

Modification of carbon paste with congo red supported on multi-walled carbon nanotube for voltammetric determination of uric acid in the presence of ascorbic acid

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Abstract A chemically modified carbon-paste electrode (CPE) is prepared by incorporating congo red (CR) immobilized on multi-walled carbon nanotube (MWCNT). The results show that CR is effectively immobilized on the surface of MWCNT under the ultrasonic agitation in aqueous solution and further incorporating the nafion. The prepared electrode, due to the electrostatic repulsions between the CR and ascorbate anion, is capable to mask the response of the ascorbic acid (AA) completely and provide an effective method for the detection of minor amounts of uric acid (UA) in the presence of high concentrations of AA. On the other hand, an increase in the microscopic area of the electrode by addition of MWCNT together with the electrocatalytic activity caused to a significant enhancement in the voltammetric response to UA. Optimization of the amounts of composite modifier in the matrix of CPE is performed by cyclic and differential pulse voltammetric measurements. The modified electrode shows a linear response to UA in the range of 1.0×10^{-7} – 1.0×10^{-4} M with a detection limit of 1.0×10^{-8} M. The electrode exhibits excellent accuracies for the determination of UA in the presence of high concentrations of AA (a

recovery of 97.6%). The response of the electrode toward sulfhydryl compounds such as cysteine, penicillamine, and glutathione is not considerable. This reveals a good selectivity for the voltammetric response toward UA. The effective electrocatalytic property, ability for masking the voltammetric responses of the other biologically reducing agents, ease of preparation, and surface regeneration by simple polishing together with high reproducibility and stability of the responses make the modified electrode suitable for the selective and sensitive voltammetric detection of sub-micromolar amounts of UA in clinical and pharmaceutical preparations.

Keywords Carbon-paste electrode · Chemically modified electrode · Congo red · Multi-walled carbon nanotube · Uric acid · Ascorbic acid

Introduction

Carbon nanotubes (CNTs) have been the subject of intense research since their discovery in 1991 [1] because of their high aspect ratio, electrical conductivity, chemical stability, and extremely high mechanical strength and Young's modulus [2, 3]. These remarkable mechanical and electrical properties of CNTs endow them with a wide range of potential applications, including their use as probe tips for scanning probe microscopy [4, 5], materials of the electrode in batteries [6], field-effect transistors [7], molecular memory devices [8], etc. In addition, the ability of CNTs to promote the electron transfer in the electrochemical reactions of important molecules such as cytochrome *c* [9], ascorbic acid [10–12], neurotransmitters [13], NADH [14, 15], and proteins [16–18] has made them attractive for the construction of various electrochemical biosensors. Elec-

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trochemical sensors based on CNTs represent a new and interesting alternative for the quantification of different analytes [19–23].

However, as a result of the strong inter-tube interactions, CNTs generally existed as highly tangled ropes and were insoluble in all solvents, which greatly hindered their promising practical applications [23]. To overcome this limitation, CNTs were either treated with acids [23–25] or heated in air [26, 27] to produce hydroxyl, carboxyl, and carbonyl functional groups at both the sidewall and the terminus and to exfoliate them into small bundles or individual nanotubes. The resulting CNTs can be dispersed in organic solvents [25, 27], concentrated sulfuric acid [23], polymers [28], or the suspension of poorly soluble surfactants [29]. The selection of dispersion strategy is on the basis of the interactions between the solvents and CNTs [23, 25, 27] or the wrapping of CNTs by polymers [28] or surfactant aggregates [29]. The CNTs' suspensions were then cast on the electrode surfaces to form various electrochemical sensors [30–32]. However, previous dispersing methods of CNTs usually suffered from some inevitable disadvantages due to the weak interactions between CNTs and the dispersing agents, such as low loadings and poor stability (e.g., surfactants or solvents dispersed CNTs), low exfoliation (e.g., polymers wrapped CNTs), and the presence of free additives, which greatly limited their applications in practice, especially for CNTs. Due to the smaller size, the larger specific area, the stronger inter-tube attractions, and the composition of only one layer of carbon atoms for CNTs, the dispersing methods of multi-walled carbon nanotubes (MWCNTs) that were widely used in electroanalytical chemistry were usually unsuitable for CNTs [33]. For instance, the treatments of MWCNTs with mixed acids or concentrated nitric acid can effectively produce functional groups on the outer sidewalls of MWCNTs and exfoliate them into individual tubes while preserving their inherent structures because of the retaining of the inner sidewalls, whereas the treatments of CNTs by similar procedures cannot be precisely controlled as a long period of treatment might seriously destruct their structures, and a short period of treatment would produce low solubility and exfoliation efficiency [33]. This contradiction might account for the rare applications of CNTs in the electroanalytical chemistry. Thus, the development of new dispersing methods with high solubility and exfoliation efficiency that can be directly employed in electroanalytical chemistry has become great challenges. As expected, the starting CNTs are assembled into bundles or ropes that contain numerous well-aligned CNTs, and the long ropes further entangle into networks, resulting in their insolubility in all solvents. Obviously, some irregular impurities are strongly attached to the sidewall of CNTs ropes and cannot be removed even for a long period of sonication in water.

With the noncovalent functionalization by a small planar and conjugated diazo dye such as CR, CNTs are readily dissolved in water with high solubility and efficiently exfoliated into individual nanotubes [34]. In addition, most of the irregular impurities on the sidewall of CNTs are removed, indicating the cleaning role of adsorbed CR. Another interesting feature of CR decorated CNTs is that one of the individual nanotube is bended to nearly a right angle at the middle part and tightly bound with another individual tube at the terminus via sidewall coupling, foreseeing the ability of water-soluble CNTs to form stable and complicated network nanostructures. In fact, by introducing into the matrix of electrode and air-drying, water-soluble CNTs form uniform films on the electrode surface, which possess three dimensional network structures with plenty of interstices inside the films. In contrast to the additive-containing CNTs-based sensors, e.g., the nafion-containing CNTs modified electrode [28], these porous network structures allow the entry of various molecules with a wide range of sizes inside the films and uses all active sites in the CNTs films. The stability of the water-soluble CNTs films was examined. Due to the strong attachment of CR onto the sidewall of CNTs and the chemical inertia of CR under ambient conditions, these films had excellent stability in air or water. When used in electrochemical measurements, the water-soluble CNTs films remained stable in neutral and acidic media but would peel off from the electrode surface if used in basic solutions.

Recently, Hu et al. have reported a new noncovalent approach for preparing highly water-soluble carbon nanotubes by CR [35]. By using a simple physical grinding treatment, the mixture of HNO₃ purified CNTs and CR was readily dissolved in water with high solubility (~3.5 mg/mL). The complete elimination of free CR from the mixture hardly changed this excellent solubility. The strong π -stacking interaction between adsorbed CR and the sidewall of CNTs was ascertained, which was regarded to be responsible for the high solubility of CNTs in water.

Uric acid (UA) and other oxypurines are the principal final products of purine metabolism in the human body [36]. Abnormal levels of UA causes symptoms of several diseases, including gout, hyperuricemia, and Lesch–Nyan disease [37]. However, UA and ascorbic acid (AA) are present in biological fluids such as blood and urine. It is important to detect them selectively and conveniently in routine analysis. Several electrochemical procedures have been developed to determine them based on their electrochemical activities. However, the direct electro-oxidation of UA and AA at bare electrodes requires high overpotentials [38]. On the other hand, these compounds can be oxidized at very similar potentials, which results in rather poor selectivity. In the present work, noncovalent immobilization

of CR on MWCNT (via ultrasonic agitation in aqueous solutions) and its further incorporation within a nafion matrix is described. The prepared composite is used for the modification of carbon-paste electrode (CPE). The modified electrode is applied as a selective and sensitive voltammetric sensor for the detection of UA in the presence of great amounts of AA.

Experimental

Chemicals

Multi-walled carbon nanotubes (purity more than 95%) with O.D. between 10 and 20 nm, I.D. between 5 and 10 nm, and tube length from 0.5 to 200 nm was prepared from Nanostructured & Amorphous Materials (Houston, TX, USA). Graphite powder (<20 μm) and spectroscopic mineral oil (Nujol) for the preparation of conventional CPE were purchased from Aldrich (St. Louis, MO, USA). A 5% nafion solution (Aldrich, EW, 1100, dissolved in a lower aliphatic alcohol and water) was used for a better dispersing of MWCNT-supported CR and immobilization in the matrix of CPE. AA, UA, and CR were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). All aqueous solutions were prepared with doubly distilled deionized water.

Stock solutions of AA and UA were freshly prepared as required in 0.10 M of appropriate buffer solution. In these experiments, 0.1 M acetate was used for pH values 4 and 5, and 0.1 M phosphate for 6 and 7. The prepared solutions were purged with pure nitrogen gas (99.999%) for 2 min before the voltammetric measurements. Voltammetric experiments were carried out in the buffered solutions deoxygenated by purging the pure nitrogen (99.999% from Roham Gas Company, Tehran, Iran). During the experiments, nitrogen gas was passed over the surface of the test solutions in order to avoid entering oxygen into the solution.

Apparatus

Voltammetric experiments were performed with a Metrohm Computrace Voltammetric Analyzer model 757VA (Herisau, Switzerland). 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 CyberScan model 2500 (Eutech Inst., Pte, Ayer Rajah Crescent, Singapore) was applied for preparing the buffer solutions, which were used as the supporting electrolyte in the voltammetric experiments.

Pretreatment of MWCNT and immobilization of CR

In order to remove the probable amorphous carbons and catalytic impurities, 500 mg of the prepared MWNT was heated in an oven with 400 °C in nitrogen atmosphere for 2 h. The heat-processed MWNT was dispersed in 50 mL of 6.0 M HCl for 2 h under ultrasonic agitation under the nitrogen atmosphere, filtered on a Wattman 42 filter paper, and washed with doubly distilled water until the pH of the solution was neutral. The MWNT was dried under the IR lamp. In order to immobilize CR on the surface of MWCNT, a 200-mg portion of pretreated nanotube was dispersed in a 5-mL doubly distilled deionized water containing 20 mg CR and sonicated for 2 h. The prepared suspension was filtered on a Wattman 42 filter paper and washed thoroughly with doubly distilled water. The mixture was dried under the IR lamp.

Preparation of the modified electrode

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 wt/wt.%). A portion of the composite mixture was packed into the end of a Teflon tube (ca. 2.5 mm i.d.). Electrical contact was made by forcing a copper pin down into the Teflon and into the back of the composite. To prepare the modified electrode, a portion of 2.0 mL of nafion solution (5%) was added to an appropriate amount of the MWCNT-supported CR and hand-mixed in a mortar and pestle. Then, an appropriate amount of this composite (MWCNT-supported CR/nafion; in a ratio of 10% w/w) was transferred to 2.00 mL of dichloromethane and mixed thoroughly with unmodified carbon paste. The solvent was evaporated at room temperature for 24 h. A portion of the composite mixture was packed into the end of a Teflon tube (ca. 2.5 mm i.d.) and was used as MWCNT-supported CR/nafion carbon-paste electrode (CNTCRNCPPE). MWCNT-supported CR carbon-paste electrode (CNTCRCPE) was prepared by mixing an appropriate amount of the MWCNT-supported CR with unmodified carbon paste and 2.00 mL of dichloromethane and was then dried, and a portion of the composite mixture was packed into the end of a Teflon tube (ca. 2.5 mm i.d.). The MWCNT modified carbon-paste electrode (CNTCPE) was prepared by mixing the unmodified mixture with MWCNT (10% w/w), and the resulting composite (for better homogeneity and reproducibility by polishing the electrode surface) was dissolved in dichloromethane. The MWCNT/nafion modified carbon-paste electrode (CNTNCPPE) was prepared by mixing an appropriate amount of nafion solution with MWCNT composite. Then, the solvent was evaporated at room temperature for 24 h and was packed into the end of

a Teflon tube. Table 1 represents the various prepared paste electrodes together with the response properties toward AA and UA.

Result and discussion

Cyclic and differential pulse voltammetric studies

The potential application of carbon-paste electrodes containing MWCNT as electrochemical sensing films was examined. First, cyclic and differential pulse voltammograms for the oxidation of UA and AA in 0.1 M acetate buffer (pH 5) at unmodified carbon-paste electrode (A) were studied. As illustrated in Fig. 1a, the cyclic voltammograms of AA and UA show totally irreversible processes. The anodic peak potential of AA and UA was 0.340 and 0.398 V, respectively. These results show a significant overlapping of the voltammetric responses of AA and UA. The anodic peak current for the oxidation of 1.0 mM AA and UA at a scan rate of 100 mV s^{-1} was 31.2 and 44.6 μA , respectively. As can be seen in Fig. 1b, the differential pulse voltammograms of the anodic oxidation of AA and UA at unmodified carbon-paste electrode also shows a significant problem for the determinations of these compounds in mixture samples. However, the voltammetric responses for these biomolecules are very different at the prepared modified carbon-paste electrodes. The results of cyclic voltammetric studies of solutions containing 1 mM AA and 1 mM UA in 0.1 M acetate buffer solution with pH 5.0 on the surface of six different electrodes are summarized in Table 1. The results revealed that the presence of MWCNT in the matrix of carbon-paste electrode (B) caused a decrease in the peak potential of AA and considerable enhancement in the corresponding peak current. On the other hand, by the presence of MWCNT/CR composite in the matrix of carbon-paste electrode (D), the peak potential of AA shifted to more positive values, and the

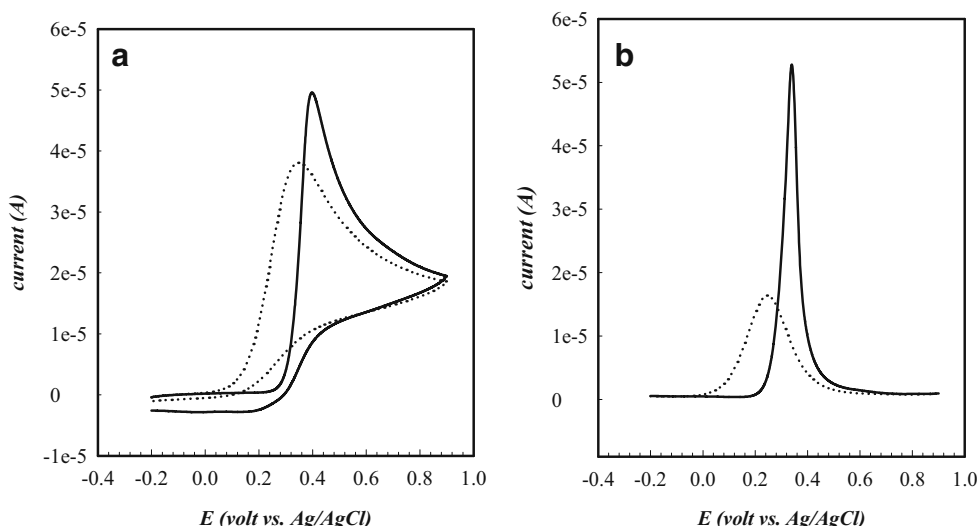
peak current decreases until it reaches to the background current. In fact, AA with a $\text{p}K_{\text{a}}$ of 4.17 exists mainly as anionic form in the experimental conditions (pH 5.0) and therefore has an electrostatic repulsion with anionic sites (from CR) at the surface of the electrode. The oxidation of AA at UCPE shows a peak current of 31.2 μA with the peak potential of 340 mV. By using the electrode B, a decrease of 50 mV was resulted in the anodic peak potential of AA, and the oxidation peak current was 54.5 μA , which shows an increase of 77%. The reason for the higher peak current may originate in the large surface area created by the presence of MWCNT and enhancement of the kinetics of the electron transfer process on the surface of CNT-modified electrode. Such improvements in the electrochemical responses of the electrodes by the presence of various forms of CNT are reported for some biologically important compounds [39–41]. A better catalytic effect of MWCNT in the electro-oxidation of AA is seen in the presence of nafion in the matrix of carbon paste containing MWCNT. The anodic oxidation peak of AA at the electrode C appeared in 286 mV, and the current of this peak reached to 68.3 μA . In fact, nafion causes a better dispersion of MWCNT in the matrix of carbon-paste electrode and enhances the effect of MWCNT on the response of electrode toward AA. On the other hand, by the presence of the MWCNT-supported CR in the matrix of electrode (D), the anodic oxidation peak of AA is broadened, and the oxidation peak current is decreased to 7.2 μA . By dispersion of MWCNT-supported CR in nafion solution, the matrix of electrode became much uniform, and MWCNT-supported CR appeared more effective for the elimination of AA response. The anodic oxidation peak of AA at carbon-paste electrode containing 5% of MWCNT/CR/nafion (E) was very weak and appeared in ~ 318 mV. The carbon-paste electrode containing 10% MWCNT/CR/nafion (F) is not sensitive to AA, and the oxidation peak current of this compound comes to the capacitive background current. The reason of this behavior can be described based on the electrostatic repulsion of AA in anionic form (in pH 5) with the negative groups of CR supported on MWCNT in the matrix of electrode. Voltammetric studies using four different electrodes, A, B, D, and E, in buffered solution (blank) showed that in the entire potential range, no considerable peak can be seen. Only on the surface of the paste electrodes containing MWCNT (D and E) is a weak anodic peak seen about 0.2 V. This peak can be assigned to the oxidation of hydroxyl groups in the structure of nanotube, which are produced during the acid and heat treatment (Fig. 2).

A better description for the response of the prepared modified electrodes can be seen using the differential pulse voltammetric (DPV) experiments. The DPVs for 1 mM AA in 0.1 M acetate buffer (pH 5) at different modified carbon-paste electrodes are shown in Fig. 3a. The anodic peak current of AA at the surface of A, B, and C was 15.8, 25.5,

Table 1 Cyclic voltammetric response properties for AA and UA on the surface of different electrodes

Electrodes	Compound			
	AA		UA	
	$I_{\text{p,a}}$ (μA)	$E_{\text{p,a}}$ (mV)	$I_{\text{p,a}}$ (μA)	$E_{\text{p,a}}$ (mV)
UCPE (A)	31.2	340	44.6	398
CNT-CPE (B)	54.5	291	94.5	361
CNT-N-CPE (C)	68.3	286	110	350
CNT-CR-CPE (D)	7.2	291	39	393
CNT-CR-N-CPE (5%, E)	9.2	318	160	352
CNT-CR-N-CPE (10%, F)	2.3	275	162	350

Fig. 1 **a** Cyclic and **b** differential pulse voltammograms of 1 mM AA (.....) and 1 mM UA (—) at the surface of unmodified carbon-paste electrodes. Supporting electrolyte was 0.1 M acetate buffer solution with pH 5.0, and sweep rate was 100 mV s⁻¹



and 25.3 μA in peak potentials of 243, 201, and 190 mV, respectively. However, the electrode D shows a broad and relatively weak oxidation peak for AA with the anodic oxidation current 3.3 μA. The anodic peak current of AA shows a significant decrease at E and completely has destroyed and reached to the capacitive background current at the electrode F. Therefore, the modified carbon-paste electrode containing 10% of MWCNT/CR–nafion was selected for the determination of UA in the presence of high concentrations of AA.

Results of previous works have shown that UA (with pK_{a,1}=5.75) in solutions of pH≤6 mainly exists as a neutral form. Therefore, UA in its neutral form (in 0.1 M acetate solution buffer with pH 5.0) does not show any electrostatic interaction with the negatively charged site on the surface of electrode. As can be seen in Table 1, the anodic peak for UA has appeared on the surface of A with a peak current of 44.6 μA and peak potential of 398 mV. By applying the electrode B, the peak current was enhanced to 94.5 μA (an

increase of 111%), and the peak potential was decreased to 361 mV. These results show a slight catalytic effect for CNT on the electro-oxidation of UA. Oxidation of UA at the electrode C occurred at 350 mV with the anodic oxidation peak current similar to that of the B. This compound was oxidized at 393 mV at the surface of D with an anodic oxidation current same as A. By dispersion of MWCNT/CR in nafion, the oxidation peak of UA at the E appeared at 352 mV by a peak current of 160 μA. As previously described, UA in solutions with pH 5 mainly exists as a neutral form and does not show any electrostatic interaction with the negatively charged surface of electrode. However, oxidation of this compound can be catalyzed by MWCNT or MWCNT-supported CR in the matrix of electrode.

The voltammetric response characteristics of the prepared modified electrodes toward UA can be seen in the DPV experiments. Figure 3b shows DPVs of 1 mM UA in 0.1 M acetate buffer (pH 5) obtained at unmodified and

Fig. 2 **a** Cyclic and **b** differential pulse voltammograms for different paste electrodes; A (—), B (.....), C (—), and E (—) in 0.1 M phosphate solution of pH 7.0

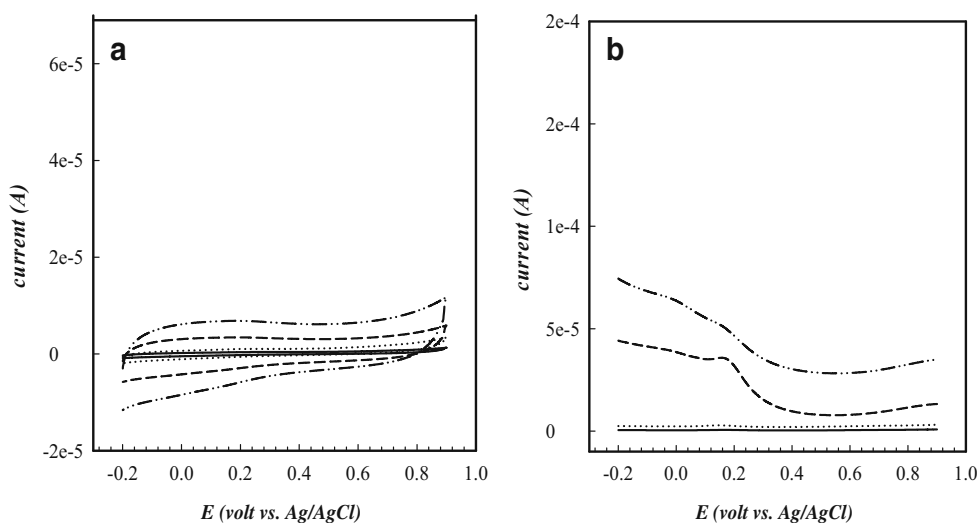
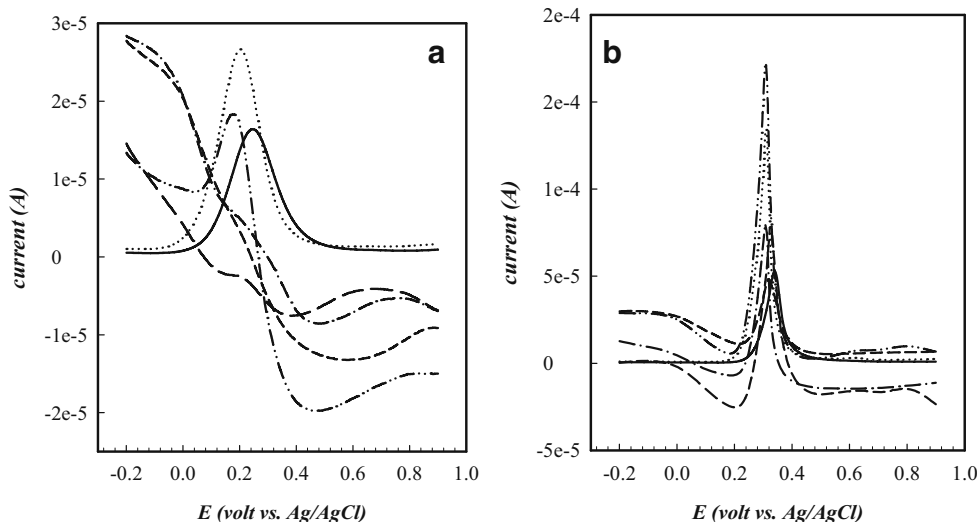


Fig. 3 **a** Differential pulse voltammograms of 1 mM AA at the surface of various electrodes; A (—), B (.....), C (---), D (---), E (---), and F (—). **b** Differential pulse voltammograms 1 mM UA at the surface of A (—), B (.....), C (—), D (---), E (---), and F (---). Supporting electrolyte in these experiments was 0.1 M acetate solution with pH 5.0, and pulse amplitude was 50 mV



different modified carbon-paste electrodes. As can be seen by modification of carbon-paste electrode with MWCNT/nafion, the electrocatalytic oxidation of UA caused to decrease the peak potential and enhancement of the anodic current for this biomolecule. The best electrochemical responses for UA can be obtained by dispersion of MWCNT/CR in nafion, at the surface of the electrode F. It can be concluded that the carbon-paste electrode modified with 10% of a composite of MWCNT/CR/nafion provides the most catalytic effect and microscopic area for the electro-oxidation of UA. Moreover, this electrode is capable to completely eliminate the voltammetric response of AA and provide an effective procedure for the determination of UA in mixture samples.

Effect of pH and potential sweep rate

In order to determine the optimum pH for the determination of UA in the presence of high concentrations of AA,

voltammetric studies were performed in various pH values of the buffered solutions. In these investigations, 0.1 M acetate was used for pH 4.0 and 5.0 and 0.1 M phosphate for 6.0 and 7.0. The results showed that the anodic peak for AA in solutions with pH 3.0 and 4.0 is large. On the other hand, this anodic peak in the solution with pH 5.0 becomes broad. By increasing the pH to 6.0 and higher, the anodic peak for AA is completely disappeared. In fact, AA with a pK_a of 4.17 exists mainly as neutral form in the solutions with $pH \leq 4.0$ and therefore has poor electrostatic interactions with the anionic sites on the surface of the electrode F. However, AA in the solutions with $pH \geq 5$ exists in the anionic form and has repulsive electrostatic interactions with anionic sites on the surface of the modified electrode (F). The anodic oxidation peak current of AA in solutions with pH 4.0, 5.0, and 6.0 is 27, 10, and 4 μA , respectively (Fig. 4), and in solution with pH 7.0, the anodic peak current comes to the background current. On the other hand, UA apparently shows an enhanced oxidation

Fig. 4 **a** Differential pulse voltammograms of 0.1 phosphate buffer solution (pH 7.0) containing various concentrations of UA: 0.2, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0 μM (downward to upward). Pulse amplitude in all measurements was 50 mV. **b** Linear calibration curves of anodic peak currents versus UA concentration in the range 0.1 to 100.0 μM

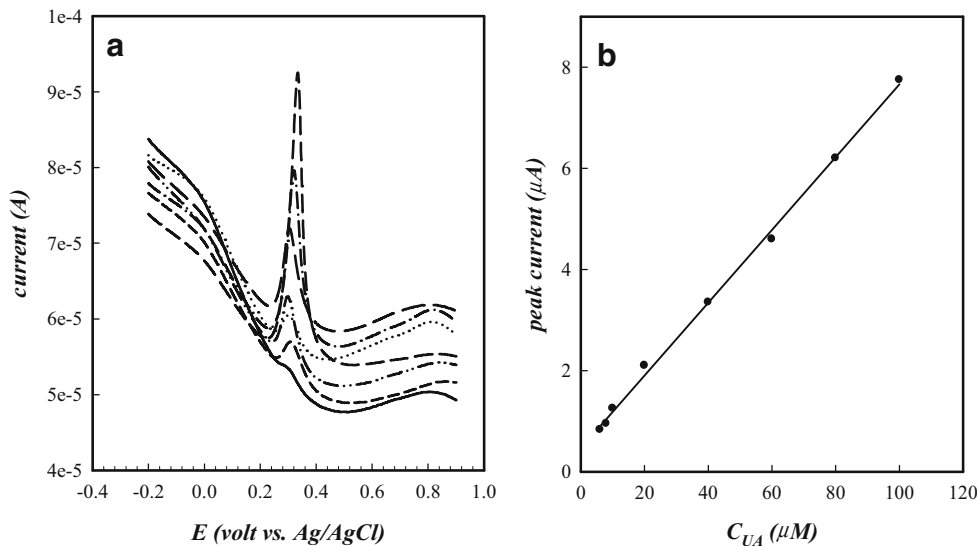
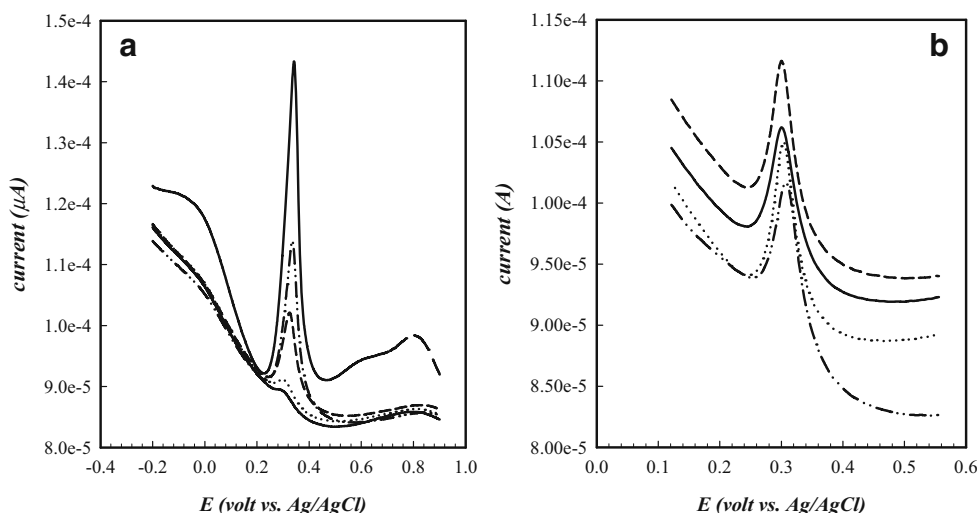


Fig. 5 Differential pulse voltammograms of **(a)** solutions containing 0.1 mM AA (constant) and various concentrations of UA: 0.2, 0.4, 2.0, 4.0, 10.0 μM (downward to upward); **b** solutions containing 0.01 mM UA (constant) and various concentrations of AA: 0.2, 0.4, 2.0, 4.0, 10.0 μM (downward to upward). Supporting electrolyte in these experiments was 0.1 M phosphate buffer (pH=7.0)



response at F in 0.1 M phosphate buffer (pH 7.0). Therefore, in order to measure UA in the presence of high concentrations of AA, a pH 7.0 was selected for measuring all solutions. The results of cyclic voltammetric studies in pH 5.0 for UA in various potential scan rates showed that the peak currents ($i_{p,a}$) vary linearly with the square root of scan rate ($v^{1/2}$). These results revealed that the anodic oxidation of UA at the surface of the electrode F is diffusion controlled.

Analytical characterization

The DPV method using electrode F was used as a very sensitive electrochemical method with sub-micromolar detection limits for the determination of UA in a wide concentration range. DPV peaks for UA were obtained in 0.1 M phosphate buffer (pH 7.0) as supporting electrolyte. Some typical DPV curves for these measurements are shown in Fig. 4a. The calibration curves of the anodic peak current versus the concentration exhibit a wide linear range of 1.0×10^{-7} – 1.0×10^{-4} M for UA (Fig. 4b). The slope of

the linear curve for UA was $0.0706 \mu\text{A}/\mu\text{M}$. The correlation coefficient (R^2) for the linear calibration curve of UA was 0.996. The voltammetric detection limit (based on 3σ) using the modified electrode F for UA was 1.0×10^{-8} M. The detection system was highly stable, and the relative standard deviation for the slope variations, based on the ten calibration measurements during 2 months, was 3.6% for UA.

One of the most remarkable advantages of the presented method was its low detection limit. Furthermore, the sulfhydryl compounds (e.g., cysteine, penicillamine, and glutathione) did not show any electrochemical reactivity on the surface of the electrode F, which improves the selectivity and accuracy of the measurements in clinical samples. Figure 5a displays DPV recordings of various concentrations of UA (from 2×10^{-7} to 10×10^{-6} M) in the presence of a constant concentration of 1×10^{-4} M of AA. A comparison between the slopes of the calibration curves of peak current versus UA concentration in the absence and presence of AA shows a correlation of 97.6%. These observations reveal excellent ability of the prepared sensor

Table 2 Recovery results for UA spiked to human urine and serum samples

Sample no.	Preparation of sample ^a	UA found (μM) ^b	Recovery (%)
1	Human urine	30.5 (± 0.5)	–
2	Sample 1+5.00 μM UA	36.0 (± 0.9)	110
3	Sample 1+10.0 μM UA	40.6 (± 1.2)	106
4	Sample 1+20.0 μM UA	49.7 (± 1.7)	96.0
5	Sample 1+50.0 μM UA	78.8 (± 2.1)	96.6
6	Human serum	3.93 (± 0.12)	–
7	Sample 6+2.00 μM UA	5.97 (± 0.14)	102
8	Sample 6+5.00 μM UA	8.98 (± 0.18)	101
9	Sample 6+8.00 μM UA	11.73 (± 0.25)	97.5
10	Sample 6+10.0 μM UA	13.63 (± 0.36)	97.0

^a Biological sample (1.00 mL) spiked with various amounts of UA and diluted to 10.0 mL with phosphate buffer solution (0.1 M, pH 7.0)

^b Concentrations are presented in primary samples, and values in parentheses are related to the SD based on five replicates

for masking the effect of AA in the voltammetric detection of UA. Due to the overlapping of the voltammetric signals and high relative concentration of AA, presence of the AA in biological samples is always considered as a serious interference for the determination of UA. Figure 5b shows DPVs of various AA concentrations (same as Fig. 5a for UA) in the presence of 1×10^{-5} M of UA at the surface of the electrode F. All measurements are performed in 0.1 M phosphate buffer (pH 7.0) as background electrolyte. The modified electrode in this study showed a remarkable selectivity for the determination of UA in the presence of high concentrations of AA, which is considerably better than previous reports. Our previous reports for the detection of UA using cobalt salophen-modified CPE incorporation tetra-*n*-octylammonium bromide showed the response of the electrode suffers from the interferences of sulfhydryl compounds [10].

The prepared modified electrode F was applied for obtaining the recovery results of the amounts of UA added to the human serum and urine samples. The biological preparations were diluted with phosphate buffer solution (0.1 M, pH=7.0) and spiked with UA. These results for various spiked amounts of UA in phosphate buffer as background electrolyte were shown in Table 2. Experimental conditions for these DPV measurements were similar as described for previous measurements in buffered solutions, which were near to physiological conditions. The excellent recovery results indicate that the complex matrix of the human serum and urine samples do not show a considerable interference with the detection of the minor amounts of UA.

Conclusions

In the present study, a composite of MWCNT-supported CR/naion is applied for the modification of CPE. The immobilization of CR on the surface of MWCNT together with the application of naion showed a very effective procedure and exhibits an acceptable reproducibility and long-time stability for the voltammetric responses of the electrode. The modified electrode in this work showed an effective and selective electrocatalytic activity for the anodic oxidation of UA and masking the effect of AA and sulfhydryl compounds, which is usually considered as interferences in most of the reported modified electrodes. Incorporation of CNT in the matrix of the modified composite enhances the microscopic area and currents in the voltammetric investigations. On the other hand, presence of CNT, due to π - π and electrostatic interactions, causes a better immobilization of CR in the matrix of the modified electrode. With the noncovalent functionalization by CR, CNTs are readily dissolved in water with high solubility and efficiently exfoliated into individual nano-

tubes. In addition, most of the irregular impurities on the sidewall of CNTs are removed, indicating the cleaning role of adsorbed CR. Another interesting feature of CR decorated CNTs is that one of the individual nanotube is bended to nearly a right angle at the middle part and tightly bound with another individual tube at the terminus via sidewall coupling, foreseeing the ability of water-soluble CNTs to form stable and complicated network nanostructures. In fact, when the matrix of the electrode containing naion is air-dried, water-soluble CNTs form uniform films on the electrode surface, which possess three dimensional network structures with plenty of interstices inside the films. One can name the advantages of the prepared sensor as high sensitivity and selectivity and very low detection limit (0.01 μ M) together with the very easy preparation and surface regeneration of the modified electrode and reproducibility of the voltammetric responses. These characteristics make the prepared sensor very useful in the construction of simple devices for the determination of UA in the presence of large amount of AA in clinical and pharmaceutical preparations.

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