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Electrochemical methods for simultaneous determination of trace amounts of dopamine and uric acid using a carbon paste electrode incorporated with multi-wall carbon nanotubes and modified with α-cyclodextrine

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Abstract In this work, we investigate the electrochemical activity of dopamine (DA) and uric acid (UA) using both a bare and a modified carbon paste electrode as the working electrode, with a platinum wire as the counter electrode and a silver/silver chloride (Ag/AgCl) as the reference electrode. The modified carbon paste electrode consists of multi-walled carbon nanotubes (>95%) treated with α-cyclodextrine, resulting in an electrode that exhibits a significant catalytic effect toward the electro-chemical oxidation of DA in a 0.2-M Britton–Robinson buffer solution (pH 5.0). The peak current increases linearly with the DA concentration within the molar concentration ranges of 2.0×10^{-6} to 5.0×10^{-5} M and 5.0×10^{-5} to 1.9×10^{-4} M. The detection limit (signal to noise >3) for DA was found to be 1.34×10^{-7} M, respectively. In this work, voltammetric methods such as cyclic voltammetry, chronoamperometry, chronocuolometry, differential pulse and square wave voltammetry, and linear sweep and hydrodynamic voltammetry were used. Cyclic voltammetry was used to investigate the redox properties of the modified electrode at various scan rates. The diffusion coefficient (D, cm^2 s^{-1} = 3.05×10^{-5}) and the kinetic parameters such as the electron transfer coefficient ($\alpha = 0.51$) and the rate constant (k, cm^3 mol^{-1} s^{-1} = 1.8×10^3) for DA were determined using electrochemical approaches. By using differential pulse voltammetry for simultaneous measurements, we obtained two peaks for DA and UA in the same solution, with the peak separation approximately 136 mV. The average recovery was measured at 102.45% for DA injection.

Keywords Dopamine · Uric acid · α-Cyclodextrine · Multi-walled carbon nanotube · Carbon paste electrode · Voltammetry methods

Introduction

Dopamine (DA) is an important neurotransmitter that belongs to the catecholamine group and plays a very significant role in the central nervous, renal, hormonal, and cardiovascular systems [1, 2]. Neurotransmitters (NTs) are chemical messengers that transmit a message from one neuron to the next. This transmission proceeds by the secretion of NTs from one neuron followed by their binding to the specific receptor located on the membrane of the target cell [3]. The determination of DA is thus a subject of great importance, both for fundamental investigation of physiological function and for better diagnosis and understanding of central nervous diseases resulting from abnormal DA metabolism such as epilepsy, senile dementia and Parkinson, as well as HIV infection [4, 5].

Uric acid (2, 6, 8-trihydroxypurine, UA) is the primary product of purine metabolism [6]. Typical physiological UA serum levels range from 41 to 88 mg ml^{-1} and urinary excretion is typically 250–750 mg per day [7]. An abnormal concentration level of UA in the human body is a symptom of several diseases such as gout, hyperuricemia, and Lesch–Nyhan syndrome. Leukemia and pneumonia are also often associated with enhanced urate levels as well [8, 9].

Various methods such as colorimetric [10, 11], enzymatic [12, 13], electrophoretic [14, 15], and chromatographic [16–

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18] techniques have been used for the analysis of DA and UA in human body fluids. Electrochemical methods (voltammetric methods) exhibit higher selectivity and sensitivity than other commonly employed methods and have the inherent advantage of lower cost and rapid sensing [19–21]. The major problem in the electrochemical determination of DA is the interference of uric acid, which is often present in biological samples.

Carbon nanotubes (CNTs) are a unique form of elementary carbon composed of graphitic sheets rolled into closed concentric cylinders with diameters on the order of nanometers and lengths on the order of micrometers. Since the discovery of CNTs in 1991 [22] they have been exploited for a number of electroanalytical and sensing purposes [21, 23, 24] due to their unique electrical conductivity, chemical stability, and high mechanical strength and elastic modulus [25–27]. CNTs can be found as single graphene layer tubes, referred to as single-walled carbon nanotube, or with multiple graphene layers, referred to as multi-walled carbon nanotube (MWCNTs). The subtle electronic properties of carbon nanotubes suggest that they have the ability to promote electron transfer when used as the electrode material in electrochemical reactions. These properties provide a new manner of electrode surface modification in the design of new electrochemical sensors [28–31] and novel electrocatalytic materials [32]. It has been reported that CNT-modified electrodes have been successfully applied to both study and identify many biological and organic molecules [33–37].

Cyclodextrins (CDs) belong to the family of cyclic oligo-saccharides formed by various D-glucopyranose units that are held together by (1–4)-glucosidic bonds. The better-known members of the family are α-, β-, and γ-CDs, which have six, seven, and eight D-glucopyranose units, respectively. CDs are widely employed in host–guest recognition of diverse substrates [38, 39]. The molecular structure of CDs resembles a hollow, truncated cone or basket with a cavity that displays receptor-like features and allows for unique features such as selectivity and sensitivity, and the ability to interact with other chemical species to form inclusion complexes (due to their different cavity sizes). These unique features allow for a wide range of applications in the pharmaceutical and food industries [40].

The use of CNTs, with their interesting electrocatalytic activity, and other compounds that have known advantages, may serve as an important solution for new electroanalytical challenges. Molecules such as cyclodextrin are used to stabilize, dissolve, retain, and liberate in a controlled way a large number of organic or inorganic chemicals through the formation of inclusion complexes. Moreover, CD has been used as a recognition agent for different compounds and to better investigate the adsorption phenomena related to electrode interactions. With the unique properties of CNTs

and the known advantages of CD in mind, this work proposes a novel strategy based on the simultaneous modification of a carbon paste electrode and a novel CNT/cyclodextrine matrix. The ability of CD to recognize DA and UA is enhanced with the faster electron transfer process inherent with the CNT electrode interface. In this work, DA oxidation is enhanced by fast diffusion through CD cavities, the large surface area of the dispersed MWCNTs, and the CNTs' fast electron transfer.

Experimental

Apparatus and materials

All electrochemical experiments were carried out using an Autolab potentiostat-galvanostat PGSTAT 35 (Eco chemie Utrecht, Netherlands), equipped with GPES 4.9,006 software. The electrochemical cell was equipped with a modified carbon paste disk as the working electrode, a platinum electrode as the counter electrode, and a silver/silver chloride (Ag/AgCl/KCl$_{sat}$) as the reference electrode. A personal computer (Pentium IV) was used for data storage and processing. The body of the carbon paste working electrode was a Teflon syringe with a rode (2 mm diameter and 5 mm deep) bored at one end, and filled with paste. A copper wire was placed through the center of the rod to make contact. The working electrode was pretreated by pushing paste out of the tube, removing the excess, and mechanically polishing the surface with weighing paper. A digital pH meter (Metrohm model 691) was used when preparing buffer solutions that served as the supporting electrolyte in the voltammetric experiments. A rotating electrode system, from Pine instrument, was employed. A Pine Instrument Company (Grove City, PA, USA) AFMSRX 1270 rotator and MSRX speed controller were used.

Chemicals

All solutions were freshly prepared using double-distilled water. DA, UA, α-cyclodextrin, and other reagents were analytical grade, purchased from Merck and used without any further purification. Pure fine graphite powder (Merck) and paraffin oil (DC 350, Merck, $\rho=0.88\ gcm^{-3}$) were used as binding agents for the graphite pastes. Before use, flasks and containers were soaked in an aqueous solution of HNO$_3$. The MWCNTs were obtained from China and had an outside diameter of 10–20 nm, a length <1–2 μm, and purity over 95% while for more purification an acid treatment was used. DA and UA solutions were prepared immediately prior to use and all experiments were carried out at ambient laboratory temperature (ca. 25 °C). All solutions were deaerated by flushing with highly pure nitrogen (99.9%) prior to experiments. A continuous flow

of nitrogen was maintained over the sample solution during the experiments. Briton–Robinson buffer solutions (B-R, 0.2 M) of different pH values were prepared from stock solutions of 0.2 M H_3PO_4, CH_3COOH, H_3BO_3, and a saturated solution of NaOH.

Preparation of electrode with MWCNT/α-CD

To prepare the MWCNT/α-CD solution, 4.0 mg of MWCNTs with 2.0 mL of α-CD solution (2% w/v) was dissolved with ultrasonication, and subsequently added to 0.45 g of graphite powder. After drying the mixture, eight to nine drops of paraffin oil was added to make a paste. The paste was packed into the end of the Teflon syringe (inside diameter of 2 mm). Electrical contact to the paste was established by inserting a copper wire down the syringe, making contact to the back of mixture. The surface was mechanically polished with a piece of weighing paper.

Results and discussion

pH dependence study

The effect of pH on the DA oxidation signal was investigated by differential pulse voltammetry, using 0.2 M buffer solutions with pH values ranging from 2.0 to 8.0 and a scan rate of 25 mV s^{-1} (see Fig. 1 (A)). For the case of DA, there is an electrochemical oxidation by a two-electron, two-proton process (see Scheme 1), resulting in a DA derivative, such as dopaquinine. Within the pH range of 2 to 8, with 2.0×10^{-5} M DA in 0.2 M B-R buffer solutions, the oxidation potential of the electrochemical cell

decreased when the pH of DA solution increased. The maximum anodic current for DA was obtained at a pH of 5.0, using 0.2 M B-R buffers. Thus, solutions of pH 5.0 were used in all subsequent experiments (Fig. 1 (B)). According to the Nernst equation (Eq. 1), where n and m represent the number of electrons and protons involved in reaction, a and b represent the coefficients reagents in reaction equation, a slope of −63 mV pH^{-1} indicates that the proportion of the electron and proton involved in the reaction is 1:1 (Fig. 1 (C)).

Also, from the intercept of curve of Fig. 1 (C), the standard formal potential of DA was obtained to be 0.575 V.

$$E_p = E^o + (0.0591/n)\log\left[(Ox)^a \big/ (R)^b\right] - (0.0591m/n)\text{pH}$$
$$E_{pa}(\text{V vs. Ag/AgCl}) = 0.5745 - 0.063[\text{pH}]$$
(1)

Kinetic investigations of modified electrode

According to our knowledge, there are no reports concerning the electrochemical properties and especially the electrocatalytic attributes of α-CD/MWCNT/CPE in aqueous media. Thus, for electrocatalytic investigations of modified electrode, the cyclic voltammograms of this electrode are drowned in presence of probes solution of Fe^{+2}/Fe^{+3} in pH 5 of B-R buffer solution at various scan rates (Fig. 2 (A)). In addition to the experiments done, the redox responses of probes in the bare electrode have weak current by $\Delta E = 0.305$ V, whereas in the surface of modified electrode, the responses are 2.07 times bigger by $\Delta E = 0.190$ V. Therefore, this amount of increasing in reversibility of redox probe can be ascribed to

Fig. 1 *A* DPV of a solution of 20.0 μM DA at various buffered pHs, performed with a scan rate of 25 mVs^{-1}. The letters *a* to *g* correspond to pH values of 2, 3, 4, 5, 6, 7, and 8, respectively. *Insets B* Intensity, *I* (μA) vs. pH and C electrochemical potential, E_{pa} (V) vs. pH
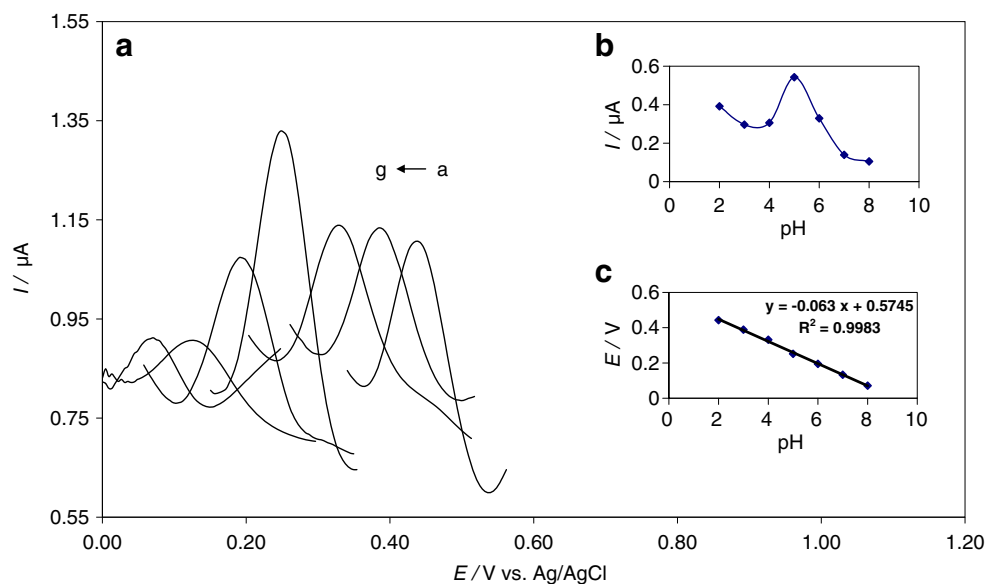
Scheme 1 Mechanism of DA oxidation at α-CD/CNT/CPE

$$-2H^+ \longrightarrow + 2H^+ + 2e$$
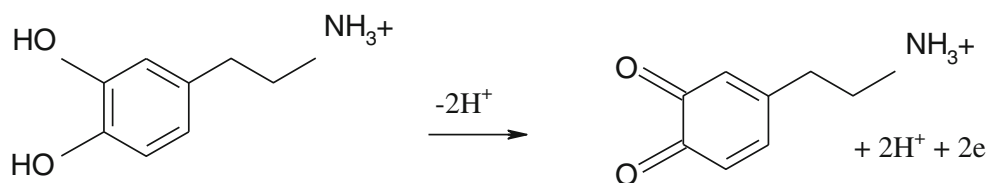
presence of mediator in the surface of electrode. An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp et al. [41]. According to this method, the peak current is related to the surface concentration of mediator, Γ, by the following equation:

$$I_p = n^2 F^2 A \Gamma v / 4RT \tag{2}$$

where n represents the number of electrons involved in reaction, A is the surface area (0.0314 cm^2) of the modified carbon paste electrode (MCPE), Γ (mol cm^{-2}) is the surface coverage of mediator, and other symbols have their usual meanings. From the slope of anodic peak currents versus scan rate (Fig. 2 (A)) the calculated surface concentration of α-CD/CNT is 5.2×10^{-6} mol cm^{-2} for $n=1$. The apparent charge transfer rate constant, k_s, and the charge transfer coefficient, α, of a surface-confined redox couple can be evaluated from cyclic voltammetric experiments and using the variation of anodic peak potentials with scan rate, according to the procedure of Laviron [42]. Inset C of Fig. 2 shows the variations of peak potentials (E_p) as a function of logarithm of the potential scan rate.

We found that the E_p values are proportional to the logarithm of the scan rate, for scan rates higher than

300 mV s^{-1} (Fig. 2 (C)). Under these conditions, the following equation can be used to determine the electron transfer rate constant between probes and MCPE:

$$\text{Log } k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha$$
$$- \log(RT/nFv)$$
$$- \alpha(1 - \alpha)n_\alpha F \Delta E_p / 2.3RT \tag{3}$$

where $(1-\alpha) n_\alpha = 0.69$ (see below), $n=1$, $\Delta E_p = E_{pa} - E_{pc}$, v is the scan rate, and all other symbols have their conventional meanings. From the values of ΔE_p corresponding to different scan rates, an average value of k_s was found to be 0.1317 s^{-1}. Also the average value obtained for the charge transfer coefficient, from the slopes of the inset D plots, was found to be 0.31.
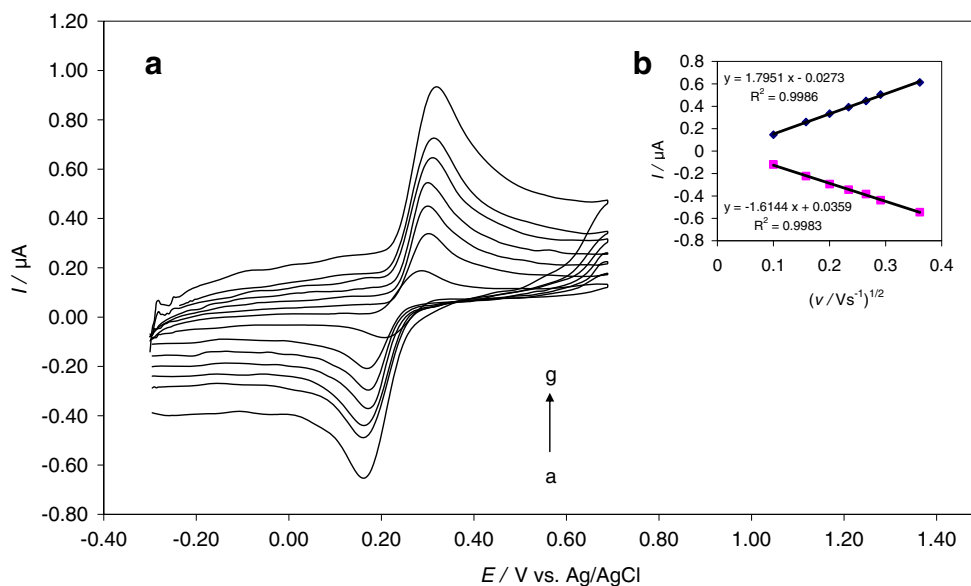
Effect of scan rate

The influence of scan rate (v) on the electrochemical behavior of DA on the α-CD/CNT/CPE system was investigated (Fig. 3 (A)). According to Randles–Sevick equation (Eq. 4) for a quasi-reversible process [43]:

$$I_p = 2.69 \times 10^5 \, n^{3/2} A \, D^{1/2} \, C \, v^{1/2} \tag{4}$$

Fig. 2 *A* CV of α-CD/CNT/CPE in Fe^{+3}/Fe^{+2} probes solution in 0.2 M B-R buffer (pH 5.0), at various scan rates. *a* to *g* correspond to 25, 45, 65, 85, 105, 125, and 145 mV s^{-1} scan rates, respectively. *Insets B* Variations of I_p versus scan rates and *C* variation of E_p versus the logarithm of the scan rate. *Inset D* Magnification of the same plot for high scan rates
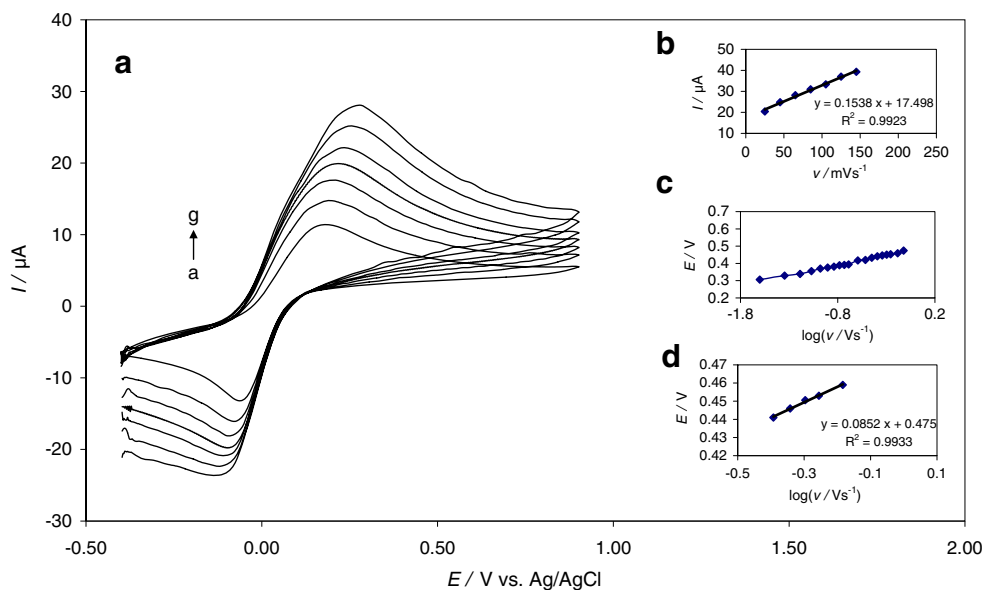
Fig. 3 CV of α-CD/CNT/CPE
in a buffer solution with pH 5.0
for 25 μM DA, using various
scan rates: 10, 25, 40, 55, 70,
85, and 130 mVs^{-1} (from inner
to outer). *A* Peak current vs. *E*
(mV). *Inset B* Intensity,
I (μA) vs. $V^{1/2}$

where D is the diffusion coefficient and C is the
concentration of reagent, in the scan rate range of 10.0–
130 mV s^{-1}, the redox peak current increases linearly with
the square root of scan rate, typical of a diffusion-controlled
process of DA on the modified electrode (Fig. 3 (B)).
Therefore the linear regression equation for DA was found
to be:

$$I_{pa} = 1.7951\nu^{1/2} - 0.0273 \quad R^2 = 0.9986$$
$$I_{pc} = -1.6144\nu^{1/2} + 0.0359 \quad R^2 = 0.9983$$

Modifier effect in oxidation of DA

The cyclic voltammogram responses from a 30-μM DA
solution with bare CPE (a), MWCNT/CPE (b), α-CD/

CPE (c), and α-CD/CNT/CPE (d) electrodes were
investigated (Fig. 4). As can be seen in Fig. 4, the total
differential potential, $\Delta E_p = E_{pa} - E_{pc}$, for the oxidation of
DA at three modified electrodes is approximately 52 mV,
while at the bare CPE, ΔE is approximately 70 mV. From
this result, it is concluded that the acceptable electro-
catalytic effect for DA oxidation is observed at the α-CD/
CNT/CPE. The results show that the peak potential of
DA oxidation at this modified electrode shifts by
approximately 12 mV toward more negative values, and
the peak potential of DA reduction shifts by approxi-
mately 6 mV toward more positive values, compared to
the bare CPE.

According to the differential pulse voltammetry
responses from the electrochemical oxidation of 5.0 μM
DA solutions for chosen ratio concentration of carbon
nanotubes and α-CD used in the modified carbon paste

Fig. 4 CV curves for a 30-μM
DA solution in a B-R buffer
solution of pH 5.0 at scan rate of
35 mVs^{-1}. (a) Bare-CPE, (b)
MWCNT/CPE, (c) α-CD/CPE,
and (d) α-CD/CNT/CPE
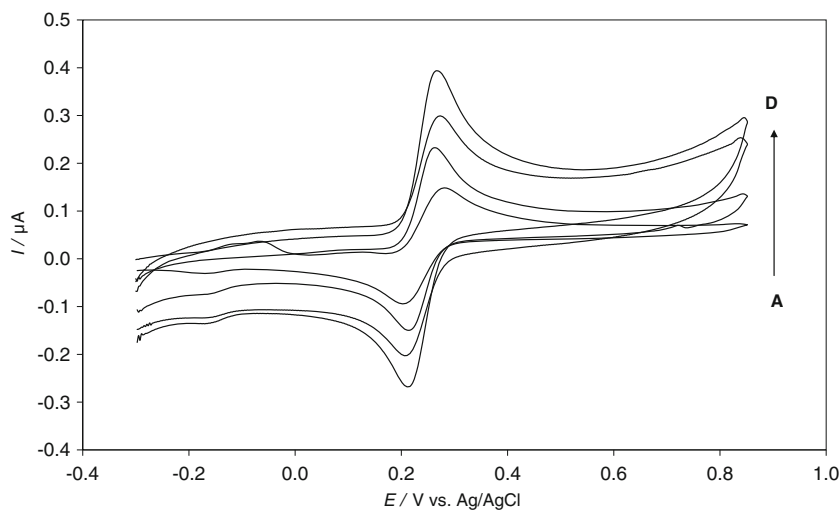
electrodes, the optimum proportion of CNT to α-CD for 0.45 g graphite was found to be 0.004 g/2 ml of 2% w/v.

A drastic enhancement of the voltammetric background current at the α-CD/CNT/CPE compared to the bare CPE was also observed. This large background current should be caused by the complex impedance of the modified electrode/electrolyte interface compared to the non-modified electrode [44].

Chronoamprometric and chronocoulometry measurements

The catalytic oxidation of DA by α-CD/CNT/CPE was studied by chronoamprometry and chronocoulometry. Chronoamprograms obtained for DA solutions in concentrations ranging from 3 to 26 μM, using a potential step of 500 mV are shown at Fig. 5 (A). In chronoamprometric studies, the diffusion coefficient of DA in the modified electrode can be determined. For an electroactive material (such as DA) with a diffusion coefficient of D (cm^2 s^{-1}), the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation (Eq. 5).

$$I = nFAD^{1/2} C_b \pi^{-1/2} t^{-1/2} \quad (5)$$

where D (cm^2 s^{-1}) and C_b (mol cm^{-3}) are the diffusion coefficient and the bulk concentration, respectively. Under diffusion limited transport (mass transport), a plot of I versus $t^{-1/2}$ (s$^{-1/2}$) will be linear, and the value of D can be extracted from the slope. Figure 5 (B) and (C) shows the fitted experimental plots for different concentrations of DA. The slopes of the resulting straight lines were plotted versus the DA concentration, and the mean value of D was found to be 3.05×10^{-5} cm^2 s^{-1}. The electrochemical oxidation of DA with a modified electrode was also investigated by

chronocoulometry. The values of the diffusion coefficient of DA obtained using chronocoulometry was very close to the values obtained via the chronoamprometry method. According to Eq. 6, the diffusion coefficient D (cm^2 s^{-1}) obtained via chronocoulometry is as follows:

$$Q = 2nFAD^{1/2} t^{1/2} C_b \pi^{-1/2} \quad (6)$$

Linear sweep voltammetry measurement in oxidation of DA

The linear sweep voltammograms of a α-CD/CNT/CPE in 0.2 M B-R buffer solution (pH 5.0) containing different concentrations of DA, with a sweep rate of 25 mV s^{-1} was estimated (not shown). The rising part of the voltammogram is known as the Tafel region, which is affected by the electron transfer kinetics between DA and modified electrode. If deprotonation of DA is a sufficiently fast step, the number of electrons involved in the rate-determining step can be estimated from the slope of the Tafel plot. The average Tafel slopes of 60.10 mV per decade obtained in this case agrees well with the involvement of two electron in the rate-determining step of the electrode process, assuming a charge transfer coefficient of $\alpha=0.51$. Also, the ionic exchanging current density ($i_\circ$) by average of Tafel intercepts for DA was found to be 1.6 μA cm^{-2}.

Differential pulse and square wave voltammetry investigations for the measurement of DA

Since differential pulse voltammetry, DPV, has a much higher current sensitivity and better resolution than cyclic voltammetry, it was used to estimate the lower limit of detection of DA. In addition, the charging current contri-

Fig. 5 *A* Chronoamperograms obtained at α-CD/CNT/CPE in 0.2 M B-R buffer solution (pH 5.0) for different concentrations of DA, with a potential step at 500 mV. *a* to *e* correspond to 3.0, 8.0, 14.0, 20.0, and 26.0 μM of DA. Inset *B* Plots of *I* vs. $t^{-1/2}$ (s$^{-1/2}$) obtained from chronoamprograms *a–e*. Inset *C* A plot of the slope of the straight lines versus the concentration of DA
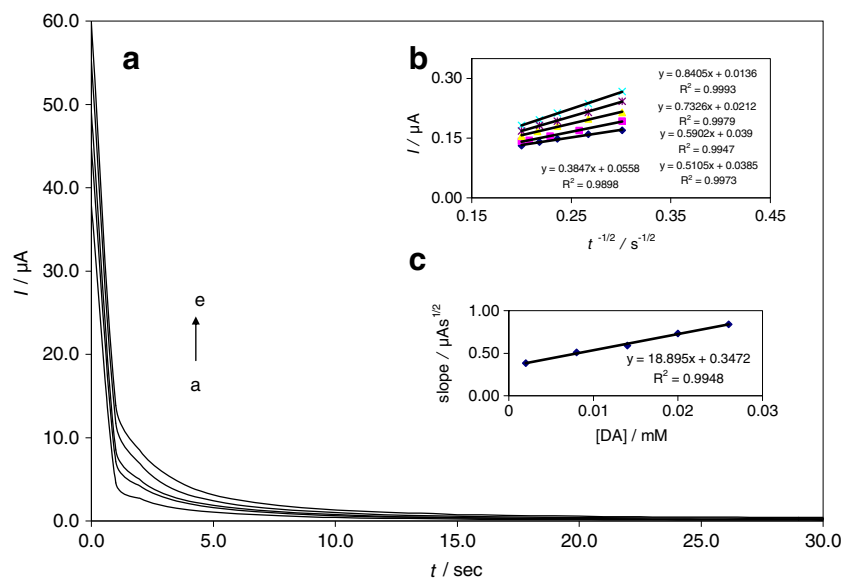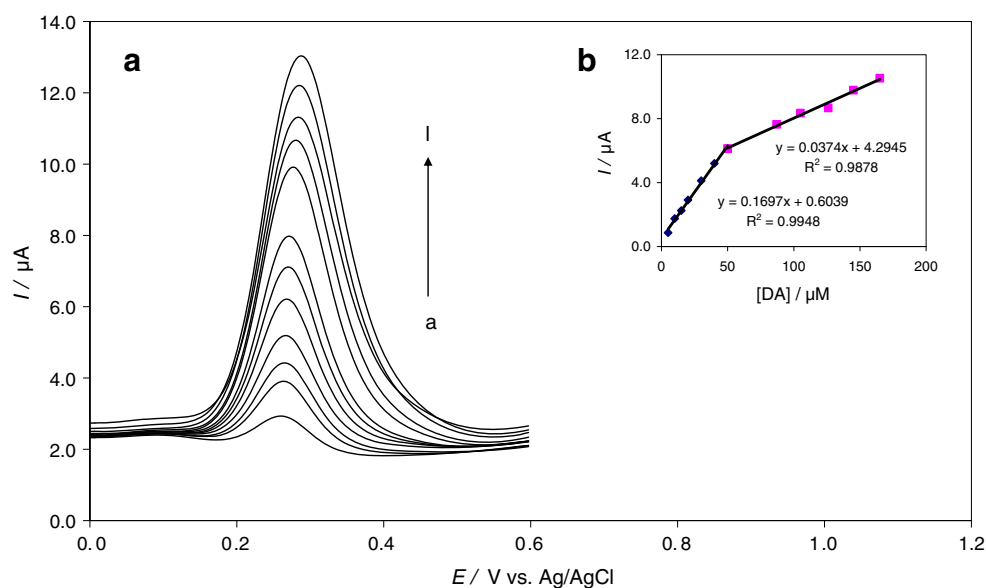
Fig. 6 *a* DPV (at 25 mV s^{-1}) of α-CD/CNT/CPE in a 0.2-M B-R buffer solution (pH 5.0), containing different concentrations of DA. The *letters a to q* correspond to 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 70.0, 90.0, 110.0, 130.0, 150.0, 170.0, and 190.0 μM of DA. *Inset b* Plot of the electrocatalytic peak current as a function of DA concentration in the range of 2.0 to 15.0 μM ($y=0.1226X+0.2003$, $R^2=0.9851$) and 15.0 to 190.0 μM ($y=0.0358X+1.7643$, $R^2=0.9932$)
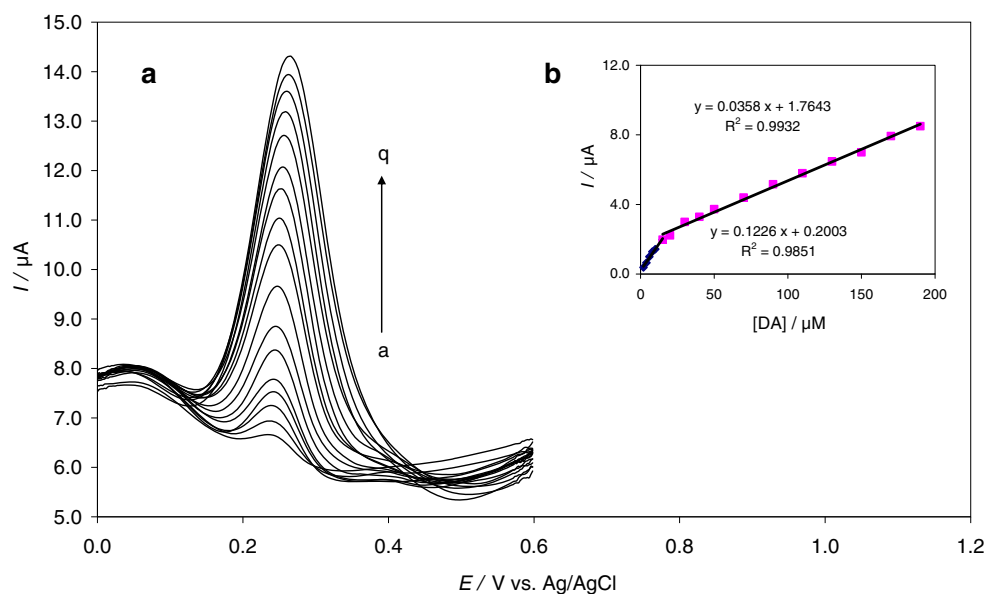
bution to the background current, which is a limiting factor in the analytical determination, is negligible in DPV mode. Figure 6 (A) shows the DPVs obtained for the oxidation of different concentrations of DA at the MCPE in 0.2 M B-R buffer solutions (pH 5.0) at a scan rate of 25 mV s^{-1}. The dependence of the peak current on the DA concentration is shown in inset B of Fig. 6. Inset B of this figure clearly show that the plot of peak current versus DA concentration is constituted of two linear segments with different slopes, corresponding to two different ranges of substrate concentration. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation. From the analysis of this data, we estimate that the lower limit of detection of DA is of the order of 0.134 μM.

In addition, the quasi-reversible response of DA in surface of α-CD/CNT/CPE, also square wave voltammetry (SWV) technique was used to estimate the detection limit of DA. Figure 7 (A) show the SWV results obtained for the oxidation of different concentrations of DA at the modified electrode. The peak currents were detected in two DA concentration ranges, 5–50 μM and 50–165 μM (Fig. 7 (B)). Under the optimized experimental conditions, with pH 5.0 and a frequency of 50 Hz, detection limits for DA was 0.097 μM, respectively. The relative standard deviation of 3.55% in oxidation peak current and 0.39% in peak potential for five repeated detections of $4.0×10^{-5}$ M DA suggests excellent reproducibility of results using the modified electrode.

Fig. 7 *A* SWV of α-CD/CNT/CPE in 0.2 M B-R buffer solutions (pH 5.0) containing different concentrations of DA at the frequency of 50 Hz. The letters *a–l* corresponds to: 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 90.0, 105.0, 125.0, 145.0, and 165.0 μM of DA. *Inset B* Electrocatalytic peak current as a function of DA concentration in the ranges of 5.0 to 50.0 μM ($y=0.1697X+0.6039$, $R^2=0.9948$) and 50.0 to 165.0 μM ($y=0.0374X+4.2945$, $R^2=0.9878$)

Fig. 8 **a** DPV (at 25 mV s^{-1}) of α-CD/CNT/CPE in 0.2 M B-R buffer solution (pH 4.5) containing 20.0 μM UA and various concentrations of DA containing *a–i* corresponding to 0, 10.0, 20.0, 30.0, 50.0, 70.0, 90.0, 110.0, and 130.0 μM. *Inset*: *I* (μA) as a function of DA concentration (μM). **b** DPV of α-CD/CNT/CPE in 0.2 M B-R buffer solution (pH 4.5) containing 40.0 μM DA and different concentrations of UA containing *a–g* corresponding to: 0, 5.0, 10.0, 15.0, 20.0, 30.0, and 40.0 μM. *Inset*: *I* (μA) as a function of UA concentration (μM)
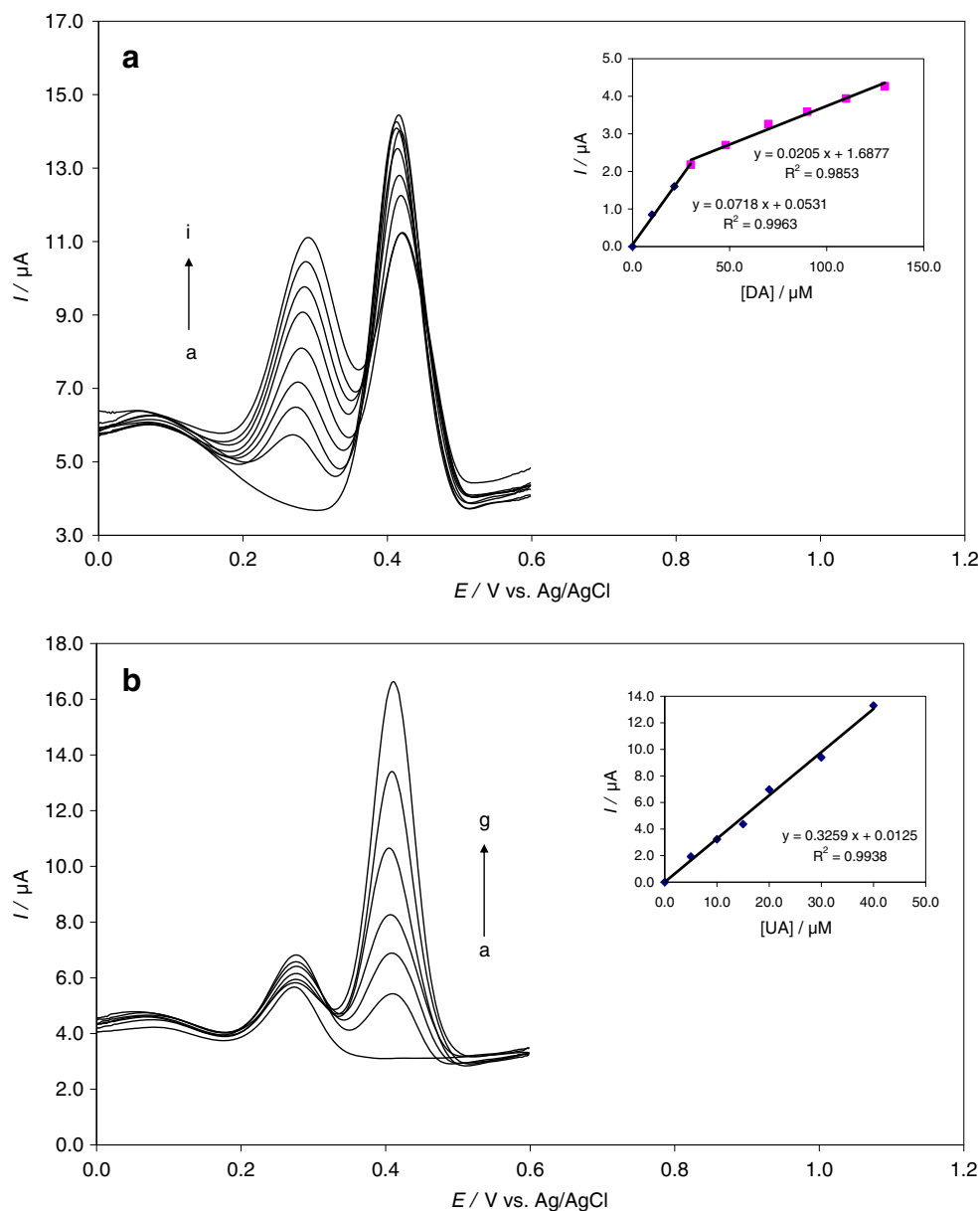
The results obtained in this study clearly show that the combination of carbon nanotubes with a mediator enhances DA oxidation and results in a strong electrocatalytic effect. Which values of detection limit is comparable to values reported by other research groups [45–50].
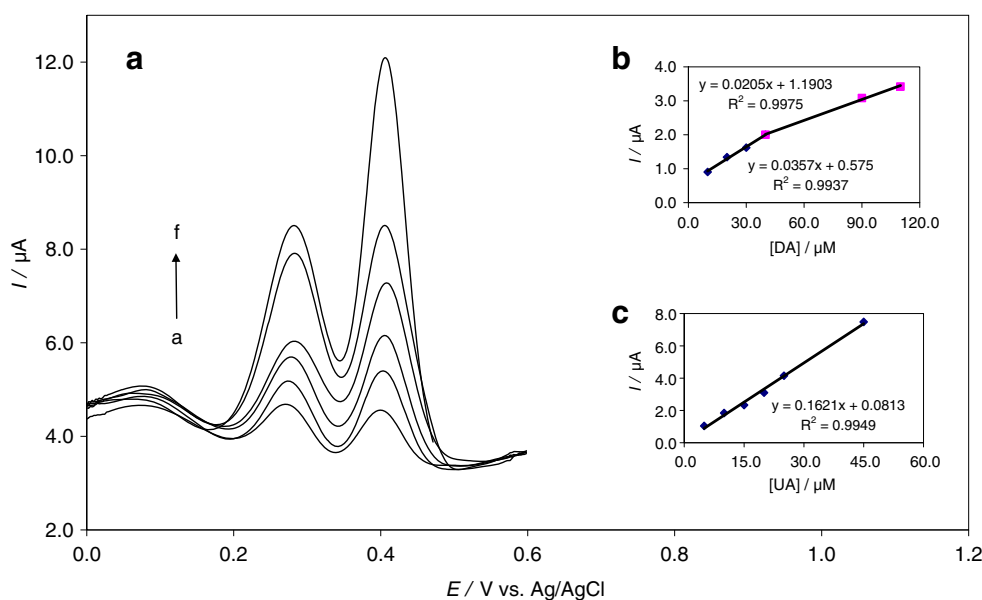
Simultaneous determination of DA and UA at α-CD/CNT/CPE

One of the main objectives of this study was to develop and test a modified electrode, capable of the electrocatalytic oxidation of DA and UA and separately detecting electrochemical responses From DA and UA. Differential pulse voltammetry was used to estimate the simultaneous determination of DA and UA. In the first stage, according to the curve of *I* vs. pH for DA and UA, the optimum pH for maximum response of two drugs was found to be 4.5. So in the first test, in mixtures of DA and UA, the concentration of one species was changed, while the concentration of the other species remained constant and the results are shown in Fig. 8a. From the figures, it can be seen that the peak current of DA is proportional to the DA concentration in the range of 0.0 to 130.0 μM, when the concentration of UA was kept at 0.02 mM. In the second test, in mixtures of DA and UA, the concentration of DA was kept at 40.0 μM, while the concentration of UA was changed. The peak current of UA increases proportionally to its concentration in the range of 0.0 to 40.0 μM, without

influencing the peak corresponding to DA concentration (Fig. 8b). As seen in Fig. 8a, higher concentrations of DA do not strongly interfere with the detection of UA. The peak current for the two chemicals increases linearly with their concentration within a range of concentrations. Calibration parameters for the simultaneous determination of DA and UA are shown in Fig. 9. The sensitivity of the modified electrode towards the oxidation of DA was found to be 0.0357 μA μM^{-1}, whereas the sensitivity towards DA in the absence of UA was found to be 0.0358 μA μM^{-1}. It is interesting to note that the sensitivities of the modified electrode towards DA in the absence and presence of UA are virtually the same, which indicates that the oxidation

processes of DA and UA at the α-CD/CNT/CPE are independent. This indicates that simultaneous or independent measurements of the two analysts are possible without any interference. If the DA signal was affected by the presence of UA, the above-mentioned slopes would be different.

Rotating disk electrode voltammetry

In this paper, we report the first time uses of rotating disk electrode, RDE, voltammetry for investigation of the electrocatalytic activity of α-CD/CNT/CPE toward oxidation of DA. Steady-state I–E curves were recorded for the
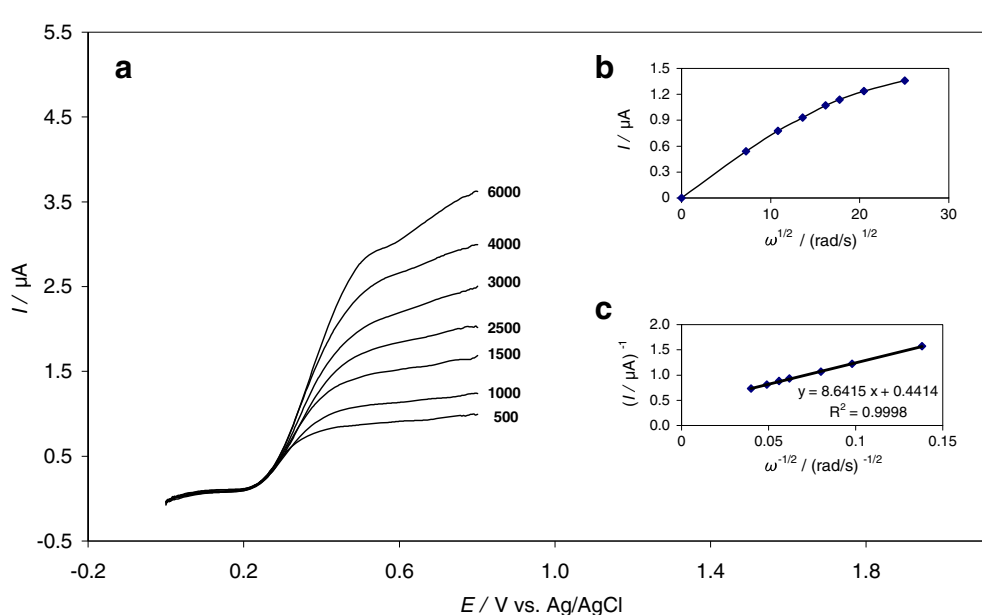
Table 1 Determination of DA in dopamine hydrochloride injection and human blood serum samples

Sample	Added (μM)	Obtained (μM)	Recovery (%)
DA inject	–	3.95	98.7
	15.0	20.8	109.8
	30.0	33.9	99.8
	40.0	44.6	101.5
Human blood serum	–	–	–
	20.0	19.7	98.5
	30.0	28.9	96.3
	50.0	50.6	101.2

oxidation of DA at α-CD/CNT/CPE under various experimental conditions. A typical example of the I–E curves (RDE voltammograms) at rotation speed ranging from 500 to 6,000 rpm for 0.04 mM solution of DA is shown in Fig. 10 (a). In the case where oxidation of DA at the surface α-CD/CNT/CPE is controlled solely by mass transfer in the solution, the relationship between the limiting current and rotating speed should obey the Levich equation [51]:

$$I_l = I_{Lev} = 0.62nFAD^{2/3}\upsilon^{-1/6}\omega^{1/2}C \qquad (7)$$

where D (cm^2 s^{-1}), υ (cm^2 s^{-1}), ω (rad s^{-1}), and C (mol cm^{-3}) are the diffusion coefficient, the kinematics viscosity, the rotation speed, and the bulk concentration of the reactant in the solution, respectively, and all other parameters have their conventional meanings. Based on Eq. 7, the plot of limiting current I_l as a function of $\omega^{1/2}$, $\omega^{1/2}$/(rad s^{-1})$^{1/2}$ should be a straight line. According to the Levich plot (inset B of Fig. 10); the current increases with increasing electrode rotation speed, but were found to be nonlinear due to kinetic limitation. For an irreversible reaction, the relation between the limiting current and rotating speed has been given by the Koutecky–Levich equation [51]:

$$I^{-1} = (nFAkCT)^{-1} + (0.62nFAD^{2/3}\upsilon^{-1/6}\omega^{1/2}C)^{-1} \qquad (8)$$

where A (cm^2), C (mol cm^{-3}), k (cm^3 mol^{-1} s^{-1}), Γ (mol cm^{-2}), D (cm^2 s^{-1}), υ (cm^2 s^{-1}), and ω (rad s^{-1}) are the electrode area, substrate concentration, catalytic rate constant, surface coverage, diffusion coefficient, kinematics viscosity, and rotation speed, respectively. It can be seen that the intercept of linear plot is positive, clearly indicating the kinetic limitation of the electrode process. In addition, the slope and intercept are inversely proportional to the bulk concentration of DA, suggesting

that the current is not limited by the rate of electron transport within the electrode.

According to Eq. 8 the plot of Γ^{-1} vs. $\omega^{-1/2}$ gives a straight line (as shown in inset C of Fig. 10). The rate-determining step must be the catalytic process at electrode surface or the electron diffusion process within the electrode. The value of the rate constant (k, cm^3 mol^{-1} s^{-1}) for the catalytic reaction can be obtained from the intercept of the Koutecky–Levich plot. The value of the rate constant for the MCPE was found to be 1.8×10^3 cm^3 mol^{-1} s^{-1} for DA in concentration of 0.04 mM. For the determination of diffusion coefficients with an RDE, it is common to change the rotation rate (ω in s^{-1}) and plot I_l vs. $\omega^{1/2}$. The slope of the resulting best-fit line can be used to determine D:

$$D = \left(slope_{Lev}\Big/0.62nFAC\upsilon^{-1/6}\right)^{3/2} \qquad (9)$$

As with chronoamperometry, there are limits to the range of experimental conditions under which Eq. 7 applies, but with the RDE the important parameter is the rotation rate, ω. If in RDE experiments, ω has been applied in the range of 10–10,000 rpm, the diffusion coefficient of DA may be obtained from the slope of the Levich plot [51]. So the mean value of D (cm^2 s^{-1}) was found to be 1.08×10^{-5}.

Real sample analysis

Determination of DA in dopamine hydrochloride injection

In order to test the reliability of our DA detection method in a pharmaceutical product, the modified electrode was used to detect the DA concentration in a dopamine hydrochloride injection (40 mg ml^{-1}, 0.21 M). The 1.0 mL dopamine hydrochloride sample was diluted 50 times with double-distilled water. A small portion (2 mL) of this solution was diluted an additional five times. Appropriate amounts of the diluted solution were transferred into the electrochemical cell for DPV analysis. The analytical results are summarized in Table 1. The recovery ranged from 98.7% to 101.5%. The results are acceptable, showing that the proposed method could be effectively used for the determination of DA in commercial samples.

Determination of DA in human blood serum samples

The application of the proposed method in real sample analysis was also investigated by direct analysis of DA in human blood serum samples. Prior to measurement, all serum samples were diluted 100 times with double-distilled water. The standard addition method was used for testing recovery. The recovery rates of the spiked samples ranged 98.5% and 101.2% (Table 1), indicating the serum sample matrix does not interfere with the detection procedures.

Conclusions

Voltammetric methods were used to measure trace amounts of DA and UA by detecting the oxidation at the surface of a carbon paste electrode, modified with α-CD and CNT (α-CD, MWCNT). The results show that the oxidation of dopamine is dependent on pH, and the peak potential of DA is shifted by -372 mV at the surface of modified electrode. Overall, this modified MWCNT electrode is sensitive, selective, and simple to fabricate and gives rapid response and the surface renewal is easy. Using chronoamprometry, chronocoulometry, and rotating disk electrode, the diffusion coefficient (D) of DA in MCPE was estimated. Using DPV to simultaneously detect the presence of both DA and UA, we obtained two separate peaks for DA and UA, with a separation between peaks of approximately 136 mV. The detection limit for DA (with signal to noise >3) was found to be 1.34×10^{-7} and 9.7×10^{-8} M by DPV and SWV, respectfully. Our results indicate that a modified CNT electrode is highly sensitive, with great potential for electrochemical sensor applications.

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