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Potentiometric Urea Biosensor Based on Carbon Nanotubes and Polyion Complex Film

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Enzymatic electrode for a potentiometric urea sensor was prepared by sequential coating of carbon nanotube (CNT), urease (Urs) and polyion complex (mixture of poly-L-lysine hydrobromide and poly (sodium 4-styrenesulfonate), PIC) on an ITO glass. The prepared electrode (ITO/CNT/Urs/PIC) was characterized by potentiometric measurements at different urea concentrations in Tris-HCl buffer (pH 7.0). The potentiometric response of the electrode was linear in the range of 1×10^{-5} to 3×10^{-3} M with a correlation coefficient of 0.999 and a sensitivity of 59.1 mV/decade. It was found that the addition of CNT caused considerable improvement of the sensitivity of the electrode to urea. The response time was approximately 60–90 s. A half of the initial sensitivity was retained for 15–17 d at room temperature.

Keywords: Urea Sensor, Urease, Carbon Nanotube, Polyion Complex.

1. INTRODUCTION

Urea is the waste product of protein metabolization in human body and its analysis has been an important operation in a clinical chemical laboratory because the level of urea in blood is a measure of renal functioning. Monitoring of urea levels is also performed in food and environmental industries.[1,2] The concentration of urea can be measured by chromatographic and spectroscopic methods.[3,4] However, these methods require a complicated sample pretreatment and are inapplicable to on-site monitoring. As alternative methods, various types of biosensors based on urease enzyme have been developed due to their simplicity and capability for on-site urea analysis.

Urea is converted enzymatically by urease to ammonia and carbon dioxide. The concentration of urea is then measured by monitoring the NH_4^+ and HCO_3^- ions in the sample. These ions can be detected using various tranducers including amperometric,[5,6] potentiometric,[7,8] conductometric,[9,10] optical,[11,12] thermal,[13,14] and piezoelectric tranducers.[15,16] Since the enzymatic reaction of urea is not electroactive, potentiometric urea biosensors using pH-sensitive conducting polymers are widely used. However, the slow response for steady state potential values is often problem for its practical applications. To enhance speed and sensitivity of the urea biosensors, carbon nanotubes are also used as a component in the bioelectrode architecture due to their large surface area, high electrical conductivity, and chemical stability.[17,18] Electrochemical property of CNTs for detecting pH and ammonia was reported earlier.[19]

For the enzymatic electrode fabrication, electrode architecture components such as enzyme and CNT should be well dispersed and integrated on the electrode surface without leakage during use to achieve fast response and stability. Most immobilization methods are based on adsorption, covalent binding, entrapment, encapsulation, and/or cross-linking.[20] Recently, polyion complex (PIC) has been used as a matrix for the enzyme immobilization in the preparation of bioelectrodes.[21,22] It is a permselective membrane and has molecular sieving ability.[23]

In this study, we attempted to combine the CNT and urease together on a base electrode using PIC to prepare an enzyme electrode. The simply integrated CNT/Urease/PIC-layered electrodes were characterized for the application in urea sensors.

2. EXPERIMENTAL DETAILS

2.1. Chemicals and Materials

Urease (Type III, 31660 units/g solid), poly-L-lysine (PLL), and poly-sodium-4-styrensulfonate (PSS) were purchased from Sigma–Aldrich. Carbon nanotube (CNT) (multiwalled, > 95 wt% purity, 10–15 nm in diameter) was

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purchased from Iljin Nanotech Co., Ltd. and used without purification. All other chemicals were of reagent grade.

Indium tin oxide (ITO) glass (< 20 X/square) was used as a base electrode. ITO glass was cut to a rectangular shape of 5 mm in width and 30 mm in length. The working area of the ITO glass for the electrochemical tests was controlled to 0.25 cm^2 using a masking tape.

2.2. Preparation of Enzyme Electrodes

CNT/Urs/PIC-layered electrodes were prepared on the ITO glass. First, the ITO was coated with 15 μL of CNT suspension (0.335 mg/mL in *N*,*N*-dimethylformaide) and dried at room temperature. Onto the CNT layer, urease solution of 10 mg/mL in a Tris-HCl buffer solution (Tris, pH 7.0) was dripped and dried at room temperature. Finally, 5 μL of PLL aqueous solution (60 mmol/L monomer unit) and 5 μL of PSS aqueous solution (60 mmol/L monomer unit) were successively dropped on the surface and dried in air to form the PIC film. It was store at 4 °C.

2.3. Analysis

Ammonia concentrations were analyzed by UV-Vis spectrophotometer (UV/Vis, Varian) using Nessler's reagent. Potentiometric measurements were carried out using a potentiostat (VSP, Bio-Logic) with a conventional three-electrode system: a working electrode, an Ag/AgCl (3M NaCl) electrode as a reference and a platinum wire as a counter electrode. All measurements were performed at about 25 °C in Tris–HCl buffer solution (pH 7.0).

3. RESULTS AND DISCUSSION

Enzymatic activity of the immobilized urease was evaluated by measuring reaction rate of urea hydrolysis. Ammonia is produced by the hydrolysis of urea. The ammonia then reacts with Nessler's reagent to form a colored product as shown in the following reactions:

$$NH_2CONH_2(\text{urea}) + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 \quad (1)$$

$$2NH_3 + 2Hg^{II}I_4^{2-} \longrightarrow NH_2Hg_2I_3 + NH_4^+ + 5I^- \quad (2)$$

By measuring the spectrophotometric absorbance for the product $NH_2Hg_2I_3$ (λ_{max} = 380 nm), urea concentration are determined. Figure 1 shows reaction rates of urea hydrolysis catalyzed by free and immobilized ureases. The activity of the immobilized urease, as calculated from the slope, exhibited about 40% of that of free enzyme.

In order to analyze the analytical performance of the ITO/CNT/Urs/PIC electrode, the electrode was immersed in urea solutions of different concentrations and then the UV absorbances of the resulting solutions were observed. Figure 2 depicts the absorbance response to varying concentrations of urea. Inset of Figure 2 represents a typical calibration curve for urea. In the range of 1×10^{-5} to 3×10^{-4} M, the correlation coefficient for linear curve was 0.992.

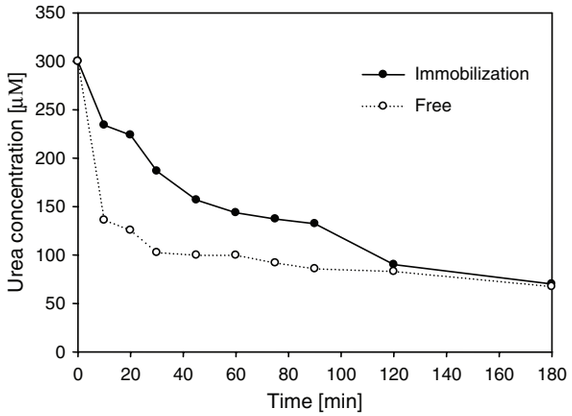

Figure 1. The comparison of enzymatic activities of free urease and immobilized urease on electrode (ITO/CNT/Urs/PIC) as measured by UV absorbance (λ = 380 nm).

The effect of urease enzyme and CNT loading and PIC loading (film thickness) on the electrochemical response was investigated to optimize the composition of the electrode architecture. As shown in Figure 3, the potential response of the electrode with 0.75 mM/cm^2 of PIC loading was highest at the early stage of the measurement, however, it decreased later on, indicating the PIC film is too thin and thus unstable for the entrapment of urea.

On the other hand, the electrode with 3.0 mM/cm^2 of PIC loading showed unstable potential response, probably due to the a high diffusion barrier of the thick film. Therefore, 1.5 mM/cm^2 was chosen as optimum PIC loading and used throughout the present study.

CNT loading also affected the potential response of the ITO/CNT/Urs/PIC electrode as shown in Figure 4. Comparing CNT loading of 0.32, 0.16 and 0.24 μg/cm^2,

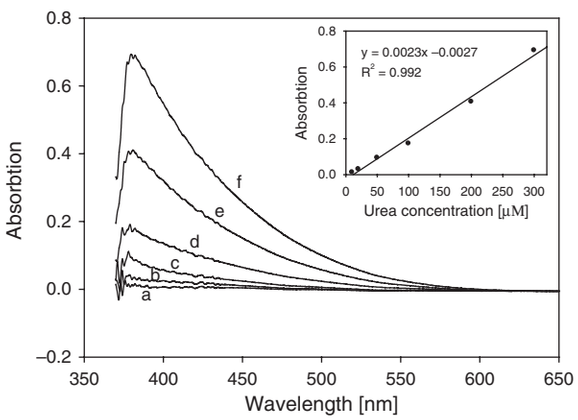

Figure 2. Spectrophotometric response to varying concentrations of urea (a) 1×10^{-5} M, (b) 2×10^{-5} M, (c) 5×10^{-5} M, (d) 1×10^{-4} M, (e) 2×10^{-4} M, and (f) 3×10^{-4} M in the presence of ITO/CNT/Urs/PIC electrode. Inset: linear regression curve for UV absorbance (λ = 380 nm) versus urea concentration.

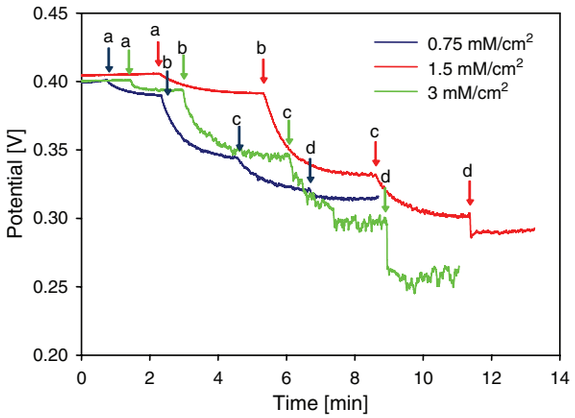

Figure 3. Effect of PIC loading (film thickness); Chronopotentiometric responses of ITO/CNT/Urs/PIC electrode with different PIC loadings to the subsequent addition of urea; (a) 1×10^{-5} M, (b) 3×10^{-5} M, (c) 7.5×10^{-5} M, (d) 3×10^{-4} M.

considerable improvement of the sensitivity of the electrode to urea was obtained at CNT loading of 0.32 μg/cm^2. The electrode showed no significant enhancement of the sensitivity by further addition of CNT more than 0.32 μg/cm^2.

The potentiometric response of the enzyme electrode was measured for urea analysis. Figure 5 shows a typical chronopotentiometric response of ITO/CNT/Urs/PIC electrode to increasing urea concentration from 1×10^{-5} to 3×10^{-3} M. The response time of the enzyme electrode was in the range of 60–90 s. This response time is comparable with those of previously reported biosensors.[8] The calibration curve for the urea concentration is shown in Figure 6. It was linear in the range of 1×10^{-5} to 3×10^{-4} (i.e., $1 \times 10^{-3.5}$) M with a correlation coefficient of 0.999. This linearity range is well comparable with that obtained in the spectrophotometric response studies. The slope of the linearity, i.e., the sensitivity of the enzyme electrode towards urea concentrations was 59.1 mV per decade.

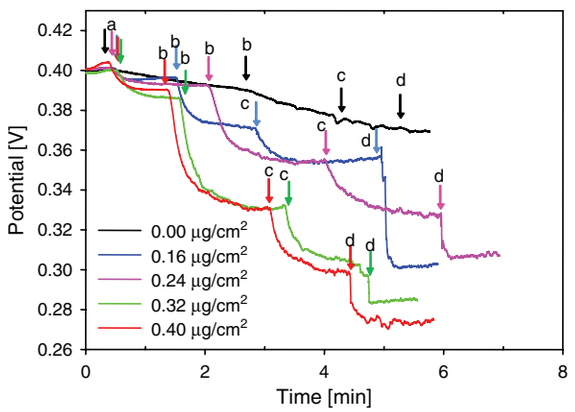

Figure 4. Effect of CNT loading; Chronopotentiometric responses of TO/CNT/Urs/PIC electrodes with different CNT loadings to the subsequent addition of urea; (a) 1×10^{-5} M, (b) 3×10^{-5} M, (c) 7.5×10^{-5} M, and (d) 3×10^{-4} M.

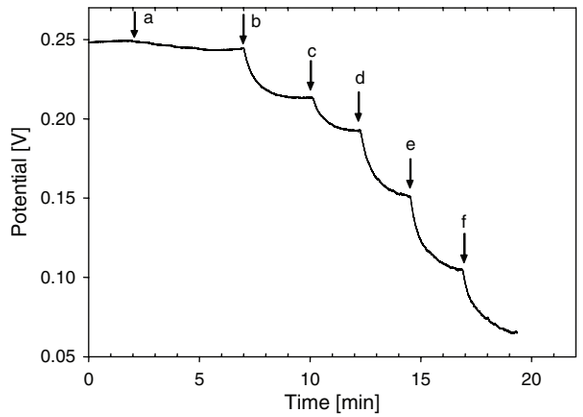

Figure 5. Chronopotentiometric response of ITO/CNT/Urs/PIC electrode to the subsequent addition of urea; (a) 1×10^{-5} M, (b) 3×10^{-5} M, (c) 7.5×10^{-5} M, (d) 3×10^{-4} M, (e) 1×10^{-3} M, and (f) 3×10^{-3} M.

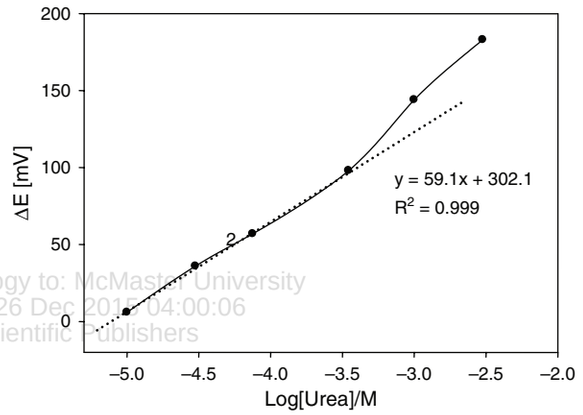

Figure 6. Calibration curve for response of ITO/CNT/Urs/PIC electrode to urea in the range 1×10^{-5} to $1 \times 10^{-3.5}$ M.

The electrode was examined for the enzyme stability. As shown in Figure 7, the potential response to 5 mM of urea was measured every for 20 d. The electrode exhibited good operation stability for 10 d. The potential response started

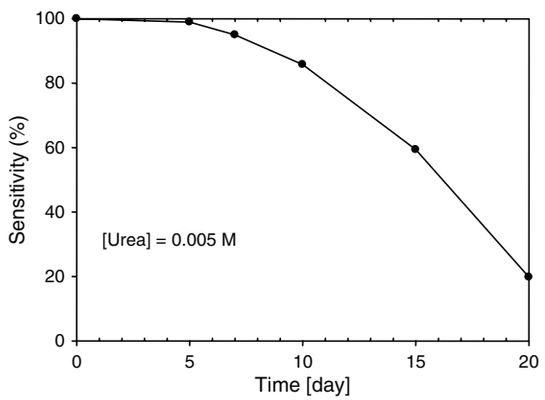

Figure 7. Variation of potential response of ITO/CNT/Urs/PIC electrode to 5 mM of urea with repeated use after storage at room temperature.

to decrease slowly after 10 d due to the enzyme denaturation and loss of enzyme from the electrode surface. A half of the initial sensitivity was retained for 15–17 d at room temperature.

4. CONCLUSIONS

An enzymatic electrode for a potentiometric urea sensor was successfully prepared by sequential coating of CNT, urease, and polyion complex on an ITO glass. The prepared electrode responded linearly to the changes of urea concentration in the range of 1×10^{-5} to 3×10^{-4} M with a correlation coefficient of 0.999 and a sensitivity of 59.1 mV/decade. The presence of CNT caused considerable improvement of the sensitivity of the electrode to urea. The response time was approximately 60–90 s. A half of the initial sensitivity was retained for about 15–17 d at room temperature. The result indicates that an enzymatic electrode for a potentiometric urea sensor can be prepared by simply entrapping urease with polyion complex.

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