A Polymer Film Modified Sensor for Voltammetric Determination of Uric Acid in the Presence of Ascorbic Acid and Its Application in Urine

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A novel uric acid electrochemical sensor was fabricated by electropolymerization on glassy carbon electrode (GCE) with 4-(2-pyridylazo)-resorcinol (PAR) and its electrochemical property was investigated through cyclic voltammetry. The effect of film thickness on the oxidation response was also studied by electropolymerized scan times. The voltammetric behavior of uric acid (UA) was studied with the poly PAR modified GCE. The modified GCE was used to electrochemically detect the individual of UA and the mixture of UA and ascorbic acid (AA) by cyclic voltammetry (CV) or differential pulse voltammetry (DPV) method. For the ternary mixture containing UA and AA, the two compounds can well be separated from each other at a scan rate of 100 mV s^{-1} with a potential difference of 345 mV in DPV between UA and AA. The peak currents of UA oxidation increase linearly with the concentration in ranges of 1.0×10^{-8}—5.0×10^{-5} mol l^{-1}, and the detection limits (S/N=3) was 1.0×10^{-9} mol l^{-1}. This method was successfully applied to the determination of uric acid in human urine samples.

Key words uric acid; uric acid sensor; polymer film modified electrode; 4-(2-pyridylazo)-resorcinol

Polymer films modified electrodes have received great attention and are widely used in the fields of chemical sensors and biosensors.[1,2] They can improve electrocatalytic properties, decrease the overpotential, increase the reaction rate and sensitivity, heighten the stability and reproducibility of the electrode response in the area of electroanalysis.[1—4] Different methodologies have been used for depositing polymeric films. Electropolymerization has demonstrated to be a very convenient way to immobilize polymers because the deposition can be controlled by adjusting the electrochemical parameters and the process is located at the electrode surface. Thus, the thickness, permeation and charge transport characteristics of the polymeric films can be controlled by the potential applied.

Uric acid (UA) is an important biological substance present in body fluid. It is produced by the metabolism of purine. Extreme abnormalities of the UA level will cause many diseases such as pneumonia, fatal poisoning with chloroform or methanol, or toxemia of pregnancy.[5] So the determination of the concentration of UA in human blood or urine is a powerful indicator in diagnosing diseases. Hence it is important to develop techniques to detect UA in body fluid.

The development of a simple and rapid methodology for the determination of UA has therefore attracted attention in recent years.[6—10] Among various methods, electrochemical determination of UA shows a higher selectivity than other methods and it is less costly and less time consuming.[11—14] Electrochemical methods are very important due to their simple procedure and high sensitivity. However, the direct electrochemical determination of UA in urine is still rather painstaking due to the poor selectivity. It is well-known that there are other compounds in urine which exhibit redox peaks near that of UA. Thus, they will interfere with the determination of UA. For example, both UA and ascorbic acid (AA) are present in urine, they can be oxidized at a similar potential.[15] The electrochemical procedures used initially could not determine them at the same time because they interfere with each other. On the other hand, the sensitivity should be further enhanced to meet the requirement of detecting UA at lower concentration levels.

Therefore it is necessary to develop an electrochemical uric acid sensor, which is free from the above mentioned problems. The development of a long-term and stable electrode would be desirable. Various approaches have been attempted to solve these problems, including the use of ion-exchange membrane coating,[16,17] chemical[18—21] and/or enzyme[22,23] modification. And most of these are also based on chemically modified electrodes, for the determination of uric acid in biological samples.[24—27] But each method has its advantages and limitations. Thus, there is an expanding demand for the development of facile, sensitive and efficient uric acid electrochemical sensor.

In the present paper, we report a polymer film of 4-(2-pyridylazo)-resorcinal (PAR) (chemical structure of the monomer is illustrated in Chart 1) to modify glassy carbon electrode (GCE) and describe the electrochemical behavior of UA. The result shows that a sensitive and rapid method for the determination of UA in the presence of AA was established. More important, it's worth to mention that UA exhibit reversible peaks in modified electrode when compared with other approaches. Furthermore, the practical application was demonstrated to determine UA in urine samples with satisfied result. Therefore, this advantage has a significant attraction in biological and chemical researches.

Experimental

Reagents and Apparatus The PAR was purchased from Shanghai Chemical Reagents Company (China). Uric acid were obtained from Fluka (Switzerland). Ascorbic acid was from Beijing Chemical Factory (China). All reagents were of analytical grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.05 mol l^{-1} NaCl and 0.05 mol l^{-1} NaH_2PO_4–Na_2HPO_4, and then adjusting the pH with 0.05 mol l^{-1} H_3PO_4 or 0.05 mol l^{-1} NaOH. All solutions were prepared with double-distilled water. UA and AA solutions were prepared freshly and used. A pH 2.0 aqueous PAR solution, adjusted with a 0.05 mol l^{-1} H_2SO_4 solution, was used for electrochemical polymerization on the clear bare GCE.

CHI 660C Electrochemical Workstation (Shanghai CH Instruments, China) was used for electrochemical measurements. A conventional three-electrode system was used throughout the experiments. The working electrode was a bare or poly PAR modified GCE (3.0 mm in diameter), the auxil-

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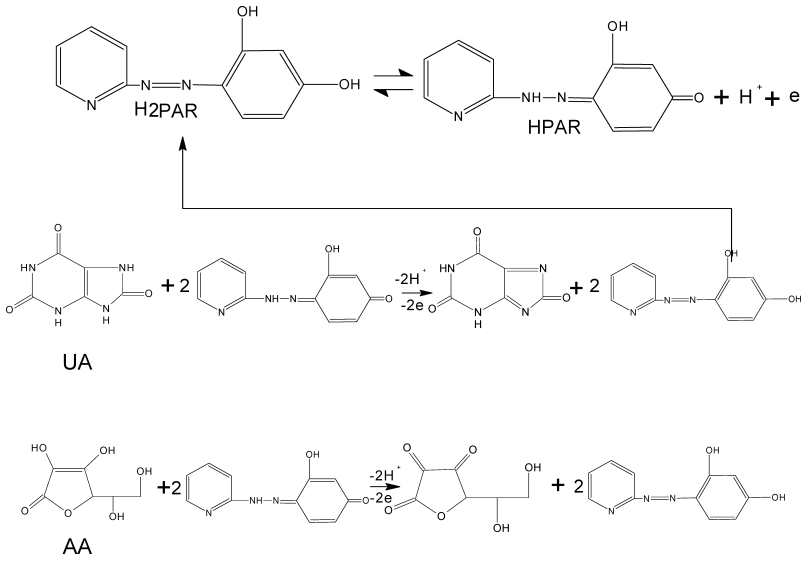

Chart 1. Proposed Electrocatalytic Reaction of UA and AA at a Poly PAR Modified GCE

iary electrode was a platinum wire and an Ag/AgCl electrode was used as a reference. All potentials mentioned in this paper were referred to this reference electrode. The experiments were conducted in PBS (0.05 mol l^{-1}, pH 2.0) at room temperature (25±1 °C). All cyclic voltammetric experiments were carried out with a scan rate of 100 mV s^{-1} unless otherwise stated.

Preparation of Poly PAR Modified GCE The bare GCE was polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on silk. Then it was rinsed with doubly distilled water, and sonicated in 1 : 1 HNO_3, acetone and doubly distilled water for 10 min, respectively. After being cleaned, the electrode was immersed in 0.05 mol l^{-1} H_2SO_4 and was conditioned by cyclic sweeping from 0.4 to 1.6 V at 100 mV s^{-1} for 20 scans. Then the pre-treated GCE was obtained.

The modified electrode was fabricated on the same conditions with the pre-treated electrode but in the presence of 0.3 mmol l^{-1} PAR with the sweeping from −0.8 to 1.3 V at 100 mV s^{-1} for 30 scans. After electropolymerized, the modified electrode was rinsed thoroughly with distilled water.

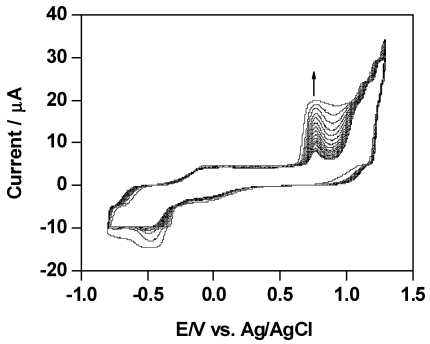

Fig. 1. Repetitive Cyclic Voltammograms of 0.3 mmol l^{-1} 4-(2-Pyridylazo)-Resorcinal (PAR) in PBS (pH 6.0) on the Clean Bare GCE

30 scans, scan rate: 100 mV s^{-1}.

Results and Discussion

Electropolymerization of PAR Film on the Clear Bare GCE Repetitive cyclic voltammograms (CVs) of 0.3 mmol l^{-1} 4-(2-Pyridylazo)-resorcinol (PAR) in PBS (pH 6.0) on the clean bare GCE was illustrated (Fig. 1). During this process, the anodic peak at about 0.76 V corresponding to the oxidation of PAR monomer increases quickly with increasing scan number. The anodic peak increased upon continuous scanning, implying the continuous growth of the film by electropolymerization of PAR. A uniform adherent deep yellow film was formed on the electrode surface by cyclic potential scan between −0.8 V and 1.3 V at 100 mV s^{-1} for 30 scans with 0.05 mol l^{-1} PBS as a supporting electrolyte. The film did not dissolve in HCl, HNO_3, acetone, methanol, or ethanol, *etc*. The film electrode was carefully rinsed with doubly distilled water, then kept in pH 7.4 PBS and was used within 4 weeks.

Electrochemical Properties of Poly PAR Film Modified GCE The cyclic voltammograms of poly PAR film on the GCE in PBS (pH 2.0) were shown in Fig. 2A. Figure 2A shows that a poly PAR film on the GCE had a chemically reversible redox couple in a 0.05 mol l^{-1} H_2SO_4 solution and the peak current increased with increasing the scan rates. The plot of anodic peak current *versus* scan rate (shown Fig. 2B) yielded straight lines in the range 20—500 mV s^{-1} ($I_{pa}(\mu A)$= 0.01840v (mV s^{-1})+0.02130, r=0.9995), and the ratio of the anodic peak current to the cathodic peak current is almost equal to unity (I_{pa} : I_{pc}=1). Therefore, the surface-controlled played a more important role in the electrode process. Separation of the peak potentials, ΔE_p ($=E_{pa}-E_{pc}$), was 60 mV. ΔE_p is close to 2.3RT/nF (or 59/n mV at 25 °C),[28] so that the number of electrons involved in the reaction was 1 (n≈1.01).

The effect of pH on the electrochemical behavior of poly PAR modified GCE in 0.05 mol l^{-1} phosphate buffer solutions with different pH values was studied by cyclic voltammetry (not shown). The linear segment was found with slope values of −59.6 mV/pH in the pH ranges of 2—8, following the Nernst equation slope in Fig. 2C. It can therefore be concluded that equal number of electron and proton is involved in the electrode reactions. Based on Chart 1, the above results could be explained. And the effect of the scans time on the electrochemical response of the PAR modified GCE towards the determination of UA was also investaged. The anodic peak currents of UA increase with scan time from 10 to 30 times. With scan time for more than 30 times, the oxidation current of UA decreases significantly. The results demonstrate that 30 times of scan time is best and enough to obtain stable results. Therefore, the 30 scan times of the CVs was used to electropolymerize PAR film in the following

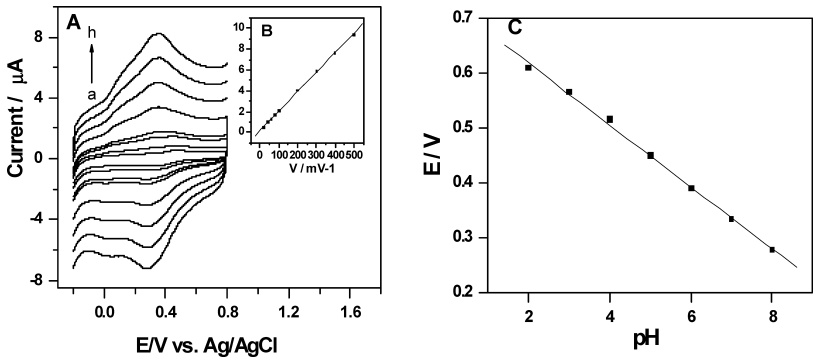

Fig. 2. (A) Cyclic Voltammograms of the Poly PAR Modified GCE in 0.05 mol l^{-1} H_2SO_4 at Various Scan Rates
(a) 20, (b) 40, (c) 80, (d) 100, (e) 200, (f) 300, (g) 400, (h) 500 mV s^{-1}.
(B) Plots of Peak Currents *vs.* Scan Rates
(C) The Effect of pH on the Electrochemical Behavior of Poly PAR Modified GCE in 0.05 mol l^{-1} Phosphate Buffer Solutions with Different pH
Scan rate: 100 mV s^{-1}.

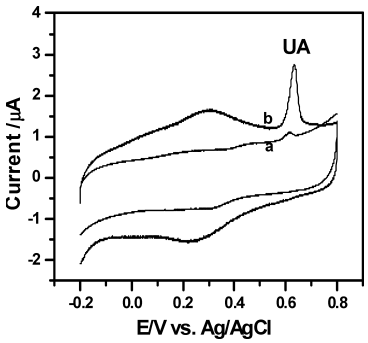

Fig. 3. Cyclic Voltammograms of 1.5 μmol l^{-1} UA at a Bare GCE (a) and a Poly PAR Modified GCE (b) in pH 2.0 PBS
Scan rate: 100 mV s^{-1}.

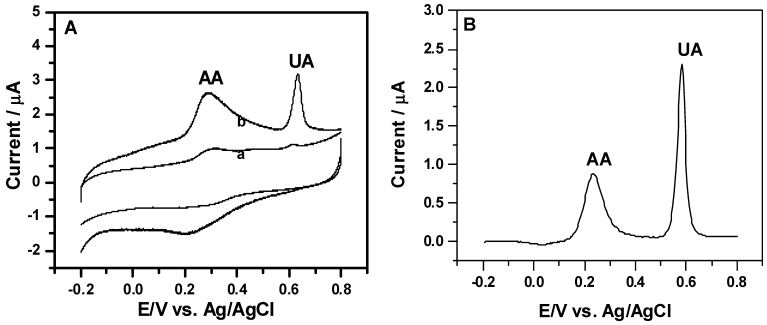

Fig. 4. (A) Cyclic Voltammograms of 20 μmol l^{-1} AA and 1.5 μmol l^{-1} UA at a Bare GCE (a) and a Poly PAR Modified GCE (b) in pH 2.0 PBS and (B) the DPV of 20 μmol l^{-1} AA and 1.5 μmol l^{-1} UA at a Poly PAR Modified GCE in pH 2.0 PBS
Scan rate: 100 mV s^{-1}.

measurements.

Electrochemical Oxidation of UA at the Poly PAR Modified GCE Figure 3 shows the CVs of UA at a bare (curve a) and the poly PAR modified GCE (curve b). The cyclic voltammetric peak of UA oxidation in the pH 2.0 PBS appeared at about 575 mV at the bare GCE. It's obvious to see a sharp and eight-fold enhanced oxidation peak at the poly PAR modified GCE. Thus, the poly PAR modified film has a strong catalytic effect upon UA. The anodic peak current was proportional to the scan rate in the range of 10—500 mV s^{-1} ($I_{pa}(\mu A)=0.01660v$ (mV s^{-1})+0.6595, $r=0.9998$), showing a adsorption-controlled surface adsorption kinetics.

Based on the discussion mentioned above, the mechanism for oxidation of UA at the poly PAR modified GCE can be illustrated (Chart 1). UA diffusing to the surface-immobilized HPAR are oxidized by the HPAR to produce to uric aicd-4,5-diol, the HPAR is reduced to produce H2PAR. Then the reduced H2PAR at electrode surface is re-oxidized rapidly by the electrode to complete the catalytic cycle.

Electrochemical Oxidation of the Ternary Mixture Containing UA and AA The cyclic voltammetric responses to a mixture of 20 μmol l^{-1} AA and 1.5 μmol l^{-1} UA at a bare (curve a) and the poly PAR modified GCE (curve b) in 0.05 mol l^{-1} PBS (pH 2.0) at 100 mV s^{-1} was shown in Fig. 4A. From Fig. 4A, the CV of the mixture solution of AA and UA shows two broad and overlapped anodic peaks (0.320 V, 0.604 V) at bare GCE (curve a). So the indistinguishable oxidation peak potentials of AA and UA, it is impossible to individually or simultaneously determine AA and UA from the ternary mixture. However, as shown in Fig. 4A (curve b), the overlapped peak of AA and UA oxidation is resolved into two well-defined CV peaks at about 281 mV and 624 mV or differential pulse voltammetry (DPV) peaks (Fig. 4B) at about 230 mV and 575 mV for AA and UA, respectively, which were large enough to determine UA in the pres-

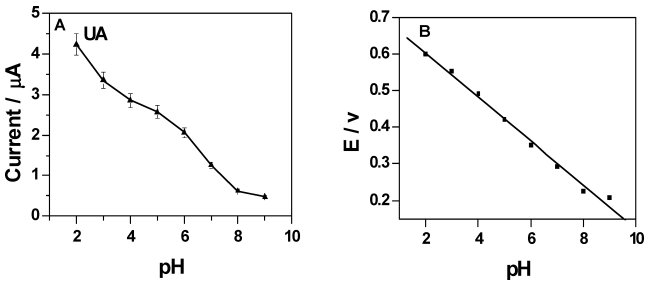

Fig. 5. The Effect of pH on the Peak Current (A) and on the Anodic Peak Potentials (B) for the Oxidation of UA (1.5 μmol l^{-1}) on the Poly PAR Modified GCE
Scan rate: 100 mV s^{-1}.

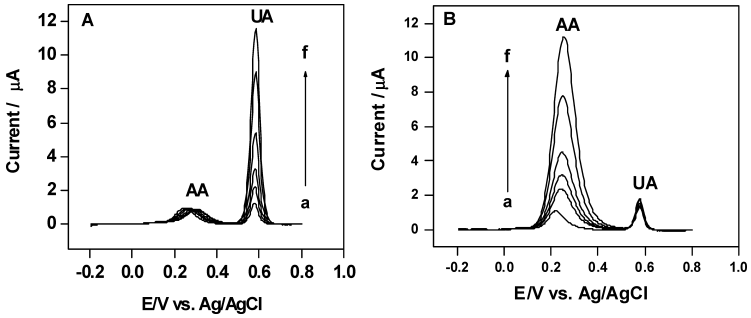

Fig. 6. (A) DPVs of UA at PAR Modified GCE in the Presence of 20 μmol l^{-1} AA in pH 2.0 PBS
UA concentrations (from a to f): 1.5, 3, 5, 10, 20, 30 μmol l^{-1}.
(B) DPVs of AA at PAR Modified GCE in the Presence of 1.5 μmol l^{-1} UA in pH 2.0 PBS
AA Concentrations (from a to f): 20, 40, 60, 80, 160, 240 μmol l^{-1}. Scan rate: 100 mV s^{-1}.

ence of AA. The good separation in peak potential for AA and UA could be attributed to the different adsorption affinity of these compounds on the structure. More investigation is undergoing in our laboratory.

The Effect of pH on the Oxidation of UA at the Poly PAR Modified Electrode The effect of the pH value of the supporting solution on the electrochemical response of the poly PAR modified GCE towards the determination of UA was studied, and variations of peak current with respect to the change in pH of the electrolyte in the pH range from 2.0 to 9.0 are shown in Fig. 5A. It can be seen from Fig. 5A that the anodic peak current of UA decreases slightly with an increase in the solution pH (2.0—9.0), for UA, a highest anodic peak current was get at pH 2.0, and then the anodic peak decreases when the pH increases further. So the pH 2.0 was selected as the optimum pH value in the subsequent process, which was best to determine UA. In addition, all the anodic peak potentials for the oxidation of UA (Fig. 5B), shifted towards negative direction with an increase in pH, showing that protons have taken part in their electrode reaction processes.

Determination of UA in the Presence of AA We carefully investigated the electro-oxidation currents of UA in the presence of AA at the poly PAR modified GCE when the concentrations of one species were kept constant, whereas the other one species changed. The results are shown in Fig. 6. From Fig. 6A, No obvious change in the AA oxidation currents was observed while varying the concentration of UA in DPV. When the concentrations of AA were kept constant, the oxidation peak current of UA increased linearly with increasing UA concentration. Similarly and obviously, as shown in Fig. 6B, keeping the concentrations UA constant, the oxidation peak current of AA was positively proportional to its concentration. Thus the good separation in peak potential for AA and UA could be due to the different adsorption affinity of these compounds on the polymer structure. More investigation is undergoing in our laboratory.

Under the optimum conditions (30 scans times and pH 2.0), using the DPV mothods to determine the UA, the catalytic current peak was linearly relating to UA concentration between 1×10^{-8} and 5×10^{-5} mol l^{-1} ($I_{pa}(\mu A)=0.4852+0.06903C_{UA}$; $r=0.9974$). The detection limit of this method was 1×10^{-9} mol l^{-1} (S/N=3).

From our experimental results depicted above, it can be obtained that the electrochemical response peaks for UA and AA oxidation at the poly PAR modified GCE were clearly separated from each other when they consist in PBS (pH 2.0). It is therefore possible to individually or simultaneously determine UA and AA in samples at a poly PAR modified GCE, which indicates the fact that the oxidation processes of UA and AA at the PAR modified GCE are independent and therefore AA does not influence the voltammetric measurement of UA.

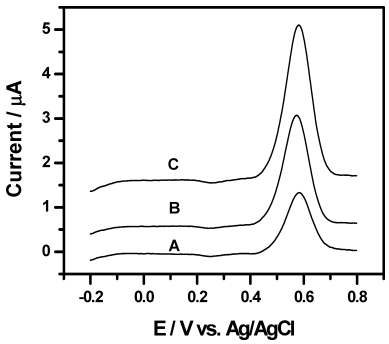

Fig. 7. DPVs of UA in Human Urine Sample at PAR Modified GCE in pH 2.0 PBS
UA samples: A (heath adult), B and C (patient B and patient C). Scan rate: 100 mV s^{-1}.

Interferences For investigating the interference, several compounds also were selected. If the tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately +5% relative error, for 15 mmol l^{-1} AA, no interference was observed for the following ions and compounds (μmol l^{-1}): Ca^{2+} (200), Mg^{2+} (200), citric acid (100), lysine (50), glucose (50), cysteine (50), purine (100), nucleosides (100), oligonucleotides (100), urea (50).

Samples Analysis Three human urine samples (A, B and C) were selected to analyze the contents of uric acid by the proposed method using the standard addition method. Sample A is from the human health subject; sample B and C are from two gout patient of First-accessory Hospital of Fujian Medical University. Then 10 μl of each diluted solution was injected into each of a series of 10 ml volume flasks and made up to volume with 0.05 mol l^{-1} PBS (pH 2.0). Then this test solution was placed in an electrochemical cell for the determination of UA using above DPV method. The results are shown in Fig. 7 and listed in Table 1.

Table 1. Determination of UA in Human Urine Sample (n=6)

Analyte	Labeled (μl)	Added (μM)	Found (μM)	R.S.D. (%)	Recovery
UA	Heath adult A	0	15.21	2.0	
	(20 μl)	5	20.28	2.1	101.4
		10	25.14	1.9	99.3
		15	30.31	2.3	100.7
		20	35.11	2.6	99.5
UA	Patient B	0	18.24	2.0	
	(20 μl)	5	23.16	2.2	98.4
		10	28.40	2.4	101.6
		15	33.18	1.9	99.6
		20	38.38	2.5	100.7
UA	Patient C	0	24.30	2.1	
	(20 μl)	5	29.44	2.0	102.8
		10	34.26	2.2	98.6
		15	39.52	2.7	101.5
		20	44.81	2.5	102.5

Conclusion

This work demonstrated that a novel poly PAR modified glassy carbon electrode by electropolymerization immobilized method. The modified electrode not only improved the electrochemical catalytic oxidation of uric acid (UA), but also resolved the overlapping oxidation peaks of UA and AA into two well-defined peaks at potentials 230 mV and 575 mV in DPV, respectively. In differential pulse voltammetric determination, the lower limit of detection of UA was estimated to be 1.0×10^{-9}. Thus, the poly PAR modified glassy carbon electrode electroanalysis of UA. The presence of cations and anions does not affect much on the redox properties of the poly PAR modified electrode, indicating that the PAR modified GCE facilitates the determination of UA in the presence of large excess of AA with good stability, sensitivity and selectivity. The proposed method can also be applied to the determination of UA in real samples with satisfactory results. These results are of great significance from the viewpoint of practical applications.

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