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Electrochemical characterization of redox polymer modified electrode developed for monitoring of adenine

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ABSTRACT

Electrochemical characterization of redox polymer for monitoring of adenine was described in this study using poly(vinylferrocenium) (PVF^+) modified platinum (Pt) electrode. Scanning electron microscope (SEM) was used for the surface characterization. The electrochemical behaviors of polymer modified and adenine immobilized polymer modified electrodes were investigated by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). In order to obtain more sensitive and improved electrochemical signals, analytical parameters such as the effects of polymeric film thickness, immobilization time of adenine, pH and adenine concentration were examined on the response of the polymer modified electrode. Alternating current (AC) impedance spectroscopy was used for the characterization of polymer modified and adenine immobilized polymer modified electrodes. The effect of possible interferents on the response of the electrode was examined.

1. Introduction

Adenine is one of the two purine nucleobases found in nucleic acids which plays a fundamental role in many biological systems. It is also found free or as a component of coenzymes [1,2]. The concentration levels of adenine were proposed as significant markers for diseases and metabolic disorders, such as AIDS, cancers, myocardial cellular energy status and disease progress [3]. Therefore, there are considerable efforts for the detection of adenine [4]. Different methods including spectroscopic [5,6], and chromatographic methods [7,8] were used for this purpose. Electrochemistry received also considerable attention in this field [9–11].

The direct electrochemistry of adenine, which exhibits slow electron transfer on bare working electrode with low sensitivity, received great interest in the bioanalytical chemistry community [12]. Numerous attempts to enhance the sensitivity, to decrease the overpotential of oxidation and to improve the stability of the electrode were performed [4]. The use of electroactive polymers as the transduction element was described in the literature [13–15]. The immobilization of biomolecules using polymer film is very simple, and the adsorption, electrostatic interaction or covalent binding methods can be simultaneously applied for effective immobilization [16,17].

In this study, an electroactive redox polymer, poly(vinylferrocenium) (PVF^+), modified platinum (Pt) electrode was used for the electrochemical monitoring of adenine. The surface morphologies of polymer modified electrode and adenine immobilized polymer modified electrode were characterized by scanning electron microscope (SEM). The electrochemical behavior of polymer modified electrode was investigated by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in the absence/presence of adenine. In order to obtain more sensitive and selective electrochemical signals, experimental parameters were investigated: the effect of polymeric film thickness, adenine immobilization time, pH and adenine concentration. Characterization of polymer modified electrode and adenine immobilized polymer modified electrode was carried out by using alternating current (AC) impedance spectroscopy. The effect of possible interferents, guanine and uric acid, on the response of the electrode was investigated.

In our previous works, this polymer was also used for specific DNA hybridization [17–19]. However, in this work the interaction of adenine and polymer was investigated directly for the detection of adenine. The electrochemical signal of adenine was obtained at low potential with improved current.

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2. Experimental

2.1. Apparatus

Electrochemical studies were carried out with CH Instruments System, Model 660 B. SEM images were obtained by Zeiss Evo 50 EP-SEM.

A Pt (Aldrich) disc working electrode (area: 0.00785 cm^2), a saturated calomel reference electrode (SCE) (BAS, USA) and a Pt (Aldrich) counter electrode were used in the study. Ag/AgCl (Aldrich) reference electrode was used for the electrooxidation of polymer performed in methylene chloride. SEM studies were performed with Pt foil (Aldrich) electrode (area: 0.25 cm^2).

2.2. Reagents and preparation of solutions

$NaH_2PO_4{\cdot}2H_2O$, $Na_2HPO_4{\cdot}2H_2O$ and NaOH were purchased from Merck. Methylene chloride was obtained from Riedel de Haen. $NaClO_4$ was purchased from Sigma–Aldrich. Vinylferrocene was obtained from Aldrich. 2,2′-Azo-bis(2-methyl-propionitrile) (AIBN) was purchased from Alfa. CH_3COONa and CH_3COOH were obtained from Fluka. Adenine, guanine and uric acid were purchased from Sigma. Other chemicals were supplied from Sigma and Merck.

Phosphate buffer solution (50 mM PBS, pH: 7.0) containing 0.1 M $NaClO_4$ was prepared from $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}2H_2O$ using triple distilled water. Acetate buffer solution (50 mM ABS, pH: 4.80) was prepared from CH_3COONa and CH_3COOH. Adenine stock solution was prepared with ultrapure tri-distilled water and kept frozen. The diluted solutions of adenine were prepared by using PBS containing 20 mM NaCl.

2.3. Supporting electrolytes

Tetra-n-butyl ammonium perchlorate (TBAP) was used as the supporting electrolyte of the polymer solution, in nonaqueous medium [20]. PBS containing 0.1 M $NaClO_4$ and ABS were used as the supporting electrolyte in aqueous medium.

2.4. Chemical polymerization of vinylferrocene and preparation of polymer solution

Poly(vinyferrocene) (PVF) was prepared by the chemical polymerization of vinylferrocene with AIBN initiator [21]. 1.0 mg mL^{-1} PVF polymer solution was prepared in methylene chloride/TBAP solvent/supporting electrolyte system.

2.5. Procedure

All the experiments were done at room temperature. Each test was repeated three times.

2.5.1. The preparation of poly(vinylferrocenium) (PVF^+) modified platinum electrode by potential-controlled coulometry

The PVF^+ modified electrode was prepared electrochemically by electrooxidation at +0.7 V vs. Ag/AgCl reference electrode in PVF solution. Perchlorate ion (ClO_4^-) in the structure of TBAP inserted into the polymeric structure as a counter ion [19].

The thicknesses of polymer films were controlled by the charge passed during the electroprecipitation. This charge was considered as an indication of polymeric film thickness [22].

2.5.2. The preparation of adenine immobilized PVF^+ modified electrode

The preparation of adenine immobilized electrode was accomplished by immersing the PVF^+ modified electrode into 1000 μg mL^{-1} adenine solution for 1 h. The electrode was washed using buffer solution after immobilization of adenine onto the polymer electrode.

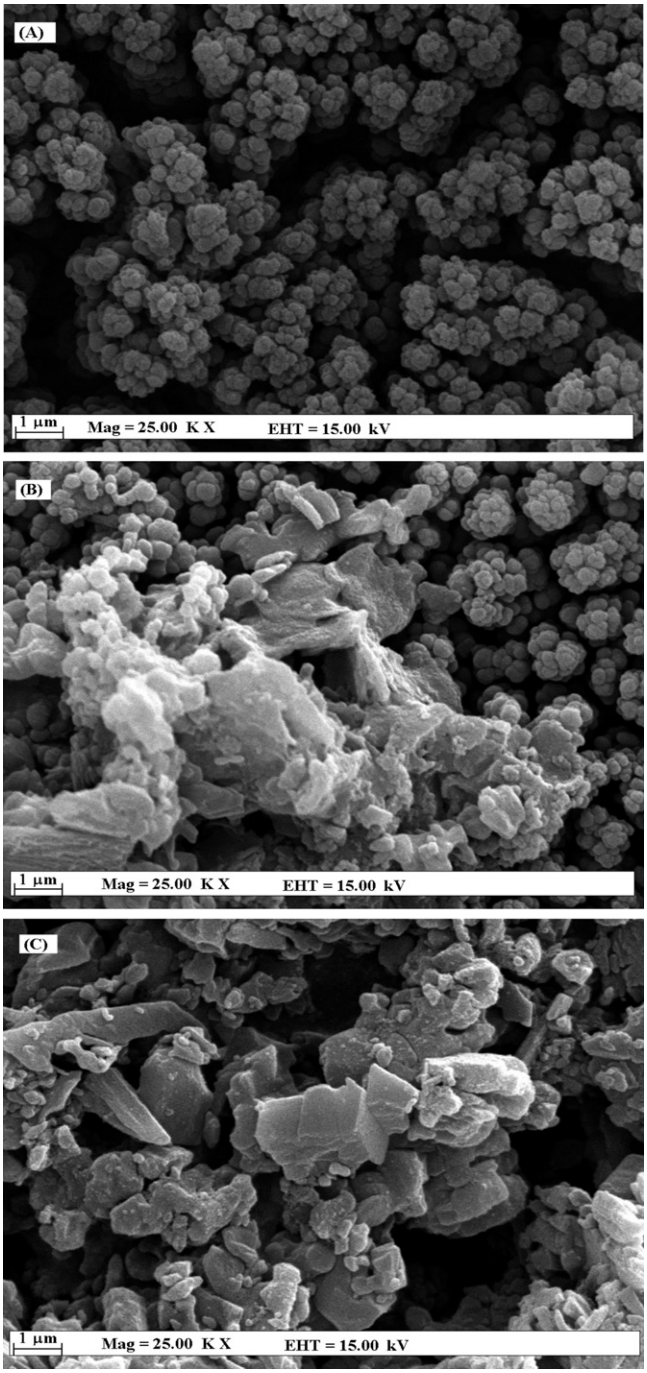

Fig. 1. SEM images of (A) polymer film, (B) adenine immobilized polymer film after 1 h immobilization, and (C) adenine immobilized polymer film after 2 h immobilization.

2.5.3. Voltammetric transduction

The oxidation signal of polymer in the absence/presence of adenine was measured in buffer solution by using cyclic voltammetry (CV) scanning between −0.1 V and +1.2 V vs. SCE and by using differential pulse voltammetry (DPV) scanning between 0.0 V and +1.0 vs. SCE at pulse amplitude of 50 mV.