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Amperometric urea biosensor based on urease and electropolymerized toluidine blue dye as a pH-sensitive redox probe

Igor Vostiar [a,*], Jan Tkac [a,b], Ernest Sturdik [a], Peter Gemeiner [b]

[a] *Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinskeho 9, SK-812 37 Bratislava, Slovak Republic*

[b] *Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-842 38 Bratislava, Slovak Republic*

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Abstract

The electropolymerized toluidine blue film deposited on the glassy carbon electrode show amperometrically detectable pH sensitivity. This feature of polytoluidine blue (PTOB) film was used for a construction of an amperometric urea biosensor. We have observed a linear shift of the formal redox potential with increasing pH value between 4 and 8 giving the slope of 81 mV^{Δ} pH^{-1}. Polytoluidine blue film has had a significantly increased stability and higher electrochemical activity compared to the adsorbed monomeric dye. The polytoluidine blue urea biosensor has been operating at a working potential of −200 mV vs. SCE. The sensitivity of the biosensor was 980 nA mM^{-1} cm^{-2}. The biosensor showed linearity in concentration range up to 0.8 mM with the detection limit of 0.02 mM (S/N = 3).

Keywords: Amperometric pH sensing; Biosensor; Polytoluidine blue; Urease

1. Introduction

Redox dyes have many applications in a construction of mediated amperometric biosensors. Phenotiazine redox dye toluidine blue has been successfully used in a biosensor construction employing NAD-dependent dehydrogenase [1–3] and peroxidase [4].

Electropolymerization of the redox dyes is a simple procedure for the preparation of stable layers on an electrode surface [5]. Moreover, electropolymerization yields in a three-dimensional mediator structure, which is more effective than the mediator adsorbed in a monolayer.

Recently Stred'anský et al. demonstrated the feasibility of the use of monomeric methylene blue, lauryl gallate [6] and hematein or electropolymerized *o*-phenylenediamine [6] as a pH-sensitive redox-active compounds for the construction of a simple amperometric biosensors based on the use of pH changing enzymes.

Like in the case of monomeric form of redox dyes, electrochemical properties of polyredox dyes is also strongly affected by a pH of the environment. The formal redox potential of polytoluidine blue (PTOB) film is shifted to the more negative values with increasing pH [7].

The aim of the present work is to investigate a possibility of the application of the electropolymerized PTOB as a pH-sensing layer for the amperometric detection of pH changes. Successively, the PTOB film with immobilized urease has been applied for the amperometric urea assay.

2. Experimental

2.1. Reagents

Urease (Ur) and dialysis membrane was purchased from Sigma and toluidine blue (TOB) from Aldrich. All other reagents were of analytical grade and were supplied by Lachema (Brno, Czech Republic). McIlvine buffer solutions (pH 4.0–8.0) consisted of 0.1 M citric acid and 0.2 M solution of Na_2HPO_4 containing 0.1 M KCl.

2.2. Apparatus

Biosensor measurements were carried out on Amperometric Detector ADLC2 (Laboratorní přístroje, Prague, Czech Republic) using a glassy carbon electrode (GCE)

* Corresponding author. Fax: +421-2-52967085.
E-mail address: vostiar@chelin.chtf.stuba.sk (I. Vostiar).

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($d = 7$ mm) as a working electrode and saturated calomel electrode (SCE) as a reference electrode. Cyclic voltammetry was performed using the GCE as a working electrode, the platinum wire as an auxiliary electrode and the SCE as a reference one.

2.3. Procedures

GCE was polished on a 0.05-μm aluminium powder, washed with acetone and redistilled water.

The TOB adsorption takes place when the potential was cyclically swept from −0.4 to +0.3 V at the sweep rate of 50 mV s^{-1} in McIlvine buffer (pH = 4.0) containing 5 mM TOB for 10 min. The electropolymerization of TOB was carried out by cyclic voltammetry in a potential range from −0.6 to +1.2 V vs. SCE at the sweep rate of 50 mV s^{-1} in McIlvine buffer (pH = 4.0) containing 5 mM TOB for 10 min. Electrode surfaces were thoroughly rinsed with redistilled water before use.

Stability of the fixation of adsorbed TOB and PTOB on the electrode surfaces was tested by using of cyclic voltammetry in the range from −0.4 to 0.3 V at the sweep rate of 50 mV s^{-1} in McIlvine buffer (pH = 4.0).

For biosensor construction, 10 ml of the stock solution of Ur in McIlvine buffer (pH 7.0 180 U ml^{-1}) was pipetted on the PTOB-modified electrode. Water was allowed to evaporate and a dialysis membrane (cut-off 12,000 Da) was applied and on the electrode surface rubbed with an O-ring.

3. Results and discussion

3.1. Electropolymerization of toluidine blue

When the potential was swept in the range between −0.4 and +0.3 V vs. SCE, a typical electrochemical behaviour of TOB monomer was observed with well defined cathodic and anodic peaks at potentials −0.160 and +0.110 V, respectively. After repetitive cycling, no significant changes in electrochemical behaviour were observed. After application of the extended potential range from +0.6 to +1.3 V, changes in electrochemical characteristics with repetitive cycling appeared. The anodic peak detected at +1.110 V represents the formation of radical cations of TOB. A slow disappearing of the cathodic and the anodic current peaks of TOB monomer was observed. Slow rising of cathodic current at +0.025 V was ascribed the formation of the PTOB film. In a coarse of polymerization, a slight shift of the anodic peak of PTOB to more positive potentials (+0.135 V) was observed.

3.2. Stability of electropolymerized polytoluidine blue vs. adsorbed toluidine blue

Stability of adsorbed TOB vs. electropolymerized PTOB was tested by repetitive scans as it was mentioned in the Experimental. Stability of the adsorbed TOB on the electrode surface was insufficient for biosensor construction because of the gradual decrease of electrode response. The current peaks of TOB at potentials of −0.160 and +0.110 V disappeared fast and after 20 min of repetitive cycling, only a 20% of initial current response was detected (Fig. 1). Stability and electrochemical activity of PTOB was much higher when compared to the adsorbed one. Initial anodic peak current value in the case of PTOB (57.5 μA) was significantly higher compared to the anodic peak current value of TOB (0.75 μA). After 80 min of cycling, current peaks of PTOB at potentials of +0.025 and +0.135 V showed almost 80% of initial height (Fig. 1). A relatively stable current response was observed in the next 24 h of repetitive cycling in McIlvine buffer solution (pH = 4.0).

3.3. Amperometric pH sensing with polytoluidine blue

A pH change significantly influences formal redox potential of the PTOB film, which is shifted to more negative potentials with the slope of 81 mV^{Δ} pH^{-1}. When the polymer covered electrode is polarized at a constant potential, the pH change is accompanied by a detectable current change.

3.4. Urea biosensor based on urease and polytoluidine blue

During urea hydrolysis, urease immobilized on the PTOB-modified electrode produced a local pH change resulted in a detectable steady-state electrode response:

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^-$$

The influence of the working potential on the amperometric urea biosensor response was tested between −250 and +50 mV. The sensitivity of the biosensor was almost

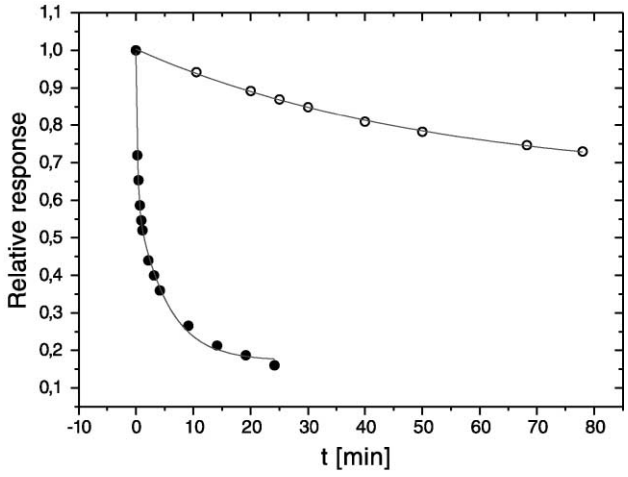

Fig. 1. Stability of adsorbed TOB ● vs. electropolymerized PTOB ○. Modified glassy carbon electrode immersed in a phosphate buffer (pH 4.0) containing 5 mM of a monomer. Measurement was carried at a scan rate of 50 mV s^{-1} from −0.4 to +0.3 V vs. SCE.

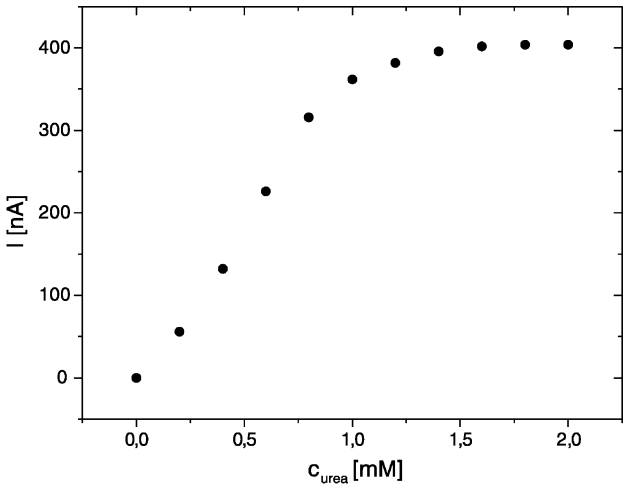

Fig. 2. A typical calibration curve of urea detection under optimized conditions.

constant (200–300 nA mM^{-1} cm^{-2}) in a potential range between +50 and −50 mV. When the working potential decreased further, the sensitivity of biosensor rose very rapidly up to −200 mV. At this potential, the signal was leveled-off at the maximum sensitivity of 980 nA mM^{-1} cm^{-2}. For further work, a working potential of −200 mV was chosen. The sensitivity of urea biosensor in a quasi-linear region was 980 nA mM^{-1} cm^{-2} with the linear range up to 0.8 mM (Fig. 2) and the detection limit of 0.02 mM (S/N = 3). Response time of the biosensor needed to attain 90% of steady state current was 20–30 s.

4. Conclusion

It can be concluded that the electropolymerized toluidine blue is useful for the amperometric detection of a pH changes. This principle is applicable in the amperometric urea biosensor construction. The polymeric form of toluidine blue has significantly improved the stability compared to the use adsorbed one on the surface of the electrode. The prepared urea biosensor has acceptable analytical characteristics. Further improvement of the biosensor characteristics could be expected after a more systematic optimization of a biosensor construction and operation parameters.

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