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ORIGINAL PAPER

Simultaneous voltammetric determination of uric acid and ascorbic acid using carbon paste/cobalt Schiff base composite electrode

Mandana Amiri · Abolfazl Bezaatpour · Zohreh Pakdel · Khadijeh Nekoueian

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Abstract Differential pulse and cyclic voltammetry were applied for the oxidation of mixture of uric acid and ascorbic acid at the surface of carbon paste/cobalt Schiff base composite electrode. The electrooxidation of these compounds at bare electrode is sluggish, and there is no suitable peak separation between them. However, using cobalt methyl salophen as modifier, two well-defined anodic waves with a considerable enhancement in the peak current and a remarkable peak potential separation near 315 mV are obtained. It can improve the kinetics of electron transfer for both compounds remarkably. All these improvements are created because of the electrocatalytic property of cobalt Schiff base complex. The effect of some parameters such as pH and scan rates were studied. All the anodic peak currents for the oxidation of ascorbic acid and uric acid shifted toward more negative potential with an increase in pH, revealing that protons have taken part in their electrode reaction processes. The best peak separation with appropriate current was obtained for pH 4.0. A linear range of 5.0×10^{-4} to 1.0×10^{-8} and 1.0×10^{-3} to 1.0×10^{-8} M with detection limit of 8.0×10^{-9} and 8.0×10^{-9} M was obtained for ascorbic acid and uric acid using differential pulse voltammetry at the surface of modified electrode, respectively. Analytical utility of the modified electrode has been examined successfully using human urine samples and vitamin C commercial tablets.

M. Amiri (✉) · Z. Pakdel · K. Nekoueian
Department of Chemistry, Payame Noor University (PNU), Ardabil, Iran
e-mail: mandanaamiri@yahoo.com

M. Amiri · A. Bezaatpour
Department of Chemistry, University of Mohaghegh Ardabili, Ardabil, Iran

Keywords Uric acid · Ascorbic acid · Carbon paste electrode · Schiff base complex · Voltammetric determination

Introduction

Uric acid (UA), 2, 6, 8-trihydroxypurine, and other oxypurines are the principal final products of purine metabolism in the human body in a concentration range of 2.5–8.8 mg/100 mL in serum for normal, healthy humans [1]. The elevated UA concentration in serum causes kidney damage and cardiovascular disease [2]. Extreme abnormalities of UA levels are symptomatic of several diseases, including gout, hyperuricemia, and Lesch–Nyan disease [3]. Thus, the determination of the concentration of UA in human blood or urine is a powerful indicator in diagnosing diseases.

On the other hand, ascorbic acid (AA), vitamin C, is an essential nutrient found mainly in fruits and vegetables and one of the most important cellular antioxidants (http://www.baobab-supply.com, accessed 12 July 2011). The body requires it to form and maintain bones, blood vessels, and skin. Ascorbic acid helps to produce collagen, a protein needed to develop and maintain healthy teeth, bones, gums, cartilage, vertebrae disks, joint linings, skin, and blood vessels (http://www.vitamins-supplements.org, accessed 12 July 2011). There is evidence that large doses of ascorbic acid contribute to the development of kidney stones (http://www.medicinenet.com, accessed 12 July 2011). In addition, the concentration of AA in foodstuffs, beverages, and pharmaceuticals can be an index of quality since it varies during production and storage stages [4].

UA and AA commonly coexist in biological fluids of humans, mainly in serum, blood, and urine. The major problem with simultaneous determination of UA and AA

by electrochemical methods is the closeness of the oxidation potentials of these two compounds which results in an overlapped voltammetric response which makes their signal discrimination very difficult [4]. In addition, AA oxidation at bare electrode suffers fouling effects [5]. Until now, sensitive and selective methods still need to be developed for the detection of UA in the presence of AA due to its clinical significance.

Carbon paste electrodes (CPEs) belong to promising electrochemical or bioelectrochemical sensors of wide applicability because of their easy renewability, reproducibility, simplicity, compatibility, and ability to be modified using different materials and methods [6–8]. One of the most important properties of chemically modified electrodes (CMEs) is their ability to catalyze the electrode process via significant decreasing of overpotential respect to unmodified electrode. With respect to relatively selective interaction of the electron mediator with the target analyte in a coordination fashion, these electrodes are capable to considerably enhance the selectivity in the electroanalytical methods.

Application of transition metal complexes, as the electron mediators for lowering the overpotential of the electrode processes and improvement of the sensitivity of the voltammetric responses, is considered as an alternative for the electrochemical determinations of biologically important compounds [9]. Metallophthalocyanines [10], metalloporphyrins [11], and salen-type complexes, in particular, provide an attractive option because of their versatility, high catalytic activity, and low cost of raw materials [9, 12].

On the basis of our previous works, Schiff base complexes of cobalt are well-known as effective redox mediators and are capable to catalyze the electrochemical oxidation of various organic and biologically important compounds [12, 13]. The electrocatalytic oxidation of uric acid and ascorbic acid using modified electrodes such as highly oxidized electrodes [14], self-assembled monolayers of heteroaromatic thiol [15], ordered mesoporous carbon modified electrodes [16], and plane pyrolytic graphite electrodes [17] has been reported.

There are a lot of attempts to obtain a suitable method for determination of AA and UA in the presence of each other recently. In this approach, we have applied carbon paste electrode modified by using methyl cobalt salophen as electron mediator for simultaneous detection of AA and UA at nanomolar concentration in biological samples. Table 1 compares some of these methods with the presented research. As can be seen, the detection limit of the presented work is much better and the dynamic linear range is wider compared to the other methods. The aim of this work is to determine those compounds in the presence of each other with good analytical results. The easy renewability, reproducibility, and simplicity and minimum fouling effect of presented electrode could be very interesting for analytical means.

Experimental

Materials

Uric acid was purchased from Alfa Aesar; all other chemicals were analytical reagent grade from Merck. All aqueous solutions were prepared with double-distilled deionized water. Voltammetric experiments were carried out in the buffered solutions and deoxygenated by purging the pure nitrogen. Deionized and filtered water was taken from a Millipore water purification system.

Instruments

Voltammetric experiments were performed using a Metrohm Computrace Voltammetric Analyzer model 797 VA. A conventional three-electrode system was used with a carbon paste working electrode (unmodified or modified), a saturated Ag/AgCl reference electrode, and a Pt wire as the counter electrode. A digital pH/mV/Ion meter (JENWAY) was applied for the preparing of the buffer solutions, which

Table 1 The characteristics of some sensors for simultaneous determination of UA-AA are summarized

LOD (UA) (mol/L)	LOD(AA) (mol/L)	ΔE (mV)	Electrode	Reference
6.4×10^{-6}	4.4×10^{-5}	400	Organoclay film modified glassy carbon electrodes	[18]
-	3.2×10	350	TiO2 nanoparticles modified carbon paste electrode^{-7}	[19]
190×10^{-9}	200×10^{-9}	200	Enlarged gold nanoparticles modified electrode	[20]
2.0×10^{-7}	3.0×10^{-6}	306	Gold nanoparticles-modified glassy carbon electrode	[21]
12×10^{-6}	7.5×10^{-6}	230	Polyaniline (PAN) nano-networks/p-aminobenzene sulfonic acid (ABSA) modified glassy carbon electrode	[22]
8×10^{-9}	8×10^{-9}	315	CPE-Me-Cosal	This Work

were used as the supporting electrolyte in voltammetric experiments.

Cobalt salophen complex synthesis

The complexes *N*,*N*-bis (salicylidene)-4-methyl 1,2-phenylenediamino cobalt(III) acetate (cobalt(III) salophen; Me-CoSal) was synthesized and purified as reported previously [13]. Identification of the structure of synthesized complex was performed by infrared, ^{1}H and $^{13}CNMR$, UV–vis, and elemental analysis. The structures of Schiff base complex of cobalt is shown in Scheme 1.

Modified electrode preparation

The unmodified carbon paste electrode (UCPE) was prepared by mixing graphite powder with appropriate amount of mineral oil (Nujol) and thorough hand mixing in a mortar and pestle (~75:25, *w*/*w*), and a portion of the composite mixture was packed into the end of a Teflon tube (about 3.0 mm i.d.). Electrical contact was made by forcing a copper pin down into the Teflon and into the back of the composite. The modified carbon paste electrode (MCPE) was prepared by mixing unmodified composite with Me-CoSal (2%, *w*/*w*), and then the composite was being dissolved in dichloromethane. The mixture was stirred until the solvent evaporated completely. The modified composite was then air-dried for 24 h and used in the same way as unmodified electrode.

Results and discussion

Electrochemical oxidation of AA at the surface of UCPE and MCPE

Figure 1 shows the cyclic voltammograms at the surface of unmodified (UCPE, dotted line) and modified carbon paste electrode with Me-Cosal (MCPE, solid line) in the presence of 1 mM AA in phosphate buffer of pH 4.0. At the UCPE, the electrooxidation of AA occurs at approximately 0.38 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 AA is irreversible at glassy carbon electrode and metal electrodes (http://www.vitamins-supplements.org, accessed 12 July 2011) [5]. However, as can be seen in Fig. 1, a very sharp anodic wave at a lower positive potential is obtained for this compound at the surface of modified electrode. A significant increase of the anodic peak current in conjunction with the sharpness of the peak, which is related to a reduction of the overpotential of the process at the surface of the modified electrode, revealed that the modified electrode could act as a very effective promoter to enhance the kinetics of the electrochemical process.

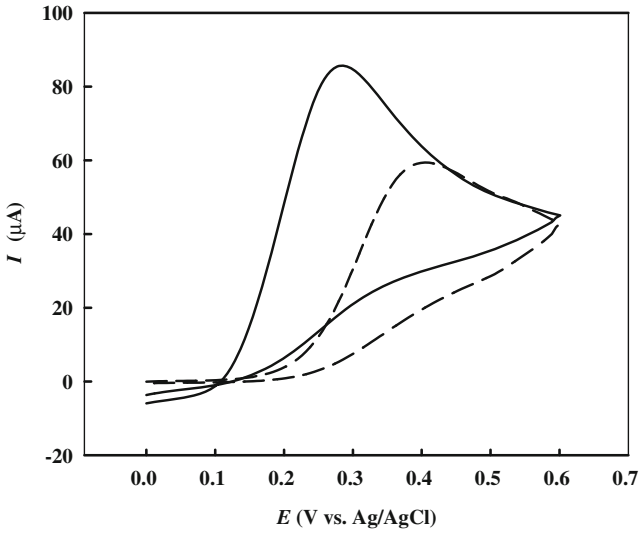
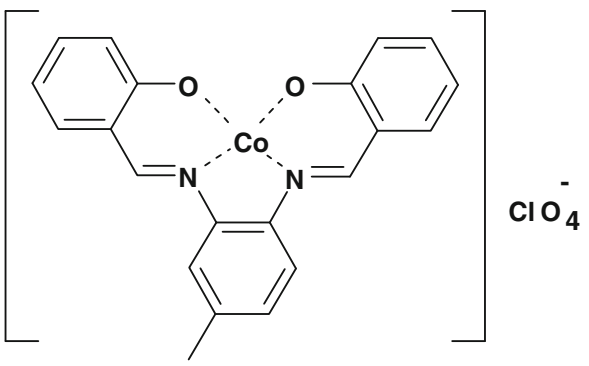


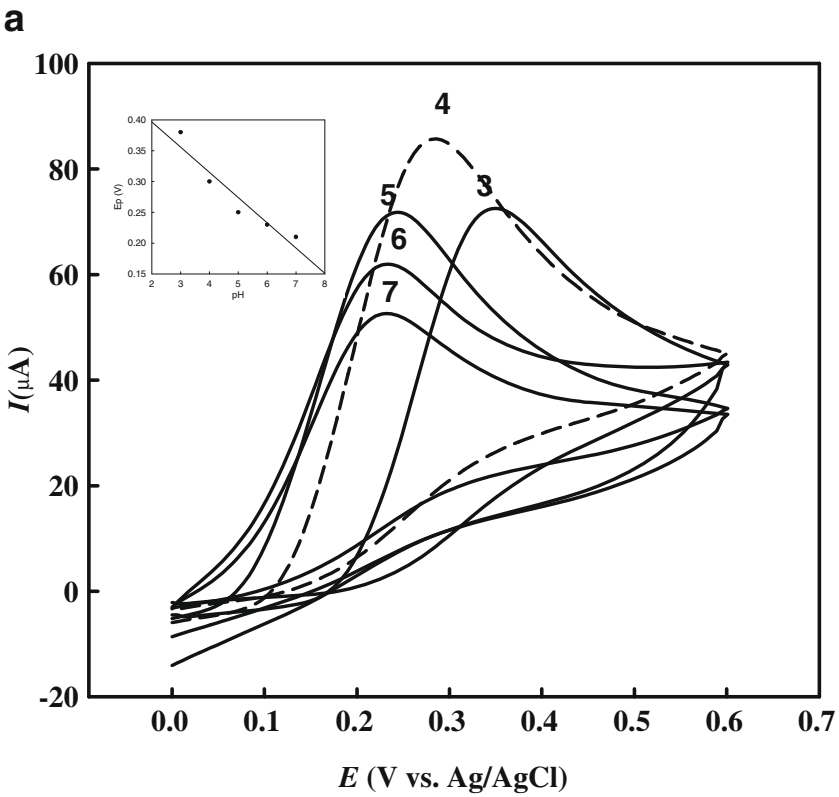
Fig. 1 Cyclic voltammograms for 1.0 mM AA at MCPE (*solid line*) and UCPE (*dashed line*) in 0.1 M acetate buffer solution pH 4.0; sweep rate was 100 mV s^{-1}

The influence of pH on the catalytic oxidation of ascorbic acid was investigated in the range 3–7. The results indicated that the anodic peak potential, $E_{p,a}$, shifts to more negative values with increasing pH. Such a behavior suggests that the acidic dissociation of ascorbic acid occurs at or before the rate-determining step [3]. Figure 2a shows the pH dependency of oxidation potential of AA. Mechanism of AA oxidation exhibited in Scheme 2.

The cyclic voltammograms were recorded for 1 mM AA at the surface of the MCPE in buffered solution of pH 4.0 at different potential sweep rates in the range of



Scheme 1 The structures of cobalt Schiff base complex



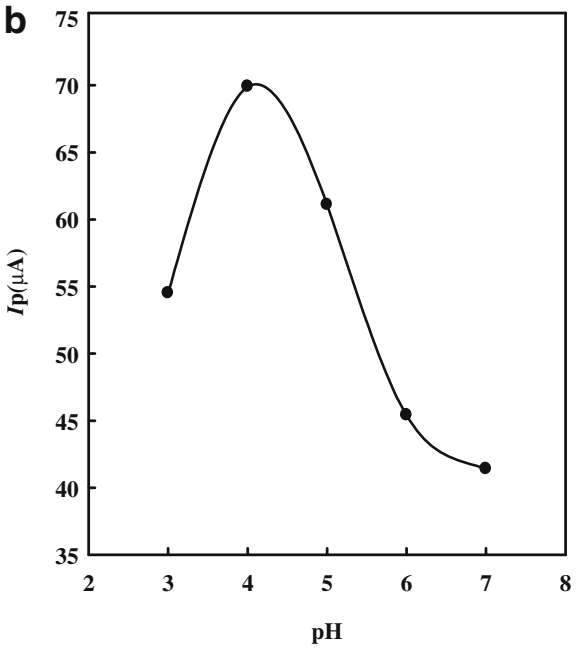
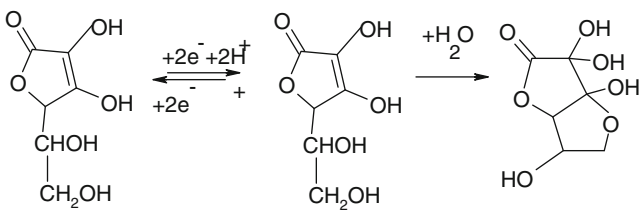


Fig. 2 **a** Cyclic voltammograms of 1.0 mM AA solution at MCPE in various pHs (3–7). Sweep rate was 100 mV s^{-1}. *Inset* variation of anodic peak potential vs. pH. **b** Variation of the anodic peak current with pH for 1 mM AA solution at MCPE. Sweep rate was 100 mV s^{-1}



Scheme 2 The mechanism of AA oxidation

20–200 mV s^{-1}. No cathodic peak is observed on the reverse scan in various potential sweep rates. Such a behavior confirms a catalytic EC mechanism, which coupled irreversible chemical reaction hindered to the electron transfer step. The oxidation peak current exhibited a linear relation to

the square root of the scan rate, with the following linear equation (Fig. 3):

$$i_{p,a}/A = 5 \times 10^{-6} v^{1/2}/\left(\text{mV s}^{-1}\right)^{1/2} + 7 \times 10^{-6} \left(r^2 = 0.9959\right) \quad (1)$$

The result indicates that the oxidation of AA at the modified electrode is a diffusion-controlled process.

Electrochemical oxidation of UA at the surface of UCPE and MCPE

Electrocatalytic oxidation of 1 mM UA was investigated at the surface of UCPE (dotted line) and MCPE (solid line; Fig. 4). It exhibits at the surface of MCPE, a sharp well-defined voltammogram, and remarkable current increase was obtained for UA electrooxidation. The mechanism of UA oxidation was shown in Scheme 3 [5].

Investigations in various pHs between 3 and 7 (Fig. 5) shows 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 (Scheme 3) [3].

The scan rate is systematically varied in the range of 20–200 mV s^{-1} (Fig. 6). The obtained results were similar to AA and confirm diffusion-controlled mechanism for UA.

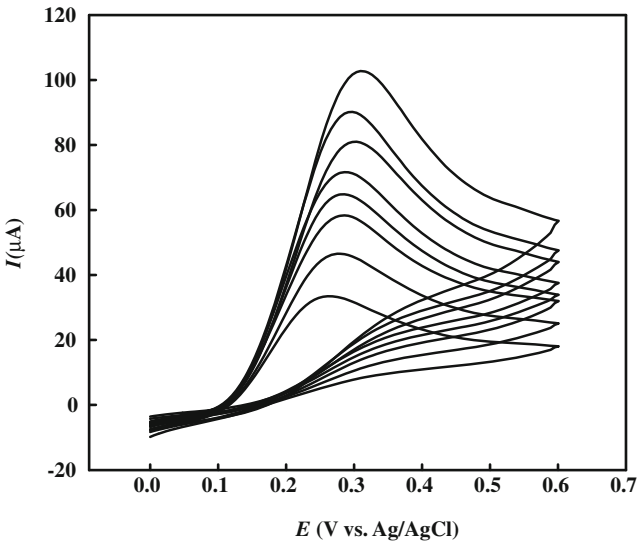


Fig. 3 Cyclic voltammograms of MCPE for 1.0 mM AA at various scan rates in 0.1 M acetate buffer solution pH 4.0. *Inset* variation of the anodic peak current with square root of the sweep rate

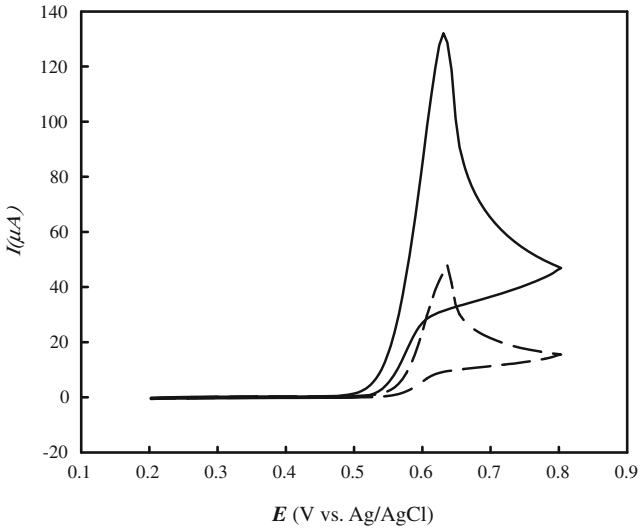


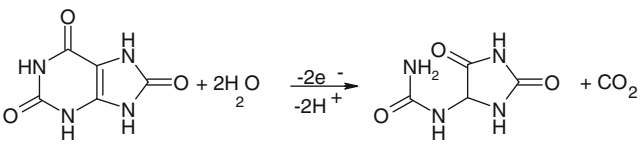
Fig. 4 Cyclic voltammograms for 1.0 mM UA at MCPE (*solid line*) and UCPE (*dashed line*) in 0.1 M acetate buffer solution pH 4.0. Sweep rate was 100 mV s^{-1}

The following equation shows the linear relation between square root of scan rate and peak current:

$$i_{p,a}/A = 3 \times 10^{-4} v^{1/2}/\left(\text{mV s}^{-1}\right)^{1/2} + 10^{-5} \qquad \left(r^2 = 0.9976\right) \quad (2)$$

Polarization measurements

In the elucidation of the mechanism and rate-determining step in a multi-step reaction, the Tafel plot and its slope play a prominent role. The results of polarization studies for the electrooxidation of AA and UA were obtained in two ranges of potential sweep rate. The plots show Tafel slopes equal to 0.99–0.91 mV s^{-1} for AA and 0.89–0.99 mV s^{-1} for UA. Considering a value of about 0.5 for α in these symmetric waves, this could indicate that a two-electron transfer process is the rate-determining step. Results of the previous studies on the catalytic effect of complexes of cobalt in the electrochemical oxidation of thiols have been shown that Co is capable to catalyze the thiol oxidation in both +2 and +3 oxidation states. The results showed that in acidic media, it is more probable that the oxidation process is caused by catalytic effect of Co (III)/Co(II) redox system as a mediator couple [12].



Scheme 3 The mechanism of UA oxidation

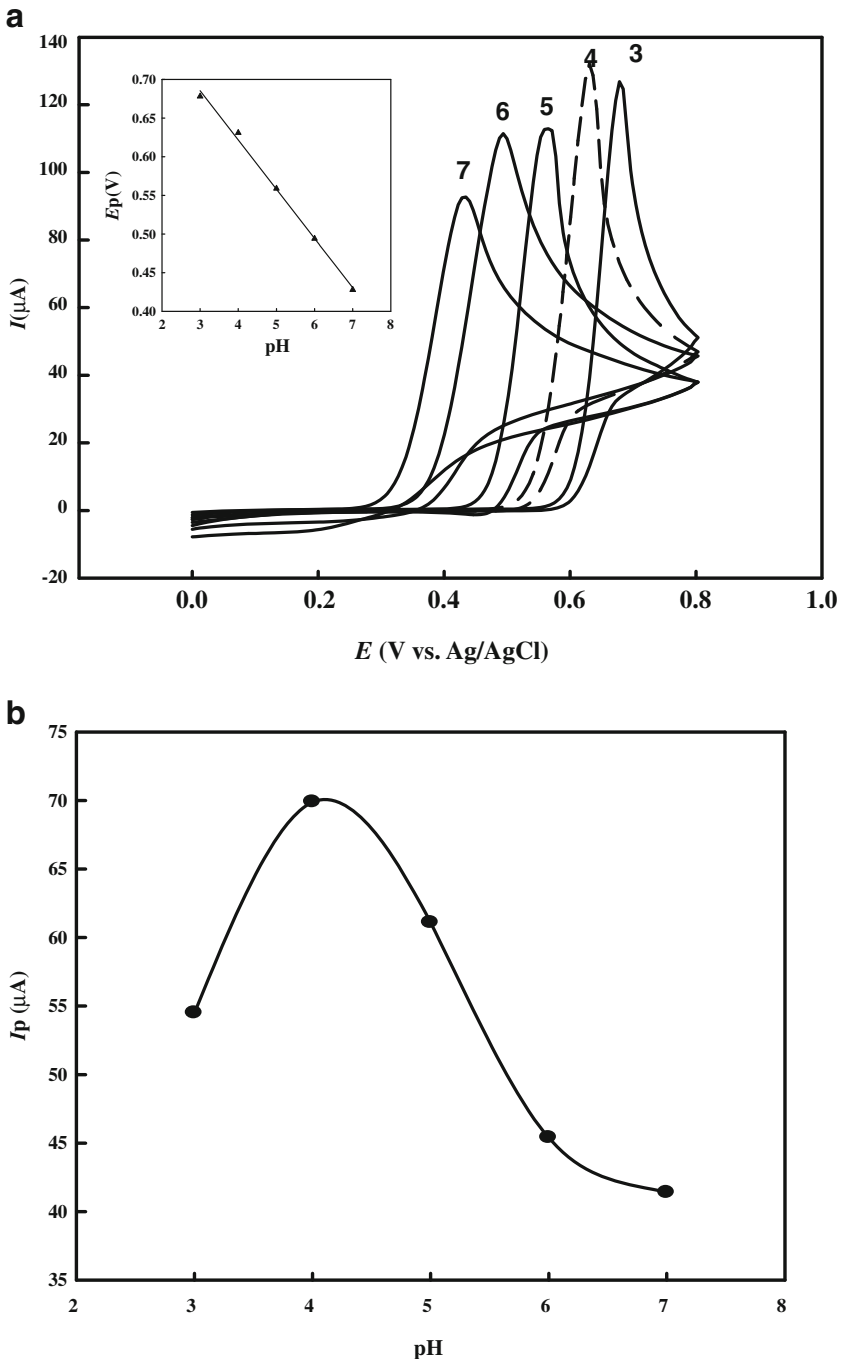


Fig. 5 Cyclic voltammograms of 1.0 mM UA solution at MCPE in various pHs. Scan rate was 100 mV s^{-1}. *Inset* variation of the anodic peak current (**a**) and anodic peak potential (**b**) with pH for 1 mM UA solution at MCPE at scan rate of 100 mV s^{-1}

Separating voltammetric peaks of binary mixture (AA–UA) at the surface of MCPE

Differential pulse voltammograms (DPVs) recorded for the oxidation of mixture of 1 mM UA and AA in 0.1 M acetate buffer of pH 4.0 at the surface UCPE (dotted line) and MCPE (solid line) are shown in Fig. 7. As can be seen, the electrooxidation of AA and UA at UCPE are sluggish, and there is no suitable peak separation between them. On the other hand, using MCPE, two well-defined anodic waves with a considerable enhancement in the peak current and a remarkably peak potential separation near 315 mV are obtained. Such a large enhancement of anodic currents revealed that by lowering the anodic overpotential of the electrode process, the kinetics of electron transfer for both AA and UA improves remarkably at the MCPE. All these improvement are created because of the electrocatalytic property of cobalt Schiff base complex. Cobalt compounds have been widely used for many years as a mediator for reducing the overpotential of the electrochemical oxidation of many compounds [12].

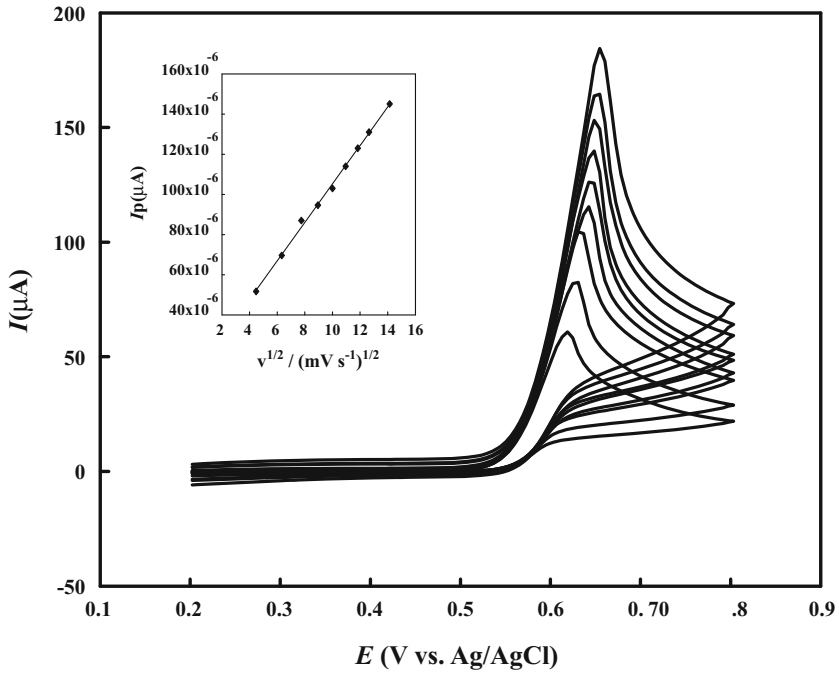


Fig. 6 Cyclic voltammograms of MCPE for 1.0 mM UA at various scan rates from inner to outer 20–200 mV s^{-1} in 0.1 M acetate buffer solution pH 4.0. *Inset* variation of the anodic peak current with square root of the sweep rate

The cobalt Schiff base complex acts as electron mediator for passing electron from UA and AA to electrode surface. The mechanism of electron transfer using Me-Cosal at the surface of MCPE is exhibited in Scheme 4.

The voltammetric investigations of variations of peak current with respect to pH of the electrolyte in the pH range from 3.0 to 7.0 in a solution containing 1 mM AA and UA using MCPE were performed. All the anodic peak currents for the oxidation of AA and UA shifted toward more negative potential with an increase in pH, revealing that protons have taken part in their electrode reaction processes. The best peak separation with appropriate current obtained for pH 4.0.

The cyclic voltammetric studies for 1 mM AA and UA were performed on the surface of the MCPE in buffered solution of pH 4.0 at different potential sweep rates in the range of 20–200 mV s^{-1}. The best peak separation with appropriate current was obtained for 20 mV s^{-1}.

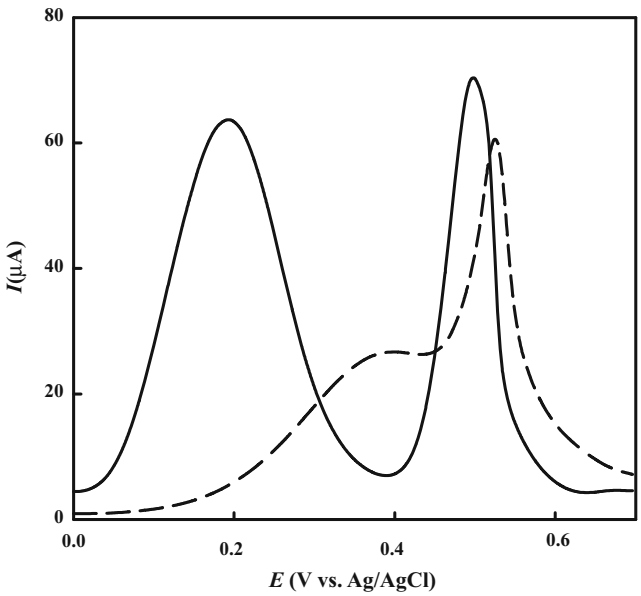
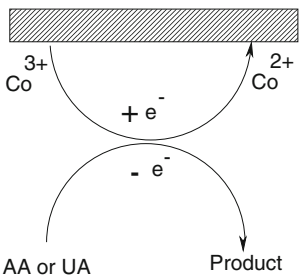


Fig. 7 Differential pulse voltammograms for 1.0 mM UA and AA at MCPE (*solid line*) and UCPE (*dashed line*) in 0.1 M acetate buffer solution pH 4.0; Scan rate 50 mV s^{-1}; pulse amplitude 50 mV; step potential 5 mV

Analytical measurements

Figure 8a shows the DPVs for solutions containing a constant background of 1.0×10^{-4} M of AA and different concentrations of UA in buffered solutions with pH 4.0. These results for solutions with a constant background of UA and different concentrations of AA (in a reverse manner) are presented in Fig. 8b. The results clearly express a high

Scheme 4 The mechanism of electron transfer using cobalt salophen at the surface of MCPE



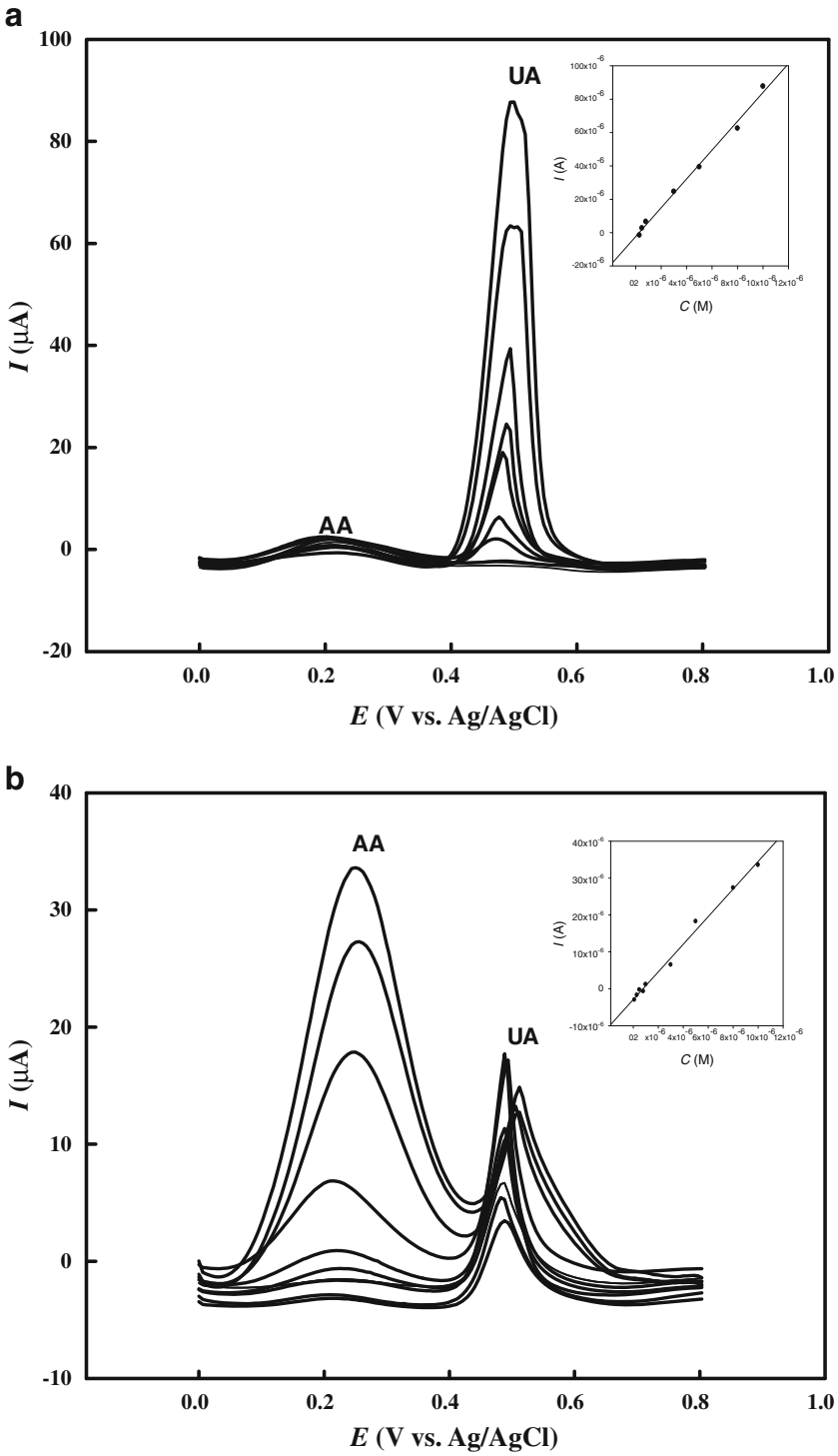


Fig.8 **a** DPVs of UA and AA at modified electrode in 0.1 M phosphate buffer solution (pH 4.0), constant [AA]= 0.1 mM and different [UA]= 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 mM. The *inset* shows the relationship between the anodic peak current and the concentration of UA. **b** Constant [UA]=0.1 mM and different [AA]=0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 mM. The *inset* shows the relationship between the anodic peak current and the concentration of AA. Scan rate 50 mV s^{-1}; pulse amplitude 50 mV; step potential 5 mV

efficiency for the prepared modified electrode for simultaneous determination of AA and UA in pharmaceutical and clinical preparations.

A linear range of 5.0×10^{-4} to 1.0×10^{-8} and 1.0×10^{-3} to 1.0×10^{-8} M with a slope of 0.045 μA/μM and 0.168 μA/μM with a correlation coefficient (R^2) of 0.997 and 0.994 was obtained for AA and UA using differential pulse voltammetry, respectively. Detection limit for both compounds is estimated 8.0×10^{-9} M (for S/N=3). The repeatability of the modified electrode was investigated in the presence of 1 mM UA by voltammetric measurements. The electrode showed an acceptable repeatability with a relative standard deviation (RSD) of 2.9% for three successive assays.

The day-to-day stability of the electrode was monitored for 2 months. After 2 months of storing in air, the electrode retained 96.8% of its initial peak current response for mixture solutions. The results indicated that only 3.2% peak current was decreased which shows the long-term stability of the CP modified electrode during the electrochemical determinations in aqueous samples. The easily renewable nature of the electrode and the fact that the response of the renewed surface is identical to the original electrode make this CPE attractive for electroanalytical applications and hence can be used in routine analysis. The detection system is very stable and the RSD (in percent) for the slope variation, based on five measurements during 2 months, was less than ~3.9%. The modified electrode was used for detection of AA and UA in real samples. Analytical utility of the modified electrode has been examined using human urine samples and vitamin C commercial tablets.

Human urine is diluted 100 times in acetate buffer of pH 4.0 and subjected to electrochemical analysis. The recovery equal to 96.60% was calculated by comparing the slope of calibration curve and standard addition.

Tablet samples of vitamin C (with labeled value of 250 mg AA per tablet) were powdered, and an aliquot equal to 5.0×10^{-6} M of vitamin C was prepared in 0.1 M acetate buffer with pH 4.0. The standard addition method was applied for drawing the calibration curves of current versus AA concentration and also determination of recovery in spiking of AA to the pharmaceutical samples. The accuracy of the method for AA, based on the labeled value, was 96.30% for tablet samples. The precision of method for AA determination in drug samples, based on the five replicates of analysis, was between 3.8%.

The characteristics of other sensors for this purpose are summarized in Table 1. Compared with the other modified electrodes, our sensor features a lower detection limit and lower oxidative potential.

Conclusions

The present study demonstrates an excellent approach for the development of a voltammetric UA sensor modified with methyl cobalt salophen. Fast electron transfer, excellent sensitivity, reproducibility, easy preparation of electrode, and antifouling properties can separate oxidation peaks toward UA and AA, which are indistinguishable at the bare electrode. As the voltammetric signals of UA and AA are well distinguished at the modified electrode, the simultaneous sensitive detection of UA and AA can be achieved.

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