

Electrocatalytic oxidation of uric acid on a carbon ionic liquid paste electrode

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Abstract : An ionic liquid (IL) modified carbon paste electrode (CILE) was fabricated by using N-butylpyridinium hexafluorophosphate (BPPF₆) as binder to substitute the traditional paraffin and the voltammetric behaviors of uric acid (UA) on CILE were studied in pH 7.0 phosphate buffer solution. Due to the presence of the IL on the surface of the modified electrode, CILE showed strong electrocatalytic activity to the oxidation of UA. On cyclic voltammogram a pair of redox peak appeared with the anodic peak potential (E_{pa}) as 345 mV and the cathodic peak potential (E_{pc}) as 270 mV (vs SCE). Compared with that on traditional carbon paste electrode (CPE), the overpotential of anodic peak was decreased for 155 mV. UA showed a quasi-reversible adsorption-controlled electrochemical process on CILE and the electrochemical parameters such as the charge transfer coefficient (α), the number of electrons transfer (n), the standard heterogeneous rate constant (k_s) etc. were calculated according to the relationship of scan rate with the peak potential. Under the selected conditions the anodic peak current was proportional to the UA concentration in the range from 2.0×10^{-6} to 6.0×10^{-4} mol/L by cyclic voltammetry with the detection limit estimated as 1.0×10^{-6} mol/L (3σ). The proposed method was further applied to the detection of UA content in human urine samples with satisfactory results.

Keywords : Uric acid, ionic liquid, carbon paste electrode, electrocatalysis, chemically modified electrode.

Introduction

Uric acid (UA) is an important biomolecule that is present in the biological fluids such as blood and urine. The content of UA is often related to some diseases such as pneumonia, toxemia of pregnancy, kidney stones or gout¹. So it is important to establish sensitive and selective detection methods for lower concentration UA. Some analytical methods had been proposed for UA determination such as colorimetry², enzymatic technique³ and chromatography⁴⁻⁶ etc. However, these methods need expensive instruments and complex operation procedure. Because UA is an electroactive compound, the electrochemical detection of UA has been carefully studied on different kinds of working electrodes⁷⁻¹¹. However, the direct oxidation of UA on the bare electrode often requires high overpotential with the possible interferences from other coexisting substances. Therefore different kinds of modified electrodes had been devised for the UA detection,

such as poly(3,4-ethylenedioxythiophene)¹², Pt-Fe^{III} nanocomposites DNA film¹³ and well-aligned carbon nanotube (CNT) modified electrode¹⁴.

Recently room temperature ionic liquids (RTILs) have been used in the field of bioelectrochemistry and electroanalysis. RTILs are composed of entirely ions and exist as liquids at room temperature or related temperature with the characteristics such as negligible vapor pressure, good solubility and chemical stability. As a new "green" media, RTILs have many unique electrochemical properties, including high ionic conductivity and wide electrochemical windows¹⁵⁻¹⁷. Dong *et al.*¹⁸ investigated the direct electrochemistry of enzyme in RTILs/carbon nanomaterials composite film modified electrode. Li *et al.*¹⁹ constructed an ionic liquid (IL) modified carbon paste electrode (CPE) using 1-butyl-3-methylimidazolium hexafluorophosphate as binder, which was bulky modified with PMO₁₂ for NO₂⁻ determination. Opallo

et al.^{20–22} studied the ion transfer processes across RTILs/ aqueous solution interface with different working electrodes as the supports for RTILs phase. Maleki *et al.*^{23–25} fabricated an octylpyridinium hexafluorophosphate (OPFP) modified CPE, which provided a remarkable increase in the electron transfer rate and decreased the overpotentials of some organic substances. Sun *et al.* also fabricated different kinds of IL modified CPE (CILE) for the investigation on protein electrochemistry or the detection of electroactive substances^{26–29}.

In this paper the voltammetric behaviors of UA were studied on the IL N-butylpyridinium hexafluorophosphate (BPPF₆) modified CPE. Compared with other kinds of chemical modified electrodes, CILE had the advantages such as easy preparation, good stability and high sensitivity. The presence of IL in the carbon paste showed good electrocatalytic ability and the electrochemical behaviors of UA on CILE were carefully investigated. The applicability of the prepared electrode was illustrated by measuring the UA content in human urine samples.

Experimental

Reagents :

Uric acid (UA, Fluka), N-butylpyridinium hexafluorophosphate (BPPF₆, Hangzhou Kemer Chemical Limited Company), and graphite powder (Shanghai Colloid Chemical Plant, average particle size 30 μm) were used without further treatment. A 1.0×10^{-4} mol/L UA solution was freshly prepared just before use. 0.1 mol/L phosphate buffer solutions (PBS) were prepared as the aqueous electrolytes. All the other chemicals used were of analytical reagent grade and doubly distilled water was used throughout.

Apparatus :

All the electrochemical measurements were carried out on a CHI 750B electrochemical analyzer (Shanghai CH Instrument, China) at the room temperature of 25 ± 1 °C. A conventional three-electrode system was used, which consisted of a CILE ($\Phi = 3.8$ mm) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode.

Procedure :

CILE was prepared with the following procedure : 3.0 g of graphite powder and 1.0 g of BPPF₆ were mixed

thoroughly in a mortar to form a homogeneous carbon ionic liquid paste, which was and further heated at 80 °C for about 1 h. Since the melting point of BPPF₆ is at 60 °C, the heating process made the mixture of IL with carbon paste more uniform and homogenous. A portion of the carbon ionic liquid paste was filled into one end of a glass tube and a copper wire was inserted through the opposite end to establish an electrical contact. The surface of CILE was smoothed on a piece of weighing paper just before use. For comparison, the traditional carbon paste electrode (CPE) was prepared by hand-mixing of graphite powder with paraffin oil at a ratio of 70/30 (w/w).

The electrochemical measurements of UA were performed with cyclic voltammetry in the potential range from -0.2 to 0.8 V.

Results and discussion

Electrochemical behaviors of UA :

As indicated in a recent report²³, CILE exhibited excellent advantages, such as large potential window, low resistance, high wettability and high sensitivity. Therefore CILE had been employed as an excellent working electrode in this paper. Fig. 1 showed the cyclic voltammograms of 1.0×10^{-4} mol/L UA on traditional CPE (curve a) and CILE (curve b) in pH 7.0 PBS. On the bare CPE a broad and small anodic peak appeared at 500 mV (vs SCE), which indicated UA took place irreversible electrode process on the CPE. As reported in reference³⁰, the oxidation of UA on glassy carbon electrode or metal electrode is an irreversible process, and at a graphite electrode is a quasi-reversible process. So the electrooxidation of UA on CPE appeared as an irreversible process with slow electron transfer kinetics. While on CILE a pair of well-defined redox peaks appeared with the anodic peak potential (E_{pa}) as 345 mV and the cathodic peak potential (E_{pc}) as 270 mV (vs SCE), indicating the electrode process changed to a quasi-reversible process. The peak-to-peak separation (ΔE) was calculated as 75 mV and the formal potential ($E^{0'}$), which is calculated by the midpoint of E_{pa} and E_{pc} , was got as 308 mV. The decrease of the anodic overpotential was 155 mV and the anodic peak current on CILE was also increased for about 10 times than that of CPE. Because the decrease of overpotential and the increase of peak current are the typical characteristics of electrocatalytic reaction

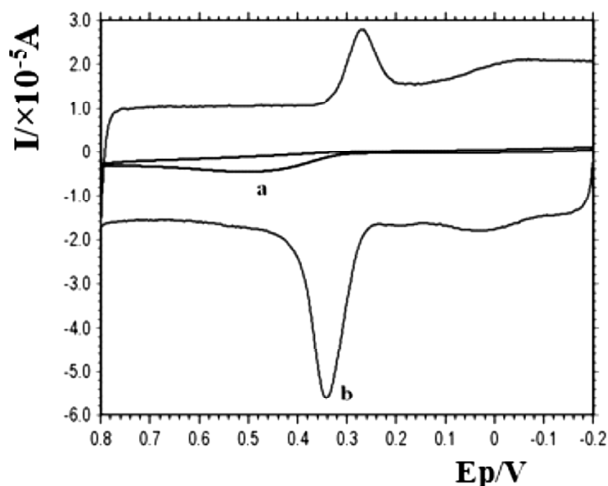


Fig. 1. Cyclic voltammograms of 1.0×10^{-4} mol/L UA on CPE (a) and CILE (b) in pH 7.0 PBS with the scan rate of 100 mV/s.

of electroactive substance, so the results indicated that CILE showed specific electrocatalytic ability to the oxidation of UA. Because of the good ionic conductivity of RTILs, the presence of ILs in the electrode can greatly enhance the electrochemical response of UA, which can be used as a superior modifier for the construction of chemically modified electrode. IL can act as the binder and the modifier in the carbon paste, and the fabricated CILE exhibits excellent electrochemical performances with a layer of IL present on the electrode surface. At the same time the background current on CILE was also much larger than that on CPE. Since CILE was composed of graphite powder and IL, the presence of IL can lay on the surface of graphite powder and fill in the void space between the graphite powder. So the increase of background current was due to the accessible capacitance of IL at the carbon surface²⁰.

Effect of scan rate :

The effect of scan rate on the electrochemical response of UA was studied and the results were shown in Fig. 2. With the increase of scan rate the redox peak currents were increased gradually. The relationships of redox peak currents with scan rate were constructed with the results shown in Fig. 3. The redox peak currents were proportional to scan rate in the range from 10 to 500 mV/s and two straight lines were got, which indicated that the electrode reaction was an adsorption-controlled process. The result may be due to the presence of IL on the carbon paste surface exhibited certain accumulation effect to the

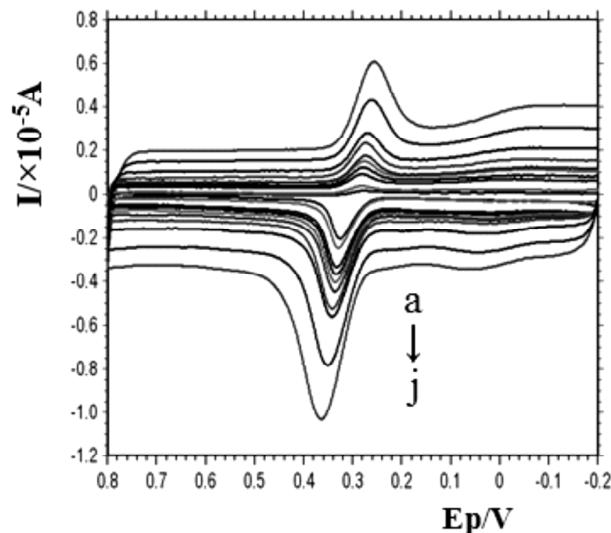


Fig. 2. Cyclic voltammograms of 1.0×10^{-4} mol/L UA on CILE with different scan rates (1–10 : 10, 20, 40, 50, 60, 70, 80, 100, 150, 200 mV/s) in pH 7.0 PBS.

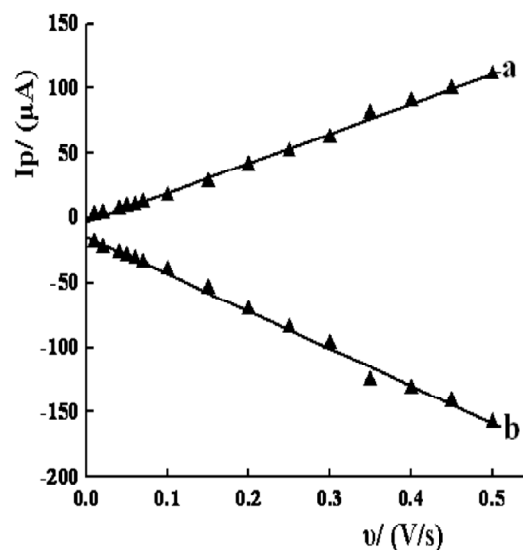


Fig. 3. The relationship of the cathodic (a) and anodic (b) peak current with the scan rate (ν).

UA in the solution.

The relationships of the redox peak potentials with scan rate were also investigated. Then the electrochemical parameters such as α , k_s and n were calculated according to the Laviron's equation³¹ for adsorption-controlled irreversible electrochemical process :

$$E_{pc} = E^{0'} - \frac{RT}{\alpha nF} \ln \nu \quad (1)$$

$$E_{pa} = E^{0'} + \frac{RT}{(1-\alpha)nF} \ln v \quad (2)$$

$$\log k_s = \alpha \log (1-\alpha) + (1-\alpha) \log \alpha - \log \frac{RT}{nFv} - \frac{(1-\alpha)\alpha F \Delta E_p}{2.3RT} \quad (3)$$

where α is the charge transfer coefficient, n is the number of electrons transfer, k_s is the standard heterogeneous rate constant, v is the scan rate, $E^{0'}$ is the formal potential and F is the Faraday constant.

The relationships of the anodic and cathodic peak potentials with $\ln v$ were shown in Fig. 4. According to the eqs. (1) and (2) the charge transfer coefficient (α) and the number of electrons transfer (n) was calculated with the results 0.57 and 2, respectively. The standard heterogeneous rate constant (k_s) was further calculated from the relationship of ΔE_p with $\ln v$ based on the eq. (3) and the result was got as 1.83 s^{-1} .

According to the reference³², the electrochemical oxidation of UA proceeds in a $2e^-$ and $2H^+$ process to lead to an unstable diimine species, which is then attacked by water molecules in a stepwise fashion to be converted into an imine-alcohol and then uric acid-4,5-diol. The uric acid-4,5-diol compound produced is unstable and decomposes to various products depending on the solution pH.

Analytical application :

Under the selected conditions the relationship of cyclic voltammetric anodic peak current and the concentra-

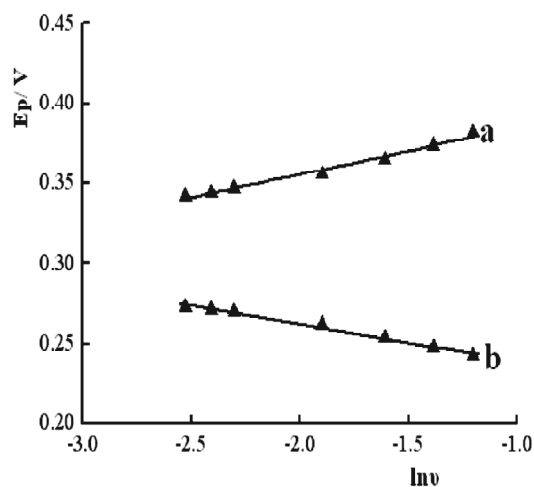


Fig. 4. The relationship of the anodic (a) and cathodic (b) peak potential with $\ln v$.

tion of UA was further investigated. The anodic peak current was linearly dependent on the concentration of UA in the range from 2.0×10^{-6} to 6.0×10^{-4} mol/L with the linear regression equation as $I_{pa}(\mu A) = -8.446 - 0.331C$ ($\mu\text{mol/L}$) ($n = 15$, $\gamma = 0.9967$). And the detection limit was got as 1.0×10^{-6} mol/L ($S/N = 3$).

The CILE was applied to 11 parallel measurements of 1.0×10^{-4} mol/L UA in pH 7.0 PBS and the relative standard deviation (RSD) value was calculated as 3.3%, which indicated that CILE showed good reproducibility. The CILE can be stored for about 3 weeks without the decrease of the response to UA, showing good stability.

The analytical parameters of CILE for UA determination was compared with other kinds of modified electrodes and the results were listed in Table 1. It can be seen that this method can provide comparable linear range

Table 1. Comparison of the performances of different modified electrodes

Electrodes	Linear range (mol/L)	Detection limit (mol/L)	Method	Ref.
PEDOT/GCE	$1.0 \times 10^{-6} - 2.0 \times 10^{-5}$	-	DPV	12
Pt-Fe ^{III} /DNA/GCE	$3.8 \times 10^{-6} - 1.6 \times 10^{-4}$	1.8×10^{-6}	DPV	13
MWNTs electrode	$2.0 \times 10^{-7} - 8.0 \times 10^{-5}$	1.0×10^{-7}	DPV	14
β -CD-PNAANI/GCE	$1.0 \times 10^{-5} - 2.0 \times 10^{-4}$	2.8×10^{-6}	SWV	33
PDMA/GCE	$1.25 \times 10^{-6} - 6.875 \times 10^{-5}$	1.25×10^{-6}	SWV	34
CILE	$2.0 \times 10^{-6} - 6.0 \times 10^{-4}$	1.0×10^{-6}	CV	This paper

PEDOT : poly(3,4-ethylenedioxythiophene); GCE : glassy carbon electrode; DNA : deoxyribonucleic acid; MWNTs : multiwalled carbon nanotubes; β -CD-PNAANI : β -cyclodextrin modified poly(N-acetylaniline); PDMA : poly(N,N-dimethylaniline); DPV : differential pulse voltammetry; SWV : square wave voltammetry; CV : cyclic voltammetry.

and detection limit by cyclic voltammetry with a simple electrode preparation procedure.

Real samples detection :

The applicability of CILE was tested by detecting the content of UA in human urine samples solution, which was collected from a normal health man in the laboratory. The human urine sample was diluted for 100 fold with 0.05 mol/L PBS and further detected by the proposed procedure without any other treatment. The recovery test was further performed with the addition of standard UA solution into urine samples. All the results were summarized in Table 2 with the recovery in the range from 97.30 to 99.52%, which indicated that this method was reliable and practical for the samples detection.

Table 2. Determination of UA in human urine samples

Urine samples	Detected ($\mu\text{mol/L}$)	Added ($\mu\text{mol/L}$)	Total ($\mu\text{mol/L}$)	RSD (%)	Recovery (%)
1	27.33	50.0	75.98	1.67	97.30
2	28.17	100.0	127.69	2.03	99.52

Conclusions

In the present work a BPPF₆ based CILE was fabricated and used to investigate the electrochemical behaviors of UA. CILE showed good electrocatalytic behavior to the UA oxidation and a pair of quasi-reversible redox peaks appeared at 345 mV (E_{pa}) and 270 mV (E_{pc}) (vs SCE) on cyclic voltammogram. Compared to that of CPE, the decrease of the anodic overpotential was calculated as 155 mV, which could be attributed to the presence of IL in the carbon paste. Under the optimal conditions the anodic peak current exhibited excellent linear relationship with UA concentration in the range from 2.0×10^{-6} to 6.0×10^{-4} mol/L. The modified electrode can be used for simple and sensitive detection of UA and the human urine samples were determined with satisfactory results.

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