Determination of Xanthine in the Presence of Ascorbic and Uric Acids on the Glassy Carbon Electrode Modified with Poly(sulfosalicylic acid) Nanorods

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Received July 24, 2008

Poly(sulfosalicylic acid) nanorods (PSSA-NRs) was prepared by electrochemical deposition in PBS solutions, and the PSSA-NRs/GCE modified electrode was investigated by voltammetric methods in buffer solution. The PSSA-NRs/GCE modified electrode demonstrated a substantially enhanced electrochemical sensitivity and selectivity towards xanthine (XA) in the presence of L-ascorbic acid (AA) and uric acid (UA). The PSSA-NRs promoted the electron transfer reaction of XA, while the PSSA-NRs restrained the electrochemical response of the negatively charged AA due to the electrostatic repulsion. The anodic peak potentials of XA, UA and AA were separated by electrochemical techniques, and the interferences from AA and UA were effectively eliminated in the XA determination. The peak currents exhibited two-step linearity according to the XA concentration over the range from 0.1-10 μM with a break point of about 1 μM. The detection limit of the XA oxidation current was 0.02 μM at a signal-to-noise ratio of 3. The results indicated that the modified electrode can be used to determine XA, without interference from UA and AA, with high sensitivity, selectivity and reproducibility.

Key Words : Sulfosalicylic acid, Electropolymerization, Nanorods, Xanthine, Electrocatalytic oxidation

Introduction

Xanthine (XA) is the metabolite of purine, and an abrupt change of XA level in the body due to the nucleoside decomposition indicates the possible presence of several diseases and the mutation of the immunological system.[1-3] The determination of XA is of great importance in medical and biological fields because XA is a precursor of uric acid (UA) which is produced as the final purine metabolite by XA oxidase (XOD).[4-6] Moreover, the conversion of XA to UA by XOD is related to the formation of superoxide anions which are one of the reactive oxygen species in the body,[7] thereby further increasing the importance of the sensitive and selective detection of XA. Chromatography and spectrophotometry have been used for the determination of XA,[8-11] but due to their expense and difficulty alternative methods are required for this particular application.

Several enzymatic methods for the determination of XA have been studied.[12-31] However, despite its inherent advantages such as high selectivity and sensitivity, the enzyme electrode is very unstable and it suffers from the inevitable disadvantage of being too costly.[32,33] Meanwhile, non-enzymatic, modified electrodes are stable and simple, and the XA detection can be performed rapidly and reproducibly.[34-42] The applied electrochemical methods for the determination of XA include several voltammetry methods such as cyclic, linear sweep, and differential pulse voltammetry. Recently poly(SSA) film was used for the determination of dopamine in phosphate buffer saline (PBS) solutions.[43]

The preparation of polymer nanoscale structures has attracted noteworthy attention due to their extensive applications. Many techniques including lithographic and chemical methods have been applied for the fabrication of nanomaterials.[44,45] The template method is a simple and resourceful approach for preparing nanomaterials of electrically conductive polymers, metals and semiconductors.[46,47] Since the pioneering work of Martin's group,[48] nanomaterials of polyaniline, polypyrrole and polythiophene have been prepared using template synthesis.[49-51] The track-etch polymeric and anodic aluminum oxide (AAO) membranes are used as the templates for the construction of nanostructure. Recently AAO membrane has attracted an increasing interest for its applications to prepare nanostructure materials.[52] The preparation methods by the AAO template are mainly electrochemical deposition and chemical synthesis. The growth rate and the dimension of the nanomaterials can be easily controlled in electrochemical preparation.[53-55] In this work, PSSA-NRs was prepared by cyclic votammetry in PBS solutions, and then the PSSA-NRs/GCE modified electrode was studied for the XA voltammetric response in the presence of UA and ascorbic acid (AA). The PSSA-NRs/GCE modified electrode enabled the film to accumulate XA by adsorption process, which largely improved the XA electrochemical response. The oxidation potentials of XA and the interferences can be separated by the modified electrode, thereby enabling the independent determination of XA in the presence of interferences such as AA and UA.

Experimental

Chemicals and electrochemical apparatus. 5-SSA, XA, UA, and AA were purchased from Aldrich. All other reagents used were of analytical grade. AAO films (d = 200 nm, Whatman) were used as a template for the PSSA-NRs.

The pH of the PBS solutions was adjusted with 0.1 M H_3PO_4 and 0.1 M NaOH. High purity argon was used for deaeration. All experiments were carried out at room temperature. Doubly distilled water with resistibility over 18 MΩ cm in a quartz apparatus was used to prepare all aqueous electrolyte solutions.

Linear sweep voltammetry (LSV) was performed with a three-electrode potentiostat [Bioanalytical Systems (BAS) 100B/W] in a ground Faraday cage. A platinum-wire electrode was used as an auxiliary electrode. A Ag/AgCl electrode supplied by BAS was used as the reference electrode. The PSSA-NRs/GCE modified electrode was used as the working electrode. GCE (3 mm in diameter) was purchased from BAS. All potentials were reported with respect to the Ag/AgCl electrode at room temperature under argon atmosphere. The pH measurements were performed with a JENCO meter. Field emission scanning electron microscope (FESEM) Image of the modified electrode was obtained on a JSM-7500F field emission scanning electron microanalyzer (JEOL, Japan).

Preparation of PSSA-NRs/GCE modified electrode. The SSA solution of methanol was sank into the pore of the Pt-coated AAO membrane slowly, and then PSSA-NRs were prepared under cyclic voltammetry conditions by sweeping the potential from −1.2 to 2.0 V versus Ag/AgCl with a scan rate of 20 mV/s at room temperature in PBS solutions. The AAO membrane was treated with 10% (w/w) NaOH solution to dissolve the AAO template, and then washed several times with distilled water carefully and filtered with polycarbonate membrane (pore size: 1 μm). PSSA-NRs were obtained and dispersed in methanol. Meanwhile, the GCE surface was highly polished with alumina paste, sonicated with ultrasonic agitation for 5 min, washed with 1.0 M HCl solution, and then rinsed with distilled water several times and methanol finally. After being cleaned thoroughly, the GCE was coated with 5 μL of PSSA-NRs methanol solution containing 0.5% Nafion, and then the methanol was evaporated in the air at room temperature. The PSSA-NRs/GCE modified electrode was used as the working electrode for the determination of XA. Before and after each experiment, the PSSA-NRs/GCE modified electrode was washed with distilled water. All experiments were carried out in a 15 mL electrolytic cell with 5 mL PBS solutions, with dioxygen being continuously removed by purging with high-purity argon.

Results and Discussion

Formation and morphology of the PSSA-NRs. Figure 1 shows cyclic voltammograms (CVs) of the Pt-coated AAO membrane including SSA in the AAO pore in PBS solutions of pH 7.4. In the repetitive cycling over 9 scans from −1.2 to 2.0 V at 20 mV/s, the oxidation peak was increased at 1.1 V, while the reduction peak was increased at −0.4 V, indicating the continuous growth of polymeric nanorods in the pore of Pt-coated AAO. The high electron density of the carbonyl and sulfonic groups in the SSA molecule increased the

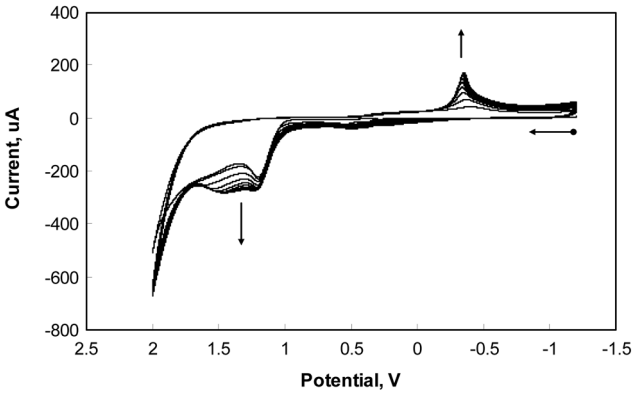

Figure 1. Cyclic voltammograms of the Pt-coated AAO membrane including SSA in the AAO pore in PBS solutions of pH 7.4.

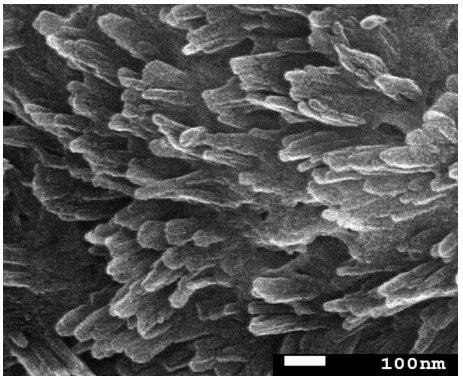

Figure 2. SEM image of the PSSA-NRs on the Pt surface bonded with carbon tape after removing the alumina template.

concentrations of negatively charged functional groups such as SO_3^- and COO^- in the PSSA-NRs obtained by electropolymerization. The typical SEM image of the PSSA-NRs on the Pt surface bonded with carbon tape after removing the alumina template is shown in Figure 2. From this image, the nanorods were observed on the Pt surface, and this result indicates that PSSA-NRs can grow in the nano-pores of the AAO membrane.

Electrochemical behavior of xanthine (XA) at the PSSA-NRs/GCE-modified electrode. The AAO membrane was treated with NaOH solution to dissolve the AAO template, and then washed several times with distilled water and filtered with polycarbonate membrane. PSSA-NRs were obtained and dispersed in methanol. After GCE was cleaned thoroughly, the GCE was coated with 5 μL of PSSA-NRs methanol solution containing 0.5% Nafion, and then the methanol was evaporated in the air at room temperature. The PSSA-NRs/GCE modified electrode was used as the working electrode for the determination of XA. Figure 3 illustrates the XA voltammograms at the bare GCE and PSSA-NRs/GCE modified electrodes in the PBS solution of pH 5.1. Figure 3a shows the voltammetric response of 10 μM XA at the bare GCE, and a small, anodic, XA peak current was observed at 0.75 V in the PBS solution. No voltammetric response was evident on the PSSA-NRs/GCE modified electrode in the blank PBS solution, as shown in

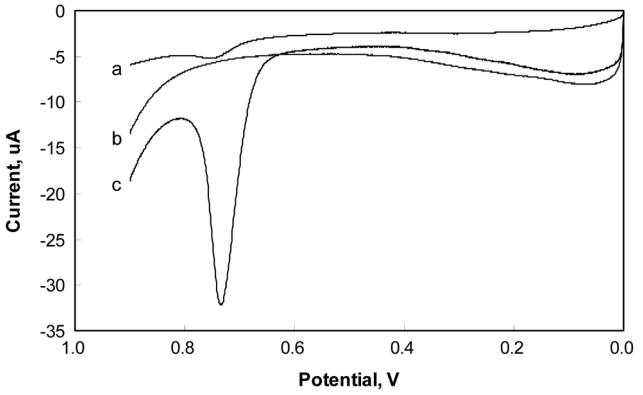

Figure 3. XA voltammograms at the bare GCE and PSSA-NRs/GCE modified electrodes in PBS solution of pH 5.1 with a scan rate of 100 mV/s, for the electrochemical response of 10 μM XA at a bare GCE (a), of no XA at the PSSA-NRs/GCE modified electrode (b), of 10 μM XA at the PSSA-NRs/GCE modified electrode (c).

Figure 3b. LSV of 10 μM XA at the PSSA-NRs/GCE modified electrode in the PBS solution is presented in Figure 3c. The anodic peak potential (0.73 V) and the current of XA (27.8 μA) are represented. The XA anodic potential at the modified electrode was slightly shifted to the negative direction compared with that at the bare GCE (0.75 V), and the XA oxidative current density (39.4 μA/mM·cm^2) at the modified electrode was markedly increased relative to that at the bare GCE (3.3 μA/mM·cm^2), indicating the XA electrocatalytic ability of the PSSA-NRs/GCE modified electrode. The electrocatalytic effect was attributed to the excellent electrical characteristics of the PSSA-NRs, and the strong interaction tendency of the PSSA-NRs to XA.

Figure 4 illustrates the effect of scan rate (0.025-0.9 V/sec) on XA oxidation at the PSSA-NRs/GCE modified electrode in the PBS solution of pH 5.1. The anodic peak current of the modified electrode in the XA solution increased linearly with the square of the scan rate, implying that direct electron transfer between XA and the modified electrode occurred on the modified electrode surface. The plot presented strong linearity, with a correlation coefficient of 0.994 on the anodic current. This indicated that the XA electrode reaction

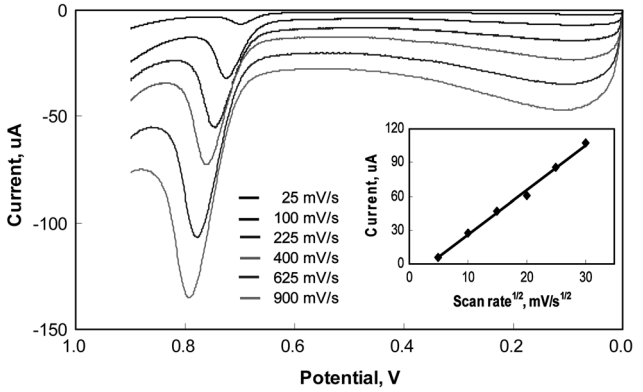

Figure 4. Effect of scan rate (0.025-0.9 V/sec) on XA oxidation at the PSSA-NRs/GCE modified electrode in the PBS solution of pH 5.1.

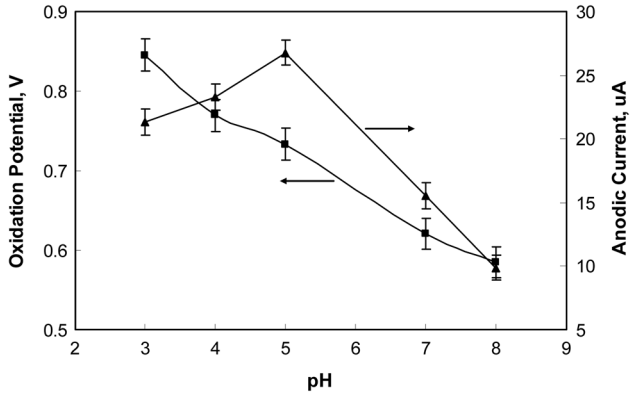

Figure 5. Effect of the buffer solution pH on the peak current and peak potential for XA oxidation.

at the modified electrode is a diffusion-controlled process.

The pH of the PBS solution significantly affected the XA oxidation at the PSSA-NRs/GCE modified electrode by influencing both the peak current and peak potential. Figure 5 shows the effect of the buffer solution pH on the peak current and peak potential for XA oxidation. The anodic peak current increased to a peak and then decreased with increasing solution pH. The pH effect on the XA peak current may have been caused by the electrostatic interaction of XA on the PSSA-NRs/GCE modified electrode. XA is known to be a protic aromatic molecule that can become deprotonated at higher pH. Likewise, PSSA-NRs has functional groups such as SO_3^- and COO^- that may become protonated or deprotonated as a function of solution pH. SSA ($pK_a = -6$)[56] is known to be a very strong acid molecule, but XA ($pK_a = 7.41$)[57] is a very weak acid. Electrostatic repulsion between XA and poly(SSA) on the electrode decreases the efficiency of the XA adsorption on the modified electrode at higher or lower pH. As the XA interaction was maximized in the PBS solution at pH 5.1, this solution was chosen for use in all experiments. The pH effect on the peak potential for the XA oxidation on the PSSA-NRs/GCE modified electrode was also investigated. The XA peak potential decreased with increasing pH. This decrease showed high linearity with a correlation coefficient of 0.991 ($y = -0.053x + 0.989$) on the peak potential. The potential shift *vs.* pH ($\Delta E/\Delta$pH) of about 53 mV/pH was close to the corresponding figure of 59 mV/pH for XA. The result indicates that the number of protons and electrons involved in the XA oxidation of XA is the same.

The effect of accumulation time on the XA peak current was investigated (Fig. 6). The peak currents increased with increasing accumulation time and then plateaued after 30 seconds, thus demonstrating the rapid interaction of XA on the surface of the PSSA-NRs/GCE modified electrode. The peak currents increased linearly with increasing accumulation time in the range 2-30 s for the XA determination, but the plot became curved with further increase in accumulation time. The curvature indicated that a limiting quantity of XA has been gained on the electrode surface under the proper condition. Further increase in the accumulation time

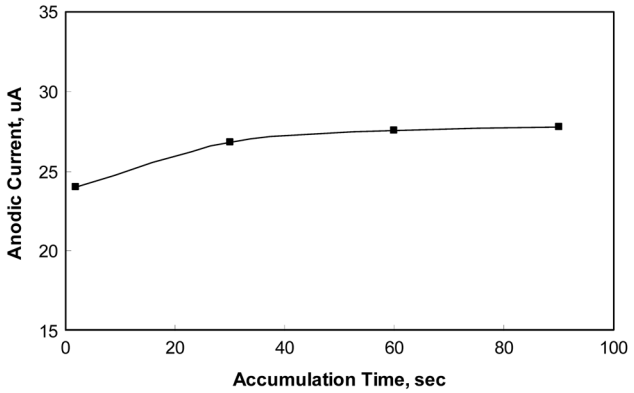

Figure 6. Effect of accumulation time on the XA peak current at the PSSA-NRs/GCE modified electrode in PBS solutions at pH 5.1 with a scan rate of 100 mV/s.

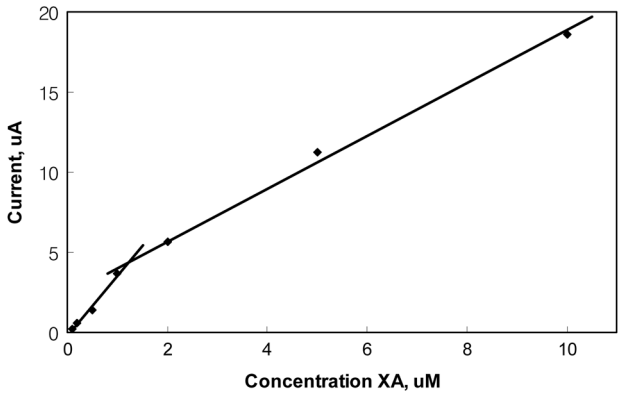

Figure 7. Peak currents *vs.* XA concentration in 0.1 M PBS solution at pH 5.1.

did not increase the XA quantity on the electrode due to surface saturation, and the peak currents were maintained almost constant. Therefore, the sensitivity for lower XA concentrations was enhanced by increasing the accumulation time, but the linear ranges were reduced. To reduce analysis time in subsequent experiments, an accumulation time of 30 sec was chosen. The effect of accumulation potentials on the XA peak current was studied. The peak current increased slightly with increasingly negative accumulation potential up to 0.0 V, but then almost plateaued. Therefore, an accumulation potential of 0.0 V was chosen in all experiments.

The reproducibility and reusability of PSSA-NRs/GCE modified electrode for XA determination were investigated. Repetitive XA determinations were carried out in a solution of 10 μM XA in 0.1 M PBS solution at pH 5.1. The PSSA-NRs/GCE modified electrode was easily regenerated by potential cycling between −0.6 V to 1.0 V at a scan rate of 100 mV/s in the blank PBS solution. In the cycle of preconcentration, determination, regeneration, and cleaning, a relative standard deviation (RSD) of 2.5% was obtained for XA for five replicate measurements. Meanwhile, the XA currents were monitored for 10 days, and the peak current was measured at 97% of the initial current. This result confirmed the good stability and reproducibility for XA determination using PSSA-NRs/GCE modified electrode.

XA determination at the PSSA-NRs/GCE modified electrode. The negatively charged polymeric nanorods should promote the selectivity and sensitivity of XA detection, and PSSA-NRs can be used as a negatively charged polymer to improve XA determination. XA determination was investigated in the presence of AA and UA in order to establish its selectivity and sensitivity for XA. LSV was used to determine the XA concentration, and Figure 7 shows the plot of the peak currents vs. the XA concentration in 0.1 M PBS solution at pH 5.1. In the plot, the peak currents exhibited a two-step linearity to the XA concentration over the range from 0.1-10 μM: the first below 1 μM XA with a correlation coefficient of 0.989 (y = 5.02x – 0.299), and the second over 1 μM with a correlation coefficient of 0.996 (y = 2.21x + 3.125). These results demonstrated different accumulation rates according to the XA concentration under a constant accumulation time. The detection limit (3:1 signal-to-noise ratio) of XA was estimated to be 0.02 μM. Various possible interferences were evaluated for their effects on the voltammetric responses of XA. In the absence of XA, the LSV of AA and UA presented two anodic oxidative peaks at 0.01 V and 0.38 V, respectively, indicating the small anodic peak currents of AA and UA. With the XA addition to the solution of AA and UA, a new anodic peak appeared, corresponding to XA oxidation. This anodic peak current (0.73 V) was increased with increasing XA concentration in the presence of AA and UA, without any variation of the anodic peak currents of AA and UA. In a separate experiment, Figure 8 illustrates a series of LSVs obtained for XA at varying concentrations (0.2 μM-10 μM) in the presence of UA (2 μM) at the PSSA-NRs/GCE modified electrode in 0.1 M PBS solution at pH 5.1. The peak current of UA remained almost constant irrespective of the XA concentration, but the oxidation potentials were slightly shifted in a negative direction. Figure 9 illustrates a series of LSVs for a constant XA concentration (2 μM) with the successive addition of UA (2 μM) and AA (1 mM and 10 mM) at the PSSA-NRs/GCE modified electrode in 0.1 M PBS solution at pH 5.1.

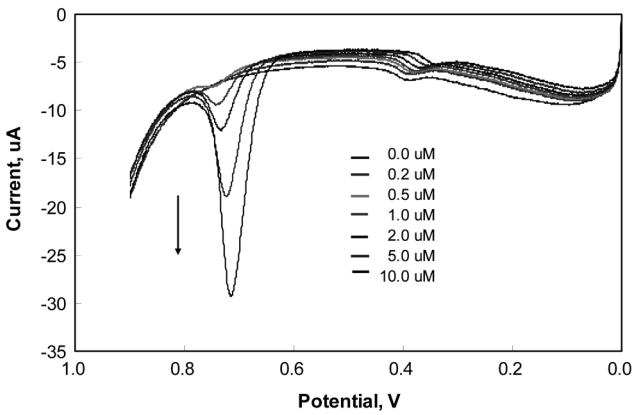

Figure 8. LSVs for XA at varying concentrations (0.0 μM-10 μM) in the presence of UA (2 μM) at the PSSA-NRs/GCE modified electrode in 0.1 M PBS solution at pH 5.1.

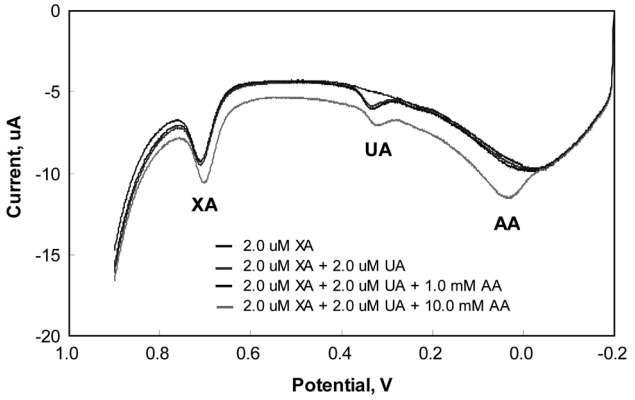

Figure 9. LSVs for a constant XA concentration (2 μM) with the successive addition of UA (2 μM) and AA (1 mM and 10 mM) at the PSSA-NRs/GCE modified electrode in 0.1 M PBS solution at pH 5.1.

The XA peak current remained constant under the addition of UA and AA. The current sensitivity of XA was higher than that of UA and AA. The interferences of UA, AA, and hypoxanthine were considered in the XA determination, but their oxidation potentials were 0.01 V, 0.38 V, and 1.08 V, respectively, in comparison with 0.73 V for the oxidation potential of XA. The responses of XA and UA were separated by a potential difference of 350 mV, as were those of XA and hypoxanthine, which rendered clearly distinguishable the anodic peak potentials of XA, AA, and UA at the PSSA-NRs/GCE modified electrode. Meanwhile, the response of hypoxanthine was poor at the PSSA-NRs/GCE modified electrode. These results support the ability of the PSSA-NRs/GCE modified electrode to determine XA concentration without any interference from AA, UA and hypoxanthine, even in a solution of excessive AA and UA concentration.

Analytical applications for xanthine (XA) determination. The applicability of the PSSA-NRs/GCE modified electrode as an electrochemical sensor was tested for the determination of XA injection, in the presence of AA and UA as interfering substances. The XA concentration in the injection was determined by applying a calibration plot using the procedures proposed in this paper. The XA determination results in the injections (n = 5) were as follows: 2.00 μM XA (labeled) in the presence of 1 mM AA and 10 μM UA, 1.97 μM DA (found), 99.9% recovery and 2.7% RSD.

Conclusions

PSSA-NRs/GCE modified electrode is a useful and effective sensing surface for selective and sensitive XA determination in the presence of UA and AA. The PSSA-NRs/GCE modified electrode was easily prepared in a rapid and simple procedure, and its application improved the sensitivity and selectivity. The negatively charged polymer PSSA-NRs in the electrode provided an efficient electrochemical sensor for the XA measurement.

Acknowledgments. This work was supported by a Research Foundation Grant funded by Chonnam National University (2008).

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