

Junjie Fei · Kangbing Wu · Yunhua Wu · Shengshui Hu

Electrocatalytic oxidation of uric acid at an electrodeposited redox polymer film-modified gold electrode

Received: 2 July 2003 / Accepted: 4 September 2003 / Published online: 13 December 2003
© Springer-Verlag 2003

Abstract A hydrated osmium complex-containing redox polymer film-modified gold electrode based on electrochemical cross-linking was developed. The amount and the characteristics of redox polymer film cross-linked on the gold electrode were investigated by using electrochemical quartz crystal microbalance (EQCM). The redox polymer film exhibited a strong electrocatalytic activity toward the oxidation of uric acid with a lowering of the overpotential by about 230 mV and a large increase in the magnitude of the oxidation peak current. Based on this procedure, an amperometric method for the determination of uric acid concentration was proposed.

Keywords Electrocatalysis · Electrochemical quartz crystal microbalance · Osmium complex · Redox polymer · Uric acid · Amperometry

Introduction

The redox hydrogel film-modified electrodes imposed by chemical cross-linking redox polymers with cross-linking agents, such as 1,5-dibromopentane, 1,10-dibromopentane, and poly(ethylene glycol) diglycidyl ether, have been used as electrocatalysts for amperometric sensors [1, 2] and as redox relays for enzyme sensor development [3, 4, 5, 6, 7, 8, 9]. However, chemical cross-linking often needs long time periods and rigorous controlled conditions. Redox hydrogel films are unique in that they have adequate electron-transfer coefficients and are permeable to water-soluble analytes and reaction products [10]. It is reported that upon illumination the redox polymer formed by coordinating $[\text{Os}(\text{bpy})_2\text{Cl}]^+ / [\text{Os}(\text{bpy})_2\text{Cl}]^{2+}$ to

poly(4-vinyl-pyridine) exchanges its inner-sphere chloride with more strongly coordinating pyridine or imidazole groups on the polymer backbones [11]. The resulting films conduct electrons by exchanging electrons between adjacent sites when they are hydrated, and their redox segments are mobile enough to collide, even though they are tethered to the cross-linked polymer. Apart from electron exchange between adjacent sites, additional processes, such as counterion and solvent movements, may be involved in the overall conduction mechanism. Any of these contributing steps may limit the rate of charge transport through the film. Determination of the rates of counterion and solvent transport through the redox polymer film are, thus, important for assessing the role that these processes play in charge transport. Such measurements can be made by electrochemical quartz crystal microbalance (EQCM). EQCM comprises a thin crystal sandwiched between two metal electrodes to establish an alternating electric field across the crystal, causing vibrational motion of the crystal at its resonant frequency. This resonant frequency is sensitive to the mass changes of the crystal and its electrode. Since the first report using QCM for in situ electrochemical measurements [12] was presented, there has been considerable interest in monitoring mass changes at the electrode surfaces and in thin films on electrodes [13, 14, 15].

It is well known that uric acid is the principal final product of purine metabolism in the human body and is present in body fluids such as blood and urine. Since abnormally high levels of uric acid in the human body are a symptom of several diseases [16], monitoring the concentration of uric acid in biological fluids may be used as an early warning signal of the presence of these diseases. Hence, there has been considerable effort made in the development of electrochemical procedures, most these based on chemically modified electrodes, for the determination of uric acid in biological samples [17, 18, 19, 20, 21]. However, even when very good selectivity and sensitivity have been achieved with the use of these modified electrodes, there are often complications, for example, the need of the renewal of the surface after

J. Fei · K. Wu · Y. Wu · S. Hu (✉)
Department of Chemistry,
Wuhan University,
430072 Wuhan, P.R. China
E-mail: sshu@whu.edu.cn
Tel.: +86-27-87218904
Fax: +86-27-87647617

each measurement [17], or a lack of stability [20, 21]. Thus, electrochemical studies of a simple electrochemical method are desired.

In this paper, a redox polymer film-modified electrode is conveniently fabricated in a one-step and easy-controlled method by applying repetitive double-potential-step chronoamperometry, and the amount of cross-linked redox polymer on the gold electrode and the small changes in mass that occurred during oxidation and reduction of the redox polymer film were studied with an electrochemical quartz crystal microbalance (EQCM). The electrocatalytic behavior of uric acid at the redox polymer film-modified electrode was described thoroughly and the redox polymer film exhibited a strong electrocatalytic activity toward the oxidation of uric acid, and an enhanced oxidation peak current was obtained. Additionally, the modified electrode showed excellent reproducibility and high stability to the determination of uric acid.

Experimental

Reagents and apparatus

[Os(bpy)₂Cl₂] (bpy = 2,2'-bipyridine) was synthesized from K₂OsCl₆ according to the procedure of Lay [22]. The synthesis of electron-conducting redox polymer (PVP-Os-EA) is described elsewhere [23]. In this preparation, poly(4-vinylpyridine) (PVP) partially complexed with [Os(bpy)₂Cl₂] (Os) and partially quaternized with 2-bromoethylamine (EA) and the PVP/Os/EA ratio was 6:1:1.2. The chemical structure of osmium complex-containing redox polymer is shown in Fig. 1. Uric acid, purchased from Sigma Chemical Co. (St. Louis, MO, USA), was used without further purification. Uric acid solutions were prepared daily, prior to use, by dissolving in nitrogen-purged doubly distilled water. A phosphate-buffer saline solution (PBS; pH 7.3) was prepared from phosphate (0.02 mol L⁻¹) and sodium chloride (0.15 mol L⁻¹). All other chemicals were of analytical grade and solutions were prepared using doubly distilled water.

EQCM was carried out with a potentiostat (EG&G Model 283) interfaced to a Model QCA-917 quartz crystal

analyzer (Seiko EG&G) which can produce $\Delta f \sim E$ plot simultaneous $E \sim i$ curve. AT-cut 9 MHz gold plated quartz crystals were used; the mass sensitivity calculated using Sauerbrey's equation [24] was 5.464 ng cm⁻² Hz⁻¹. The crystal was mounted in a Teflon electrode holder with an exposure area of 0.20 cm². The quartz crystal electrode facing solution was used as the working electrode. A platinum wire and a saturated calomel electrode (SCE) were used as counter and reference electrodes, respectively, in a conventional three-electrode cell. Repetitive double-potential-step chronoamperometry was performed with a computer-controlled Model CHI 660A electrochemical workstation (CH Instrument, Inc., Austin, USA). All potentials in this paper are quoted with respect to the SCE.

Preparation of the redox polymer film-modified electrode

The quartz crystal electrode facing solution was washed with alcohol and doubly distilled water before modification. The redox polymer film was electrodeposited onto the gold surface from a 0.5 mg mL⁻¹ osmium complex-containing redox polymer solution using 100-time repetitive double-potential-step (between 0.70 V, 2 s and -0.40 V, 2 s) chronoamperometry. The electrode was ready for use after the final washing with water.

Procedure

The estimation of the amount of cross-linked redox polymer was performed as follows: first, the steady frequency (f_1) of the fresh crystal electrode was measured. Then, the crystal was electrodeposited in a 0.5 mg mL⁻¹ PVP-Os-EA solution using repetitive double-potential-step chronoamperometry and the steady frequency (f_2) was measured in PBS. Thus, the amount of cross-linked redox polymer could be calculated from the difference between f_1 and f_2 . Before frequency measurements, the fresh crystal electrodes and the modified electrodes were cycled between -0.1 and 0.7 V in PBS buffer until the stable voltammograms of sequential cycles were identical. Amperometric detection experiments were carried out in well-stirred (rotation speed, 750 rpm) solutions at a constant potential of 0.30 V. Uric acid in human urine samples was analyzed by the standard addition method without any preliminaries.

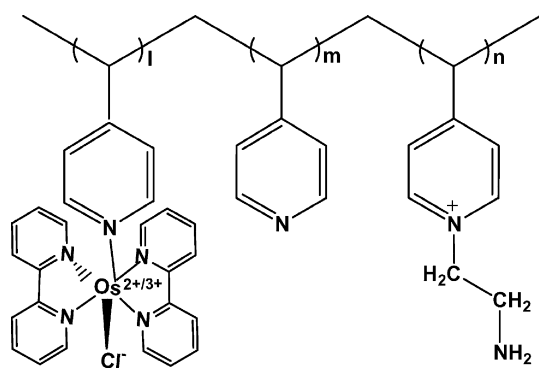


Fig. 1 Chemical structure of the redox polymer

Results and Discussion

Electrodeposition of redox polymer film on the gold electrode

In order to obtain a satisfactory redox polymer film, experimental conditions of the electrodeposition were optimized. Since cross-linking occurs in the ligand-

exchange process, the surface density of the adsorbed redox polymer must be high; otherwise, the fraction of cross-linked polymer will be small [11]. It was found that a redox polymer concentration of 0.50 mg mL^{-1} was best for our purpose. The square-wave potentials applied were expected to affect the properties of the film. The reducing potential was examined in the range from 0.1 V to -0.6 V and when the potential was more positive than 0 V, no obvious response in terms of the formation of redox polymer film was observed. The rate of deposition increased with a lowering of the reduction potential and reached a plateau at -0.4 V. A slight decrease in the total amount of material deposited was observed when the potential became more negative than -0.5 V. Since the purpose of the oxidative half-cycle, where the electrode surface is oxidized, is to increase the surface density of the redox polymer prior to its cross-linking in the reductive half-cycle, the oxidizing potential may affect the fraction of cross-linked polymer. The rate of electrodeposition increased when the oxidizing potential increased from 0.3 V to 0.7 V and began to decrease when the potentials were more positive than 0.7 V.

The effect of the numbers of the repetitive double-potential-step chronoamperometry applied to the electrodeposition of the redox polymer film was studied using QCM and cyclic voltammetry (Fig. 2). Fig. 2a shows that the difference in the resonance frequency between the fresh crystal and the modified electrode increases linearly with the cyclic number of the repetitive double-potential-step chronoamperometry, which indicates that the amount of redox polymer cross-linked on the electrode increased linearly with the cyclic number. Fig. 2b shows that the redox couple surface coverage (Γ) of the redox polymer film increased linearly with the number of cycles applied at first and then leveled off when it reached more than 100, which is due to the fact that the redox couple surface coverage reached its maximum value and no longer increased with the thickness of the redox polymer film. The redox couple surface coverage was calculated integrating either the

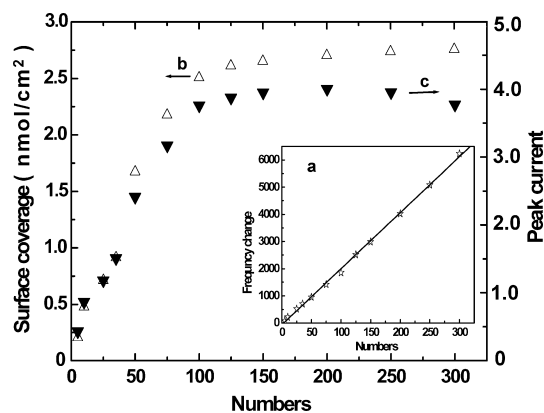


Fig. 2a–c The effect of the number of double-potential-step chronoamperometry applied on the electrodeposition of the redox polymer film. **a** (Insert), Frequency change; **b** (Δ), osmium redox couple surface coverage; **c** (∇), Peak current. Scan rate: 20 mV s^{-1}

reduction or the oxidation peak of a cyclic voltammogram. The peak current of the redox polymer film underwent a similar change with the increased redox couple surface coverage, at first and began to decrease slowly when the numbers were higher than 200 (Fig. 2c). This is probably due to the thickness of the film which affects the electron transfer rate of the film. On applying 100 square waves, the surface coverage of the hydrated redox polymer was calculated to be about $11 \mu\text{g cm}^{-2}$, according to the frequency change, and $2.5 \times 10^{-9} \text{ mol cm}^{-2}$ $\text{Os}^{2+}/\text{Os}^{3+}$ redox centers on the electrode surface was obtained by integrating of the cyclic voltammogram. The reproducibility of the electrode was examined by measurement of the redox centers' surface coverage of redox polymer film for 5 electrodepositions and the relative standard deviation (RSD) was 6.2 %.

Electrochemical characteristics of the redox polymer film-modified electrode

A typical frequency and current response of the redox polymer film in blank PBS buffer by cyclic voltammetry is shown in Fig. 3. The frequency response is stable at a level of ± 0.1 Hz with the absence of any noise influences. In Fig. 3a, a decrease in frequency is observed during oxidation process which is essentially recovered during re-reduction, and indicates that the redox polymer film increases in mass as the Os^{2+} is oxidized to Os^{3+} and decreases in mass by the same amount on reversion to the initial value. The mass change between oxidation and reduction per faraday of charge, calculated from the observed frequency change, is 350 g mol^{-1} . This value is larger than the chloride anion molecular mass, which indicates that the chloride anion is not alone responsible for changes in mass within the redox film and that it involved the transport of neutral molecules (water). This result suggests that water is being taken up into the film at the same time as the chloride anions are being accumulated during oxidation. On reduction, chloride anions are expelled from the film and water is simultaneously released, while the mass of the film returns to its starting value. The effect of the

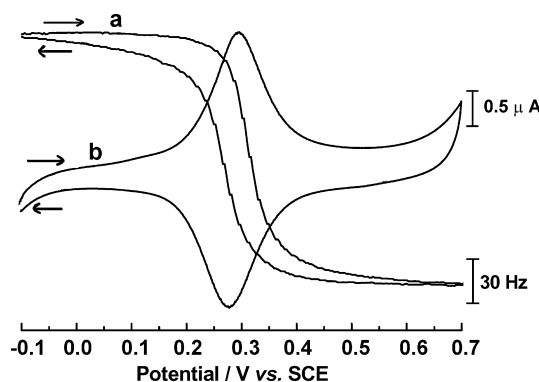


Fig. 3 Frequency (**a**) and current (**b**) responses of cyclic voltammetry on a redox polymer film at a scan rate of 20 mV s^{-1} in PBS

scan rate on the frequency response during cyclic voltammetry was also studied and the results showed that this effect was little, which indicated that the rates of chloride ions and the water transport through the redox polymer film was high.

Cyclic voltammograms of the modified electrode at the various scan rates are shown in Fig. 4. It can be seen that a pair of redox peaks with nearly equal charges for the reduction and oxidation processes and same formal potential, based on the average value of the anodic and cathodic peak potential, are around 290 mV, corresponding to the oxidation and reduction of the $\text{Os}^{2+}/\text{Os}^{3+}$ redox couple in the film. Both anodic and cathodic peak currents increased linearly and the separation between the anodic and cathodic peak potentials (ΔE_p) increased only marginally, as the scan rate increased from 10 to 250 mV s^{-1} , which indicates that the redox polymer film-modified electrode exhibits a reversible surface-bound redox process and the charge transfer from the film to the electrode is rapid [25].

Electrocatalytic oxidation of uric acid at the redox polymer film-modified electrode

The excellent electrocatalytic property of the modified electrode towards the oxidation of uric acid in the pH 7.3 PBS buffer is demonstrated in Fig. 5A. It can be seen from Fig. 5A (curve c) that the onset of the uric-acid oxidation occurs at about 0.30 V, and the current reaches a maximum value at about 0.51 V at a bare gold electrode, indicating sluggish electrode kinetics for the uric-acid oxidation on a gold electrode. However, on a redox polymer film-modified electrode, the oxidation of uric acid starts at about 0.18 V and a well-defined anodic current peak can be seen at around 0.28 V (curve b). The obvious increase in the oxidation peak current and the decrease in the anodic overpotential of about 230 mV for uric-acid oxidation was observed which demonstrates that an efficient catalytic reaction occurs between the redox polymer film and uric acid in the solution. The uric-acid oxidation reaction occurs in the potential region of the $\text{Os}^{2+}/\text{Os}^{3+}$ redox couple and the reduction

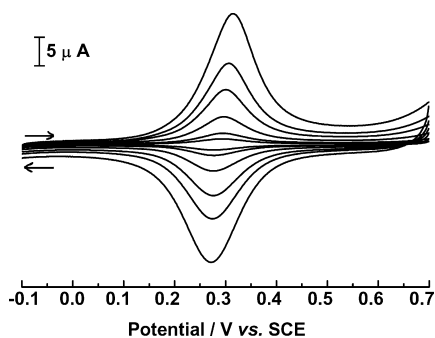


Fig. 4 Cyclic voltammograms of the modified electrode in the PBS buffer. Scan rates from the innermost to the outermost waves: 10, 20, 50, 100, 150, 250 mV s^{-1}

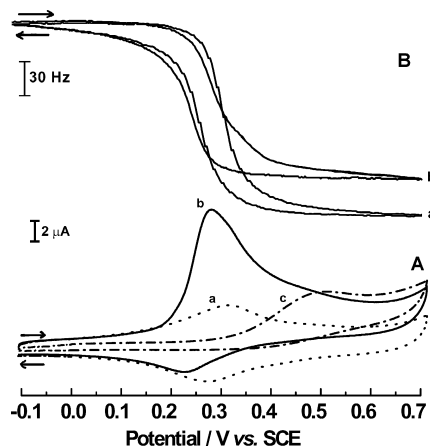


Fig. 5A,B Cyclic voltammograms of a bare gold electrode (c) and a redox polymer film-modified electrode (a, b) in the absence of uric acid (a) or in the presence of $5.0 \times 10^{-5} \text{ mol L}^{-1}$ uric acid (b, c). **B** Frequency response of cyclic voltammetry on a redox polymer film in the absence of uric acid (a) or in the presence of $5.0 \times 10^{-5} \text{ mol L}^{-1}$ uric acid (b). Scan rate: 20 mV s^{-1}

peak of the $\text{Os}^{2+}/\text{Os}^{3+}$ redox couple decreases, indicating that the uric-acid oxidation is mediated by the $\text{Os}^{2+}/\text{Os}^{3+}$ redox couple. The shift in the overpotential is due to a kinetic effect attributed to the improvement in the reversibility of the electron-transfer process [26]. The frequency response of the redox polymer film in the presence of uric acid during the cyclic potential scan was also studied, as shown in Fig. 5B. The decrease in frequency during the oxidation in the presence of uric acid (curve b) is less than that in the absence of uric acid (curve a). It can be expected that the Os^{2+} is fully oxidized to Os^{3+} during the oxidation process when the redox polymer film is placed into a blank solution. The uric acid was oxidized by the surface bound Os^{3+} sites when uric acid was added. Thus, a part of $\text{Os}^{2+}/\text{Os}^{3+}$ redox couple of the redox polymer film existed in the Os^{2+} state in the presence of uric acid (UA) during the oxidation and the quantities of the counterions that moved into the film are correspondingly smaller than those in the absence of UA.

Figure 6 presents the dependence of the oxidation current of uric acid on the pH at the redox polymer film-modified electrode was studied for a range of 3.5–8.5. It can be seen that the peak current increases at first and then reaches a maximum value over the range of 7.0–7.7, which shows that the redox polymer is an efficient electrocatalyst for the oxidation of uric acid at high pH values. Essentially, as the formal potential for the uric-acid oxidation shifts cathodically with increasing pH, the free-energy difference (ΔG) between the uric acid and the electrocatalyst increases, resulting in increasingly efficient electrocatalysis, as expected based on the linear free energy relation in Marcusian theory [2]. The effect of pH on peak potentials (E_p) for the oxidations of UA was also studied. The slope of the E_p -pH dependence was found to be -58 mV/pH , which indicates that the total number of electrons and protons involved in the

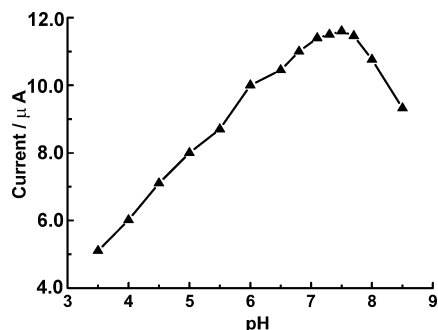


Fig. 6 The dependence of the peak current of 5.0×10^{-5} mol L^{-1} uric acid on pH. Scan rate: 20 mV s^{-1}

oxidation of UA is the same. The oxidation of uric acid is generally known to undergo a two-electron transfer process; the number of protons involved is also predicted to be two.

The influence of the potential scan rate on the oxidation peak current and peak potential has been tested by CV voltammetry. The results demonstrate that the peak currents were linearly proportional to the square root of scan rates in the range of 10 and 250 mV s^{-1} . The oxidation of uric acid at the redox polymer film-modified electrode in the PBS is controlled by the diffusion of uric acid in solution.

Based on the discussion mentioned above, the mechanism for the oxidation of uric acid at the modified electrode can be illustrated (Fig. 7). Uric acid diffusing to the film is oxidized by the surface-bound Os^{3+} sites to produce to uric acid-4, 5-diol and Os^{2+} . Then the reduced Os^{2+} sites at the electrode surface are re-oxidized rapidly by the electrode due to the high electron transfer rate associated with the redox polymer film and the electrode and the fast self-exchange rate between the redox sites within the polymer film to complete the catalytic cycle.

Hydrodynamic amperometry

Figure 8 shows the hydrodynamic voltammograms obtained for $10 \mu\text{M}$ uric acid at a redox polymer film-modified electrode. The current increases with increasing potential, and it finally reaches a steady

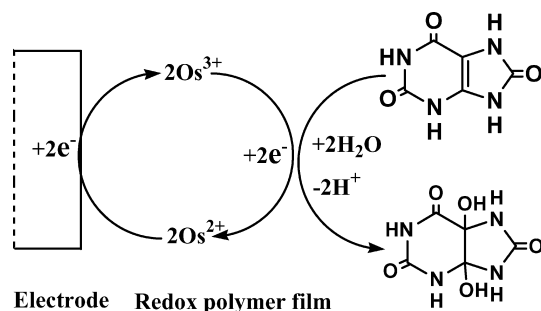


Fig. 7 Scheme of redox polymer film for the oxidation of uric acid

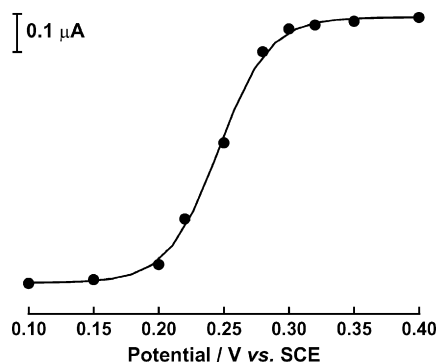


Fig. 8 Hydrodynamic voltammograms of $10 \mu\text{M}$ uric acid at the redox polymer film-modified electrode

response at about 0.30 V. Thus, the redox polymer film-modified electrode could be used as an effective amperometric sensor for the determination of uric acid with high sensitivity and relatively low detection potential.

A typical hydrodynamic amperometric experiment involving uric acid were carried out in a well-stirred solution (rotating speed, 750 rpm) by keeping the potential at 0.30 V. Amperometric tests showed that the redox polymer film-modified electrode gives a good response for fourteen sequential additions of $1 \mu\text{M}$ uric acid (Fig. 9). The oxidation current increases as uric acid is added and reaches steady-state within 2 sec. The current responses were found to be linear to the uric-acid concentration over the range of 4.0×10^{-7} to 1.0×10^{-4} mol L^{-1} , and a sensitivity of $68 \text{ nA } \mu\text{M}^{-1}$ and an intercept of 29 nA with a correlation coefficient, r , of 0.9993 were obtained. The precision was estimated from two series of 15 repetitive measurements of 1 and $10 \mu\text{M}$ uric-acid solution, and the relative standard deviations (RSD) were 3.3% and 2.7%, respectively. These results suggest that the fabricated redox polymer film-modified electrode exhibits a high accuracy for the determination of uric acid. The limit of detection (LOD), defined as a signal-to-noise ratio of 3:1, was found to be 1.5×10^{-7} mol L^{-1} . Furthermore, the modified electrode possessed a high stability. The stability tests were carried out at room temperature by measuring the response from hour

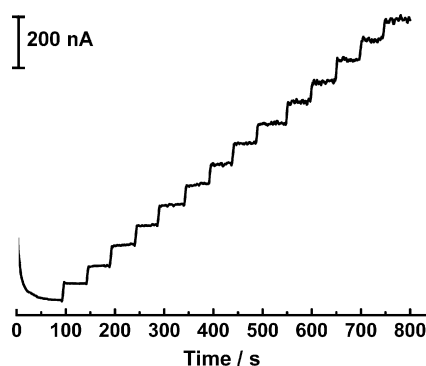


Fig. 9 Amperometric responses at 0.30 V of fourteen sequential additions of uric acid (each $1 \mu\text{M}$) at redox polymer film-modified electrode. Rotating speed: 750 rpm

Table 1 Results of uric-acid analysis in human urine samples^a

Sample no	Detected ($\mu\text{mol L}^{-1}$)	Spiked ($\mu\text{mol L}^{-1}$)	After spiked ($\mu\text{mol L}^{-1}$)	Recovered (%)	Total value ^b ($\mu\text{g mL}^{-1}$)
1	4.43	5.0	9.61	103.6	372.36
2	3.96	10.0	13.75	97.9	332.88
3	4.74	15.0	19.44	98.0	398.42

^a Average of four replicate determination

^b Total value is obtained by multiplying the detected value by the dilution factor of 500

to hour and the redox polymer film-modified electrode was kept at room temperature. It was shown that the modified electrode maintains its initial sensitivity after 72 h.

Applications

Various possible interferences were evaluated for their effects on the amperometric responses of uric acid. The interference studies were carried out by adding a specified concentration of interfering substances into a PBS (pH 7.3) containing 1.0×10^{-6} mol L⁻¹ uric acid. The experimental results showed that, at least, a 50-fold amount of progesterone, xanthine, hypoxanthine, glucose, serine, adenine, guanine, progesterone, purine, urea, theophylline, estradiol, estrone, and estriol had no effect on the amperometric current of uric acid. One drawback of this method is that ascorbic acid and dopamine can also become oxidized at the potential of 0.30 V. Fortunately, low concentrations (about 2 μM) of ascorbic acid and dopamine have little amperometric effect on the redox polymer-modified electrode. Thus, this method can be applied to real samples having lower ascorbic acid and dopamine concentrations than that of uric acid, such as human urine sample.

In order to confirm the sensitivity and generality of the proposed method, the analytical procedure was applied in determining the concentration of uric acid in human urine samples by using the standard addition method. The results are listed in Table 1. The amounts recovered were in the range of 97.9–103.6%, which indicates that this method is practically sound for the selective determination of the amount of uric acid in biological samples.

Conclusion

An easily prepared redox polymer film-modified electrode was studied for the catalytic oxidation of uric acid.

The counterions and solvent transport through the redox polymer film was investigated by using EQCM. In the PBS, the modified electrode reduced the overpotential of uric-acid oxidation by 230 mV and increased the oxidation current significantly, which clearly demonstrates the excellent electrocatalytic activity of the redox polymer film for the oxidation of uric acid. Additionally, the easy-controlled electrodeposition procedure may provide a better control to generate a coating on small electrodes of complex geometry. Experimental results showed that the method is quite valuable and seems to be of great utility for sensor development.

Acknowledgement The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (No. 60171023 and 30370397). The authors thank Dr. Zhiqiang Gao for the reagents.

References

- Doherty AP, Vos JG (1993) *Anal Chem* 65:3424
- Doerty AP, Stanley MA, Vos JG (1995) *Analyst* 120:2371
- Heller A (1990) *Acc Chem Res* 23:128
- Pravda M, Adeyoju O, Iwuoha EI, Vos JG, Smyth MR, Vytras K (1995) *Electroanalysis* 7:619
- Willner I, Heleg-Shabtai V, Blonder R, Katz E, Tao G, Bückmann AF, Heller A (1996) *J Am Chem Soc* 118:10321
- Kenaisi G, Chen Q, Heller A (1997) *Anal Chem* 69:1054
- Danilowicz C, Cortón E, Battaglini F, Calvo EJ (1998) *Electrochim Acta* 43:3525
- Calvo EJ, Etchenique R, Pietrasanta L, Wolosiuk A, Danilowicz C (2001) *Anal Chem* 73:1161
- Forzani ES, Otero M, Perez MA, Tejel ML, Calvo EJ (2002) *Langmuir* 18:4020
- Heller A (1992) *J Phys Chem* 96:3579
- Gao ZQ, Binyamin G, Kim HH, Barton SC, Zhang YC, Heller A (2002) *Angew Chem Int Ed* 41:810
- Nomura T, Iijima M (1981) *Anal Chim Acta* 131:97
- Ward MD, Buttry DA (1990) *Science* 249:1000
- Hillier AC, Ward MD (1992) *Anal Chem* 64:2539
- Schumacher R (1990) *Angew Chem Int Ed* 29:329
- Dutt VSE, Mottola HA (1974) *Anal Chem* 46:1777
- Cai X, Kalcher K, Neuhold C (1994) *Fresenius J Anal Chem* 348:660
- Zen JM, Chen PJ (1997) *Anal Chem* 69:280
- Brajter-Toth A, El-Nour KA, Cavalheiro ET, Bravo R (2000) *Anal Chem* 72:1576
- Rocheleau MJ, Purdy WC (1991) *Electroanalysis* 3:935
- Gao ZQ, Huang H (1998) *Chem Comm* 2107
- Lay PA, Sargeson AM, Taube H (1986) *Inorg Syn* 24:291
- Gregg B, Heller A (1991) *J Phys Chem* 95:5970
- Deakin MR, Buttry DA (1989) *Anal Chem* 61:1147A
- Murry RW, (1984) *Electroanal Chem* 13:191
- Osteryoung J, O'Dea JJ (1988) In: Bard AJ (ed) *Electroanalytical Chemistry*, vol 14. Marcel Dekker, New York, pp 86–118