

*Full Paper*

## **Voltammetric Determination of Dopamine in Human Serum with Amphiphilic Chitosan Modified Glassy Carbon Electrode**

**Cheng Yin Wang\*, Zhi Xian Wang, Ai Ping Zhu and Xiao Ya Hu\***

Department of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, China

E-mails: yzswcy@yzcn.net (Cheng Yin Wang), xiaofeixia1982@163.com (Zhi Xian Wang),

zapyz@yahoo.com (Ai Ping Zhu), xyhu@yzu.edu.cn (Xiao Ya Hu).

Phone: 086-0514-7975590-9217. Fax: 086-0514-7975244.

\* Authors to whom correspondence should be addressed.

*Received: 14 August 2006 / Accepted: 3 November 2006 / Published: 8 November 2006*

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**Abstract:** An improvement of selectivity for electrochemical detection of dopamine (DA) with differential pulse voltammetry is achieved by covalently modifying a glassy carbon electrode (GCE) with O-carboxymethylchitosan (OCMCS). The amphiphilic chitosan provides electrostatic accumulation of DA onto the electrode surface. In a phosphate buffer solution (pH 6.0), a pair of well-defined reversible redox waves of DA was observed at the OCMCS/GCE with a  $\Delta E_p$  of 52 mV. The anodic peak current obtained from the differential pulse voltammetry of dopamine was linearly dependent on its concentration in the range of  $6.0 \times 10^{-8}$  to  $7.0 \times 10^{-6}$  M, with a correlation coefficient of 0.998. The detection limit ( $S/N = 3$ ) was found to be  $1.5 \times 10^{-9}$  M. The modified electrode had been applied to the determination of DA in human serum samples with satisfactory results.

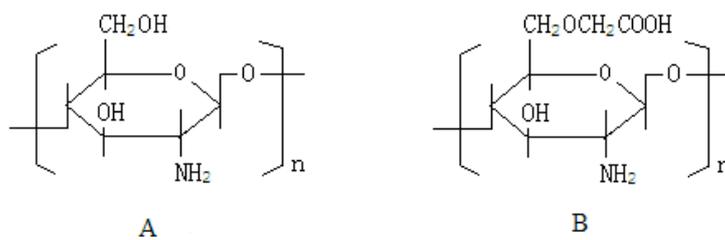
**Keywords:** O-carboxymethylchitosan; chitosan; dopamine; voltammetry; human serum

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### **1. Introduction**

Chitosan (Scheme 1A) is a de-N-acetylated derivative of chitin. Polysaccharides chitosan and chitin are structural components of the cell walls of many fungi and the exoskeletons of lots of arthropods such as crabs and shrimps [1]. Chitosan is one of the most promising natural polymers for use as an immobilization matrix, with advantages such as biodegradability, chemical inertness, nontoxicity, biocompatibility, high mechanical strength, good film-forming properties, and low cost

[2]. Some biomolecules can be immobilized in chitosan membrane in order to make different biosensors. In recent decades, several different biosensors with chitosan supports. For example, immunosensors [2, 3] and enzymatic sensors [4-8] have been reported for the determination of  $\alpha$ -1-fetoprotein [2], *Schistosoma japonicum* antigen [3], glucose [4, 5], hydrogen peroxide [6, 7], and urea [8]. Moreover, chitosan has an ability to chelate with metal ions owing to the existence of hydroxyl groups and amino groups [9]. In previous studies, the chitosan/GCEs were prepared for determination of lead ions [9], EDTA species [10], and total iron [11]. In these cases, the methods are simple and reliable. However, there has been no report for the determination of neurotransmitters with the chitosan/GCEs. Among the numerous water-solution chitosan derivatives, O-carboxymethylchitosan (OCMCS, Scheme 1B) is a very promising candidate for applications demanding cell and blood compatibility. The OCMCS with 100 carboxymethyl groups and 75 amino groups per 100 anhydroglucosamine units of OCMCS can be synthesized by the reaction of chitosan and monochloroacetic [12]. Although several properties of OCMCS have already been studied, the application of OCMCS in the electroanalytical chemistry has not been investigated.



**Scheme 1.** Chitosan (A) and O-carboxymethylchitosan (B).

Dopamine (DA) is one of the naturally occurring catecholamines. It is an important compound for message transfer in the mammalian central nervous system. Changes in its concentration may lead to serious diseases such as Parkinson's [13]. Quantitative determination of DA in human physiological fluids is of considerable significance in both biochemical and clinical diagnoses. Methods for the detection of DA include chemiluminescence [14], fluorimetry [15], ultraviolet-visible spectrometry [16], and capillary electrophoresis (CE-luminescence) [17]. Because of its electrochemical activity, DA can also be determined with electrochemical methods [18-20]. Electrochemical techniques have attracted great interest in many cases, and these techniques can be fast in detections, low in cost, and with merits of low detection limit and high accuracy [18]. However, a major problem frequently encountered in the electrochemical detection of DA is serious interferences caused by the ascorbic acid (AA) and uric acid (UA), which exist in body fluids in relatively high concentrations. The direct redox reactions of three substances at bare carbon electrodes regularly take place at very similar potentials [19], and often suffer from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. Thus, it is difficult to detect DA in the presence of a high level of AA and UA in real biological samples. Recently, many techniques were reported to improve the selectivity for the determination in the presence of AA. Several approaches, based on polymer-modified electrodes [20-28], nanomaterial-modified electrodes [29-32], and self-assembled monolayers [33-38] have been tried for solving this problem. One possibility is to covalently attach negatively charged functionalities to the electrode surface, for example, poly (2-picolinic acid) [20], *p*-phenylacetate [21], poly

(sulfosalicylic acid) [22], polyeugenol [23], nickel phthalocyanine polymer [24], and over-oxidized poly (N-acetylaniline) [25]. In these cases, differentiation is attributed to an electrostatic repulsion between AA and the functionalities on the electrode surface, while there is an attraction for cationic DA. Because AA exists in its anionic form ( $pK_a$  4.1) and DA is a cation ( $pK_a$  8.9) at the physiological pH (7.4), AA cannot enter the polymer film, and interference with the determination of DA is diminished. To improve the selectivity for determination of DA in real samples, Zare developed an effective method to simultaneously determine DA, AA and UA using a carbon paste electrode modified by tetrabromo-*p*-benzoquinone [39]. The modified electrode showed good selectivity and high stability. Zhao reported a selective detection of dopamine in the presence of AA and UA using a carbon nanotubes-ionic liquid gel modified electrode, and the linear range of DA was  $1.0 \times 10^{-6} \sim 1.0 \times 10^{-4}$  M with a detection limit of  $1.0 \times 10^{-7}$  M [40]. In these cases, the differentiation of DA, AA and UA was attributed to the electrocatalytic oxidation of them at the modified electrodes.

In this paper, we describe the improvement of selectivity for voltammetric detection of DA by covalently modifying a GCE with OCMCS. The modified electrode shows high selectivity and sensitivity as a result of the attraction to DA and repulsion to AA and UA. A good linear relationship was obtained between the anodic peak current and the DA concentration in the range of  $6.0 \times 10^{-8}$  to  $7.0 \times 10^{-6}$  M, with a detection limit of  $1.5 \times 10^{-9}$  M. Compared with the previous methods [39, 40], the present detection limit decreased two orders of magnitude. The modified electrode showed considerable attraction to DA and repulsion to AA and UA. The presence of 1000-fold AA and 200-fold UA did not interfere with the determination of DA. The performance of the modified electrode was illustrated by determination of DA in spiked serum samples. This method has the advantages of rapid and simple operation, high selectivity and accuracy. This modifier film is considered to be a low-cost, steady, and promising material in the modification of electrodes.

## 2. Experimental Section

### 2.1. Apparatus

Electrochemical measurements were performed using a 660A CHI (Chenhua Instruments, China) in conjunction with a computer. A three-electrode system was used with a glassy carbon electrode (3.0 mm diameter,  $0.071 \text{ cm}^2$  geometrical areas) or an OCMCS-modified glassy carbon electrode, a platinum plate electrode as the counter electrode and an Ag/AgCl as the reference electrode. All experiments were carried out at 25 °C.

### 2.2. Chemicals and Solutions

Dopamine was purchased from Sigma-Aldrich Co. (Milwaukee, Wisconsin, USA). Ascorbic acid and uric acid were obtained from the Chemical Reagent Company of Shanghai (Shanghai, China). Thionyl chloride was obtained from the Linfeng Chemical Reagent Company of Shanghai (Shanghai, China). Chitosan powder was supplied by Lianyungang Biological Inc., China. Its viscosity-averaged molecular weight is  $5.2 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$ , and the degree of acetylation is 10 mol%. Monochloroacetic acid, Isopropanol, ethyl alcohol, hydrochloric acid, formic acid and sodium hydroxide were purchased

from Sigma-Aldrich Co. They were used as received without further purification. All reagents were of analytical grade. All solutions were prepared with double-distilled water.

An oxidizing solution was prepared by dissolving about 0.3 g of  $\text{NaNO}_3$  and 4.5 g of  $\text{KMnO}_4$  in 40 mL of concentrated  $\text{H}_2\text{SO}_4$ . A solution containing 0.3 g of the OCMCS in 20 mL of formic acid was used in the modification. The DA and AA solutions were prepared with the doubly distilled water immediately prior to each experiment. The UA solution was prepared with 0.10 M NaOH solution. A 0.025 M phosphate buffer solution (PBS) at various pH values was used as a kind of supporting electrolytes for the determination of DA.

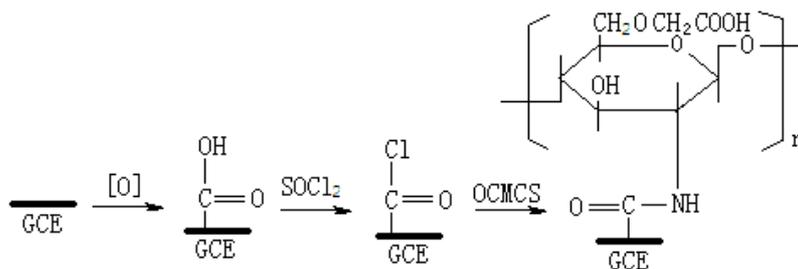
### 2.3. OCMCS Synthesis [12]

2 g chitosan was immersed in 25 mL of 50 wt% NaOH solution to swell and alkalize for 24 h. The alkalized chitosan was crushed into a filtration cake and then transferred into a flask. Five grams of monochloroacetic acid was dissolved in 25 mL of isopropanol, and then added to the flask drop-wise for 20 min. The reaction in the flask was allowed to continue for 8 h at room temperature, after which the mixture was filtered to remove the solvent. The filtrate obtained was dissolved in 100 mL of water, and 2.5 M HCl was added to it to adjust its pH to  $\sim 7$ . After this solution was centrifuged to remove the precipitate, 400 mL of anhydrous ethanol was added to it to precipitate the product. Finally, the product was filtered, rinsed thrice with anhydrous ethanol, and vacuum-dried at room temperature. The molar fractions of carboxymethylated groups and amino groups inside an anhydroglucosamine unit of OCMCS were found to be about 100 % and 75 %, respectively. The viscosity-averaged molecular weight of the OCMCS is  $2.0 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$ .

### 2.4. Fabrication of the OCMCS-modified Glassy Carbon Electrodes

Prior to each fabrication, the glassy carbon electrode was polished before each experiment with 1, 0.3 and 0.05  $\mu\text{m}$   $\alpha$ -alumina powder, consecutively rinsed thoroughly with doubly distilled water between each polishing step, sonicated in 1:1 nitric acid, acetone, and doubly distilled water in that order. The GCE was immersed in the oxidizing solution for 60 min at room temperature. Having been rinsed with doubly distilled water, the GCE was immersed immediately in a 3 % solution of  $\text{H}_2\text{O}_2$  for 30 min in order to reduce the excess of  $\text{KMnO}_4$ . The GCE was rinsed with doubly distilled water again, and then it was dried under an infrared lamp. Subsequently the GCE was immersed in thionyl chloride for 60 min. Finally, the GCE was soaked in the OCMCS solution for 40 min, and then it rinsed thoroughly with formic acid and doubly distilled water to remove the OCMCS not adsorbed on the electrode surface. The procedure can be presented as follows (Scheme 2).

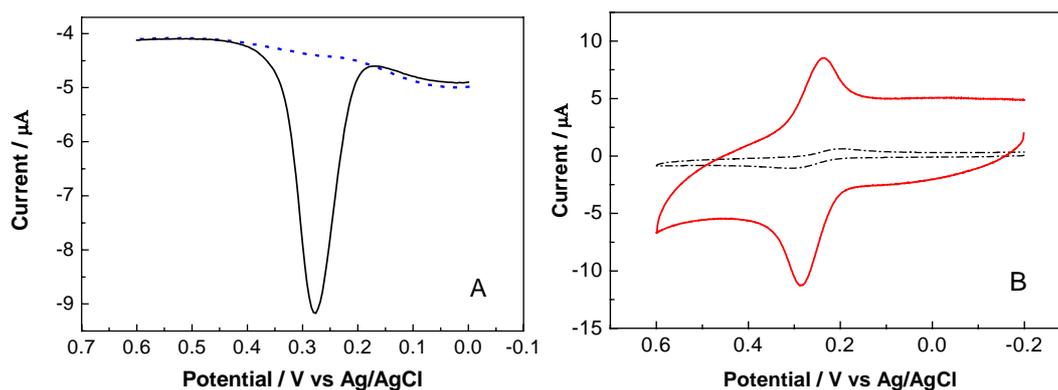
Prior to determination of DA, it is necessary to pretreat the electrode. Having been dried under an infrared lamp, the modified electrode did not stop electroactivating by cyclic scanning from + 0.00 V to + 0.60 V (vs. Ag/AgCl) in pH 6.0 phosphate buffer solution (PBS, 0.025 M) until a steady cyclic voltammogram was obtained. Finally, the electrode was dried with a stream of high purity nitrogen.



**Scheme 2.** The procedure of modification of the GCE.

### 2.5. Determination of DA

The modified glassy carbon electrode, the platinum wire counter electrode, and the reference electrode were immersed in 20.00 mL 0.025 mol·L<sup>-1</sup> PBS (pH 6.0). A certain amount of the DA was added to the solution, with stirring by a magnetic stirrer. The stirring was stopped after the electrochemical accumulation for 60 s was at - 0.20 V. Then the differential pulse voltammetry (DPV) was immediately performed to scan from + 0.00 to + 0.60 V after quiet time of 30 s. To establish the optimum conditions for the determination of DA by means of DPV technique, various instrumental variables were studied, and the optimum conditions were as follows: scan rate, 5 mV·s<sup>-1</sup>; sampling width, 0.05 s; pulse amplitude, 50 mV; and pulse period, 0.2 s. The anodic peak currents of DA at + 0.276 V were recorded (Figure 1(A)). The standard addition method was applied to quantitative determine DA. After each determination, a renewal of the electrode was easily accomplished by soaking the modified electrode in the solution of 6.0 M HNO<sub>3</sub> and cyclically scanning between - 0.10 and + 0.60 V for about 10 cycles.



**Figure 1.** (A) Differential pulse voltammograms of DA at the modified electrode in the PBS (0.025 M, pH 6.0). Dot line: blank solution; Solid line:  $5.0 \times 10^{-7}$  M DA. Accumulation potential under stirring, - 0.20 V; accumulation time, 60 s; quiet time, 30 s; scan rate, 5 mV·s<sup>-1</sup>; sampling width, 0.05 s; pulse amplitude, 50 mV; pulse period, 0.2 s. (B) Cyclic voltammograms of  $2.0 \times 10^{-5}$  M DA at the bare GC electrode (dash line) and the modified electrode (solid line) in the PBS (0.025 M, pH 6.0). Scan rate: 100 mV·s<sup>-1</sup>.

### 3. Results and Discussion

#### 3.1. The Role of the OCMCS at the Modified Electrode

Figure 1(B) shows the cyclic voltammetric behavior of  $2.0 \times 10^{-5}$  M DA at bare GCE and the OCMCS/GCE in the 0.025 M PBS solution (pH 6.0). At bare GCE, there was irreversible electrochemical behavior of DA with  $\Delta E_p$  of 110 mV at a scan rate of  $100 \text{ mV}\cdot\text{s}^{-1}$ , which  $\Delta E_p$  is the difference between the anodic peak potential ( $E_{pa}$ ) and the cathodic peak potential ( $E_{pc}$ ). However, a pair of well-defined redox waves of DA was observed at the OCMCS/GCE with  $\Delta E_p$  of 52 mV, thus the reversibility of DA improved significantly. Moreover, the peak current of DA at the modified electrode was more than that at the bare GCE. The reason for this is as follows: In PBS solution of pH 6.0, there are negatively-charged functional groups on the electrode surface. At pH 6.0, DA exists in the cationic form. Hence, the surface-active group of OCMCS can attract DA into the modifier film, and improve favorable accumulation of DA to result in a pair of higher sensitive and well-defined redox waves of DA.

The scan rate effect on the peak current of  $4.0 \times 10^{-6}$  M DA at the modified electrode was also investigated. The both anodic and cathodic peak current increased with increasing the scan rate. A good linearity between  $i_p$  and the scan rate  $\nu$ , was obtained from the range of  $40 \sim 600 \text{ mV}\cdot\text{s}^{-1}$ , which demonstrates that the electrode reaction was an adsorption-controlled processes [41]. The linear equations were  $i_{pa} (\mu\text{A}) = -7.40 - 0.06\nu (\text{mV}\cdot\text{s}^{-1})$  with a correlation coefficient  $r = 0.998$ , and  $i_{pc} (\mu\text{A}) = 5.86 + 0.03\nu (\text{mV}\cdot\text{s}^{-1})$  with  $r = 0.996$ .

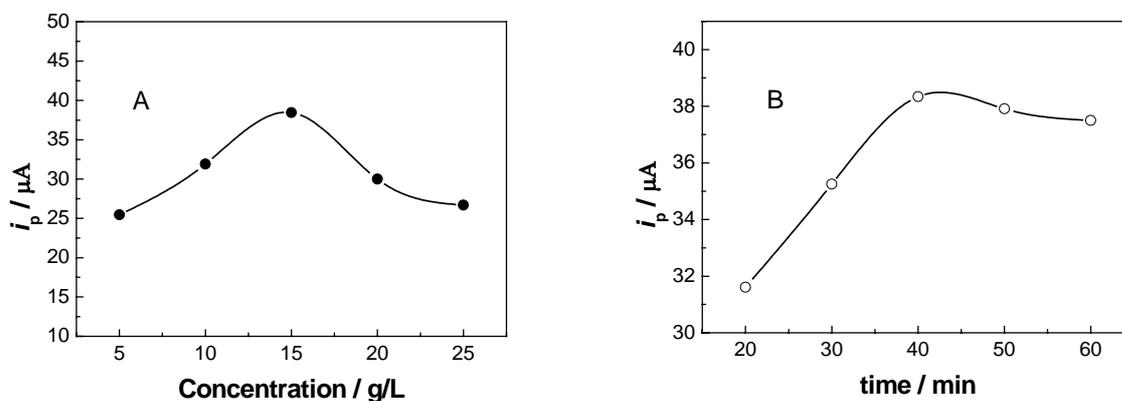
#### 3.2. Optimization of the GCE Modification Conditions

The anodic peak currents of DA were considerably related to the amount of OCMCS on the surface of the GCE. Figure 2(A) and 2(B) show remarkably effect of the concentration of the OCMCS solution and time of the modification on the anodic peak current of DA, respectively. The anodic peak current gradually increased with increasing the amount of OCMCS on the surface of the electrode. In general, the higher concentration of OCMCS in the modifier solution, the more OCMCS was modified on to the GCE surface by covalent-bond reaction. Similarly, the longer time for the bare GCE being immersed in the OCMCS solution, the more OCMCS was combined with the GCE surface. However, the anodic peak current can decrease when excessive OCMCS was modified on to the electrode surface. We owed it to the decline of the electrode conductivity. Therefore a solution containing  $15 \text{ g}\cdot\text{L}^{-1}$  OCMCS and the time of 40 min were chosen for the modification.

#### 3.3. Optimization of Experimental Conditions of the Determination

To establish the optimum conditions for the determination of DA by means of DPV technique, various instrumental variables were studied. The high sensitive and well-defined peaks of DA in voltammograms were obtained in the PBS (0.025 M) compared with other supporting electrolytes, such as NaOH, HCl,  $\text{H}_2\text{SO}_4$ , HAc-NaAc,  $\text{NH}_4\text{Cl}\cdot\text{NH}_3\cdot\text{H}_2\text{O}$ , and KCl solution. The effects of accumulation potential and accumulation time on the DPV current response of DA were studied,

respectively. The accumulation step proceeded in a constantly stirred solution and the voltage-scanning step was performed after 30 s of quiet time. Figure 3(A) shows that the peak current of DA was the highest at - 0.20 V as the accumulation potential. This is attributed to the fact that DA exists in the cationic form at pH 6.0 and the negative potential is more favorable to the accumulation. Therefore an accumulation potential of - 0.20 V was chosen in all the subsequent work.



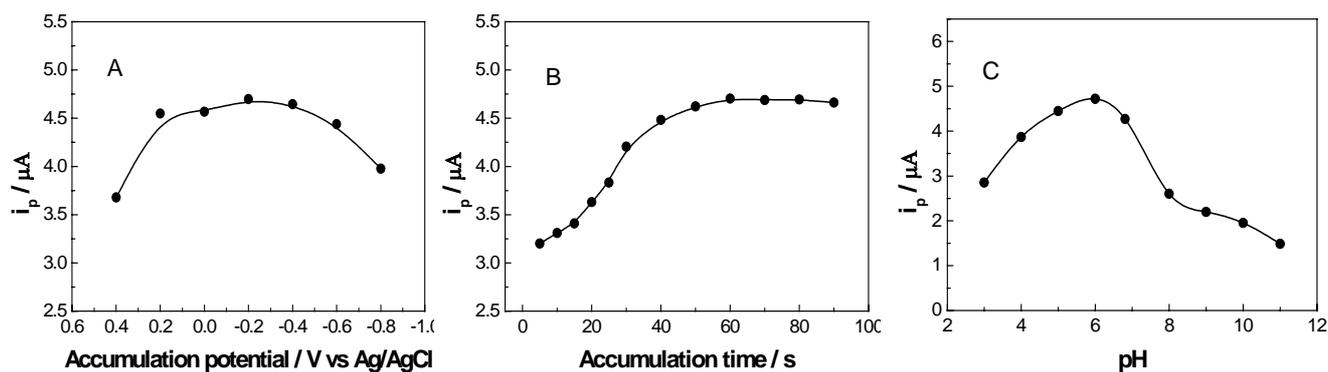
**Figure 2.** Effect of the OCMCS concentration (A) and the modification time (B) on the anodic peak current of DPV for  $5.0 \times 10^{-6}$  M DA at the modified electrode. The experimental parameters are similar to Figure 1(A).

The effect of accumulation time on the peak current was also investigated. Figure 3(B) shows that the peak current of DA increased with increasing accumulation time within 60 s, which indicates that DA on the modified electrode surface was rapidly adsorbed. Further postponement of the accumulation time did not increase the response of DA on the electrode after 60 s, owing to the surface adsorption saturation, and the peak current remained almost constant. For practical purposes, a 60 s accumulation time was sufficient for the determination of DA.

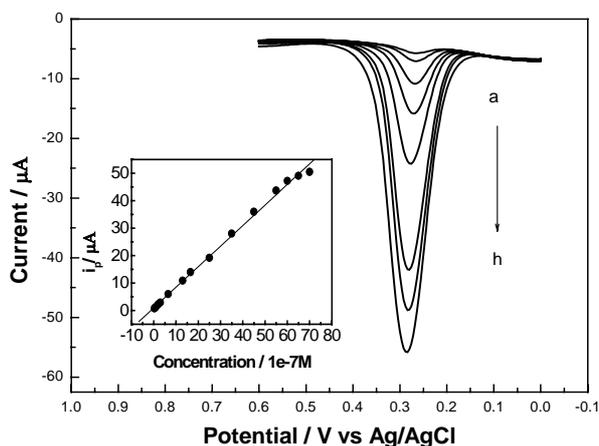
The pH value of the base solution has a significant influence on the oxidation of DA at the OCMCS/GCE. Figure 3(C) shows the effect of solution pH on the anodic peak currents examined by recording differential pulse voltammograms of DA, in a series of PBS buffers (0.025 M) with varying pH, in the range of 3.0 ~ 11.0. The peak current response of DA was the highest when the pH value was 6.0. This was attributed to the existence of DA ( $pK_a$  8.9) in the cationic form at pH 6.0. Because the OCMCS/GCE undertook negative charges, the modified film can attract and accumulate the cationic DA onto the electrode surface. Therefore the PBS buffer solution (0.025 M, pH 6.0) was selected for the determination.

### 3.4. Calibration Curve and Detection Limit

Under the preceding optimum detection conditions, the anodic peak currents were proportional to DA concentrations in the range of  $6.0 \times 10^{-8}$  ~  $7.0 \times 10^{-6}$  M. The linear equation is  $i_{pa} (\mu A) = 0.972 + 0.749C (10^{-7} M)$  with a correlation coefficient,  $r = 0.998$  (Figure 4). The detection limit of DA was  $1.5 \times 10^{-9}$  M in terms of signal to noise ratio of 3:1 ( $S/N = 3$ ).



**Figure 3.** (A) Effect of accumulation potential on the anodic peak current of the DPV. (B) Effect of accumulation time on the anodic peak current of the DPV. (C) Effect of pH on the anodic peak current. DA:  $5.0 \times 10^{-7}$  M, the other experimental parameters are similar to Figure 1(A).

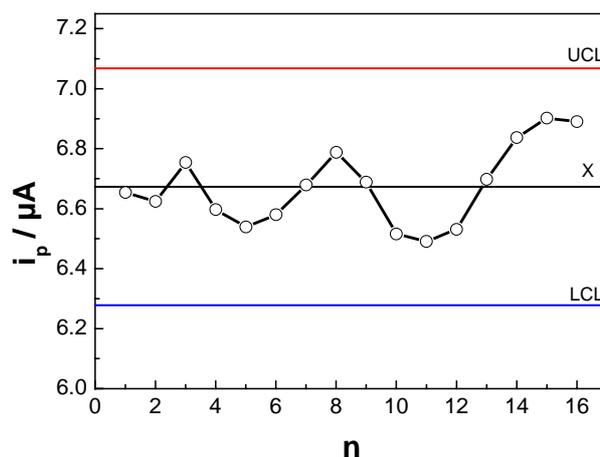


**Figure 4.** Differential pulse voltammograms of DA at the modified electrode in the PBS (0.025 M, pH 6.0). The concentrations of DA: (a)  $6.0 \times 10^{-8}$  M; (b)  $2.0 \times 10^{-7}$  M; (c)  $6.5 \times 10^{-7}$  M; (d)  $1.3 \times 10^{-6}$  M; (e)  $2.5 \times 10^{-6}$  M; (f)  $4.5 \times 10^{-6}$  M; (g)  $5.5 \times 10^{-6}$  M; (h)  $6.5 \times 10^{-6}$  M. Inset: Plot of the anodic peak current of dopamine versus the DA concentration. The experimental parameters are similar to Figure 1(A).

### 3.5. Reproducibility and Lifetime of the OCMCS/GCE

Because the modified electrode can adsorb the cation DA, it was necessary to renew the electrode surface. We found that the renewal of the electrode surface is easily accomplished by soaking the modified electrode in a 6 M  $\text{HNO}_3$  solution and cycling its potential between - 0.10 and + 0.60 V for about 10 cycles. In order to estimate the precision of determination, a quality control chart (X-chart) has been constructed. Figure 5 shows the quality control (X-chart) of  $7.5 \times 10^{-7}$  M DA applying the proposed method, where the X-axis represented the number of analyses while the Y-axis represented the anodic current of DA. There is a series of 16 points over the average score and all of them fall in between the upper control limit (UCL) and the lower control limit (LCL), which indicates that the

analysis progress was under the statistical control. For the special samples detection, after detecting DA in every five serum samples, the electrode was renewed in the 6 M HNO<sub>3</sub> solution under stirring, and then transferred into a standard solution of  $7.5 \times 10^{-7}$  M DA to record its oxidation peak current. The average current was 6.6  $\mu$ A with a relative standard deviation (RSD) of 1.7 % ( $n = 5$ ). Such stability seems to be acceptable for most practical applications. We also examined the lifetime of the OCMCS/GCE, and demonstrated that the OCMCS film retained 98.3 % of its initial peak current response after one month of storage in PBS buffer solution (0.025 M, pH 6.0).



**Figure 5.** Quality control chart (X - Chart) of  $7.5 \times 10^{-7}$  M DA.

### 3.6. Interference

For the following special detection in body fluid, a few other common coexisting substances were examined previously. When  $5.0 \times 10^{-7}$  M DA was determined under the optimum experimental conditions, interferences response were hardly observed in the presence of  $5.0 \times 10^{-4}$  M of KCl, NaNO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, urea, glucose, *D*-fructose, *L*-alanine, Vitamin B<sub>1</sub>, Vitamin B<sub>2</sub>, Vitamin B<sub>6</sub>,  $5.0 \times 10^{-5}$  M of tartaric acid, citrate, cysteine, caffeine, *DL*-tyrosine, MgCl<sub>2</sub>, CaCl<sub>2</sub>, or Fe(NO<sub>3</sub>)<sub>3</sub>. The results obtained were summarized in Table 1.

Furthermore, the influence of AA and UA, as co-existing electroactivated substances, was examined in more detail for the following serum samples analyses. Figure 6 (A) shows the differential pulse voltammograms in response to different concentrations of AA in the base solution. There was no evident oxidation peaks observed at the modified electrode except that the anodic current increased only a little with increasing the concentration of AA. This is attributed to the fact that AA exists in the anionic form ( $pK_a$  4.1) in PBS buffer solution (0.025 M, pH 6.0). The OCMCS/GCE undertook negative charges, so the thin film can repulse anionic AA. Figure 6 (B) shows that the coexistence of lower than  $5.0 \times 10^{-4}$  M AA only slightly increased the background current, but the values of DA peak currents were constant when the  $5.0 \times 10^{-7}$  M DA was detected. However, when the AA concentration was higher than  $8.0 \times 10^{-4}$  M, the value of DA peak current was affected, and its relative error was more than 5 %. This content of the coexistence is considered to interfere with the determination of DA.

Therefore the presence of 1000-fold AA did not interfere with the determination of  $5.0 \times 10^{-7}$  M DA. Similarly, UA ( $pK_a$  5.5) exists in its anionic form at the pH 6.0, and it would be repulsed by OCMCS film. However, a peak current response of UA can be observed because it can weakly adsorb on the electrode via hydrogen bonding [42, 43]. Figure 6(C) shows the differential pulse voltammograms of  $5.0 \times 10^{-7}$  M DA in the presence of different concentrations of UA. There was no current response for UA at the modified electrode when the concentration of UA was lower than  $6.0 \times 10^{-6}$  M. When the concentration of UA was not exceed  $1.1 \times 10^{-4}$  M, it did not interfere with the measurement of DA because of a separation of two anodic peak potentials, which was enough for the selective determination of DA in the presence of common concentration of UA, such as human serum samples.

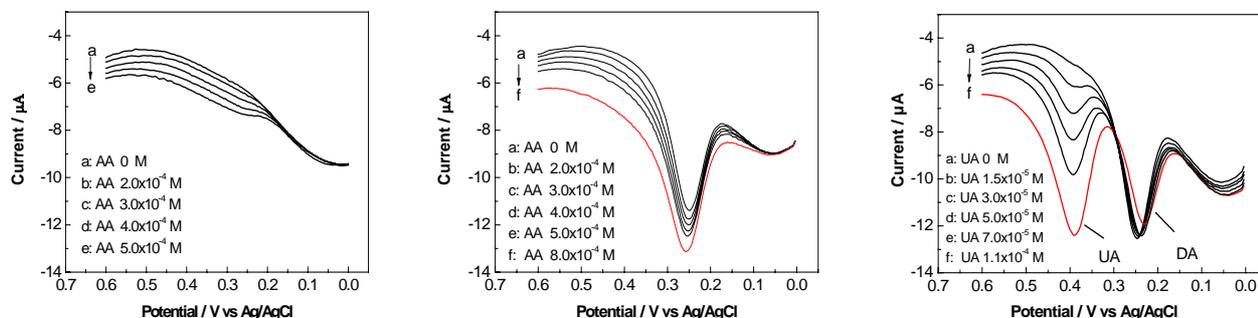
**Table 1.** Influence of potential interferents on the voltammetric response of 0.5  $\mu$ M DA.

Interferent	Concentration / mM	Signal change % ( $i_{DA} = 100$ %)
AA	0.30	+ 2.4
	0.40	+ 3.0
	0.50	+ 3.5
UA	0.03	- 1.8
	0.05	- 2.4
	0.07	- 3.1
urea	0.50	+ 2.7
tartaric acid	0.05	- 0.5
D-fructose	0.50	- 2.8
citrate	0.05	+ 2.1
glucose	0.50	- 0.7
cysteine	0.05	- 2.9
DL-tyrosine	0.05	- 2.3
L-alanine	0.50	- 0.5
caffeine	0.05	+ 1.5
vitamine B <sub>1</sub>	0.50	+ 2.2
vitamine B <sub>2</sub>	0.50	+ 2.7
vitamine B <sub>6</sub>	0.50	+ 2.0
NaNO <sub>3</sub>	0.50	- 3.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.50	- 2.3
KCl	0.50	- 1.9
MgCl <sub>2</sub>	0.05	- 2.9
BaCl <sub>2</sub>	0.05	- 3.0
Fe(NO <sub>3</sub> ) <sub>3</sub>	0.05	- 3.4

### 3.7. Detection of DA in the Human Serum Sample

Six human serum samples from healthy volunteers were determined with the modified electrode. To fit into the linear range and reduce the matrix effect, the serum samples were diluted 200 times with 0.025 M PBS (pH 6.0) before analyses, without further pretreatments. A standard addition method was employed under the previously described optimal experimental conditions. A sharp anodic peak was observed at + 0.267 V when the serum sample was added in the PBS base solution. And the height of the peak quantificationally increased after the standard DA solution was added sequentially, which indicates conclusively that the anodic peak was caused by DA in the serum sample. The results

obtained are listed in Table 2. The recoveries determined by spiking the serum samples with an amount of standard DA solution was found to be in the range of 96.8 % to 103.3 %, indicating the modified electrode can be applied to the determination of DA in human serum samples with satisfactory results.



**Figure 6.** (A) Differential pulse voltammograms of AA at the modified electrode. (B) DA:  $5.0 \times 10^{-7}$  M in the presence of different concentrations of AA. (C) Differential pulse voltammograms of  $5.0 \times 10^{-7}$  M DA in the presence of different concentrations of UA. The experimental parameters are similar to Figure 1(A).

**Table 2.** Determination of the DA in spiked serum samples with OCMCS/GCE.

Serum	Spiked / $\mu\text{g}$	Detected / $\mu\text{g}$	Recovery / %
Sample 1	0.95	0.92	96.8
Sample 2	1.71	1.71	100.0
Sample 3	4.55	4.54	99.8
Sample 4	9.48	9.79	103.3
Sample 5	13.39	13.50	100.8
Sample 6	18.02	18.15	100.7

#### 4. Conclusions

Consequently, this paper described a sensitive method for the detection of DA in serum samples by differential pulse voltammetry using a glassy carbon electrode modified by amphiphilic chitosan, O-carboxymethylchitosan (OCMCS), via covalent-bond reaction. The electrode demonstrated strongly accumulation of DA, drastically suppressed the response of AA and resolved an overlapping voltammetric response of DA and AA into well-defined peaks of DA with a large anodic peak current. Significant advantages were achieved by rapid determination, excellent sensitivity and selectivity. This novel modifier is considered to be a promising, low-cost, and steady material of electrode modifiers. The success of this strategy suggests that OCMCS film will have significant electroanalytical utility in the future. It is hope that the OCMCS/GCE will be in application for further sensor development.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20375034), the Natural & Scientific grant of Jiangsu Province (BK2006072) and the Novel Foundation of Jiangsu Provincial Education Department for Doctors (2005).

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