. Selectivity was good with no observed response to other common amino acids or to L-ornithine. The optimum pH (6.0), enzyme concentration (50 mg/ml), and buffer concentration (0.1M PO<sub>4</sub>) required for maximum electrode response were determined. Response time and recovery time of

the electrode were diffusion-limited and total time for analysis varied from 15 to 25 min depending on L-lysine concentration. Results of analysis of grain and feed samples using the L-lysine electrode compare favorably with lysine values obtained from automated amino acid analysis using ion exchange. The technique should prove useful as an inexpensive method of analyzing grain and feed samples for L-lysine.

Much emphasis has been placed on determination of L-lysine since it is usually the nutritionally limiting amino acid in cereal grains and feedstuffs based on cereal grains. Several methods for quantifying L-lysine using the chemical properties of the free epsilon amino group have been devised (Bodwell 1976, Carpenter and Booth 1973, Finley and Friedman 1973, Friedman and Finley 1975, Holsinger and Posati 1975, Lakin 1973, Morrison and McLaughlen 1972, Mossberg 1970, Ostrowski et al 1970, Ramirez et al 1975, Watz and Ford 1973). These methods tend to be highly variable from one grain or feed to another and subject to interference by high levels of carbohydrate in samples. The most widely accepted and accurate method for L-lysine analysis remains the amino acid analyzer employing ion-exchange chromatography. High capital equipment and maintenance costs prohibit routine use of an amino acid analyzer to characterize the nutritional quality of cereal grains. A complete amino acid analysis by a private laboratory may exceed \$100 per assay.<sup>1</sup>

Since the concept of the enzyme electrode was first introduced by Updike and Hicks (1967), there have been many applications of this technique (Berezin and Klesov 1976, Bowers and Carr 1976, DeClercq 1973, Ferris 1974, Fishman 1976, Gough and Andrade 1973, Gray 1977, Guilbault 1972, Kessler 1976, Olson and Richardson 1974, Rechnitz 1975). Analysis for specific amino acids was accomplished by using various enzymes in association with the appropriate sensor (Guilbault and Shu 1972, Hsiung 1977, Johansson 1976, Nanjo 1974).

Enzymatic analysis of L-lysine using a microbial L-lysine decarboxylase was initially reported by Gale in 1944, whereby CO<sub>2</sub> produced by the enzyme was measured manometrically. Tong and Rechnitz (1976) recently used an electrode to measure the CO<sub>2</sub> produced by the enzyme.

This article describes the physical membrane entrapment of L-lysine decarboxylase at the surface of a CO<sub>2</sub> electrode, thereby allowing simple preparation of a sensitive specific enzyme electrode. This electrode responds linearly from 10<sup>-4</sup> M to 10<sup>-2</sup> M to L-lysine in buffer and in protein hydrolysates yielding results that compare favorably with conventional L-lysine analysis employing ion-exchange chromatography.

## MATERIALS AND METHODS

### Reagents and Materials

Pyridoxal-5'-phosphate, L-lysine decarboxylase Type II powder

(from *Bacterium cadaveris*) (Lot 109B-6881), L-lysine HCl, and all other L-lysine and D-amino acids were obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals were reagent grade.

Samples of grain and feed were obtained from the Departments of Dairy Science and Poultry Science of the University of Wisconsin-Madison. These samples were analyzed previously on a NJEOL Model JLC-6AH amino acid analyzer, and the lysine values were compared with those obtained with the enzyme electrode.

### Apparatus

Potentiometric measurements were obtained using a Radiometer model pH 26 meter appropriately interfaced to a Barber Coleman strip chart recorder model 8700-26000. The CO<sub>2</sub> electrode (IL 20334) was purchased from Instrumentation Laboratories and equipped with a Silastic® membrane (IL 19030). The external membrane used to entrap the enzyme solution was Cupraphan® (Type 150 PM) cellulose-hydrate membrane, obtained from Enka Glanzstoff A6, Wuppertal 2, W. Germany.

### Preparation of Electrode

The external cylinder of the disassembled CO<sub>2</sub> electrode was inverted and a clean Silastic membrane was placed over the end of the cylinder. To the center of the Silastic membrane surface was applied 20 µl of enzyme solution containing 50 mg/ml of L-lysine decarboxylase in 0.1M phosphate buffer, pH 6.00. A sheet of Cupraphan membrane was then placed over the drop of enzyme solution causing it to spread over the end of the CO<sub>2</sub> cylinder. Precautions were taken to exclude air bubbles from between the Silastic and Cupraphan membranes. A rubber "O" ring was used to secure the Silastic and Cupraphan membranes with a thin film of enzyme solution entrapped between in place over the end of the CO<sub>2</sub> cylinder. Excess membrane was trimmed from behind the "O" ring. The electrode surface and folded membrane remaining behind the "O" ring were rinsed with 0.1M phosphate buffer to remove any adhering enzyme solution. The internal electrolyte was then added to the upright CO<sub>2</sub> chamber, and the pH electrode was carefully inserted into the chamber while the membrane surface of the chamber was submerged in distilled water. This prevented dehydration of the Cupraphan membrane, which may rupture when it is stretched, and the pH electrode is inserted.

The electrode was operated at room temperature by immersing it beyond the "O" ring into a 10-ml beaker containing the sample, which was stirred with the aid of a magnetic flea. The end point was taken as the millivolt response after the electrode had come to equilibrium with the sample (the plateau representing a steady state response on a response vs. time curve). The electrode surface was rinsed with distilled water after exposure to each sample and

<sup>1</sup>Personal communications, WARF Institute, Madison, WI.

carefully dried with a tissue before placing it in a solution of buffer with added  $1.0 \times 10^{-5} M$  pyridoxal-5'-phosphate, but no L-lysine, to allow the recorder pen to return to baseline. The electrode was stored in buffer containing  $1.0 \times 10^{-5} M$  pyridoxal-5'-phosphate when not in use to assure that response of the electrode did not become cofactor limited.

### Sample Preparation

A 1.000-g sample of grain or feed was placed in a 300-ml round-bottom flask with a 24/40 standard taper joint, and 50 ml 6*N* HCl was added. A stopcock joined to a male 24/40 standard taper joint, as used by Stahmann and Woldegiorgis (1975) was inserted into the flask, which was evacuated and flushed with  $N_2$  three times. The stopcock was closed and the flask placed in a positive draft oven at 110°C for 21–22 hr. The hydrolysates were evaporated to dryness on a rotary evaporator at 45°C, resuspended in 50 ml of distilled water, and dried again. The hydrolysates were filtered with a sintered glass funnel and the filtrates again evaporated to dryness. Forty milliliters of 0.1*M* phosphate buffer, pH 6.00, in four 10-ml aliquots, was used to transfer the hydrolysate to a 50-ml volumetric flask. The pH of the solution was adjusted to pH 6.00 using a pH meter and 1.0*N* HCl or 1.0*N* KOH. The sample volume was then adjusted to 50 ml by addition of 0.1*M* phosphate buffer, pH 6.00. Samples were stored frozen and thawed just before analysis to prevent microbial growth.

## RESULTS AND DISCUSSION

### Theory

An enzyme selective membrane electrode for L-lysine was prepared by immobilizing a layer of L-lysine decarboxylase at the active surface of a  $CO_2$  electrode (Fig. 1). The enzyme specifically decarboxylated L-lysine liberating  $CO_2$ , which diffused through the gas-permeable Silastic membrane causing a change of pH in the electrolyte solution between the Silastic membrane and the internal pH electrode. This change in pH of the internal electrolyte was stoichiometrically related to the L-lysine concentration of a solution in contact with the L-lysine electrode.

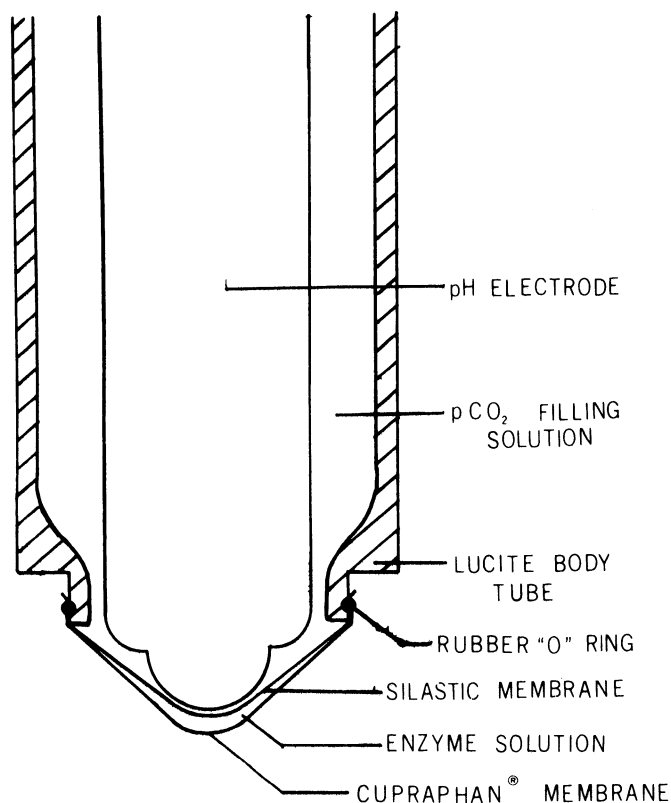


Fig. 1. Design of the L-lysine electrode.

### Response

Response curves of the L-lysine electrode were linear over the range of  $10^{-2} M$  to  $10^{-4} M$ . To assure that lysine concentrations were within the linear range of the electrode, the hydrolysate samples were diluted so as to be in an appropriate range of  $4.10 \times 10^{-4} M$  to  $5.05 \times 10^{-3} M$  L-lysine. These sample ranges are characteristic of corn and soybean meal hydrolysates and, therefore, represent realistic extremes of L-lysine concentrations to be expected in routine use of the electrode. The theoretical slope of the response curve should correspond to one pH unit per decade of L-lysine concentration difference; a slope of 0.5 pH per decade was realized for this electrode, however. Deviation from the theoretical slope is most probably the result of measuring only a portion of the  $CO_2$  produced by the enzyme reaction. Presumably, a large portion of the  $CO_2$  produced diffused back into the bulk solution being analyzed. In practice, the slope of the response curve is determined by the amount of enzyme activity, pH equilibrium, and the geometry of the electrode surface. The limits of detection of the enzyme electrode are nearly the same as those for the  $CO_2$  electrode itself and are determined by the same factors. The lower limit of detection is determined by the internal electrolyte concentration and the  $pCO_2$  in the atmosphere, whereby the upper limit of detection is determined by the solubility of  $CO_2$  at the operating temperature and pH of the electrodes.

Stability of the enzyme electrode is also the same as of the  $CO_2$  electrode alone, exhibiting a drift of  $\pm 1$  mV at equilibrium.

### Specificity

The L-lysine electrode gave no detectable response to a mixture

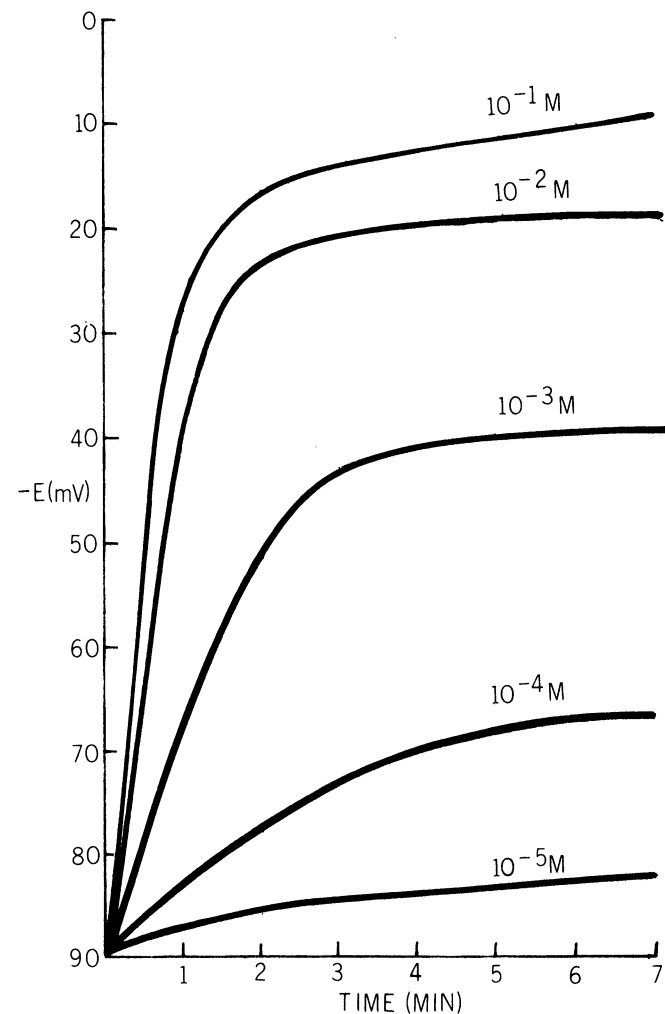


Fig. 2. Response of the L-lysine electrode with respect to time with increasing concentrations of L-lysine.

of D-amino acids or to the L-isomers of alanine, arginine, asparagine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Neither did the electrode respond to solutions of L-ornithine. This demonstrates the specificity of the L-lysine electrode to those species that would be common in food or feed samples.

### Response Times

Typical response curves of the L-lysine electrode for solutions of L-lysine are shown in Fig. 2. Equilibrium response ( $\pm 1$  mV) was achieved in all samples within 6 min. More concentrated samples exhibited a faster response time probably because the increased concentration gradient enhanced mass transport across the Cupraphan membrane. Equilibrium was attained when rates of diffusion of substrate into the soluble enzyme layer, diffusion of products out of the enzyme layer, and diffusion of  $\text{CO}_2$  to the electrode surface reached a steady state. Diffusion of L-lysine across the membrane and establishment of an equilibrium with the finite volume of enzyme solution entrapped between the membranes were the primary rate-limiting steps in establishing an equilibrium response from the electrode. Thickness of this diffusion layer formed by the outer membrane and the enzyme solution and permeability of the outer membrane layer determined the response time of the electrode. Recovery time of the electrode on completion of analysis was also related to thickness of this diffusion barrier and was usually longer than the response time. Vigorous stirring of the sample and relatively large volumes of

solution during the recovery period decreased the time required to approximately 1.5–2 times the response time. Therefore, the total time to complete an analysis and await recovery of the electrode was 15–25 min, depending on the L-lysine concentration in the sample.

### pH Optimum

The pH for L-lysine determination was optimized using phosphate buffer as described by Soda and Moriguchi (1969). Response curves in Fig. 3 obtained at several pH values indicated maximal linearity at pH 6.20 and pH 6.00. Values for the pH optimum of the L-lysine decarboxylase of 6.20 and 6.00 have both been reported (Gale 1944, Soda and Moriguchi 1969). The electrode was operated at pH 6.00 because of a positive pH shift in the enzyme solution at the electrode surface caused by accumulation of products in the enzyme layer. This pH shift was not seen in the bulk of the solution because the concentration of products was relatively dilute. A small pH shift in the bulk solution did occur if the electrode was kept in a weakly buffered solution of substrate for several hours.

### Ionic Strength

The primary effects of ionic strength are on buffering capacity of the buffer and the activity of the ions in solution. As shown in Fig. 4, the optimum buffer concentration was 0.1M. As the ionic strength of the substrate solution was increased, the response of the electrode decreased, as would be predicted by the Debye-Huckel equation. Consequently, the entire response curve shifted toward a

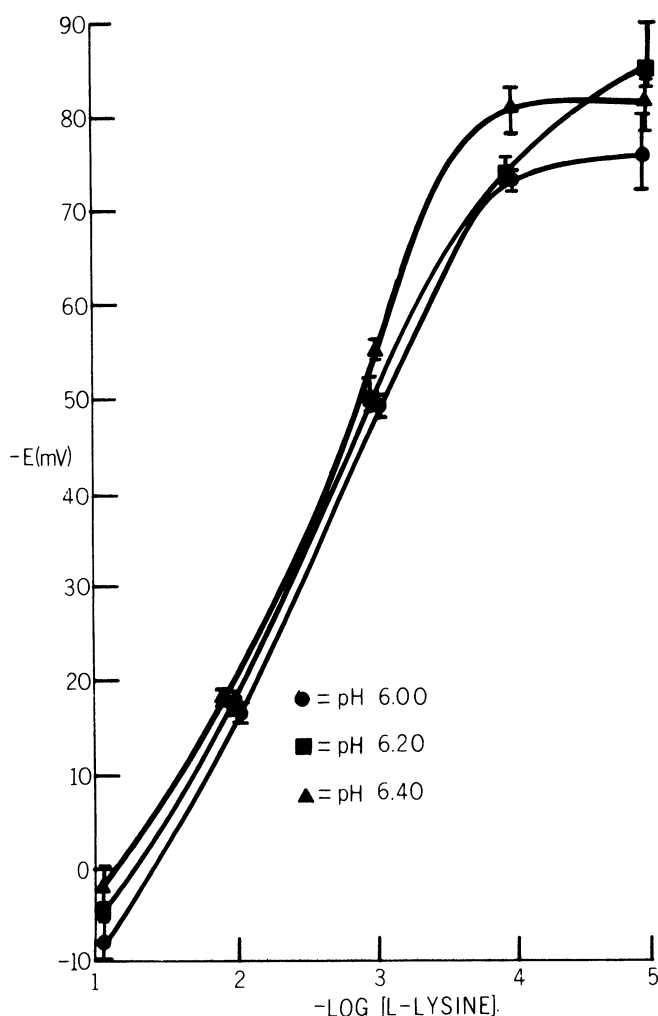


Fig. 3. Effect of pH on electrode response as a function of L-lysine concentration.

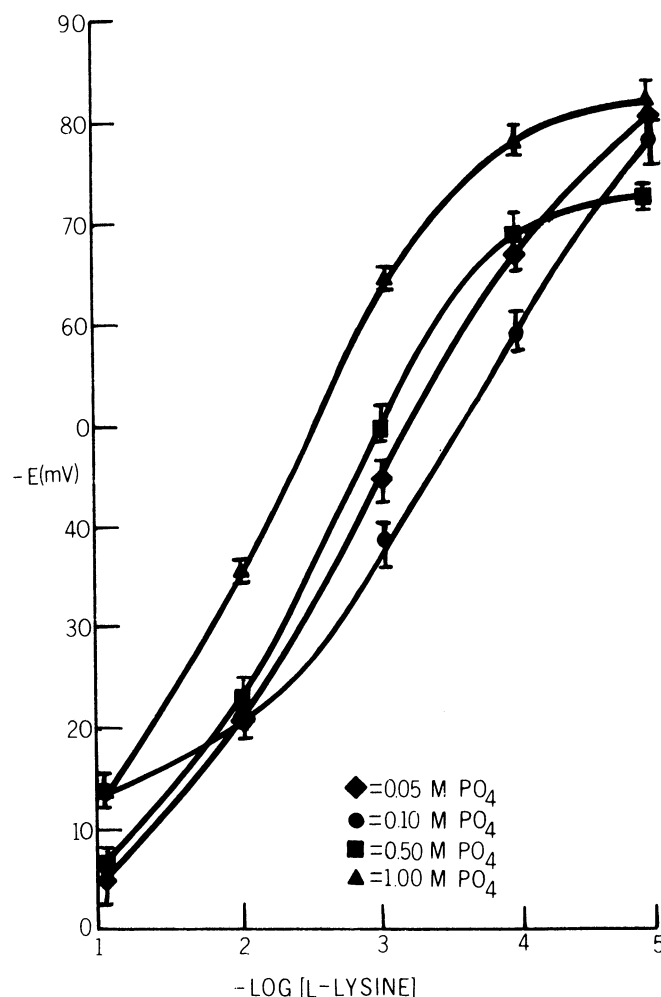


Fig. 4. Effect of ionic strength of phosphate buffer on electrode response as a function of L-lysine concentration.

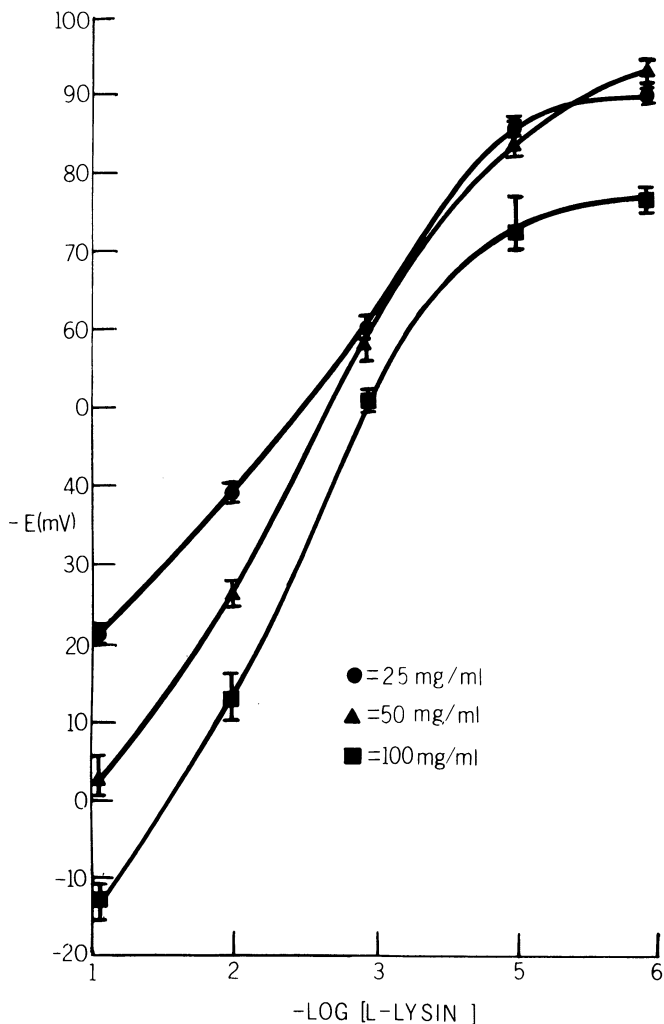


Fig. 5. Effect of enzyme concentration on electrode response as a function of L-lysine concentration.

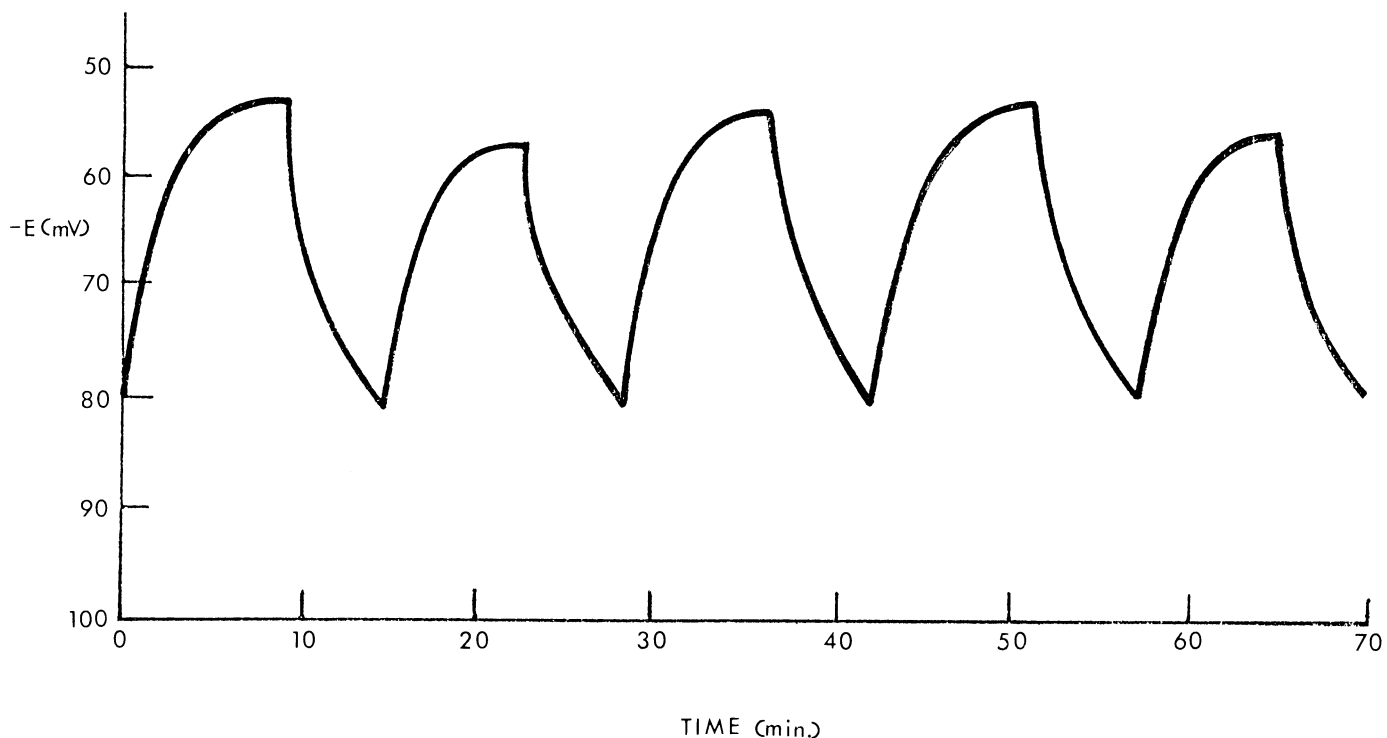


Fig. 6. Electrode response and recovery curves of L-lysine electrode.

lower electrode response and the lower limit of detection changed at higher substrate concentrations. Decreases in ionic strength caused deviations from linearity at the upper limit of detection as the concentration of substrate approached the concentration of buffer and the ionizable groups on the substrate became major contributors to the pH of the system. Actual changes in pH occurred as a result of high substrate concentration exceeding the buffer capacity of buffering species.

#### Enzyme Concentration

The L-lysine decarboxylase Type II powder (lot 109B-6881) used for preparation of L-lysine electrodes had a specific activity of 1.5 units (where 1 unit = 1  $\mu$ mol of  $\text{CO}_2$  released from L-lysine per minute at pH 6.0 at 37°C per milligram of protein). Various concentrations of this enzyme were used to prepare electrodes and the responses of these probes are compared in Fig. 5. The optimum concentration of enzyme was 50 mg of enzyme powder per milliliter of buffer. Lesser concentrations of enzyme produced response curves with decreased slopes. On the other hand, doubling the enzyme concentration to 100 mg/ml produced a greater response at all concentrations of substrate, thereby providing no increase in slope of the response curve. This increased enzyme concentration also increased the response time, probably owing to an increase in the viscosity of the enzyme solution.

Because of the method of immobilization of the enzyme, it is difficult to control the exact volume of the enzyme entrapped between the membranes. Therefore, changing the concentration of the enzyme solution is the only practical method of varying the enzyme activity without using an enzyme preparation of different specific activity.

#### Analysis of Grain and Feed Hydrolysates

Results for analysis of L-lysine in acid hydrolysates of grain and feed samples are shown in Table I. The data obtained with the L-lysine electrode are compared with the results obtained with an amino acid analyzer. The grand mean represents the mean of nine determinations (three replicates on three subsamples). The mean percent relative error obtained is 4.64% for the 11 samples. The percent coefficient of variance within three replications of a single subsample ( $\%CV_w^3$ ) and the percent coefficient of variance between three subsamples of a given sample ( $\%CV_b^3$ ) were calculated

TABLE I  
Analysis of L-Lysine in Acid Hydrolysates of Grain and Feed Samples

Sample Identification	Amino Acid Analysis (molar concentration)	Enzyme Electrode Mean <sup>a</sup> (molar concentration)	% Relative Error	% CV <sub>w</sub> <sup>b</sup>	% CV <sub>b</sub> <sup>b</sup>
Larker barley	$7.25 \times 10^{-4}$	$7.73 \times 10^{-4}$	+6.62	5.24	12.11
Steptoe barley	$5.06 \times 10^{-4}$	$5.06 \times 10^{-4}$	0	5.21	6.57
Corn meal	$4.10 \times 10^{-4}$	$4.28 \times 10^{-4}$	+4.39	5.13	18.33
Soybean meal	$5.05 \times 10^{-3}$	$5.21 \times 10^{-3}$	+3.17	8.57	8.57
Chicken feed	$1.68 \times 10^{-3}$	$1.77 \times 10^{-3}$	+5.36	7.67	2.05
Cattle feed	$7.32 \times 10^{-4}$	$7.16 \times 10^{-4}$	-2.23	2.64	13.60
12% Distillers grain					
Cattle feed	$8.40 \times 10^{-4}$	$7.87 \times 10^{-4}$	-6.73	7.78	1.37
15% Distillers grain					
Cattle feed	$9.45 \times 10^{-4}$	$8.79 \times 10^{-4}$	-7.51	4.64	5.22
18% Distillers grain					
Cattle feed	$1.13 \times 10^{-3}$	$1.18 \times 10^{-3}$	+4.42	5.53	3.83
21% Distillers grain					
Sheep feed 1	$1.13 \times 10^{-3}$	$1.18 \times 10^{-3}$	+4.42	5.53	3.83
Sheep feed 2	$6.02 \times 10^{-4}$	$6.39 \times 10^{-4}$	+6.15	14.10	10.06
			$\bar{X} = 4.64$	$\bar{X} = 6.55$	$\bar{X} = 7.78$

<sup>a</sup> Mean of triplicate determinations on triplicate subsamples by comparison to lysine standard curve.

<sup>b</sup> % CV<sub>w</sub><sup>3</sup> = percent coefficient of variance within three replicates of the same subsample. % CV<sub>b</sub><sup>3</sup> = percent coefficient of variance between three subsamples of a sample as calculated by method described by Rodbard (1974).

according to the method described for radioimmunoassays by Rodbard (1974).

As expected, the % CV<sub>w</sub><sup>3</sup> was less than % CV<sub>b</sub><sup>3</sup>, indicating that the major source of error in the analysis was sampling error. The overall agreement of the electrode measurements and the amino acid analyzer data is very good. Typical response and recovery curves obtained during analysis of grain and feed hydrolysates are shown in Fig. 6 and illustrate the total time of analysis of a sample to be about 15 min.

### CONCLUSIONS

The L-lysine electrode described possesses good precision and accuracy, compared with an amino acid analyzer. No interfering or inhibiting substances were observed in the feed and grain hydrolysates. Attempts to preserve the hydrolysates with bacteriocides such as NaN<sub>3</sub> and pentachlorophenol, however, resulted in interference with electrode response and enzyme inhibition, respectively.

The relatively long response times compared with that for other electrode systems represents a disadvantage of this technique. However, the low capital investment in equipment and high degree of accuracy compared with L-lysine analysis on an amino acid analyzer should compensate for this shortcoming.

Long-term stability of the electrode has not been adequately evaluated. Storage studies are in progress and preliminary findings indicate that the electrode is relatively stable for several weeks. The data shown in Table I represent analyses of 150–170 samples including periodic measurements of standard L-lysine solutions. This indicates that the electrode is relatively stable in use with protein hydrolysates since no adjustments in slope were necessary during the 48 hr duration of the analysis, and the standard curve obtained at the end of the analyses was identical to the initial standard curve.

Use of membrane entrapment behind a film such as Cupraphan had definite advantages over chemical immobilization on membranes or in gels. This method of electrode preparation is simple and rapid. The cost of materials is low, and enzyme preparations of high specific activity are not required; enzyme isolation and purification are unnecessary because adequate commercial L-lysine decarboxylase preparations of sufficient

activity are available. Use of enzyme entrapment also is a flexible technique and should be applicable to many other enzyme electrode systems. Because there is no chemical reaction between the enzyme and a binding material, there is also no decrease or change in activity (pH optimum specificity, temperature optimum).

The L-lysine enzyme electrode provides an attractive alternative to existing methods for measurements of L-lysine in grain and feed samples.

### ACKNOWLEDGMENT

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### LITERATURE CITED

- BEREZIN, I. V., and KLESOV, A. A. 1976. Enzyme electrodes. *Usp. Khim.* 45(2):180.
- BODWELL, C. E. 1976. Status of chemical methods to determine biologically available lysine in wheat proteins. Proc. IX Nat. Conf. on Wheat Utilization Res., Oct. 8–10, 1975. Seattle, WA, ARS-NC-40, p. 181. U.S. Dep. Agric.: Washington, DC.
- BOWERS, L. D., and CARR, P. W. 1976. Applications of immobilized enzymes in analytical chemistry. *Anal. Chem.* 48(7):545A.
- CARPENTER, K. J., and BOOTH, V. H. 1973. Damage to lysine in food processing: Its measurement and significance. *Nutr. Rev.* 43:424.
- DeCLERCQ, H. 1973. Enzyme electrodes. *Farm. Tijdschr. Belg.* 50(4):291.
- FERRIS, C. D. 1974. Introduction to Bioprobes. Plenum Press: New York.
- FINLEY, J. W., and FRIEDMAN, M. 1973. Chemical methods for available lysine. *Cereal Chem.* 50:101.
- FISHMAN, M. M. 1976. Enzymes in analytical chemistry. *Anal. Chem.* 48:322R.
- FRIEDMAN, M., and FINLEY, J. W. 1975. Vinyl compounds as reagents for available lysine in proteins, p. 503. In: FRIEDMAN, M. (ed.). Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods—Biological, Biochemical, and Chemical. Marcel Dekker: New York.
- GALE, E. F. 1944. Studies on bacterial amino acid decarboxylase. *Biochem. J.* 39:46.
- GOUGH, D. A., and ANDRADE, J. D. 1973. Enzyme electrodes. *Science* 180(4084):380.
- GRAY, D. N. 1977. Immobilized enzymes in analytical chemistry. *Anal.*

- Chem. 49:1067.
- GUILBAULT, G. G. 1972. Analytical uses of immobilized enzymes. *Biotechnol. Bioeng. Symp.* 3:361.
- GUILBAULT, G. G., and SHU, F. R. 1972. Enzyme electrode based on the use of a CO<sub>2</sub> sensor. *Anal. Chem.* 44:2161.
- HOLSINGER, V. H., and POSATI, L. P. 1975. Chemical estimation of available lysine in dehydrated dairy products, p. 479. In: FRIEDMAN, M. (ed.). *Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods—Biological, Biochemical, and Chemical.* Marcel Dekker: New York.
- HSIUNG, C. P. 1977. A specific enzyme electrode for L-phenylalanine. *Anal. Chim. Acta* 90:45.
- JOHANSSON, G. 1976. An enzyme reactor electrode for determination of amino acids. *Anal. Chim. Acta* 85:55.
- KESSLER, M. 1976. Ion and enzyme electrodes, p. 450. In: *Electrodes in Biology and Medicine.* Univ. Park Press: Baltimore, MD.
- LAKIN, A. L. 1973. Evaluation of protein dye-binding procedures, p. 179. In: PORTER, J. W. G., and ROLLS, B. A. (eds.). *Proteins and Nutrition.* Academic Press: New York.
- MORRISON, A. B., and McLAUGHLIN, J. M. 1972. Availability of amino acids in foods, p. 389. In: BIGWOOD, E. J. (ed.). *Protein and Amino Acid Functions.* Pergamon Press: Oxford.
- MOSSBERG, R. 1970. Estimation of protein content and quality by dye-binding, p. 203. In: BENDER, A. E., KILBERG, R., LOFGVEST, B., and MUNCK, L. (eds.). *Evaluation of Novel Protein Products.* Proc. Int. Biological Programme and Wenner-Gun Center Symposiums, Stockholm, Sept. 1968. Pergamon Press: New York.
- NANJO, M. 1974. Enzyme electrode for L-amino acids and glucose. *Anal. Chim. Acta* 73:367.
- OLSON, N. F., and RICHARDSON, T. 1974. Symposium: Immobilized enzymes in food systems. *J. Food Sci.* 39:653.
- OSTROWSKI, H., JONES, A. S., and CADENHEAD, A. 1970. Availability of lysine in protein concentrates using Carpenter's method and modified Silcock method. *J. Sci. Food Agric.* 21:103.
- RAMIREZ, J. E., CAVANAUGH, J. R., SCHWEIZER, K. S., and HOAGLAND, P. D. 1975. The determination of free epsilon-amino groups of lysine in proteins using <sup>19</sup>F NMR spectroscopy. *Anal. Biochem.* 63:130.
- RECHNITZ, G. A. 1975. Membrane bioprobe electrodes. *Chem. Eng. News* 53(4):29.
- RODBARD, D. 1974. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin. Chem.* 20(10):1255.
- SODA, K., and MORIGUCHI, M. 1969. Crystalline lysine decarboxylase. *Biochem. Biophys. Res. Commun.* 34:34.
- STAHMANN, M. A., and WOLDEGIORGIS, G. 1975. Enzymatic method for protein quality determination, p. 211. In: FRIEDMAN, M. (ed.). *Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods—Biological, Biochemical, and Chemical.* Marcel Dekker: New York.
- TONG, S. L., and RECHNITZ, G. A. 1976. Enzymatic l-arginine and l-lysine detection using pCO<sub>2</sub> electrode. *Anal. Lett.* 9(1):1.
- UPDIKE, S. J., and HICKS, G. P. 1967. The enzyme electrode. *Nature* 214:986.
- WATZ, O. P., and FORD, J. E. 1973. The measurement of "available lysine" in protein foods. A comparison of chemical biological and microbiological methods. *Z. Tierphysiol. Tierernähr. Futtermittelkde. Beih.* 30:304.

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