Direct Determination of Uric Acid in Human Serum Samples Using Polypyrrole Nanoelectrode Ensembles

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Polypyrrole (PPy) nanotubes have been synthesized by chemical oxidative polymerization of pyrrole within the pores of polycarbonate membrane using the technology of diffusion of solutes. The nanotubes array prepared by the proposed method can be considered as nanoelectrode ensembles (NEEs). An amperometric uric acid sensor based on PPy NEEs has been developed and used for determination of uric acid in human serum samples. The electrode can direct response to uric acid at potential of 0.60V vs. SCE with wide linear range of 1.52×10^{-6} to 1.54×10^{-4} M. The detection limit is 3.02×10^{-7} M. This sensor has been used to determine uric acid in real serum samples. PPy NEEs is thought of as a good application in the foreground.

Key Words: Uric acid, Sensor, Polypyrrole nanotubes, Polycarbonate membrane, Diffusion

Introduction

Nanostructured conductive polymers have attracted growing attention due to their potential applications in biosensors,[1,2] biomedicines,[3] actuators,[4] and fuel cell.[5,6] Nanostructured polypyrrole (PPy) is one of the most extensively used conducting polymers in design of enzyme sensors,[7-9] immunosensor[10] and DNA sensor[11] because of its high environmental stability, electronic conductivity, ion exchange capacity, and biocompatibility. Up to now, nanostructured PPy (nanofibers, nanotubes, nanowires) with various morphologies have been synthesized by electrochemical methods and chemical methods combining the technology of solid template, self-assembly, surfactant, molecularly print. For instance, Martin *et al.*[12-14] have investigated the electrochemical and chemical template synthesis of PPy within the pores of polycarbonate membranes. Hernández *et al.*[14] have synthesized protein-functionalized Gold-PPy-Gold segmented nanowires by using porous aluminum oxide as a template. Chen *et al.*[15] have used oriented carbon nanotubes as the template to electrochemically deposit a thin PPy coating on the surface of the carbon nanotubes. PPy nanowire networks have been synthesized in high yield by chemical oxidative polymerization of pyrrole in the presence of hexadecyltrimethylammonium bromide (HTAB) and organic diacids.[16] Nanostructured PPys with controlled morphologies have been synthesized on atomically flat surfaces using adsorbed surfactant molecules as template.[17] However, most of these synthesized methods are time-consuming,[15,16] complex[14,17] and not easy to synthesize oriented nanostructures.[12,16] The method of synthesizing nanostructured PPy with simple procedure and oriented nanostructures is still in need of exploration.

The development of simple and rapid methods for the determination of uric acid in urine and serum is attracted because the abnormal levels of uric acid in urine and serum are symptoms of several diseases like gout, hyperuricaemia and Lesch–Nyhan syndrome. Different methods have been reported for the detection and estimation of uric acid levels, including enzymatic methods,[18] HPLC,[19] HPCE[20] and electrochemistry methods.[21,22] The enzymatic methods for determination of uric acid is promising due to their high selectivity, but this method inherently more expensive, need more technology of the enzymatic immobility. HPLC and HPCE are sensitive, but they are time-consuming and complex. Alternatively, the determination of uric acid by electrochemical methods have received much interest because they are more selective, less expensive and less time consuming than those based on enzymatic methods, HPLC and HPCE.

In this study, PPy nanotubes have been synthesized by chemical oxidative polymerization of pyrrole in the presence of polycarbonate membrane using the technology of diffusion of solutes. The diameter of PPy nanotubes is about 200 nm and the length is about 1.8 μm. The nanotubes array prepared by the proposed method can be considered as nanoelectrode ensembles (NEEs). An amperometric uric acid sensor based on PPy NEEs has been developed and used for determination of uric acid in human serum samples. The as-prepared sensor displays high sensitivity, quick response to uric acid, good stability and wide linear range. Besides, the lifetime of this sensor is much longer than that of biosensor based on uricase.

Experimental Section

Apparatus and reagents. Cyclic voltammetric and amperometric measurements were carried out on XJP-821(C) polarograph (Jiangsu, China). Scanning electron microscopy (SEM) analysis was performed by using XL30ESEM-TMP microscope (Philips Ltd., Holand). The three-electrode cell (10 mL) was constituted of the PPy NEEs modified glassy carbon (GC) electrode as the working electrode, a saturated calomel electrode (SCE) as reference electrode and platinum foil electrode as counter electrode. All potentials were measured and reported versus the SCE.

Nucleopore PC (0.2 μm) membrane was provided by

Whatman (Anodisc 47, 0.2 µm); uric acid was purchased from Sigma (USA). The pyrrole monomer was purified by distillation. The other chemicals, such as ammonium persulfate ($(NH_4)_2S_2O_8$, APS), chloroform ($CHCl_3$), ethanol (C_2H_5OH), were of analytical-reagent grade and used as received without further purification, and doubly distilled water was used throughout. The supporting electrolyte was 0.2 M borate buffer solution, which was prepared with H_3BO_3 and $Na_2B_4O_7 \cdot 10$ H_2O.

Synthesis of polypyrrole nanotubes. PPy nanotubes have been synthesized by diffusion of solutes has been reported.[12] In this paper, the device was simplified as shown in Fig. 1. A beaker (25 mL) was divided into two portions by the interlayer using epoxy resin. The PC membrane was adhibited onto the hole in the interlayer. The molar ratio of APS to pyrrole was 1:1.[16] The solution of pyrrole (10 mL, 0.008 M) was added into one side, while the solution of ASP (10 mL, 0.008 M) was added into another side at the same time. The height of two solutions was kept at the same level. After polymerization for 30 min at room temperature, the synthesis of PPy nanotubes was completed. Then the PC membrane was taken out and washed with deionized water.

Preparation of PPy NEEs / GC electrode. Glassy carbon (GC) electrode (3 mm diameter) was first polished with emery paper and alumina slurry, successively rinsed thoroughly with absolute alcohol and distilled water in ultrasonic bath, and dried in air.

Two pieces of PC membrane with PPys nanotubes with different polymerization time (30 min and 60 min) were taken and attached down on the surface of GC electrode by the graphite conductive adhesive, then the edge of PC membrane was sealed using epoxy resin. After 3 h, the PC membrane was dissolved by immersing the electrode in chloroform for 10 min and washed successively using ethanol and water, and dried in air. PPy NEEs / GC electrodes (polymerized for 30 and 60 min) have been prepared by the process. A bare GC electrode (3 mm diameter), GC electrode modified by PC membrane with PPy before dissolved by chloroform and PPy NEEs / GC electrodes (polymerized 60 min) were used as comparisons.

Results and Discussion

Morphological characterization of the prepared PPy. Fig. 2 shows the morphologies of the PPy with different polymerization time characterized by scanning electron microscopy (SEM). Comparing with the PC membrane before polymerization (Fig. 2(a)), it can be concluded that the reaction of
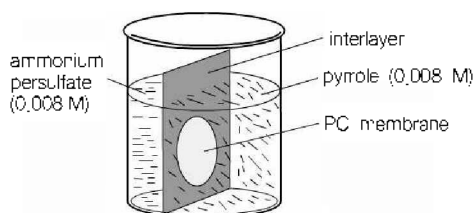

Figure 1. The illustration of the device for preparation of ordered polypyrrole nanotubes
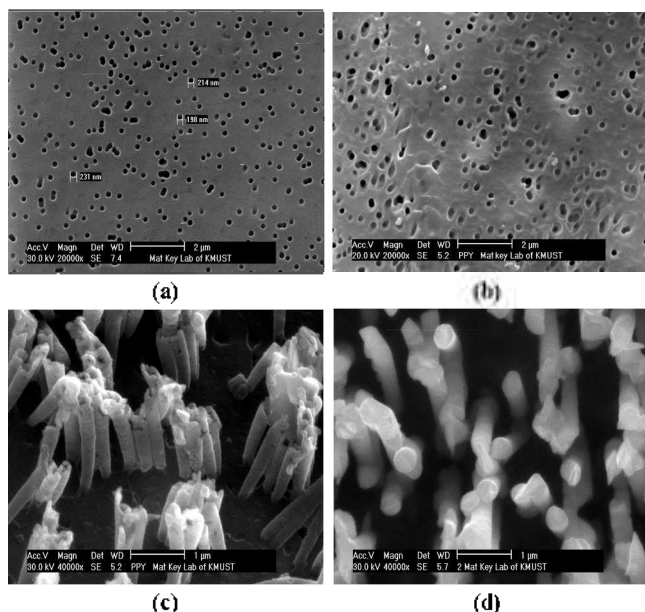

Figure 2. SEM images of the polypyrrole: the PC membrane before polymerization (a), after polymerization for 3 min (b), 30min (c) and 60 min (d).

polymerization can occur in the pore of the PC membrane after diffusion 3 min (Fig. 2(b)). After polymerization for 30 min, the PPy nanotubes were obtained by etching away the membrane. From Fig. 2(c), it can be seen that PPy nanotubes are hollow and vertically oriented with an average diameter about 200 nm, which correspond to the size of the nanopore in the membrane. The length is about 1.8 µm. Each vertical nanotube can work as an individual nanoelectrode[23,24] and entire nanotubes array can be considered as PPy NEEs. However, after polymerization for 60 min, the morphologies of the PPys have been changed to solid nanotubes (Fig. 2(d)).

Effect of dissolved time of PC membrane. Fig. 3 describes the cyclic voltammograms of different electrodes with different dissolved time by chloroform in 0.2 M borate buffer solution (pH 7.4) containing 0.80×10^{-2} M uric acid at 100 mVs⁻¹.
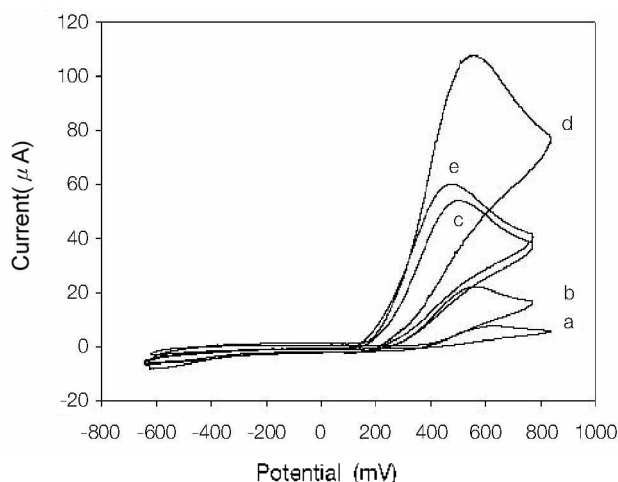

Figure 3. Cyclic voltammograms (CVs) of PPy NEEs / GC electrode dissolved by chloroform different time in 0.2 M borate buffer solution (pH 7.4) containing 0.80×10^{-2} M uric acid at 100 mV/s: (a) before dissolved, (b) dissolved 1 min, (c) dissolved 5 min, (d) dissolved 10 min, (e) dissolved 15 min.
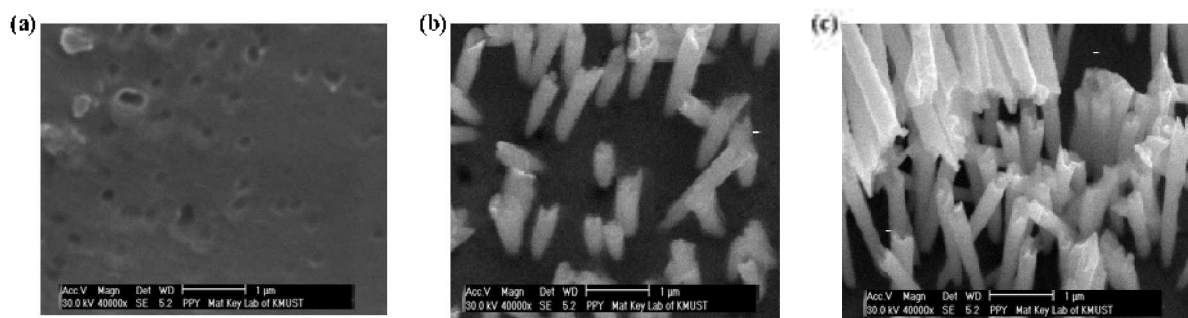
Figure 4. SEM images of p electrodes modified by PC membrane with PPy with different dissolved time by chloroform: (a) before dissolved, (b) dissolved 5 min, (c) dissolved 15 min.

It can be seen that the current of PPy NEEs / GC electrode (polymerized for 30 min) elevated as dissolved time increased. However, when dissolved time excessed 10 min, the current decreased. This fact can be ascribed that the bareness of PPy nanotubes increased with dissolved time increasing. But, when dissolved time was too long, PC membrane was dissolved so completely that PPy nanotubes released from PC membrane were not vertically oriented, and the nanotubes array was destroyed, which lead to the lower current. Additionally, Fig. 4 illustrates the SEM of different electrodes with different dissolved time by chloroform (Fig. 4(a): before dissolved, (b): 5 min, (c): 15 min, 10 min see Fig. 2(c)) which clarifies this point. Therefore, the dissolved time of 10 min was adopted in the subsequent work.

Effect of PPy NEEs / GC electrode of catalysis to uric acid and the mechanism of the amperometric detection of uric acid. Fig. 5 depicts the cyclic voltammograms of different electrodes in 0.2 M borate buffer solution (pH 7.4) containing 0.80 × 10⁻² M uric acid at 100 mVs⁻¹. It can be seen that the current of GC electrode modified by PC membrane with PPy before dissolved by chloroform (curve a) was lower than that of the bare GC electrode (curve b). PPy NEEs / GC electrodes (polymerized for 30 min, hollow nanotubes) was larger than that of the bare GC electrode, electrode modified by PC membrane with PPy before dissolved by chloroform and PPy NEEs / GC electrodes (polymerized 60 min, solid nanotubes). Therefore, polymerization of 30 min was fixed for the rest of the experiments.

In our opinion, both the increase in current and the better reversibility are related to some specific electrocatalysis of PPy.²⁵⁻²⁷ In fact, the PPy nanotubes can be oxidized electrochemically to PPy⁺, which then oxidizes uric acid. Fig. 6 clarifies this point. PPy NEEs / GC electrodes (polymerized for 30 min) have more PPy⁺ than the bare GC electrode and GC electrode modified by PC membrane with PPy before dissolved. Furthermore, PPy NEEs (hollow) can provide more surfaces containing PPy⁺ than the PPy NEEs (solid). It is known that uric acid is oxidized in a two electron process to produce allantoin as shown in the following equation:²⁸
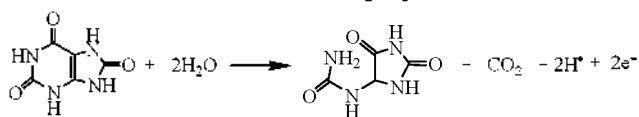
Cyclic voltammetry characterization. The cyclic voltammograms of uric acid at different electrodes were shown in

Fig. 6. It can be seen that the current of PPy NEEs / GC electrode in 0.2 M borate buffer solution (pH 7.4) containing 1.14 × 10⁻² M uric acid was larger than that of the bare GC electrode due to the larger accessible surface area of the modified electrode. The increased response current at about 0.60 V indicated the response of the catalysis of PPy NEEs to uric acid was irreversible.

Typical cyclic voltammograms of PPy NEEs / GC electrode in 0.2 M borate buffer solution (pH 7.4) containing 1.14 × 10⁻² M uric acid at different scan rates from 20 to 100 mVs⁻¹ were investigated (data not shown). The oxidation peak potentials of uric acid were observed to shift positively with the increase of scan rate, and the peak currents rose accordingly. Moreover, there was a good linear relationship between the peak currents of uric acid and the square root of scan rates. The linear regression equations were $ip = 70.07 + 7.57 v^{1/2}$ (ip: 10^{-6} A, v: mVs⁻¹, r: 0.997), showing typical diffusion-controlled electrochemical behavior.

Effect of the PPy NEEs on the response of electrode. Amperometric responses of bare GC and optimized PPy NEEs / GC electrode for successive injection of 0.5 mM uric acid at 0.6 V in 10 mL 0.2 M borate buffer solution (pH 7.4) were investigated. The electrochemistry oxidation of uric acid can
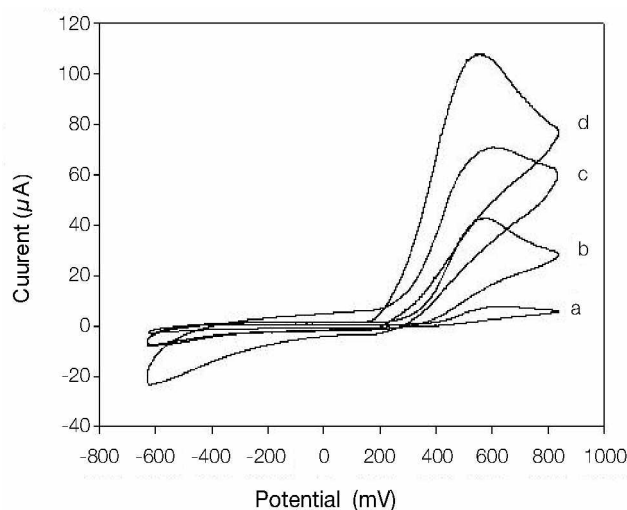

Figure 5. Cyclic voltammograms (CVs) of (a) electrode modified by PC membrane with PPy(before dissolved), (b) bare GC electrode, (c) PPy NEEs (solid nanotubes) / GC electrode and (d) the PPy NEEs (hollow nanotubes)/ GC electrode in 0.2 M borate buffer solution (pH 7.4) containing 0.80 × 10⁻² M uric acid at 100 mVs⁻¹.

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occur both at PPy NEEs / GC electrode and bare GC electrode. To study the role of PPy NEEs, the responses of the electrode with and without PPy NEEs were studied. From Fig. 7, it can be seen that the sensitivity of PPy NEEs / GC electrode toward uric acid were better than that of bare GC electrode. indicating that PPy NEEs have better catalysis to uric acid.

Optimization of experimental variables. The experimental variables, which can affect the amperometric determination of uric acid, including the pH of the supporting electrolyte and applied potential, were investigated.

Effect of pH. The influence of the pH of the assay solution over the range 6.7 to 8.2 on the amperometric response of PPy NEEs / GC electrode to uric acid at a fixed concentration of 4.9 mM in borate buffer solution at 0.6 V was investigated. The results of amperometric responses indicated that the optimum pH was 7.4 (data not shown). Therefore, pH 7.4 was fixed for the rest of the experiments.

Effect of applied potential. The effect of applied potential on amperometric response of PPy NEEs / GC electrode was investigated in 10 mL 0.2 M borate buffer solution (pH 7.4)
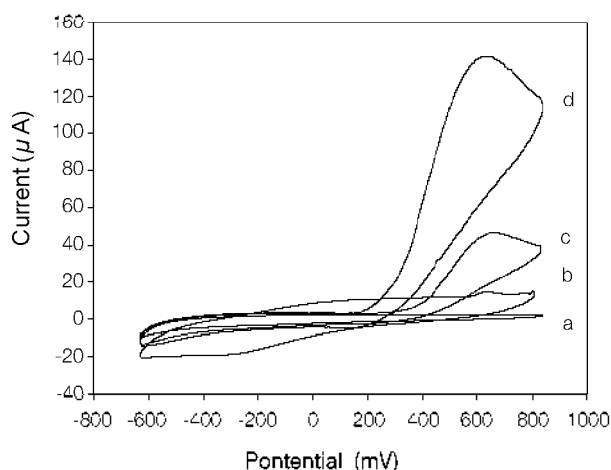
containing 4.9 mM uric acid was studied (data not shown). With the increasing of potential from 0.45 to 0.90 V, the response current of the electrode increased significantly. To avoid interference at higher applied potentials, a potential of 0.60 V (*vs.* SCE) was selected as the applied potential for amperometric measurements.

Response characteristics of PPy NEEs / GC electrode. The calibration curve is shown in Fig. 8. The responses current increases as the uric acid concentration is elevated. Over a concentration range of 1.52×10^{-6} to 1.54×10^{-3} M, the electrode provided a linear response to uric acid with a good sensitivity of 0.452 A $M^{-1}cm^{-2}$. The linear equation could be described as follows: I (μA) = 0.443 +28.73 C (mM), with a correlation coefficient of 0.999, and the detecting limit of is 3.02×10^{-7} M. The linear range is wider than the previous results reported by Zhang[29] based on uricase biosensor. Wu[21] and Lin[23] with electrochemical methods. The detecting limit is lower than previous reports using uricase biosensor[29] and electrochemical methods.[21,23,28,30]

Interference. To assess the selectivity of PPy NEEs / GC electrode. twelve possible interferences in the presence of 4.9 mM uric acid were investigated which was shown in Table 1. Before treatment. the amperometric response ratio of ascorbic

Figure 6. The cyclic voltammograms of different electrodes in various solutions: (a) the bare GC electrode and (b) PPy NEEs / GC electrode both in borate buffer solution (pH 7.4); (c)the bare GC electrode and (d) PPy NEEs / GC electrode both in borate buffer solution (pH 7.4) containing 1.14×10^{-2} M uric acid.

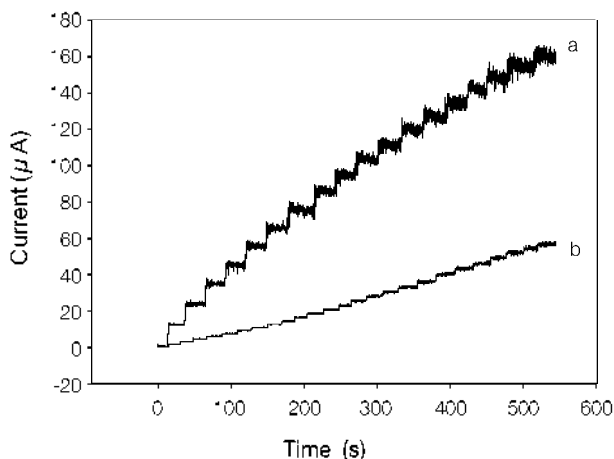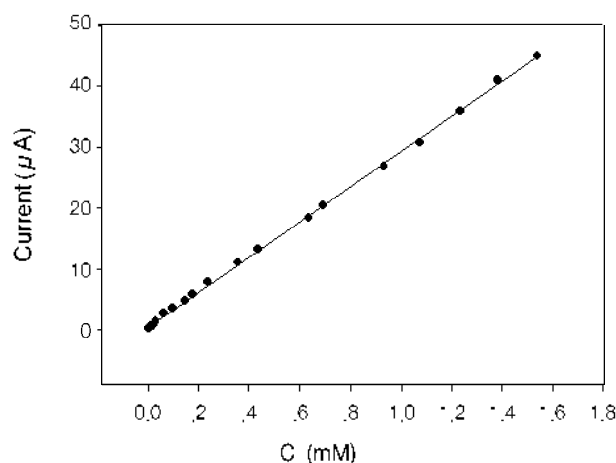**Figure 8.** The calibration curves of PPy NEEs / GC electrode

Figure 7. Amperometric response of PPy NEEs / GC electrode (a) and the bare GC electrode (b) to the successive addition of 0.5 mM uric acid at 0.60 V in 10 mL 0.2 M borate buffer solution (pH 7.4).
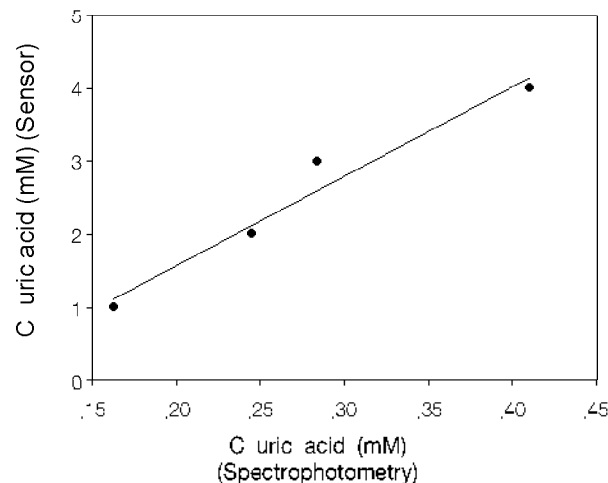

Figure 9. Comparison of the uric acid contents in serum sample determined with PPy NEEs / GC electrode and spectrophotometry.

Table 1. Possible interferences tested with PPy NEEs / GC electrode

	$C_{interferences} / C_{uric\ acid}$	$i_{interferences} / i_{uric\ acid}$
citric acid	4.4	0.12
glucose	7.4	0.03
alanine	3.4	0.08
ascorbic acid	3.4	0.15^a
tartaric acid	3.4	0.07
oxalic acid	3.4	0.10
glutamic acid	3.4	0.03
pyruvic acid	3.4	0.1
Pb^{2+}	2.2	0.03
Cd^{2+}	2.2	0.04
Hg^{2+}	2.2	0.08
Cu^{2+}	2.2	0.06

^{a}Ascorbic acid was treated with 0.001 M NaOH in water bath at 60 °C for 20 min.

acid to uric acid was 5:1 when the concentration ratio of ascorbic acid to uric acid was 3.4:1, indicating that the oxidation of ascorbic acid at the applied potential (0.60 V). After ascorbic acid was treated with 0.001 M NaOH in water bath at 60 °C for 20 min, the amperometric response ratio of ascorbic acid to uric acid was changed from 5:1 to 0.15:1 because ascorbic acid was easy to be oxidized in air, especially, in basic and heated condition. In this way, the interferences of ascorbic acid can be eliminated. Much more possible interferents were investigated in this work comparing with the the previous reports.[18,21,23,28,29] and the results showed that few substances interfere with the determination of uric acid including heavy metal ions due to urease-free at PPy NEEs / GC electrode.

Real sample analysis. Before determination, 1 mL serum sample was treaed with 20 μL 0.05 M NaOH in water bath at 60 °C for 20 min.[31] PPy NEEs / GC electrode was used to determine uric acid in the real serum samples in the borate buffer solution (pH 7.4). Results were compared with those determined by Yunnan Normal University hospital using spectrophotometry (Fig. 9). The correlation coefficient was 0.950, which indicated that uric acid contents obtained by the two methods agree well with each other.

Repeatability and stability of PPy NEEs / GC electrode. The repeatability of the response current of PPy NEEs / GC electrode was studied at a uric acid concentration of 4.9 mM. The variation coefficient was 3.05% for three successive assays. The stability of the electrode was tested by measuring the response of the sensor with 4.9 mM of uric acid over 90 days. The response current of the sensor decreased to 93.5% after 90 days. When not in use, the electrode was stored dry at room temperature.

Conclusion

In this paper, PPy nanotubes have been synthesized by chemical oxidative polymerization of pyrrole in the presence of polycarbonate membrane using the technology of diffusion of solutes. Each PPy nanotube is vertically, making each

nanotube working as an individual nanoelectrode and entire nanotubes array can be considered as PPy NEEs. The direct detection of PPy NEEs / GC electrode toward uric acid has been studied. The nanostructure improves analytical performances of the corresponding sensors compared to the bare GC electrodes. This PPy NEEs / GC electrode displays high sensitivity, quick response to uric acid, good stability and wide linear range. The lifetime of this sensor is much longer than that of biosensor based on uricase.

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