A Poly(3-acetylthiophene) Modified Glassy Carbon Electrode for Selective Voltammetric Measurement of Uric Acid in Urine Sample

Mehmet ASLANOGLU*, Aysegul KUTLUAY, Sultan ABBASOGLU, and Serpil KARABULUT

Department of Chemistry, University of Harran; Sanliurfa 63510, Turkey.
Received October 7, 2007; accepted December 3, 2007; published online December 7, 2007

A reliable and reproducible method for the determination of uric acid in urine samples has been developed. The method is based on the modification of a glassy carbon electrode by 3-acetylthiophene using cyclic voltammetry. The poly(3-acetylthiophene) modified glassy carbon electrode showed an excellent electrocatalytic effect towards the oxidation of uric acid in 0.1 M phosphate buffer solution (PBS) at pH 7.2. Compared with a bare glassy carbon electrode (GCE), an obvious shift of the oxidation peak potential in the cathodic direction and a marked enhancement of the anodic current response for uric acid were observed. The poly(3-acetylthiophene)/GCE was used for the determination of uric acid using square wave voltammetry. The peak current increased linearly with the concentration of uric acid in the range of 1.25×10^{-5}—1.75×10^{-4} M. The detection limit was 5.27×10^{-7} M by square wave voltammetry. The poly(3-acetylthiophene)/GCE was also effective to determine uric acid and ascorbic acid in a mixture and resolved the overlapping anodic peaks of these two species into two well-defined voltammetric peaks in cyclic voltammetry at 0.030 V and 0.320 V (*vs.* Ag/AgCl) for ascorbic acid and uric acid, respectively. The modified electrode exhibited stable and sensitive current responses toward uric acid and ascorbic acid. The method has successfully been applied for determination of uric acid in urine samples.

Key words voltammetry; uric acid; ascorbic acid; 3-acetylthiophene; urine sample

Uric acid is the final oxidation product of purine metabolism. Severe changes in the concentration of uric acid causes several diseases such as gout, hyperuricemia and Lesch–Nyhan syndrome.[1] Therefore, the accurate determination of uric acid in urine samples can be a powerful indicator of diagnosing diseases. Thus, many researchers have focused to develop a simple, sensitive and accurate method for determining uric acid. Various methods have been developed for the determination of uric acid including, *e.g.*, enzyme biochip system,[2] fluoroscence,[3] liquid chromatography,[4] scanning electrochemical microscopy.[5] Self-assembled monolayers have also been developed for the determination of uric acid in urine samples.[6] Recently, it has been shown that electrochemical determination of uric acid at polymer modified electrodes showed good selectivity and sensitivity.[7] Moreover, ascorbic acid which is a powerful water soluble antioxidant, has a key role in protecting against the oxidative injury and has been used for the treatment of several diseases.[8—11] Therefore, the detection of ascorbic acid has a biological importance. Several techniques have been used for the determination of ascorbic acid such as capillary zone electrophoresis,[11] spectrofluorometry,[12] flow injection,[13] voltammetry,[14] and chemiluminescence.[15] However, electrochemical methods have attracted more attention because of sensitivity, selectivity and simplicity.[16] At bare electrodes, the oxidation of ascorbic acid occurs at a potential close to that of uric acid and the bare electrode very often suffers from fouling effects.[1] Therefore it is necessary to develop an electrochemical sensor to separate the oxidation peak potentials of uric acid and ascorbic acid and for the determination of uric acid in the presence of ascorbic acid. Up to now, no articles have been reported concerning the determination of uric acid on a poly(3-acetylthiophene) or derivatives of poly(thiophenes) modified glassy carbon electrodes (GCEs). However, there are limited reports about the electropolymerization of other thiophenes used for determination of some neurotransmitters.[17—19] The electropolymerization of thiophenes on a GCE enables better electrode surfaces for the electrocatalytic oxidation of biochemical species because poly(thiophenes) have potentially more useful electronic properties and provide highly conducting surfaces.[17—19]

In this paper, a poly(3-acetylthiophene) modified glassy carbon electrode was prepared to study the electrocatalytic oxidation of uric acid. The method is based on modification of a GCE by electropolymerization of 3-acetylthiophene in acetonitrile containing 0.05 M $LiClO_4$ as the supporting electrolyte using cyclic voltammetry. Then the coated electrode was utilized for determination of uric acid using voltammetric methods. It has been shown that the oxidation potential of uric acid can be well separated from than that of ascorbic acid at the modified electrode. The results showed that the modified electrode could be used to detect uric acid in the presence of ascorbic acid. The modified electrode has successfully been applied for the determination of uric acid in urine samples.

Experimental

Chemicals Uric acid and ascorbic acid obtained from Fluka (Germany) were used as received. 3-acetylthiophene was purchased from Fluka (Germany). Solutions of 3-acetylthiophene was prepared in acetonitrile (Merck, Germany) containing $LiClO_4$ (Fluka, Germany) as supporting electrolyte. All other reagents were of analytical grade or equivalent, and obtained from Merck or Fluka. Solutions of uric acid and ascorbic acid were prepared in 0.1 M phosphate buffer solution (PBS) at pH 7.2. Aqueous solutions were prepared with doubly distilled water. Oxygen-free nitrogen was bubbled through the cell prior to each experiment. All experiments were carried out at *ca.* 25±0.5 °C.

Apparatus Electrochemical experiments were performed using an Eco-Chemie Autolab PGSTAT 12 potentiostat/galvanostat (Utrect, The Netherlands) with the electrochemical software package 4.9 or an EPSILON potentiostat (Bioanalytical Systems, Lafayette, U.S.A.) with the electrochemical software 1.6.70_XP. A three-electrode system was used: a bare or poly(3-acetylthiophene) modified glassy carbon electrode as working electrode [3 mm in diameter (Bioanalytical Systems, Lafayette, U.S.A.)], a Pt wire counter electrode and an Ag/AgCl reference electrode.

* To whom correspondence should be addressed. e-mail: maslanoglu@harran.edu.tr

Preparation of Modified GCE Prior to electrochemical modification, the bare GCE was polished with 0.05 μm alumina slurry on a polishing pad. Then it was rinsed with water, and sonicated with 1+1 HNO_3 and acetone, and water for 10 min, respectively. After being cleaned, the electrode was activated by 5 cyclic sweepings from −0.6 to +0.8 V in PBS at pH 7.2. Then, the electrode was immersed in a solution of 10 mM 3-acetylthiophene dissolved in acetonitrile and containing 0.05 M $LiClO_4$ as the supporting electrolyte and was conditioned by cyclic sweepings from −1.5 to +1.8 V for 20 scans. Afterwards, the modified electrode was electroactivated by cyclic voltammetry from −0.6 to +0.8 V at 100 mV/s in PBS at pH 7.2.

Results and Discussion

Electrocatalytic Oxidation of Uric Acid on a Poly(3-acetylthiophene) Modified Electrode Cyclic voltammograms 20 ml of 8.75×10^{-5} M uric acid at bare GCE and poly(3-acetylthiophene) modified GCE in 0.1 M PBS at pH 7.2 are given in Fig. 1. At bare glassy carbon electrode, uric acid showed an oxidation peak at +0.440 V. The voltammetric response is rather broad due to a slow electron transfer, which is caused by fouling of the electrode surface. It is reported that the oxidation of uric acid at glassy carbon and metal electrodes is irreversible while *quasi*-reversible at a carbon paste electrode.[20] It has been reported that the oxidation of uric acid proceeds in a $2e^+$, $2H^+$ process to lead to an unstable diimine species which is then attacked by water molecules. Then the product is converted into an imine-alcohol and finally uric acid-4,5 diol. The uric acid-4,5 diol compound is unstable and decomposes to various products depending on the solution pH.[21] However, as shown in Fig. 1, a sharp and well-defined voltammetric peak was obtained for the oxidation of uric acid at the poly(3-acetylthiophene)/GCE. This indicated that the electrochemical response of uric acid has greatly been enhanced at poly(3-acetylthiophene)/GCE. The anodic peak potential shifted negatively to +0.322 V. Intensive increase in peak current is observed owing to the improvement in properties of electron transfer process and the larger real area of the polymer film. This suggests an efficient oxidation reaction toward uric acid at the poly(3-acetylthiophene) modified glassy carbon electrode. This behaviour might be also attributed that the polymer film coated electrode can act as a promoter to increase the rate of electron transfer[22,23] and lowering the overpotential of uric acid at the bare electrode, and the oxidation peak shifts negatively. These two facts, the remarkable increase in the peak currents and lowering the overpotential, provide clear evidence of the electrocatalytic effect of poly(3-acetylthiophene) film toward uric acid.[24—26] Thus, the electron transfer properties of uric acid have been improved owing to the catalytic effect of the polymer film at the surface of GCE.

Figure 2 shows the effect of the scan rate on the electrochemical response of uric acid at poly(3-acetylthiophene)/GCE using cyclic voltammetry. The anodic peak current (I_{pa}) was proportional to the square root of the scan rate ($v^{1/2}$) over the range of 50—250 mV/s. The oxidation peak potential shifted positively with increasing scan rate. The results indicated that the oxidation of uric acid at polymer coated GCE is a diffusion-controlled process.

In addition, the influence of the pH value of the PBS buffer solution on peak potential of 2.5×10^{-5} M uric acid at poly(3-acetylthiophene)/GCE was also investigated. The relationship between oxidation peak potential of uric acid and

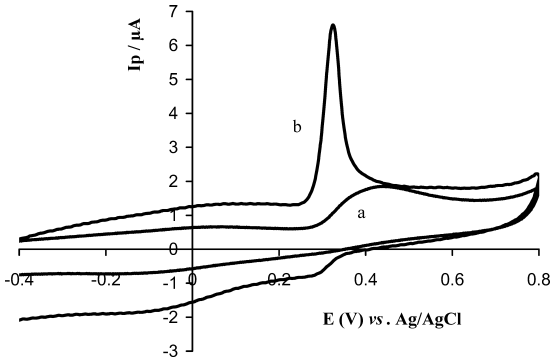

Fig. 1. Cyclic Voltammograms of 8.75×10^{-5} M UA at Bare GCE (a) and Poly(3-acetylthiophene)/GCE (b) in 0.1 M PBS at pH 7.2
Scan rate: 50 mV/s.

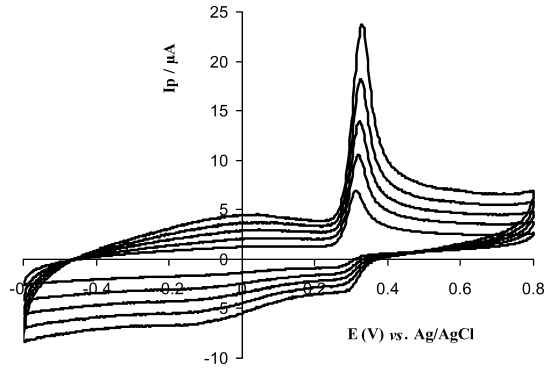

Fig. 2. Cyclic Voltammograms of 8.75×10^{-5} M UA in 0.1 M PBS at pH 7.2
Scan rates increasing from 50 to 250 mV/s.

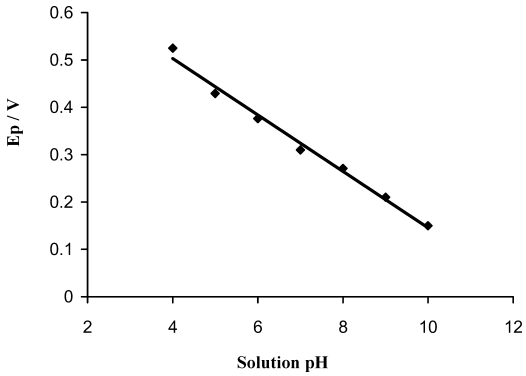

Fig. 3. A Plot of Oxidation Peak Potential of 2.5×10^{-5} M UA *versus* Solution pH
The results are the mean of five different determinations.

pH value of phosphate buffer solution is given in Fig. 3. The results are the mean of five different measurements. The oxidation peak potential of uric acid shifted in the negative direction with increasing pH. This shows that the redox couple of uric acid includes transfer of hydrogen ions in the reduction and oxidation processes. The slope of Fig. 3 was *ca.* 59.6 mV/pH which indicates that the number of electrons and protons are equal in the process.[20]

Calibration Equation for the Determination of Uric Acid Square wave voltammetry (SWV) was performed for the determination of the concentration of uric acid at poly(3-acetylthiophene)/GCE. SWVs of different concentration of

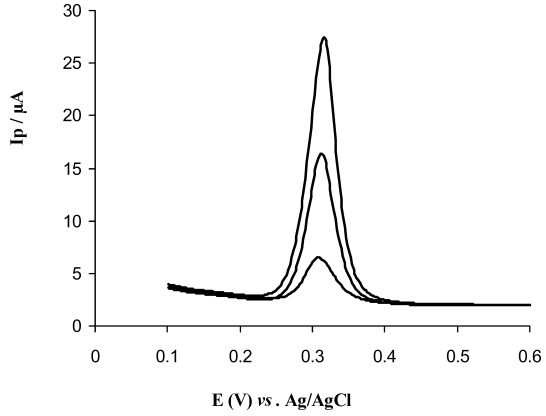

Fig. 4. Square Wave Voltammograms of Increasing Concentrations of UA at Poly(3-acetylthiophene)/GCE

UA concentrations: 2.0×10^{-5} M, 7.5×10^{-5} M, and 1.3×10^{-4} M. Equilibrium time: 5 s, frequency: 10 Hz, step potential: 20 mV, amplitude: 25 mV. Supporting electrolyte: 0.1 M PBS at pH 7.2.

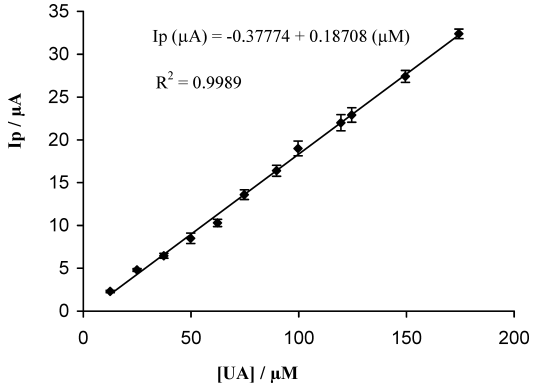

Fig. 5. Calibration Plot for UA

Error Bars were calculated at 95% confidence intervals of five different determinations. The average value of RSD for five different determinations of the each concentration of UA was 2.95%.

uric acid at poly(3-acetylthiophene)/GCE are given in Fig. 4. The anodic peak currents were plotted against the bulk concentration of uric acid as given in Fig. 5. The response of anodic peak currents of uric acid at poly(3-acetylthiophene) was proportional to the uric acid concentration over a range of 1.25×10^{-5}—1.75×10^{-4} M. The linear regression equation was I_{pa} (μA)$=-0.37774+0.18708C$ (μM) with a correlation coefficient of 0.9989. The detection limit was 5.27×10^{-7} M (S/N=3).

Reproducibility and Stability of Poly(3-acetylthiophene)/GCE The relative standard deviation (RSD) of 5 successive scans was 2.30% for 5.0×10^{-5} M uric acid (UA). This indicated that the reproducibility of the poly(3-acetylthiophene) modified GCE was excellent. However, the modified electrode should be well treated to maintain its reproducibility. It was found that 20 cycles of scanning in 0.1 M PBS in the potential range 0.0—0.8 V could regenerate clean background cyclic voltammetry (CV) curves and the modified electrode was ready for the next experiment or storage in 0.1 M PBS. Also, the current response decreased only by 5% over a week and 8% for two weeks for storage in 0.1 M PBS at 4 °C.

Electrocatalytic Oxidation of Ascorbic Acid Figure 6 shows the cyclic voltammograms of ascorbic acid at bare and poly(3-acetylthiophene) modified GCE. Ascorbic acid exhibited a broad anodic peak at $E_{pa}=+0.437$ V in 0.1 M PBS buffer pH 7.2. It has been suggested that the electron transfer kinetics of ascorbic acid is slow because of the fouling of electrode surface by oxidation product of ascorbic acid. It has also been reported that the ascorbic acid shows an irreversible oxidation process at GCE. However, a sharp and well-defined voltammogram was obtained for the oxidation of ascorbic acid at poly(3-acetylthiophene) modified GCE. Also, the oxidation peak potential of ascorbic acid shifted negatively to +0.030 V indicating that the poly(3-acetylthiophene)/GCE favours the oxidation process of ascorbic acid. The anodic peak potential of ascorbic acid shifted with increasing scan rate. The oxidation peak current was increased linearly with the square root of the scan rate which indicates a diffusion-controlled electrochemical process of ascorbic acid at the modified electrode.

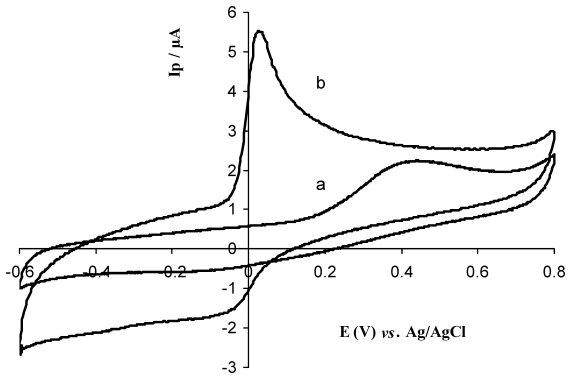

Fig. 6. Cyclic Voltammograms of 2.0×10^{-4} M AA at Bare GCE (a) and Poly(3-acetylthiophene)/GCE (b) in 0.1 M PBS at pH 7.2

Scan rate: 50 mV/s.

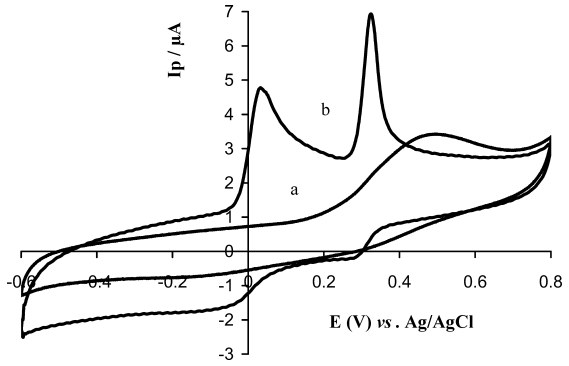

Fig. 7. Cyclic Voltammograms of the Mixture of 1.75×10^{-4} M AA and 6.25×10^{-5} M UA at Bare GCE (a) and Poly(3-acetylthiophene)/GCE (b) in 0.1 M PBS at pH 7.2

Scan rate: 50 mV/s.

Detection of Uric Acid in the Presence of Ascorbic Acid It is known that ascorbic acid is the main interferent in the voltammetric determination of uric acid. The main goal of this study was to determine uric acid in the presence of ascorbic acid. Figure 7 shows the cyclic voltammograms of the mixture of uric acid and ascorbic acid in 0.1 M PBS at bare and poly(3-acetylthiophene) modified glassy carbon electrodes. The bare GCE electrode could not separate the responses of uric acid and ascorbic acid and a broad voltam-

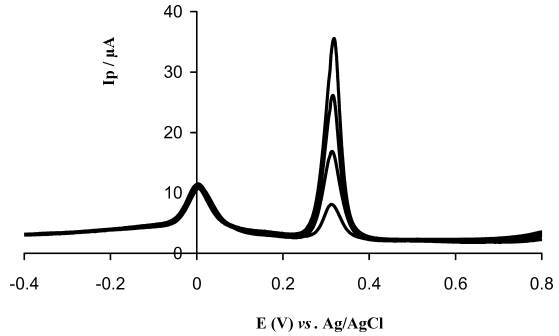

Fig. 8. Square Wave Voltammograms of Various Concentrations of UA in the Presence of 2.5×10^{-4} M AA at Poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 7.2

UA concentrations: 2.5×10^{-5} M, 7.5×10^{-5} M, 1.25×10^{-4} M and 1.75×10^{-4} M. Equilibrium time: 5 s, frequency: 10 Hz, step potential: 20 mV, amplitude: 25 mV.

Table 1. Effects of Possible Interferents on the Peak Current of 1×10^{-4} M UA at Poly(3-acetylthiophene)/GCE

Possible interferents (1×10^{-3} M)	Change in I_p (%)[a]
Glucose	−1.2
Purine	−2.0
Oxalate	+1.5
Cystine	+2.5
Urea	*ca.* 0
Adenine	*ca.* 0
Hypoxanthine	*ca.* 0
Tyrosine	*ca.* 0

a) Mean of five determinations in 0.1 M PBS at pH 7.2.

metric peak was obtained. The fouling of the electrode surface by the oxidation products results in a single voltammetric peak for uric acid and ascorbic acid. Therefore it is impossible to use a bare electrode for the voltammetric determination of uric acid in the presence of ascorbic acid. However, two well-defined oxidation peaks were obtained at the polymer modified GCE. The anodic peaks were appeared at about +0.030 V and +0.320 V for ascorbic acid and uric acid, respectively. Figure 8 shows square wave voltammograms of various concentrations of uric acid in the presence of a constant concentration of ascorbic acid. This indicated that ascorbic acid had no interference on the determination of uric acid at poly(3-acetylthiophene)/GCE. These results also showed that poly(3-acetylthiophene)/GCE could enable the determination of uric acid in the presence of ascorbic acid. Selectivity of UA was also studied in the presence of other coexisting interferents including cystine, oxalate, purine, glucose, *etc*., as shown in Table 1. We observed that the responses of these species were greatly suppressed by the modified electrode and their interferences on the detection of UA were almost negligible.

The Recovery Test of Real Samples The proposed method was utilized for the determination of uric acid in human urine samples. Figure 9 shows a cyclic voltammogram of urine sample diluted 50 times with 0.1 M PBS at pH 7.2. It should also be noted that peak potential of uric acid in urine sample shifts negatively in cyclic voltammetry as the pH increases. The samples were analyzed by the standard addition method. Urine samples were filtered and diluted by 0.1 M PBS at pH 7.2 and subjected to the square wave

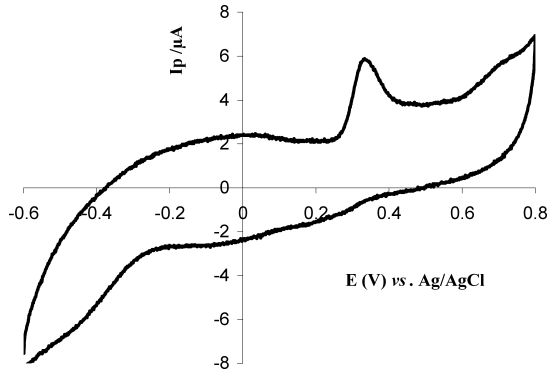

Fig. 9. A Cyclic Voltammogram of Urine Sample Diluted 50 Times with 0.1 M PBS at pH 7.2

Scan rate 50 mV/s.

Table 2. Recovery Determination of Uric Acid at Poly(3-acetylthiophene)/GCE

	Original/μM	Added/μM	Found/μM	Recovery, %
Sample 1	46.5	50	94.3	97.7
Sample 2	48.2	50	96.9	98.6
Sample 3	52.0	50	98.1	96.2

Urine samples were diluted 50 times by 0.1 M PBS at pH 7.2. The results are the average values for five determinations.

voltammetric analysis. The results are given in Table 2. The recovery was a lightly lower than 100%, which was probably due to the existence of other interferents, given in Table 2.[7,27] The data obtained by the proposed method are comparable with literature values.[7,21,28] The superiority of proposed method is mainly owing to its simplicity, high stability and long usage life. These results indicated that the proposed method could be easily used for the determination of uric acid in urine samples.

Conclusions

The results indicated that poly(3-acetylthiophene) modified GCE could be used for the determination of uric acid. The anodic peak current of uric acid is linear with the concentration range of 1.25×10^{-5}—1.75×10^{-4} M with a detection limit of 5.27×10^{-7} M. The results also proved that ascorbic acid does not interfere with the detection of uric acid at the poly(3-acetylthiophene) modified GCE. The poly(3-acetylthiophene) film modified electrode has a good stability and reproducibility. The method could also be utilized for the detection of uric acid in urine samples. The proposed method is simple, accurate and sensitive.

Acknowledgements The authors appreciate the financial support from the Scientific and Technological Research Council of Turkey for a grant (Project No. 106T404).

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