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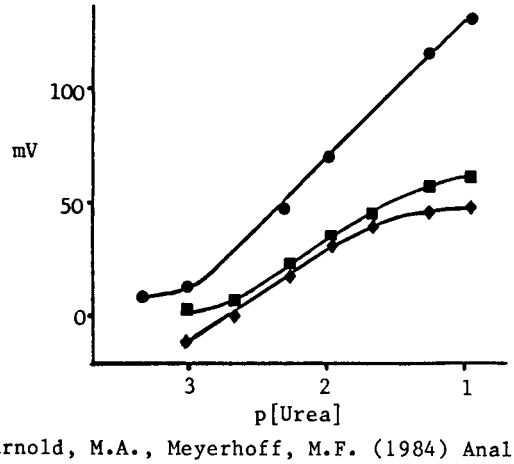
A BACTERIAL ELECTRODE FOR THE DETERMINATION OF UREA

C.J. Olliff, G.W. Hanlon, Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ, U.K.

Ion selective electrodes have been developed over the last decade for a wide range of compounds (Arnold & Meyerhoff 1984). Several electrode systems for the measurement of organic compounds have specific enzymes immobilised on an ion-selective membrane which is sensitive to the product of the enzyme-substrate reaction. Enzyme electrodes while being both sensitive and specific suffer from a number of disadvantages some of which may be overcome by using immobilised whole cells producing the enzyme of interest. In this study we report the development of a bacterial electrode for the measurement of urea which employs the urease-producing bacterium Proteus vulgaris in conjunction with a Phillips ammonium electrode. Ammonium ions resulting from the enzymatic degradation of urea were detected by the ion-selective electrode with a SCE double junction reference electrode and a Corning 135 ion selective meter. P.vulgaris was grown at 37°C in nutrient broth for 24 h after which the cells were washed with pre-warmed phosphate buffered saline and the supernatent liquid discarded. Varying amounts of the pelleted cells were then sandwiched between Visking dialysis tubing and the ion selective electrode. The number of bacterial cells trapped was between 10^{11} and 10^{12}.

The initial rate of response and equilibrium value for the ammonium electrode obtained by placing the bacterial electrode into a range of concentrations of buffered urea solutions at 37°C was monitored by recorder. A typical potentiometric 'S' shaped plot was obtained for both rate of response and equilibrium voltage (Figure). A linear section from 2×10^{-3} to 2×10^{-2} M urea was obtained with a Nernstian slope of 39.1 mV for a plot of rate of response as a function of urea concentration. The equilibrium values gave a slope of 40.0 mV and a linear range of 10^{-3} to 2×10^{-2} M urea with 95% of equilibrium response time being between 0.5 and 15 minutes depending on conditions used. Coefficient of variance of 6% (n = 5) was obtained at 5×10^{-3} M urea decreasing to 3% (n = 5) for 10^{-1} M urea. The suspending medium did not cause interference with the electrode readings. A second electrode employing a thinner layer of entrapped cells produced a similar potentiometric plot with a slope of 60.0 mV and a linear range of 2×10^{-3} to 10^{-1}. Adult serum urea concentrations are normally within the range $3.8 - 7.1 \times 10^{-3}$ M which lies on the linear region of the graph obtained. Using the initial rate of response as an indicator of concentration the electrode incorporating a thin film of bacteria gave readings within 15-30 seconds with almost theoretical Nernstian slope thus overcoming two of the major literature-quoted limitations of bacterial electrode systems.



Figure

Effect of urea concentration on:

Rate of response of electrode
- ● thin film of P.vulgaris
- ■ thick film of P.vulgaris

Equilibrium values of electrode
- ◆ thin film of P.vulgaris

Arnold, M.A., Meyerhoff, M.F. (1984) Anal. Chem. 56: 20R-48R