

Amperometric Determination of Urea and Acetic Acid Using Electrodes Coated with Tri-enzyme/Polydimethylsiloxane-Bilayer Membranes

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The determination of urea in body fluids is of importance in clinical chemistry. Most enzymatic methods for determining urea are based on its hydrolysis by urease (EC 3.5.1.5). The reaction can be followed by monitoring each product: numerous works have been carried out on the preparation of urease-based sensors using potentiometric pH-, NH₄⁺- and NH₃ gas-sensing devices. However, potentiometric methods in principle suffer from the disadvantages of low accuracy and reproducibility: e.g., 1 mV error corresponds to 4 μM urea. To avoid this drawback, a few works have been carried out for preparing urea sensors based on amperometric pH-sensing electrodes. But the responses of urea sensors based on pH-measuring systems are severely affected by variations in the buffer capacity and pH of the samples.

In this work, we have employed a tri-enzyme system based on urea amidolyase (UA, EC 3.5.1.45), pyruvate kinase (PK, EC 2.7.1.40) and pyruvate oxidase (PyOx, EC 1.2.3.3) [1]. UA catalyzes the ATP-consuming hydrolysis of urea to produce ADP, PK transfers phosphate from phosphoenolpyruvate to ADP to form pyruvate, then PyOx catalyzes the oxidation of pyruvate.

The concentration of ADP and pyruvate in serum and urine samples are quite low, which inherently advantageous for the precise measurement of urea in such samples. For monitoring the PyOx-catalyzed reaction, we have used an electrode coated with polydimethylsiloxane (PDMS) for the cathodic detection of oxygen. The cathodic detection can essentially be free from interference by oxidizable species, such as L-ascorbic acid, uric acid, acetaminophen and L-cysteine. On a bare metal (or carbon) electrode/oxidase-system, however, an ambiguous response is often obtained, since the oxidase-catalyzed reaction brings about a decrease in the current for the oxygen reduction but a simultaneous increase in the reduction current for hydrogen peroxide, a product of the enzymatic reaction. The PDMS-coating resulted in a significant decrease in the current for the hydrogen peroxide reduction, whereas it did not show such an effect on the oxygen reduction current [2,3].

A PDMS layer was formed on a platinum disc electrode by placing the polymer emulsion (Type DC84 ADDITIVE, Toray Dow Corning Silicone) on the electrode surface. The electrode was allowed to dry for 4h at room temperature. A tri-enzyme membrane was prepared from an aqueous solution containing a photocrosslinkable poly(vinyl alcohol) (PVA-SbQ) [4], UA (from *Candida* sp., Toyobo), PK (from rabbit muscle, Sigma) and PyOx (from *Aerococcus viridans*, Asahi

Chemical Industry), according to the procedure described previously [4]. The enzyme membrane (thickness, 30 μm) was placed on the PDMS layer and covered with a polyester mesh (100 mesh). The mesh was held in place with a rubber ring so that the enzyme membrane was directly contact with the PDMS-coated electrode surface. The test solution used was an air-saturated 0.1 M potassium phosphate buffer (pH 7.0, 20 ml) containing ATP, PEP, MgCl₂, KHCO₃, tyamine pyrophosphate and FAD. Here, KHCO₃ was the activators of UA; MgCl₂, that of PK and PyOx; tyamine pyrophosphate and FAD were the activator of PyOx. The solution was stirred with a magnetic bar. The potential of the enzyme electrode was set at -0.4 V vs. Ag/AgCl.

The cathodic current for the enzyme electrode decreased after the addition of urea and reached in the steady state within 30 s. The steady-state current decrease was proportional to the urea concentration up to 0.35 mM (slope, 96 μA cm⁻² mM⁻¹). The detection limit was 5 μM (signal-to-noise ratio, 5). The relative standard deviation for ten successive measurements of 0.1 mM urea was 1.9%. Uric acid, acetaminophen and L-cysteine did not virtually cause the change in the electrode current, indicating that the present electrode was useful for determining urea in biological samples. The present tri-enzyme electrode could be used for a month. Although PyOx from *Pediococcus* sp. has widely been used for preparing pyruvate- and phosphate-sensing systems, the poor stability of the enzyme often resulted in a rather rapid decrease of the electrode response, which brings about a serious problem for the practical application of the enzyme-based sensors. Hence we have employed another PyOx, PyOx from *Aerococcus viridans*. The enzyme from *Aerococcus viridans* showed much higher stability than the same enzyme from *Pediococcus* sp., which was effective for improving the stability of the enzyme-based electrode.

A variety tri-enzyme electrodes can be prepared by coupling ATP-dependent enzymes with the PK/PyOx-based, ADP-sensing system. We have prepared, for instance, an acetate-sensing electrode by using a tri-enzyme system of acetate kinase (EC 2.7.2.1). The electrode could be used for the determination of acetate in food samples such as wines, and soy sources.

References [1] F. Mizutani, Y. Sato, Y. Hirata and S. Iijima, *Anal. Chim. Acta*, in press. [2] F. Mizutani, T. Sawaguchi, S. Yabuki and S. Iijima, *Electrochemistry*, 67, 1138 (1999). [3] F. Mizutani, S. Yabuki and S. Iijima, *Electroanalysis*, in press. [4] K. Ichimura, *J. Polym. Sci., Polym. Chem. Ed.*, 22, 2817 (1984).