J. Indian Inst. Sci., 66, Sept.-Oct. 1986, pp. 609-612
© Indian Institute of Science, Printed in India

Short Communication

Development of an air-gap urea-specific electrode

R. K. SHARMA
Regional Research Laboratory, Hoshangabad Road, Bhopal 462 026, M.P., India.

Received on September 17, 1986.

Abstract

Air-gap urea-specific electrode has been developed from a combined pH electrode. Urease has been immobilized on polyacrylamide gel by the entrapment method. Urea concentration, pH, temperature, response time studies were conducted and found that optimum pH is 8.5 at temperature of 37°C in the two-minute response time. This electrode is also suitable for blood urea determination with five minutes response time giving reproducible readings.

Key words: Urease, urea estimation, air-gap electrode.

1. Introduction

It is known that some enzymes exist *in vivo* in free protein form in an aqueous medium. Majority of them are either bound to membranes, or to solid-state assemblies or are present in the gel-type surroundings. The enzyme urease, is absent in the human being but is present in Jach beans[1] and is used as a catalyst to hydrolyse urea to ammonia. In 1953, Grubhofer and Schlith[2] bounded the enzyme on the matrix. Later on, cellulose, sephodex, acrylic beads, porous glass beads and other synthetic polymer membranes were tried[3,4] for other enzymes, except urease.

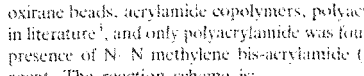
The determination of urea is required in haemodialysis. Spectrophotometric methods, being used, are time-consuming and waste free urease enzyme. The development of cation selective electrodes has made it easy to determine urea, but the electrodes also show interference of other cations and are not wholly accurate too. To eliminate interference by other cations in urea estimation, an air-gap electrode has been developed.

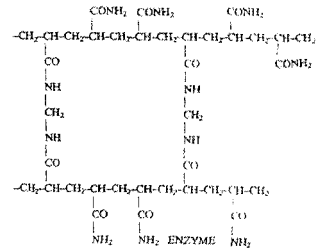
2. Materials and methods

2.1 Urease enzyme immobilization

To develop air-gap urea-specific electrode the urease enzyme is immobilized on the porous solid support. The immobilization of urease (Sigma) was tested on glass beads,

oxirane beads, acrylamide copolymers, polyacrylamide, agar agar, sephodex as reported in literature[1], and only polyacrylamide was found suitable[2]. Urease gets entrapped in the presence of N, N methylene bis-acrylamide (BIS) which is working as a cross-linking agent. The reaction scheme is:





The given reaction scheme is very simple: 0.725 g of acrylamide and 0.037 g of BIS are dissolved in 2.5 ml of 0.05 M phosphate buffer (pH 7.5) and after cooling to 0°C, 0.5 g of urease enzyme is added. After mixing rigorously, 0.05 ml of ammonium persulphate solution (0.5 g in 1 ml) is added with continuous stirring. The mixture is kept in ice for one to two minutes and then in a desiccator for 1 h under slight vacuum. Finally the mixture is washed with water and kept in phosphate buffer (pH 7.5) containing sodium azide.

The activity of polyacrylamide urease was measured and found to be 228 units/g of urease entrapped polymer at 37°C.

2.2. *Preparation of electrode*

The electrode consists of a combined glass pH electrode (fig. 1). The lower portion of the electrode is attached to a small gas-tight reaction chamber of 5 ml capacity containing 500 mg of immobilized urease. A small piece of polymethylene sponge (well soaked in the ammonium chloride solution, 1×10^{-3}M) is put on the bulb of pH electrode with the help of an "O" ring. The urea (1×10^{-2}M) solution is taken in the reaction chamber along

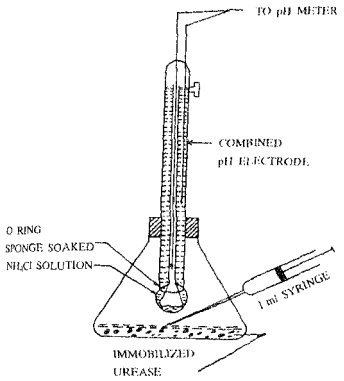


FIG. 1 Air-gap urea-specific electrode.

with immobilized urease and its pH maintained at 8.5. The urea solution, injected into the reaction chamber through a syringe, will come in contact with the immobilized urease and liberates free ammonia which is directly proportional to the pH change.

3. Results and discussion

The immobilized urease enzyme is in the reaction chamber, away from the combined glass electrode. The solution is buffered with 0.05 M phosphate buffer (pH 8.5) in the reaction chamber. Free ammonia diffuses at the electrode surface (well soaked polymethylene sponge of ammonium chloride solution, 1×10^{-3}M). Ammonia, which is highly soluble in water when it is in contact with well soaked ammonium chloride solution there, is formed by the (NH_4^+) and (OH^-) ions responding directly to the pH change. Therefore, the diffusion of free ammonia is to the electrode surface, not away from the surface. Thus, pH is directly proportional to the concentration of (NH_4^+) ion ($pH \propto CNH_4^+$) and hence is related to the concentration of ammonia and, therefore, urea.

Experimental observations (Table I): pH increases with the concentration of urea. Studies, conducted from pH 6 to 9, show that the resulting pH increases with increase in pH of sample solution, but at higher pH the enzyme is unstable. Therefore, an optimum pH of 8.5 has been chosen. Experiments with blood samples show that difference in pH change is more or less same above 210 seconds response time, indicating good reproducibility over continuous observations.

The optimum temperature for experiments was found to be 37°C. As the temperature exceeds 37°C, pH decreases due to enzyme deterioration. At room temperature the rate of reaction will not be as fast as at optimum temperature but experiments can be performed.

Table I
Analytical determination of urea at different concentrations

Sl. no.	Sample no.	I	II	III	IV	V
	Urea conc.	pH				
1.	1×10^{-6} M	0.38	0.30	0.32	0.32	0.32
2.	1×10^{-5} M	0.65	0.65	0.75	0.75	0.72
3.	1×10^{-4} M	1.45	1.30	1.40	1.30	1.30
4.	1×10^{-3} M	1.90	1.95	2.00	2.10	1.90
5.	1×10^{-2} M	2.65	2.60	2.65	2.70	2.80

(a) pH of reaction chamber : 8.5; (b) Response time 2 minutes; (c) Temperature : 24 C.

4. Conclusion

The electrode is quite stable, the response time is only about two minutes and does not change for 3–4 weeks or 200–300 operations. Experimental determination of urea concentration in blood sample requires only five minutes and is quite economic in comparison to other methods.

Acknowledgement

Thanks are due to the Director, Regional Research Laboratory, CSIR, Bhopal, and ICMR, New Delhi for financial support.

References

1. LENINGER, A. L. — *Biochemistry* Wroth, New York, 1975, p. 83.
2. GRUBHEFER, N. AND GRIFFIN, E. G. — *Naturwiss*, 1953, **40**, 508.
3. JAKOBY W. B. AND WILCHEK, M. (EDS) — *Methods in enzymology*, **34**, Academic Press, New York, 1974.
4. ZABOTSKY, O. — *Immobilized enzymes*, CRC Press, 1972.
5. MISHRA, R. K., BHARADWAJ, L. M., SHARMA. R. K., AND LAMBA, J. — *Testing of the efficiency of a haemodialyzer and immobilization techniques for improving and miniaturing it*, Technical report submitted to ICMR, 1980 (unpublished data).
6. MODDY, G. J. AND THOMAS, J. R. D. — *Selective ion sensitive electrodes*, Merrow Technical Library, Merrow, Watford, England, 1971.