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# Voltammetric Sensing of Uric Acid and Ascorbic Acid with Poly (p-toluene sulfonic acid) Modified Electrode

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Glassy carbon electrode (GCE) is modified with electropolymerized films of *p*-toluene sulfonic acid (*p*TSA). This polymer (*p*TSA) modified electrode is used to electrochemically detect uric acid (UA) in the presence of ascorbic acid (AA) and shows an excellent electrocatalyticl effect on the oxidation of UA and AA by cyclic voltammetry (CV). In differential pulse voltammetric measurements (DPV), the polymer (*p*TSA) modified electrode could separate the oxidation peak potentials of UA and AA present in the same solution by about 300 mV though the bare electrode gave a single broad response. A successful elimination of the fouling effect by the oxidized product of AA on the response of UA has been achieved at the polymer (*p*TSA) modified electrode. The detection limit of UA in the presence of AA at millimolar level was $5.0 \times 10^{-7}$ M. The modified electrode exhibits the stable and sensitive response to UA.

**Keywords:** modified electrode, uric acid, ascorbic acid, *p*-toluene sulfonic acid

## 1. INTRODUCTION

Uric acid (2,6,8-trihydroxypurine, UA) and other oxypurines are the principal final products of purine metabolism in the human body [1]. Extreme abnormalities of UA levels are symptomatic of several diseases, including gout, hyperuricemia, and Lesch-Nyan disease [2]. Thus the determination of the concentration of UA in human blood or urine is a powerful indicator in diagnosing diseases. As UA is electrochemically active at a carbon-based electrode, its electrochemical detection becomes one of the feasible methods [3]. One of the major problems in the detection of UA by electrochemical method is the presence of ascorbic acid (AA) as interference. AA has an oxidation potential close to that of UA and the bare electrode very often suffers from fouling effects [4]. Various methods, such as an adorption/medium exchange approach [5], enzyme-based techniques [6], electrochemically

pretreated electrode [7] and chemically modified electrode [8] were developed to solve the UA detection problem. Until now, sensitive and selective methods still need to be developed for the detection of UA due to its clinical significance.

Preparation of polymer films by oxidation and electropolymerization of aromatic compounds (aniline, phenol, benzene, and their derivatives) and of heteroaromatic compounds (pyrrol, tiophene, and their derivatives) has been widely used in electrode surface modification as a mean to obtain interesting electrode properties. The increasing development of new electrode materials has motivated the electropolymerization study of new organic molecules [9].

Electropolymerization is a good approach to immobilize polymers to prepare polymer modified electrodes (PMEs) as adjusting the electrochemical parameters can control film thickness, permeation and charge transport characteristics. Polymer-modified electrodes have many advantages in the detection of analytes because of its selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [10]. Selectivity of PMEs as a sensor can be attained by different mechanisms such as size exclusion [11], ion exchange [12], hydrophobicity interaction [13], and electrostatic interaction [14-15].

The basal concentration of UA and AA in biological samples varies from species to species in a wide range from $1.0 \times 10^{-7}$ to $1.0 \times 10^{-3}$ M [16]. Therefore both sensitivity and selectivity are of equal importance in developing voltammetric procedures for biological detection. The present work relates to the electrochemical deposition of *p*-toluene sulfonic acid on a glassy carbon electrode to develop a sensor for selective and sensitive detection of UA in the presence of AA by successful elimination of the fouling effect caused by the oxidation product of AA.

## 2. EXPERIMENTAL PART

### *2.1. Reagents*

Uric acid was purchased from Sigma and it was used as received. *p*-toluene sulfonic acid (*p*-TSA) was obtained from Sinopharm Chemical Reagent Co. Ltd (China); ascorbic acid was purchased from Beijing Chemical Factory (Beijing, China). All other chemicals were of analytical grade and were used without further purification. A 0.1 M phosphate buffer solution was used to control the pH. All solutions were prepared with deionized water treated in a Millipore water purification system (Millipore Corp.). All experiments were carried out at room temperature (approx. $20 \pm 1$ $^{o}$C).

### *2.2. Apparatus*

Voltammetric measurements were performed with a CHI 440 electrochemical analyzer (CH Instruments, Chenhua Co. Shanghai, China) controlled by a personal computer. A conventional three-electrode cell was used, including a saturated calomel electrode (SCE) as reference electrode, a platinum wire counter electrode and a bare or modified glassy carbon disk working electrode (GCE). The pH values were measured with a PB-10 pH meter (Satorius). Unless otherwise stated, the electrolyte solutions were thoroughly degassed with $N_2$ and kept under a $N_2$ blanket.

*2.3. Preparation of modified glassy carbon electrodes*

Prior to electrochemical modification, the bare GCE with a diameter of 3 mm was polished with diamond pastes and alumina slurry down to 0.05 μm on a polishing cloth (Buehler, Lake Bluff, IL). Then it was rinsed with water and sonicated in 1 +1 $HNO_3$, acetone and water for 10 min, respectively. After being cleaned, the electrode was immersed in 0.1 M NaCl solution containing 1.0 mM *p*-TSA and was conditioned by cyclic sweeping from -2.0 to 2.5 V at 100 mV $s^{-1}$ for 10 scans. After that, the modified electrode was electroactivated by cyclic voltammetry from -0.5 to 0.5 V at 100 mV $s^{-1}$ in pH 7.0 PBS.

## 3. RESULTS AND DISCUSSION

*3.1. The electropolymerization of p-*TSA *at the GCE surface*

Electrooxidation of alkyl substituted aromatic compounds is believed to occur by removal of an electron from the alkyl side chain or the aromatic ring and by the formation of a radical cation [9]. The reaction mechanism of the electropolymerization of *p*-TSA has been reported previously [9]. Fig. 1 shows the cyclic voltammograms of 1.0 mM *p*-TSA in 0.1 M NaCl solution at a glassy carbon electrode. In the first cycle, with the potential scanning from -2.0 to 2.5 V, three strong reduction peaks were observed at 0.231 V (peak A), -0.563 V (peak B) and -1.357 (peak C), which might be the reduction of the monomer. From the third cycle on, one obvious oxidation peak appeared with potential at 1.528 V (peak D). In the second and subsequent cycles the larger peaks were observed upon continuous scanning, which was reflecting the continuous growth of the film. It could be observed that film growth was faster for the first five cycles than for the other cycles. These facts indicated *p*-TSA was deposited on the surface of GCE by electropolymerization. A uniform adherent blue polymer was formed on the GCE surface. After modification, the poly(*p*-toluene sulfonic) (P-*p*TSA) modified electrode was carefully rinsed with water, then stored in air and was prepared to use later.

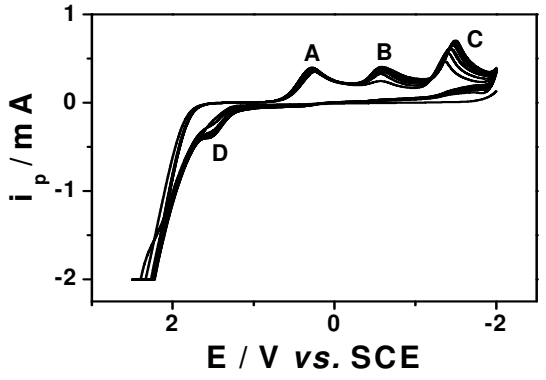


**Fig. 1.** Cyclic voltammograms for 1.0 mM *p*-TSA recorded with a GCE under high pure $N_2$ stream in 0.1 M NaCl solution; scan rate, 100 mV $s^{-1}$.

*3.2. Electrochemical oxidation of UA at the P- pTSA modified electrode*

Fig. 2A shows the cyclic voltammograms at a bare GCE (Fig. 2A curve a) and a P-*p*TSA modified electrode (Fig. 2A curve c) in presence of 0.1 mM UA in phosphate buffer of pH 7.0. At the bare electrode, the electrooxidation of UA occurs at approximately 0.36 V and the voltammetric peak is rather broad, suggesting slow electron transfer kinetics, presumably due to the fouling of the electrode surface by the oxidation product. It is reported that the oxidation of UA is irreversible at GCE and metal electrodes and is quasi-reversible at a graphite electrode [17]. The electrochemical oxidation of UA proceeds in a $2e^-$, $2H^+$ process to lead to an unstable diimine species which is then attacked by water molecules in a step-wise 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 [18]. However, as can be seen from Fig. 2A curve c, a sharp well-defined voltammogram was obtained for the oxidation of UA at the P-*p*TSA modified electrode. The oxidation peak potential shifts negatively to 0.31 V and the peak current increases significantly. P-*p*TSA, itself, is electroinactive in the potential rage from -0.2 to 0.6 V (Fig. 2A curve b). These results indicated that P-*p*TSA could accelerate the rate of electron transfer of UA by a nonmediation mechanism in pH 7.0 PBS, and may be called a promoter.

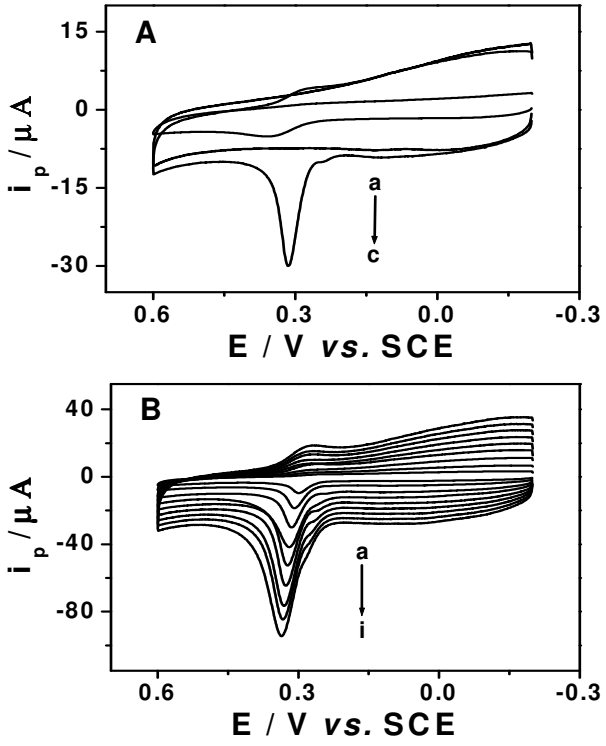


**Fig. 2. (A)** Cyclic voltammograms at a bare GCE (a) and P-*p*TSA modified electrode (b, c) in presence of 0.1 mM UA (a, c) and in the absence of UA (b) in 0.1 M PBS (pH 7.0); scan rate,100 mV $s^{-1}$. **(B)** Cyclic voltammograms of 0.1 mM UA at P-*p*TSA modified electrode in phosphate buffer (pH 7.0) at different scan rates (a) 20 mV $s^{-1}$; (b) 50 mV $s^{-1}$; (c) 100 mV $s^{-1}$; (d) 150 mV $s^{-1}$; (e) 200 mV $s^{-1}$; (f) 250 mV $s^{-1}$; (g) 300 mV $s^{-1}$; (h) 350 mV $s^{-1}$; (i) 400 mV $s^{-1}$.

Fig. 2B shows the CVs of UA at the P-*p*TSA modified electrode at different scan rates. The oxidation peak potential was observed to shift positively with the increase in scan rate, and in addition, exhibited a linear relation to the scan rate, $v$, with the linear regression equation $i_{pa}$ / μA= 5.3682 + 0.1588 $v$ / mV $s^{-1}$ (correlation coefficient, $r$=0.9985). The result indicates that the oxidation of UA at the P-*p*TSA modified electrode was controlled by the adsorption of UA rather than UA diffusing to the electrode.

The effects of the pH value of phosphate buffer solution on the anodic peak potential and peak current were investigated. The response of UA is well-behaved in phosphate buffer solution, as the solution pH increases, the anodic peak potential shifts to the negative and the potential of $E_{pa}$ *vs.* pH in phosphate buffer solution has a good linear relation in the range of pH 3.03 - 7.95. The linear regression equation $E_{pa}$ / V = 0.7195 – 0.0588 pH (correlation coefficient, $r$ = 0.9961) was obtained, which showed that the uptake of electrons is accompanied by an equal number of protons. As well known, pH 7.0 is close to the physiological pH value, thus, it was chosen for the electrochemical detection of UA.

*3.3. Electrochemical oxidation of AA at the P- pTSA modified electrode*

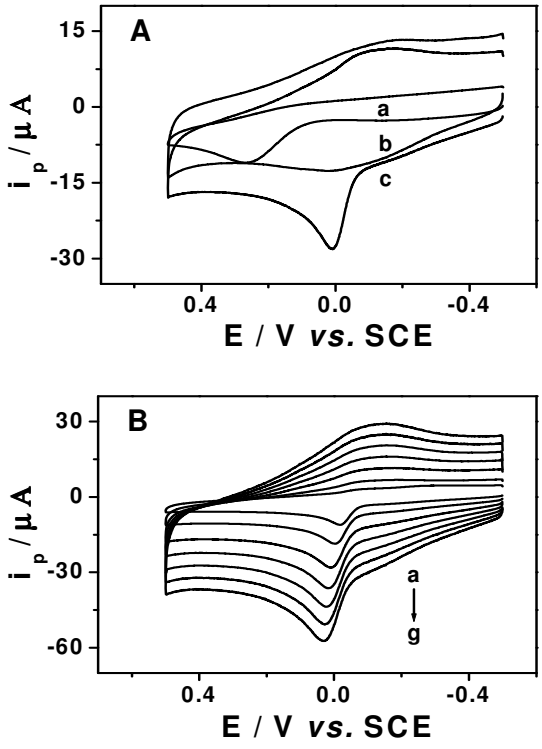


**Fig. 3. (A)** Cyclic voltammograms at a bare GCE (a) and P-*p*TSA modified electrode (b, c) in presence of 0.5 mM AA (a, c) and in the absence of AA (b) in 0.1 M PBS (pH 7.0); scan rate,100 mV $s^{-1}$. **(B)** Cyclic voltammograms of 0.5 mM AA at P-*p*TSA modified electrode in phosphate buffer (pH 7.0) at different scan rates (a) 20 mV $s^{-1}$; (b) 50 mV $s^{-1}$; (c) 100 mV $s^{-1}$; (d) 150 mV $s^{-1}$; (e) 200 mV $s^{-1}$; (f) 250 mV $s^{-1}$; (g) 300 mV $s^{-1}$.

Fig. 3A shows the cyclic voltammograms of AA at the bare GCE and the P-*p*TSA modified electrode. At the bare GCE, the oxidation occurs at around at 0.27 V *vs*. SCE and the oxidation peak is rather broad (Fig. 3A curve a). Oxidation of AA at bare electrode is generally believed to be totally irreversible and requires high overpotential and also, no reproducible electrode response is obtained due to fouling of the electrode surface by the adsorption of the oxidized product of AA [10]. However, the oxidation peak is shifted to less positive potential (ca. 10 mV) at the P-*p*TSA modified electrode (Fig. 3A curve c), indicating that the P-*p*TSA film on the electrode surface favors the oxidation process. This film prevents the fouling of the electrode surface, the electron transfer kinetics for the oxidation of AA are faster at the P-*p*TSA modified electrode. So the possible explanation for the negative shift observed in the oxidation peak potential of AA could be due to the prevention of the electrode surface fouling by the oxidation product. Because the oxidation peak of AA is shifted to less positive potential it would not interfere with the measurement of UA. The oxidation peak potential was observed to shift positively with the increase in scan rate, and in addition, exhibited a linear relation to the square root of the scan rate, $v^{1/2}$, with the linear regression equation $i_{pa}$ / μA=3.9202 + 0.9420 $v^{1/2}$ / (mV $s^{-1}$) $^{1/2}$ (correlation coefficient, $r$=0.9960) (Fig. 3B). The result indicates that the oxidation of AA at the P-*p*TSA modified electrode is a diffusion-controlled process.

### *3.4. Simultaneous detection UA and AA*

The electrochemical sensing of biomolecules using the bare electrodes suffers from the interference of AA that is present at millimolar level in biological fluids, because the fouling effect of the oxidized product of AA is not negligible and the oxidation potential for AA is fairly close to those of most of biomolecules [10].

The electrochemical behavior of a mixture of 0.1 mM UA and 0.5 mM AA at the P-*p*TSA modified electrode was investigated. Fig. 4 shows the cyclic voltammograms obtained for UA and AA coexisting at bare GCE and modified electrodes. As shown in Fig. 4, bare electrode cannot separate the voltammetric signals of UA and AA. Only one broad voltammetric signal of UA and AA was observed for both analytes and the voltammetric peak decreased in the subsequent sweeps. The fouling of the electrode surface by the oxidation products results in a single voltammetric peak for both UA and AA. Therefore it is impossible to use the bare electrode for the voltammetric determination of UA in the presence of AA.

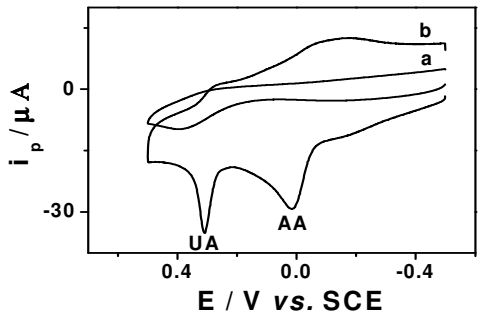


**Fig. 4.** Cyclic voltammograms at a bare GCE (a) and P-*p*TSA modified electrode (b) in presence of 0.1 mM UA and 0.5 mM AA in 0.1 M PBS (pH 7.0); scan rate, 100 mV $s^{-1}$.

However, the P-*p*TSA modified electrode resolved the mixed voltammetric signals into two well-defined voltammetric peaks at 0.31 and 0.01 V corresponding to the oxidations of UA and AA, respectively. The oxidation potential of AA is well before the oxidation potential of UA is reached. Thus the catalytic oxidation of AA by the oxidized UA is completely eliminated and the precise determination of UA in the presence of AA is possible at the P-*p*TSA modified electrode. The voltammetric signals of UA and AA remained unchanged in the subsequent sweeps, indicating that the P-*p*TSA modified electrode does not undergo surface fouling. Furthermore, the separation between the voltammetric peaks of UA and AA is large (-300 mV) and thus the simultaneous determination of UA and AA or the selective determination of UA in the presence of AA is feasible at the P-*p*TSA modified electrode.

The next attempt was taken to detect UA and AA simultaneously by using the P-*p*TSA modified electrode with the more sensitive method, differential pulse voltammetry (DPV). Fig. 5B shows the DPVs of UA and AA coexisting in a solution at bare and P-*p*TSA modified electrode. The bare electrode can not separate the responses of UA and AA and gave a large response due to homogeneous catalytic oxidation of AA by the oxidized UA (Fig. 5B, curve c). The P-*p*TSA modified electrode gave two peaks (Fig. 5B, curve d). One peak was observed at 0.249 V and the current response at this potential is approximately the same as that given by UA in the absence of AA (Fig. 5A, curve a). The another peak appeared with potential at -0.055 V and the current response at this potential is also approximately the same as that given by AA in the absence of UA (Fig. 5A, curve b). Thus, it is can be confirmed that these two peaks are for UA and AA, respectively.

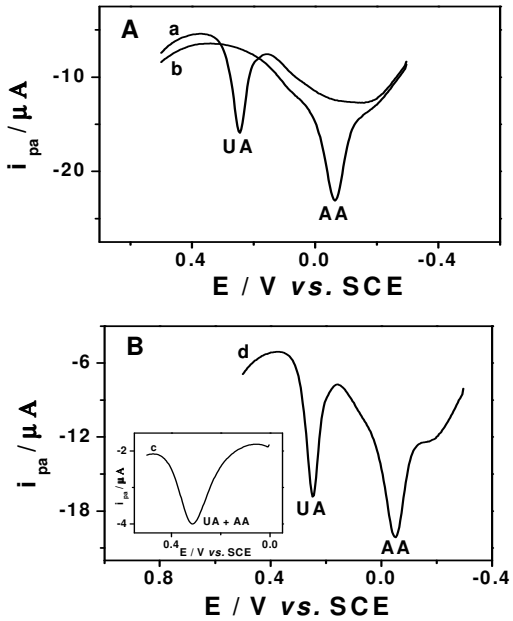


**Fig. 5. (A)** DPVs for 0.1 mM UA (a) and 0.5 mM AA (b) at P-*p*ABSA modified electrode in 0.1 M Phosphate buffer solution (pH 7.0). **(B)** DPVs for the homogeneous solution of 0.1 mM UA and 0.5 mM at bare (c) and P-*p*ABSA modified electrode (d)**.** Scan rate: 4 mV $s^{-1}$; pulse amplitude: 50 mV; pulse width: 60 ms; pulse time: 200 ms.

Fig. 6A represents the DPV recordings at different concentrations of UA where the concentration of AA was kept constant. The oxidative peak current for UA was increased linearly with the increase in UA concentration with the correlation coefficient of 0.9961 and the detection limit was $5.0 \times 10^{-7}$ M based on the signal-to noise ratio of 3. Furthermore, while UA peak current increased with the increase in UA concentration, the peak current of AA kept almost constant. Fig. 6B represents the DPV recordings at different concentrations of AA where the concentration of UA was kept constant. Here also the oxidative peak current for AA was increased linearly with the increase in AA concentration with the correlation coefficient of 0.9974 and the detection limit was $8.0 \times 10^{-5}$ M based on the signal-to noise ratio of 3. Furthermore, while AA peak current increased with the increase in AA concentration, the peak current of UA was almost constant. This suggests that the oxidation of AA mediated by the oxidized UA cannot occur at the P-*p*TSA modified electrode. It also can be noted from this result that the presence of AA at millimolar level, the electrode can sense the increase of UA at micromolar concentration which is much larger than the concentration difference of UA and AA under physiological condition [10]. Thus, the simultaneous selective and sensitive detection of UA and AA was achieved at the P-*p*TSA modified electrode. About the mechanism of the P-*p*TSA coating facilitating the oxidation of UA and AA, it needs further research.

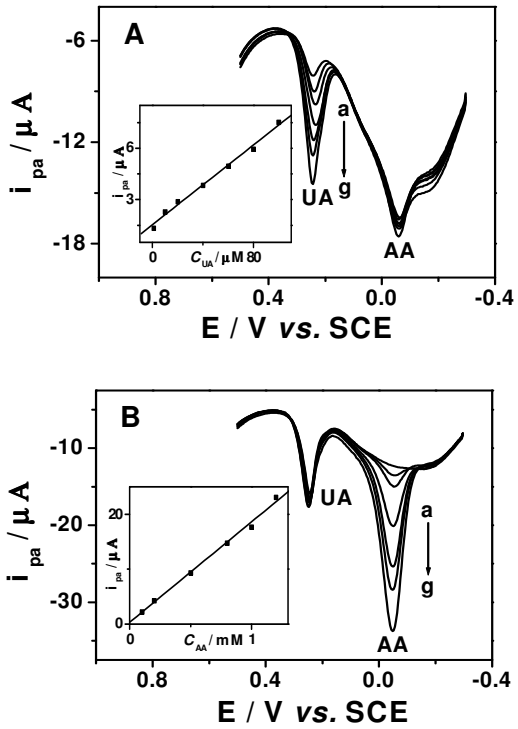


**Fig. 6. (A)** DPVs of UA and AA at P-*p*ABSA modified electrode in 0.1 M phosphate buffer solution (pH 7.0), [AA] was kept constant and [UA] was changed (i.e., [AA] = 0.2 mM, [UA]: (a) 1, (b) 10, (c)20, (d) 40, (e) 60, (f) 80, (g) 100 μM). The inset shows the relationship between the anodic peak current and the concentration of UA. **(B)** [UA] was kept constant and [AA] was changed (i.e., [UA]= 0.1 mM, [AA]: (a) 0, (b) 0.1, (c) 0.2, (d) 0.5, (e) 0.8, (f) 1, (g) 1.2 mM). The inset shows the relationship between the anodic peak current and the concentration of AA. Scan rate: 4 mV $s^{-1}$; pulse amplitude: 50 mV; pulse width: 60 ms; pulse time: 200 ms.

Analytical utility of the P-*p*TSA modified electrode has been examined using human urine samples. Human urine is diluted 100 times in phosphate buffer of pH 7.0 and subjected to electrochemical analysis. The amount of uric acid present in the urine is estimated to be 0.62 ± 0.05 g / L. This value is comparable to the reported values in the literature [19].

## 4. CONCLUSIONS

The present study demonstrates an excellent approach for the development of a novel voltammetric UA sensor based on P-*p*TSA coating. Fast electron transfer, high selectivity and excellent sensitivity for the oxidation of UA are achieved at the P-*p*TSA modified electrode. The present modified electrode showed excellent sensitivity, selectivity, reproducibility and antifouling properties and can separate oxidation peaks towards UA and AA, which are indistinguishable at the bare electrode. As the voltammetric signals of UA and AA are well separated at the P-*p*TSA modified electrode, the sensitive detection UA in the presence of AA or the simultaneous detection of UA and AA can be achieved. The electrode is stable and does not undergo surface fouling during the measurements.

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