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A Sensor for Simultaneous Determination of Dopamine and Morphine in Biological Samples Using a Multi-Walled Carbon Nanotube/Chitosan Composite Modified Glassy Carbon Electrode

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A glassy carbon electrode modified with carbon nanotube/chitosan (MWCNTs-CHT/GCE) was used for the sensitive voltammetric determination of dopamine (DA) and morphine (MO). The electrochemical response characteristics of the modified electrode toward DA and MO were investigated by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry (CA). Under optimum conditions the sensor provides a linear DPV response in the range of 1.0×10^{-6} to 2.1×10^{-4} M with a detection limit of 1.9×10^{-7} M for DA, and in the range of 2.0×10^{-6} to 1.0×10^{-4} M with a detection limit of 2.4×10^{-7} M for MO. The designed sensor was used successfully for the simultaneous determination of DA and MO in human blood serum and urine and satisfactory results were obtained.

Keywords: Dopamine, morphine, chitosan, multi-walled carbon nanotubes, modified glassy carbon electrode

1. INTRODUCTION

Carbon nanotubes (CNT) show unique structural, electronic and mechanical properties that make them a very attractive material for a wide range of applications. When CNT are used as electrode materials, they present the outstanding ability to promote electron-transfer reactions due to the nanometer dimensions of the CNT, the electronic structure and the topological defects present on the tube surfaces [1-5]. Therefore, CNT have been successfully used in the preparation of chemical or electrochemical sensors for a wide range of electroactive species in recent years [6-9].

Chitosan (CHT), a natural cationic polysaccharide with abundant primary amino groups and hydroxyl groups, can simply form a thermally and chemically inert film on a solid electrode surface. CHT displays a number of properties including biocompatibility, hydrophilicity, gel-forming ability, nontoxic behavior, doping feasibility, good mechanical stability, good permeability, cost-effectiveness, and availability of reactive functional groups for chemical modifications. Moreover, another important property of CHT that makes it one of the most widely used biopolymers in sensors is good biochemical interaction with biological samples. Some materials such as metal nanoparticles, carbon nanotubes and redox mediators have been introduced to CHT films to refine electric conductivity of the non-conducting CHT biomaterial [10-15]. In this context, much research has been done on the possibility of CNT-CHT composites as platforms to develop biosensors in various practical fields, e.g. in pharmaceutical, environmental and biotechnogical applications [16-18].

Dopamine (DA), 3,4-dihydroxyphenylethylamine is an important naturally occurring neurotransmitter in the mammalian central nervous system. A deficiency in this catecholamine level can cause some serious diseases such as Parkinson, epilepsy and senile dementia in humans [19-23]. There is considerable interest in the quantitative determination of this neurotransmitter in human physiological fluids in biochemical and clinical diagnoses. Several methods have been reported to monitor DA including UV spectrometry [24], chemiluminescence [25], capillary electrophoresis [26] and fluorimetry [27]. Due to its electrochemical activity, DA can also be studied by electrochemical techniques, but some difficulties exist in the study of its electrochemical behavior, because, its oxidation needs a high overpotential at bare electrodes and the products are often adsorbed on the electrode surface, resulting in electrode fouling and unstable analytical signal. Recently, research has been dedicated to the development of new modified electrodes for monitoring DA [28-35].

A phenolic compound and an alkaloid, morphine (MO) is a highly effective and preferred drug to relieve severe pain in patients, especially those undergoing a surgical procedure which can cause disruption in the central nervous system. To prevent overdose-induced toxicity, monitoring of the therapeutic levels of MO concentrations in blood or urine in patients is a critical subject in clinical medicine and epidemiological investigation for drug abuse control as well as in forensic cases as an indicator of heroin usage and to identify causes of intoxication or death [36-39]. The most common analytical techniques currently used to determine MO concentrations are high performance liquid chromatography (HPLC) [38,40], liquid chromatography with UV detection [41,42], GC–mass spectroscopy [43-46], LC-mass spectrometry detection [47], capillary electrophoresis with mass spectrometry [48], laser induced fluorescence detection [49] and surface plasmon resonance based immunosensors [50,51].

Research has shown that the morphine-induced hyper locomotion can be ascribed to an increase in DA turnover, release, and firing in specific mesolimbic and mesostriatal dopaminergic system areas. A possible relationship between morphine analgesia and the effect of this drug on the brain catecholamine metabolism has been suggested by many investigators. It has been found that the neuronal activity of DA is important for morphine analgesia [52,53]. In addition, it has been demonstrated that morphine can be synthesized in the body from DA via the catecholamine pathway [54-56].

To the best of our knowledge, no study has been reported so far on the simultaneous determination of DA and MO by using electrochemical methods. Thus, here we report for the first time, the application of a multi-walled carbon nanotubes/chitosan polymer composite modified glassy carbon electrode (MWCNTs-CHT/GCE) for the determination of DA and MO in an aqueous buffer solution.

2. EXPERIMENTAL METHODS

2.1. Reagents and solutions

Dopamine hydrochloride was obtained from Acros. Morphine Sulphate was acquired from Temad Company (Tehran, Iran). All other reagents were of analytical-reagent grade and were used without further purification. All solutions were prepared with triply distilled water. Stock solutions of DA and MO were freshly prepared for daily use. A 0.1 M phosphate buffer solution (PBS) was prepared using Na_2HPO_4 and NaH_2PO_4 (Merck). Multi-walled carbon nanotubes (MWCNTs) with a diameter of 5-20 nm and length 1-10 μm purchased from PlasmaChem GmbH Company. The purity of MWCNTs was >95 wt%, with the number of walls being 3-15. Chitosan (CHT) (M of $1.0–3.0\times10^5$ g mol^{-1}) was obtained from Acros and used as received. All experiments were carried out at ambient temperature. Fresh human serum samples were obtained from Razi Institute of Vaccine and Serum Company (Tehran, Iran). The serum and urine samples were filtered and diluted 50 times using a 0.1 M PBS of pH 7.0 and applied for determination of spiked dopamine and morphine in the samples.

2.2. Apparatus

Cyclic voltammetric (CV), differential pulse voltammetric (DPV) and chronoamperometric experiments were performed using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) running on GPES software coupled with a 663 VA stand (Metrohm Switzerland) with a conventional three-electrode system, a working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode filled with 3 M KCl. A modified glassy carbon electrode with a diameter of 3 mm was used as working electrode. All the potentials in this paper are given against the potential of the reference electrode. DP voltammetric experiments were conducted under the instrumental conditions of 10 mV s^{-1} scan rate, 50 mV pulse amplitude and 200 ms pulse interval. Before every measurement, the potential scan was repeated successively for 10 times in a blank solution to regenerating the electrode surface. pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode.

2.3. Preparation of MWCNTs-CHT/GC composite modified electrode

Prior to modification, the bare GCE was polished to a mirror finish on a Buehler polishing cloth using alumina slurries down to 0.05 μm. After each polishing, it was rinsed with triply distilled

water, and ultrasonicated in ethanol and doubly distilled water for 5 min, successively, in order to remove any adsorbed substances on the electrode surface. Finally, it was dried under nitrogen flow and ready for use. Different percents of CHT from 0.0 to 0.5% were studied to prepare MWCNTs-CHT/GC modified electrodes. In the presence of 0-0.1% CHT a shoulder appeared beside the dopamine peak after the first sweep and gradually increased in subsequent sweeps to interfere with the detection of DA and MO.

From 0.2-0.5% the shoulder was not observed but higher amounts of CHT caused a lower current, so 0.2% of CHT was selected to prepare the modified electrode in competition between the fouling effect at a lower amount of CHT and the decrease of peak current at higher amount of CHT. A 0.2 wt% chitosan solution was prepared similar to the reported method [57].

Accurately weighed solid chitosan was dissolved in 1% acetic acid solution and the solution pH was adjusted to ~5.0 with concentrated NaOH. 1.0 mg MWCNTs were dissolved in 500 µl of chitosan solution with the aid of ultrasonic agitation for 30 minute. This resulted in a homogeneous black suspension. The MWCNTs/chitosan coated electrode was prepared by dropping a 10 µl of MWCNTs-CHT suspension on the electrode surface. Before using the electrode in experiments, the electrode was put in air at room temperature for some time in order to evaporate the solvent. The obtained electrode was ready for use after the final wash with water and denoted as MWCNTs-CHT/GCE, as previously reported [58].

The MWCNTs-CHT/GCE was placed in the electrochemical cell containing phosphate buffer solution (pH 7.0) and several CV cycles between 0.0 and 0.6 V were applied until the response was stable. The relative electrochemical surface areas of the modified MWCNTs-CHT/GCE and bare GCE were determined by CV measurements between -0.1 and 0.6 V in 1.0 mM ferricyanide solution (PBS at pH 7.0) at several scan rates. The modified MWCNTs-CHT/GCE showed a surface area 7 times that of the GCE.

2.4. General procedure

The general procedure used to detect DA and MO was as follows. Each sample solution (10 mL) of 0.1 M PBS at pH 7.0 was pipetted into a voltammetric cell then an appropriate amount of the analytes was added to the solution.

The stirrer was switched on in the accumulation step under open circuit conditions for 50 s. Following the accumulation period, the stirrer was stopped and after 5 s quiescence time, the voltammogram was recorded by applying a positive going differential pulse scan from 0 to 0.6 V. The amounts of DA and MO were obtained using corresponding peak heights and drawing calibration curves. The modified electrode was regenerated by successive washing with 0.1 M PBS (pH 7.0) and running several CV scans between 0.0 to 0.6 V in the buffer solution. Finally the electrode washed with triply distilled water to remove all adsorbates from the surface and to provide a fresh surface before running the next experiments. All electrochemical experiments were carried out at ambient temperature.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characteristics of DA and MO at the MWCNTs-CHT/GCE

The electrochemical behavior of DA and MO were studied on modified and unmodified electrodes. Fig. 1 shows the cyclic voltammograms of 20 µM DA and MO at MWCNTs-CHT coated GCE in 0.1 M phosphate buffer solution (pH 7.0) at a scan rate of 50 mV s^{-1}. DA unlike MO shows a couple of redox peaks.

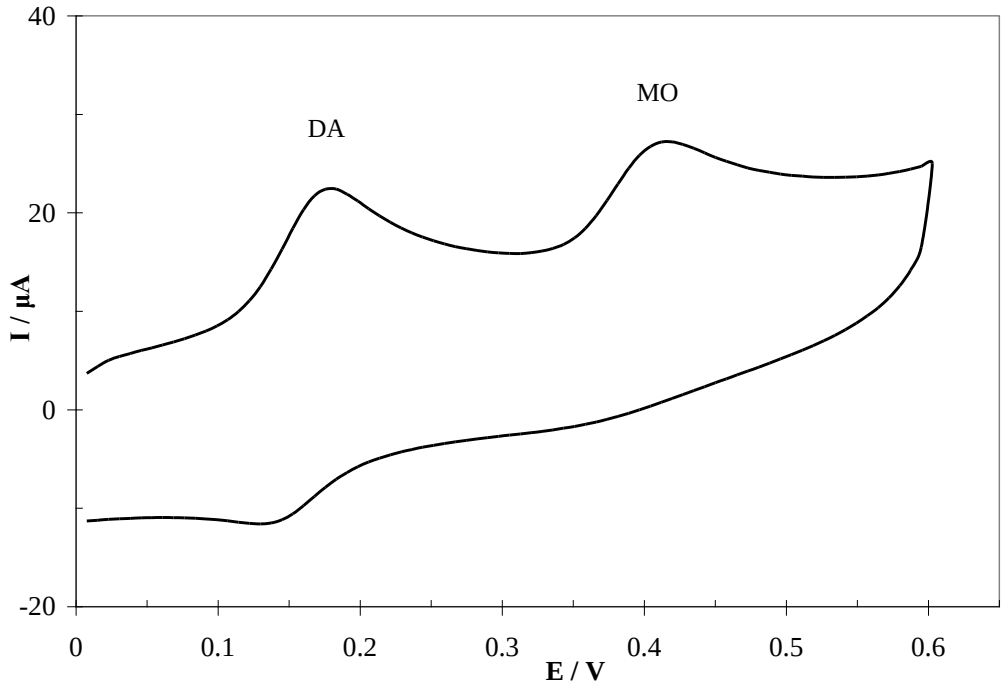

Figure 1. Cyclic voltammograms of 20 µM DA and MO at MWCNTs-CHT/GCE in 0.1 M phosphate buffer solution (pH 7.0) at scan rate of 50 mV s^{-1}.

The influence of scan rate on the oxidation peak potential (E_{pa}) and current of DA and MO at the MWCNTs-CHT/GCE in 0.1 M PBS (pH 7.0) were studied by cyclic voltammetry (not shown). The E_{pa} shifted to more positive potentials with increasing scan rate (v), confirming the kinetic limitation of the electrochemical reaction. The anodic peak current of 20 µM DA and MO was proportional to the scan rate over the range of 10 to 100 mV s^{-1} with a linear regression equations of :

$i_{pa}(\mu A) = 0.1108v + 5.9339$ (mV s^{-1}) ($R^2 = 0.9908$) **DA**

$i_{pa}(\mu A) = 0.0913v + 3.2407$ (mV s^{-1}) ($R^2 = 0.993$) **MO**

These phenomena indicate oxidations of DA and MO are adsorption-controlled processes at those scan rates. At sweep rates from 100 to 1000 mV s^{-1} values, the plot of currents vs. scan rate

deviate from linearity and the peak currents relate linearly with the square root of scan rate ($\nu^{1/2}$) that shows a diffusion-controlled mechanism with a linear regression equation of:

$i_{pa}(\mu A) = 1.3238\nu^{1/2}\ (mV\ s^{-1})^{1/2} - 4.7547$ $(R^2 = 0.9927)$ **DA**

$i_{pa}(\mu A) = 1.5397\nu^{1/2}\ (mV\ s^{-1})^{1/2} - 5.2197$ $(R^2 = 0.9941)$ **MO**

At scan rates higher than 200 mV s^{-1}, peak separations (ΔE_p) for DA begin to increase, demonstrating the limitation due to charge transfer kinetics. Based on Laviron theory [60] the charge transfer coefficient (α) and electron transfer rate constant (k_s) can be determined by measuring the variation of ΔE_p *vs*. log scan rate. The slope of the ΔE_p *vs*. log(ν), was about, -131.8 mV and the slopes of E_{pc} and E_{pa} *vs*. log(ν) are -0.0564 and 0.0626, respectively. Using the equations of:

$$E_{pc} = K - 2.303\ (RT/\alpha_c nF)\ \log(\nu)$$

$$E_{pa} = K + 2.303\ (RT/\alpha_a nF)\ \log(\nu)$$

By considering two electrons transferred for DA, cathodic (α_c) and anodic (α_a) charge transfer coefficients of 0.52 and 0.47 were obtained. Substituting the α value into the following equation, an apparent surface electron transfer rate constant, $k_s = 2.70\ s^{-1}$ was obtained.

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nF\upsilon}\right) - \alpha(1-\alpha)\frac{nFE}{2.3RT}$$

The large value of the electron transfer rate constant shows the high ability of the modified electrode for promoting electron transfer between the DA and the electrode surface. The surfaces of MWCNTs contain a large number of defects and the special nanostructure of MWCNTs may act as molecular wires, enhancing the direct electron transfer between DA and the electrode.

Fig. 2 exhibits the DPV responses from the electrochemical oxidation of DA and MO at modified and unmodified electrodes. Voltammograms **a** and **b** are DPVs recorded for the oxidation of DA (20 µM) and MO (20 µM) at GCE and MWCNTs/CHT/GCE respectively.

As can be seen the MWCNTs-CHT/GCE exhibit enhanced electrocatalytic oxidation with a less positive shift of potential and also much higher peak current for the oxidation of DA and MO in comparison to the bare GCE. According to the fact that MWCNTs can increase the surface activity noticeably, the background current and the anodic peak currents of DA and MO became considerably larger at the modified electrode in comparison to those at the GCE. Based on these results, it is inferred that MWCNTs-CHT/GC modified electrode exhibits potent and persistent electron mediating behavior followed by well-separated oxidation peaks towards DA and MO. These improvements are due to the presence of MWCNTs and CHT on the GC electrode surface which enhance the characteristics of DA and MO oxidation, accelerate the electron transfer rate and diminishes electrode fouling. Therefore, the

MWCNTs-CHT/GCE can be used for the simultaneous electrochemical determination of DA and MO and the concentrations of these analytes can be obtained using corresponding peak heights.

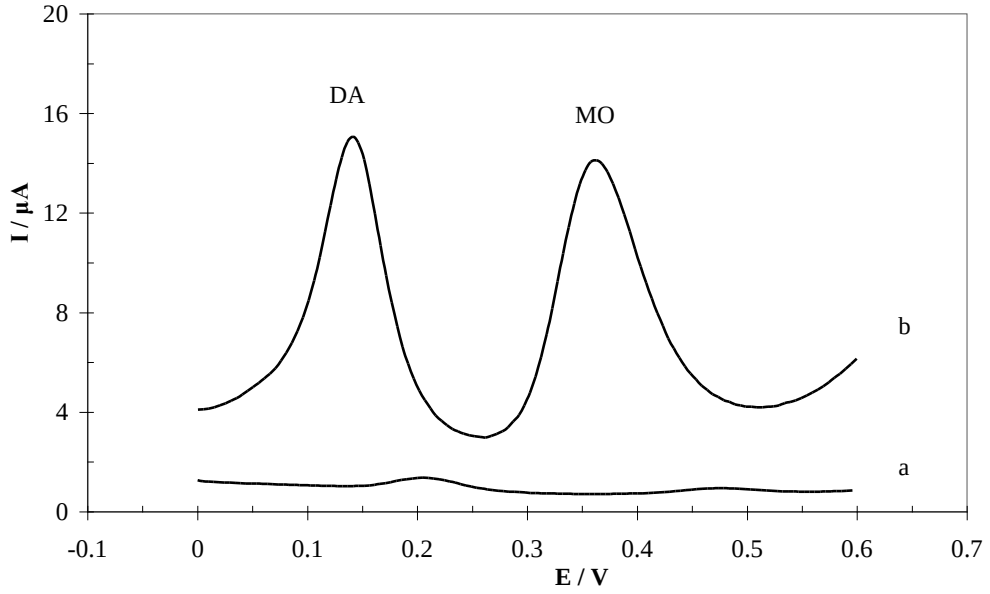

Figure 2. Differential pulse voltammograms of 20 µM DA and MO at (a) GC and (b) MWCNTs-CHT/GCE in 0.1 M phosphate buffer solution (pH 7.0). Other conditions: Open circuit, t_{acc}=50 s, pulse amplitude=50 mV, scan rate=10 mV s^{-1}, interval time=0.5 s, modulation time=0.2 s and step potential=5 mV.

3.2. Optimization of operational parameters

3.2.1. Effects of solution pH

The dependence of peak current (Ip) and peak potential (Ep) of DA (30 µM) and MO (40 µM) on pH for phosphate buffer solution at modified electrode was examined by DPV method. It was found that the oxidation peak shifted to more negative potential with increasing solution pH of 4.0–10.0 which could indicate the presence of a chemical reaction (proton-transfer reaction) preceding the electrode process. The following equation displays the correlation between peak potential and pH:

E_{pa} *vs.* Ag/AgCl (V)=0.5603 – 0.0597 pH (R^2= 0.9921) **DA**

E_{pa} *vs.* Ag/AgCl (V)=0.7966 – 0.0631 pH (R^2= 0.9904) **MO**

The slope of the variation of *E*p as a function of solution pH is 0.0592(*m*/*n*) V/pH, where *m* and *n* are the number of protons and electrons involved in the electrode process, respectively. The slope of *E*p versus pH for DA and MO is close to that expected for a Nernstian reaction which is 0.059 V at 25 °C [59], revealing that the uptake of electrons is accompanied by an equal number of protons.

Fig. 3 exhibits plots of electrooxidation peak currents of DA and MO versus electrolyte pH over the pH range from 4 to 10. It was observed that in the pH range of 4 to 7, the peak current of DA and MO was improved and at pH above 7 starts to decline. According to the results and considering the physiological environment, the pH 7.0 PBS was chosen as the supporting electrolyte for the simultaneous determination of DA and MO in the mixture in further experiments.

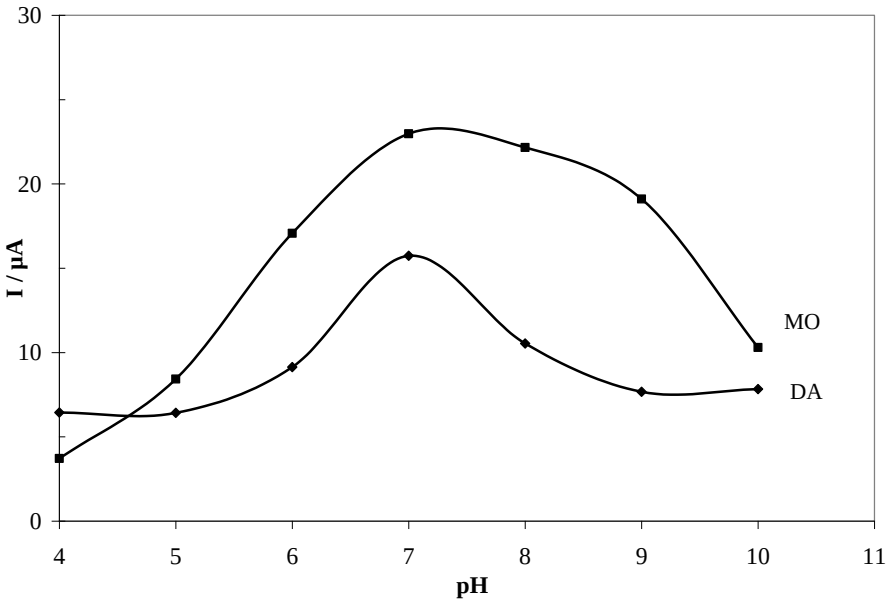

Figure 3. Effect of pH on the differential pulse voltammogram peak currents of DA and MO compounds at MWCNTs-CHT/GCE in 0.1 M phosphate buffer solutions. Concentrations: DA: 30 μM and MO: 40 μM.

3.2.2. Effects of accumulation time

Fig.4 illustrates plots of the anodic peak currents in differential pulse voltammetry versus accumulation time for 20 μM DA and 20 μM MO under open circuit condition. Initially, the oxidation peak currents of DA and MO improve with accumulation time up to 50 s for both DA and MO. At longer accumulation time, the peak currents remained almost constant. As a consequence, the accumulation time of 50 s was selected as an optimum time for subsequent analyses.

3.3. Linear dynamic range and detection limit of the method

To validate the linear relationship between oxidative peak currents of DA and MO concentrations, differential pulse voltammetry and chronoamperometry experiments were accomplished at the surface of MWCNTs-CHT/GC electrode under optimum conditions and corresponding calibration curves were constructed. The DPVs and calibration curves for DA and MO at various concentrations in pH 7.0 PBS at the nanocomposite modified electrode are displayed in Fig. 5.

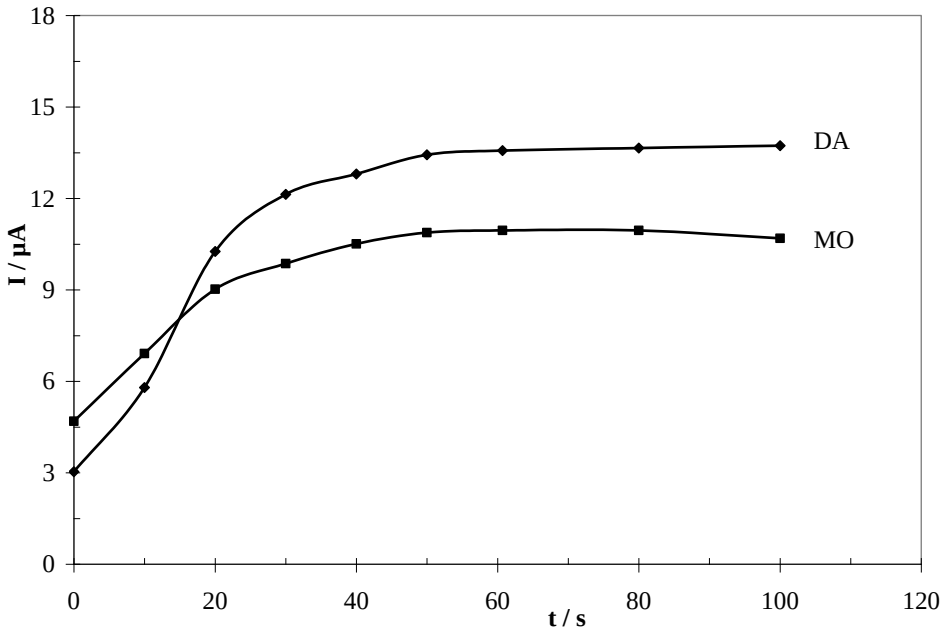

Figure 4. Effect of accumulation time on the differential pulse voltammogram peak currents of 20 µM DA and MO in 0.1 M phosphate buffer (pH 7.0) solution.

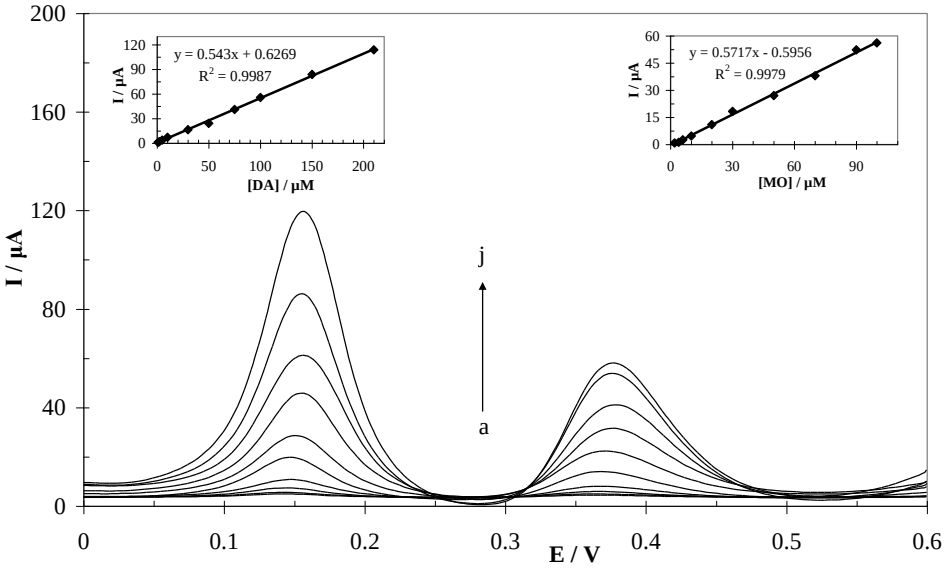

Figure 5. Differential pulse voltammograms for different concentrations of DA and MO mixture as (a) 1 + 2, (b) 2 + 4, (c) 5 + 6, (d) 10 + 10, (e) 30 + 20, (f) 50 + 30, (g) 75 + 50, (h) 100 + 70, (i) 150 + 90 and (j) 210 + 100, respectively, in which the first value is the concentration of DA and the second value is the concentration of MO in µM. Insets: (A) Plot of peak currents as a function of DA concentration. (B) Plot of the peak currents as a function of MO concentration.

The anodic peak current of DA showed a linear relationship for concentrations in the range of 1 to 210 µM. The linear regression equation was expressed as $i$pa(µA) = 0.6269 + 0.543c (µM) (R^2 = 0.9987) with a detection limit of 0.19 µM for this interval. With regard to MO, the linear range was

from 2 to 100 µM with an equation of *i*pa(µA) = 0.5956 + 0.5717*c* (µM) (R^2 = 0.9979). The detection limit for MO was found to be 0.24 µM (S/N = 3).

Fig. 6 displays the chronoamperogram response of the rotated modified electrode (3000 rpm) with successive injection of DA and MO in PBS (pH 7.0) at modified electrode. The step potential for chronoamperometry was set 0.55 V. The sensor shows linearity from 0.5 to 280 µM for DA and from 1 to 280 µM for MO with the linear regression equations as follows:

$$y = 0.3281c + 1.3605 \qquad (R^2 = 0.9981) \qquad \textbf{DA}$$

$$y = 0.2924c + 1.2894 \qquad (R^2 = 0.9975) \qquad \textbf{MO}$$

Also, the detection limit of 0.35 and 0.4 µM (S/N=3) was obtained for DA and MO, respectively.

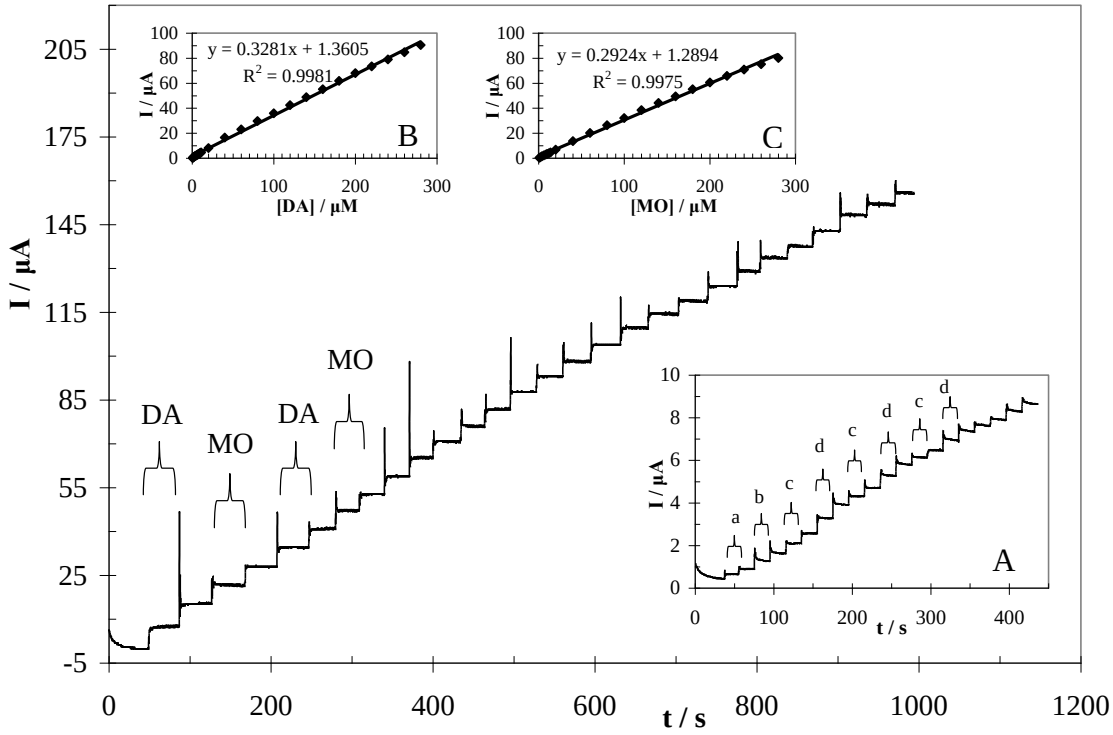

Figure 6. Hydrodynamic Amperometric response at rotating MWCNTs-CHT/GCE (rotating speed 3000 rpm) held at 0.55 V in PBS (pH 7.0) for simultaneous determination of DA and MO by successive additions of 20 µM DA and 20 µM MO. (Insets: (A) successive additions of (a) 0.5 µM and (c) 1 µM DA and (b) 1 µM and (d) 2 µM MO, (B) and (C) Plots of peak currents as a function of DA and MO concentration)

3.4. Stability and reproducibility of the MWCNTs-CHT/GCE

In order to characterize the reproducibility of the method, a series of repetitive differential pulse voltammetric measurements were carried out in 30 µM of DA and MO solution. The results of

10 successive measurements showed excellent reproducibility with a relative standard deviation (RSD) of 1.9 and 2.5 %, respectively. The small values of RSD indicate that the modified electrode is stable after consecutive measurements and does not undertake surface fouling by the oxidation products of DA or MO during the voltammetric measurements due to antifouling property of CHT [61]. These results indicate that MWCNTs-CHT/GCE electrode was stable for repeated measurements for the selective determination of DA or MO compounds.

The stability of the MWCNTs-CHT/GC modified electrode was checked over a period of operation. The performance of the MWCNTs-CHT/GCE to detect DA and MO was examined in air or phosphate buffer (pH 7.0) for a longer period by determining the decrease in peak currents during repetitive DPV measurements. When the nanocomposite modified electrode was stored in air over 7 days, the current response of DA and MO decreased by about 5 and 9.3%, respectively. When the modified electrode was subjected to 10 experiments during 36 h gave less than 8.6 and 12.2% decrease in the current response of DA and MO, respectively. It shows that the MWCNTs-CHT/GCE composite has a very stable environment for detection of these compounds and demonstrates high sensitivity for the simultaneous determination of DA and MO. The high mechanical strength and high water stability of the CNTs-interspersed CHT could be the reason for the high stability of the MWCNTs-CHT film modified electrode. These characteristics are suitable for long-term electrochemical sensing applications [62].

3.5. Effect of interferences

The influences of some possible interfering species to detect DA and MO at a MWCNTs-CHT/GC modified electrode was examined to evaluate their effect on the electrode responses by spiking of various excess amounts of each interference to sample solutions in the presence of 80 μM DA and MO in 0.1 M phosphate buffer solution at optimum conditions.

Table 1. Maximum tolerable concentration of interfering species

Interfering species	[DA]/ μM	[MO] / μM
Ascorbic acid	300	1500
Tryptophan	700	800
Tyrosine	1000	800
L-alanin	1000	3000
L-glutamic acid	1300	1500
Histidine	1100	1000
Aspartic acid	2300	2000
Uric acid	300	250
Oxalic acid	2300	2500
Caffeine	1800	3200
Tartaric acid	2000	3500
Sodium Citrate	3000	3500
Cysteine	2700	3800
Glucose	5000	5000

Table 1 exhibits the tolerance limit concentration of each interference which gives a depression of the peak response by about ≤ 10%. The results reveal that no serious interference occurred from the most common interfering species tested.

3.6. Real sample analysis

A feasibility study of the application of the proposed MWCNTs-CHT/GC electrode as the electrochemical sensor to determine DA and MO in human blood and urine was performed by differential pulse voltammetry method under the optimum conditions. In order to do this, the standard additions method used to prevent any matrix effects. In this method, known amounts of analytes were added to phosphate buffer solution (pH 7.0) containing deliberate amounts of real sample. Additionally, the recovery ratios on the basis of this method were investigated and the values were between 94.0 and 104.0%. The recovery ratios show that the determination of DA and MO using the modified electrode is effective, accurate and very reproducible. In light of these results, the prepared electrode was very reliable and sensitive and can readily be applied to determine DA and MO in real samples. The MWCNTs-CHT/GCE combined the CNTs ability to develop the adsorption and electron-transfer process with attractive permselective properties of the biocompatible CHT film.

Table 2. Estimation of DA and MO diluted (50-fold) real sample (Human blood and urine)

Sample	Spiked (µM)		Found (µM)		[a]R.S.D. (%)		Recovery	
	DA	MO	DA	MO	DA	MO	DA	MO
Urine	5.0	5.0	5.2	4.7	2.6	3.3	104.0	94.0
	15.0	15.0	14.8	15.4	2.3	2.8	98.6	102.6
Blood	5.0	5.0	4.8	4.8	3.2	3.5	96.0	95.0
	15.0	15.0	15.2	14.5	2.5	3.1	101.3	96.6

[a] Average of three determinations at optimum conditions.

4. CONCLUSION

The results obtained in this work clearly demonstrate that using glassy carbon electrode modified with a dispersion of CNT in chitosan as a detector improves the characteristics of DA and MO oxidation; such as improved electrochemical processes, high resistance to electrode fouling and sensitive, stable and reproducible voltammetric and amperometric detection of these analytes due to high water stability and high mechanical strength of MWCNTs-CHT nanocomposite and antifouling effect of Chitosan. The developed composite film combines the advantages of ease of fabrication, an efficient electron transfer reaction together with well-defined oxidation peaks for the simultaneous determination of DA and MO. Moreover, the sensor has wide linear range, sub-micro-molar detection limit, speed, low cost and simple approach for regeneration of the electrode surface. The presented method with a composite film biosensor provides an opportunity for qualitative, quantitative

characterization and simultaneous determination of DA and MO at physiologically relevant conditions without the necessity of sample pretreatments or time-consuming extraction.

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