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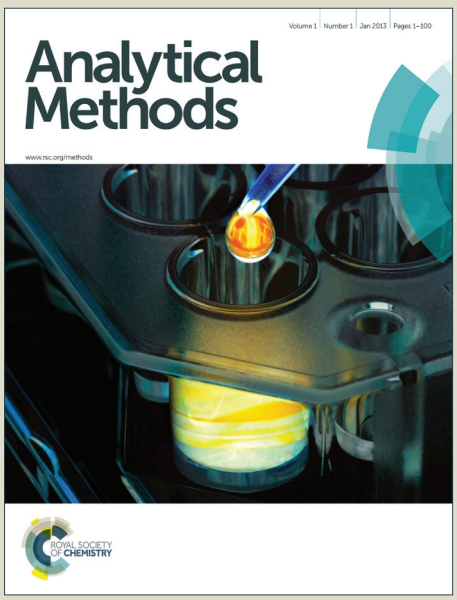

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Catechin-modified carbon paste electrode for electrocatalytic determination of neurotransmitters

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Catechin is a polyphenol antioxidant which can be found in a great abundance in the leaves of tea plants. In this study, catechin was electrodeposited on an activated carbon paste electrode for electrocatalytic determination of two neurotransmitters, dopamine (DA) and serotonin (ST). The voltammetric conditions for electrode preparation in catechin solution were optimized as follows: phosphate buffer at pH 7.4, catechin concentration of 1.0 mM, potential window of 0.2–1.6 V (vs. Ag/AgCl/KCl_{sat}), scan rate of 50 mV s^{-1} and cycle number of 15. The prepared electrode showed high electrocatalytic activity to the oxidation of both DA and ST. The highest electrocatalytic activity to DA oxidation was observed in the physiological pH (7.4) buffer solution. Amperometric detection under stirring achieved a current sensitivity of 10.29 nA·nM^{-1}·cm^{-2} to DA in the linear concentration range of 10–780 nM, and of 4.81 nA·nM^{-1}·cm^{-2} to ST in the range of 30–2340 nM, with the lowest detection limits of 0.5 and 3 nM for DA and ST, respectively. The resulting biosensor was successfully used to quantify DA and ST in commercial samples with high sensitivity and good stability. In addition, the fact that the oxidized catechin can effectively promote the electron transfer processes of DA and ST, which may help understanding the role of catechin in nervous excitement.

1. Introduction

Serotonin (ST) and dopamine (DA) are two types of monoamine neurotransmitters, their levels in brain are generally believed to have a direct influence on human's moods and emotions, thus they have been used as a standard to evaluate the conditions of emotional health.[1-9] Tea, as a popular beverage favoured by many people, contains various types of polyphenolic flavonoids, among which the main composition is catechin (CA).[10] It has been proved that CA is beneficial to people's health, and its medicinal efficacy is mainly attributed to its role as an effective antioxidant to eliminate harmful free radicals generated in the body,[11-15] thus preventing the occurrence of resultant diseases. For example, CA can shield neurotransmitters from the impairment caused by reactive oxygen species (ROS).[16, 17] Meanwhile, as a moderate stimulant, it has significant refreshing effect.[18] Also, some researchers reported that CA had an antidepressant effect, probably due to its ability to block the neurotransmitters uptake by synaptosome, and to increase the concentration of neurotransmitters in synaptic gap.[19] This indicated an underlying interaction between CA and neurotransmitters, although the mechanism has not been fully understood yet.

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CA and neurotransmitters contain at least one phenolic hydroxyl group with electrochemical activity.[2, 3, 20] This offers a possibility to evaluate their interactions from an electrochemical viewpoint, and further to develop CA-based biosensors for detecting neurotransmitters. Until recently, only a few examples on flavonoids modified biosensors have been reported, which include nafion coated glassy carbon electrode modified with catechin hydrate for DA detection[21] and rutin modified electrode for electro-oxidation of ST, epinephrine (EP) and ascorbic acid (AA).[22] On the other hand, the electro-oxidation of phenolic flavonoids have been studied extensively.[20, 23, 24] Their oxidation mechanisms are found to be very complex and the electro-oxidation products of polyphenolic flavonoids are highly pH- and potential-dependent.[25-27] Therefore, the experimental conditions must be optimized carefully based on the understanding of the oxidation mechanisms of flavonoids, for effective preparation of flavonoids-modified electrodes.

In our earlier work,[25] the electro-oxidation and deposition of CA was studied on a solid carbon paste electrode (sCPE). Here, we attempt to prepare a CA-modified CPE as a biosensor for the detection of two neurotransmitters and also as a tool for the evaluation of the interactions between CA and the neurotransmitters. Electrochemical methods are widely used for detection of biological and pharmaceutical compound due to their particularities of rapidity, high sensitivity, portability, etc.[28-33] Therefore, in our work, electrochemical methods such as differential pulse voltammetry and amperometric determination have been used for the determination of neurotransmitters.

2. Experimental

2.1. Chemicals and solutions

Specpure graphite powders (320 mesh) and paraffin wax (solidification point 46-48 °C) were purchased from shanghai chemical works for preparing the solid carbon paste electrode. CA was purchased from Fluka (Japan), AA, DA and ST (99%) were purchased from Chemical Regent Company of Shanghai (Shanghai, China), Acros Organics (Geel, Belgium) and Alfa Aesar, and were used as received. All other chemicals were of analytical grade. High purity nitrogen was used to deaerate the solutions. Water was doubly-distilled from an all-glass distillatory apparatus.

CA was dissolved in ethanol/water mixture (20:80, v/v) under ultrasonication and prepared as 1 mM solution to modify CPE electrode. 0.1 M PBS buffer solutions with different pH values were used as supporting electrolytes. DA and ST were prepared as 1.0 mM stock solutions and diluted to desired concentrations with the supporting electrolytes prior to use. All stock solutions were degassed with high purity nitrogen for 10 min and stored at 4 °C.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric determination were recorded using a CHI 660C computer-controlled potentiostat (ChenHua Instruments Co., Shanghai, China) with a three-electrode system. Bare CPE, activated CPE (ACPE) and CA-modified activated carbon paste electrode ACPE (CA/ACPE) were served as the working electrodes; a platinum wire was used as a counter electrode and saturated Ag/AgCl electrode (Chenhua Instruments Co., Shanghai, China) completed the cell assembly. All solutions were deoxygenated with nitrogen bubbling for 10 min before each measurement. All experiments were performed at a temperature of 25 ± 1°C under nitrogen.

A 10-mL volume single-compartment cell was used for the conventional voltammetric measurements. A thin-layer spectroelectrochemical cell was self-made, using a standard quartz photometric cell with 10 mm optical path length as the cell body. The schematic view of the thin-layer cell can be found in the literature.[34] The incident light beam parallels to the working electrode and goes through the thin-layer electrolyte solution (10 mm long, 0.2 mm thick) on the electrode surface.

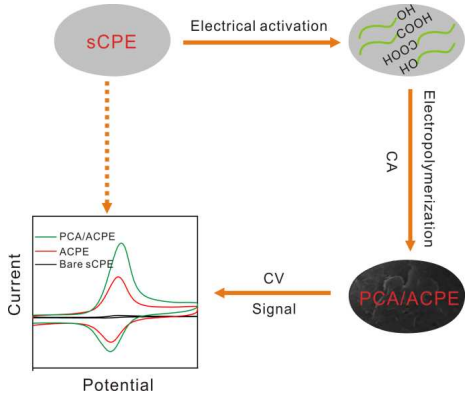
2.3. Electrode preparation

The electrode body was a hollow polystyrene tube with an inner diameter of 2.5 mm, which was impacted with a copper rod, leaving a cavity of 2 mm in depth. Solid wax was heated until molten, and mixed with the graphite powders in an agate mortar until a well-blended paste was obtained. The paste was compactly pressed into the cavity of the electrode body, forming a bare CPE with a geometric area of 4.9 mm.^{2} The bare CPE was polished with 800–4000 grit emery papers, followed by an ultrasonic cleansing in doubly-distilled water for 5 s. The electrode was then activated in 0.1 M $NaHCO_3$ solution by 75 cycle potential scans between 0–2.5 V at a scan rate of 0.5 $V{\cdot}s^{-1}$ until the background current was obtained.

The obtained ACPE was further modified by 15 cycle potential scans in 1.0 mM CA solution (pH 7.4) between –0.2–1.6 V at a scan rate of 0.05 $V{\cdot}s^{-1}$ to obtain a CA-modified film on the substrate. Finally, the CA/ACPE was rinsed with doubly-distilled water and cleaned by potential cycling in 1.0 M KCl solution to remove any adsorbed substances.

2.4. Strategy for detection of DA and ST

Scheme 1 illustrates the strategy for the detection of DA and ST based on electrodeposition of CA on ACPE. Firstly, the CPE was electrochemically activated to produce numbers of active oxygen-containing groups attached to the CPE surface,[35, 36] such as carboxyl gor hydroxyl groups. Activated carbon electrodes generally exhibit an electrocatalytic effect on the oxidation of some reactants such as dopamine, AA and uric acid.[37] Then CA was electrodeposited on ACPE to prepare CA/ACPE electrode. The CA/ACPE was used for the detection of DA and ST by DPV and amperometry.



Scheme 1 Schematic illustration of strategy for the detection of DA and ST based on electrodeposition of CA on ACPE.

3. Results and discussion

3.1. Characterization of deposits of CA on carbon paste electrodes

The morphologies of bare CPE and CA/ACPE were imaged by scanning electron microscopy (SEM) as shown in Fig. 1(A and B). The surface of the bare CPE was rather smooth. After subjected to anodization in the CA solution at physiological pH, the surface was covered by a laminated deposit. Fig. 1C shows the multi-cycle CV between -0.2–1.6 V for electrodeposition of CA on the activated electrode at physiological pH. Three anodic peaks (A_1, A_2 and A_3) and one cathodic peak (C_1) were observed in the tested potential range. The peaks A_1 and A_2 are due to the electro-oxidation of CA, while the peak A_3 corresponds to the oxygen evolution. The oxidation of CA in the peak A1 occurs at 3',4'-OH groups of the B-ring to generate the corresponding CA-*o*-quinone.[25] This *o*-quinone is not chemically stable, and subsequently, undergoes dimerization and then polymerization.[38] The small peak C_1 represents the electro-reduction of CA-*o*-quinone that did not convert. This peak showed higher current peak current when the scan was reversed at a less positive potential (e.g. 0.3 V). The peak separation (ΔE_p) between A_1 and C_1 was as small as 20 mV, suggesting one pair of quasi-reversible adsorption peaks. The resorcinol at A ring is electrochemically less active and may undergo an irreversible electro-oxidation reaction at the second peak A_2. The 3-hydroxyl at

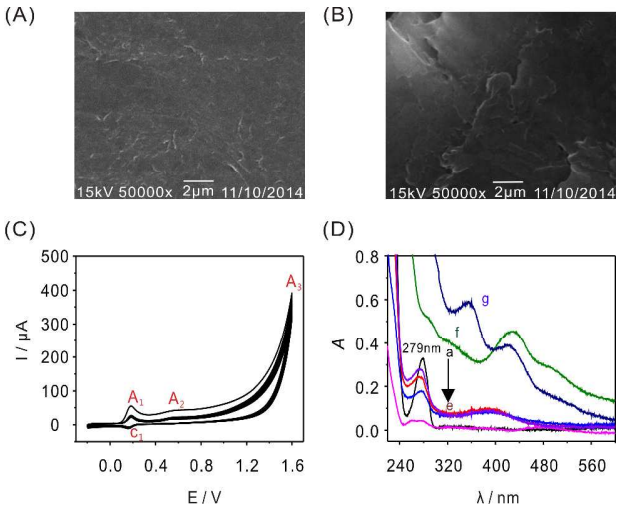


Fig. 1 SEM micrographs of (A) bare CPE and (B) CA/ACPE prepared by 15 cycle scans; (C) Fifteen cyclic potential scans on ACPE in 1.0 mM CA solution (pH 7.4) for preparation of CA/ACPE. E_{acc} = – 0.2 V; t_{acc} = 20 s; v = 50 mV·s^{-1}. (D) Thin-layer UV–Vis spectra of the (+)–CA solutions (0.1 mM) with various pHs subjected to electrolysis at different potentials. (a) pH = 1.8, open circuit; (b) pH = 1.8, E = 0.6 V; (c) pH = 3.3, E = 0.55 V; (d) pH = 5.0, E = 0.45 V; (e) pH = 7.4, E = 0.35 V; (f) pH = 9.2, E = 0.25 V; and (g) pH = 11.5, E = 0.15 V.

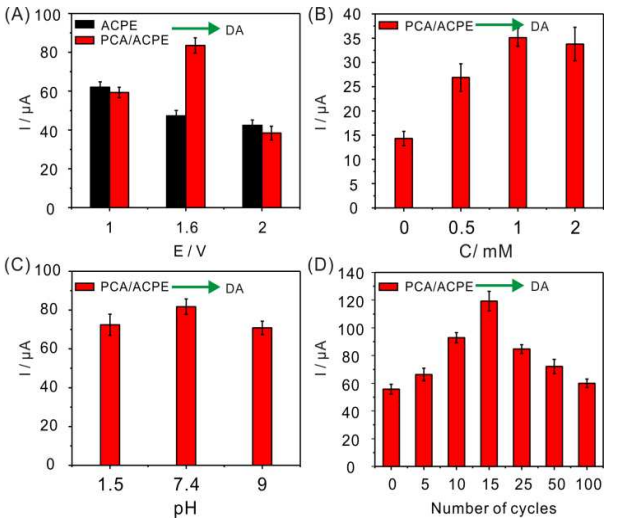


Fig. 2 Different conditions for electropolymerization of CA on ACPE for the detect of 0.1 mM DA, (A) different potential ranges for electropolymerization of 1 mM CA on ACPE for fifteen cyclic scans, pH = 7.4, E: −0.2–1.0 V, −0.2–1.6 V, −0.2–2.0 V; (B) electropolymerization of different concentrations of CA on ACPE in the potential range of -0.2–1.6 V for fifteen cyclic scans, pH = 7.4, CA concentration (c): 0, 0.5, 1, 2 mM; (C) different pH conditions for electropolymerization of 1mM CA on ACPE in the potential range of -0.2–1.6 V for fifteen cyclic scans, pH: 1.5, 7.4, 9 and (D) different cyclic scans for electropolymerization of 1 mM CA on ACPE in the potential range of -0.2–1.6 V, pH = 7.4, number of cycles: 0, 5, 10, 15, 25, 50 and 100. Error bars were based on three separate electrodes.

C ring is relatively stable and difficult to be oxidized in the test potential range.

Fig. 1D shows the in situ UV-vis spectra of the thin layer CA solutions at different pH values after subjected to oxidation at the peak potentials of peak A_1. The peak potential is pH-dependent, with a value of 0.6, 0.55, 0.45, 0.35, 0.25 and 0.15 V at pH 1.8, 3.3, 5.0, 7.4, 9.2 and 11.5, respectively. The reactant CA shows a single characteristic absorption peak at 279–288 nm, the value of which is dependent on pH. During the oxidation under acidic conditions, this absorption peak decreased in intensity, with the appearance of a new weak peak around 386 nm. The new peak with a larger wavelength suggests an extension of the conjugated double-bond system due to polymerization of the catechin *o*-quinone formed at peak A_1. A pH 9.2 and 11.5, at least three new absorption peaks occurred in the wavelength range of 300-550 nm, which indicates that alkaline pH is favorable for the subsequent polymerization step, and also for the dissolution of the polymerized products. At pH 7.4, however, the light-absorption of the electrolyte solution dramatically decreased nearly down to the background, due to deposition of the polymerized products onto the electrode surface. Therefore, pH 7.4 is the best for the depositon of CA on a substrate electrode in CA solutions.

3.2. Optimization of voltammetric polymerization of CA

The effects of modified potential range, concentration, pH, and number of cycles for electropolymerization of CA on ACPE were investigated. As shown in Fig. 2A, in the potential range from -0.2 to 1.6 V, the effect of electropolymerization of CA for the detection of DA is effective. Compared with ACPE, the current of the detection of DA on CA/ACPE in this potential range increased almost 2 times, but in other potential ranges, it was almost unchanged. This may be due to that, electropolymerization of CA on ACPE occurred in a high oxidation potential, and there will be produced with numbers of electrochemically active oxygen-containing groups,[35, 36] such as carboxyl group, hydroxyl group, quinones, ketones and phenols on electrode surface, which played a electrocatalytic role for detection of DA. But in a higher oxidation potential range, there will occur peroxidation, which may destroy the part of surface of the electrode, thus playing a strong influence for the detection of DA.

The concentrations of CA solution also influenced the electropolymerization (Fig. 2B). It was found that, companying with the increasing of CA concentration, electrocatalytic role increased. When the concentration reached to 1 mM, electrocatalytic role played the best. If the concentration of CA continued to increase, electrocatalytic role remained constant, which may be due to that, in the concentration of 1 mM, CA adsorption at the electrode surface reached equilibrium.

We investigated the electrodepostion of CA on the electrode surfaces under different pH conditions. The oxidation mechanism is very complex. In the potential range of -0.2–1.6 V, there are three oxidation peaks existed, and in different pH buffer solutions, each oxidation peak corresponds to different oxidation product. One oxidation peak also corresponds to different oxidation products, which formed a complex polymer film on the electrode surface.[20, 39] Fig. 2C shows the experimental results in acidic, weak alkaline and alkaline buffer, at pH 7.4, the deposition of CA on the electrode

surface is optimum, and the catalytic effect of DA is the best. This result is also in accordance with the thin-layer UV–Vis spectra characterization.

The cycle number of the scans also affects the electrocatalytic performance of the resulting CA/ACPE. As shown in Fig. 2D, the catalytic effect of CA/ACPE on DA initially became better and then decrease with increasing cycle number, showing an optimum cycle munber of 15. On an overall consideration of the modified condition, the potential ranges of -0.2–1.6 V, concentration of 1 mM CA, cyclic scans of 15 and the pH 7.4 buffers were selected for electropolymerization of CA on ACPE in this work.

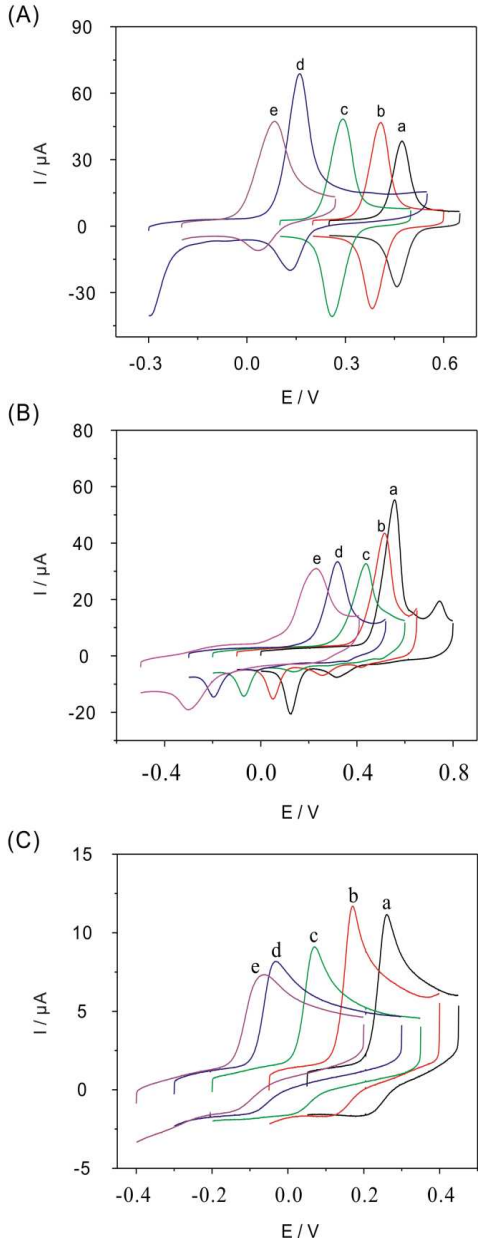


Fig. 3 CVs at CA/ACPE in (A) 0.1 mM DA, (B) 0.1 mM ST and (C) 0.1 mM AA with different pHs. (a→e): 2.0, 3.3, 5.3, 7.4 and 9.0, v = 50 mV·s^{-1}, 0.1 M PBS as buffer solution.

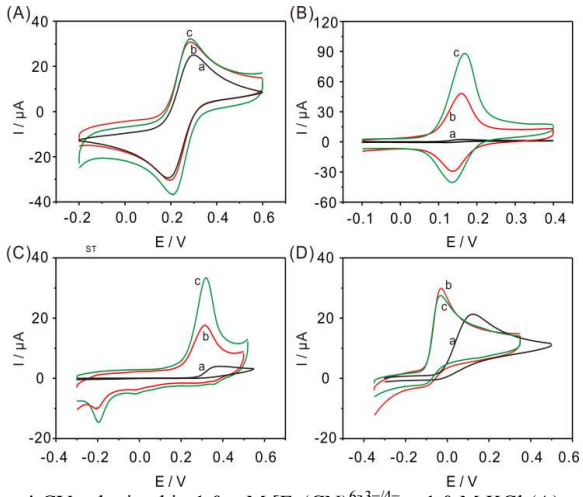


Fig. 4 CVs obtained in 1.0 mM $[Fe(CN)^6]^{3-/4-}$ + 1.0 M KCl (A), and in 0.1 M pH 7.4 PBS containning 0.1 mM of DA (B), ST(C), or AA (D), using CPE (a), ACPE (b) and CA/ACPE (c). Scan rate 50 mV·s^{-1}.

3.3. Effect of pH on the oxidation of DA, ST and AA

The pH effect of the buffer solution on the oxidation of the analytes at CA/ACPE was examined. The oxidation peak potentials of AA, DA and ST shifted negatively with increasing pH (CVs of DA, ST and AA shown in Fig. 3), due to their oxidation mechanisms all involving both electron and proton transfer.[40-42] The oxidation peak currents of DA in the physiological buffers were higher than other basic buffers with a maximum at pH 7.4 (Fig. 3A). The result is different to the result reported for PAMT/CPE electrode, at which the DA showed much lower oxidation peak current at pH 7.4,[40] indicating different electrocatalytic mechanisms between the two modified films. As for the oxidation of ST, the peak currents were higher in the acidic media, but showed little change with increasing pH in the alkaline range (Fig. 3B). However, as shown in Fig. 3C, with increasing pH in the basic buffer, the peak currents of AA decreased gradually, which indicated that the interference of AA for detecting DA and ST under physiological condition may be avoided. On an overall consideration of the three analytes, the pH 7.4 buffer was selected in this work.

3.4. Comparison of the electrocatalytic activity at bare CPE, ACPE and CA/ACPE

The redox couple of $[Fe(CN)_6]^{3-/4-}$ was used as an electrochemical probe to characterize the electric property of the modified film on the electrode substrate (Fig. 4A). It is observed that the peak current of the probe on ACPE was slightly larger than that on CPE, showing a little electrocatalytic effect of ACPE on $[Fe(CN)_6]^{3-/4-}$ probe. However, the peak current on CA/ACPE was nearly equal to that on ACPE, indicating that the deposited CA film has no accumulation or repulsion effect on the anionic redox couple $[Fe(CN)_6]^{3-/4-}$. Accordingly, the surface of CA/ACPE seems to be electroneutral. At the pH of 7.4 (pKa: AA 4.1, DA 8.9 and ST 9.8), AA existed as anions while DA and ST existed as cations. Compared to ACPE, the

peak current of electro-oxidation of AA on CA/ACPE was almost unchanged (Fig. 4D), indicating that CA/ACPE had no electrocatalytic effect to AA. The similar result had been reported.[43] However, CA/ACPE had a good electrocatalytic effect toward DA and ST. Compared to ACPE (line b), and the peak currents at CA/ACPE were both enhanced 1.8-fold for DA and ST. Meanwhile, the peak currents at CA/ACPE were enhanced 30.1-fold and 8.8-fold for DA and ST, respectively, in comparison with those at the bare CPE (line a). This phenomenon may not be due to the electrostatic attraction between film and analyte, but CA/ACPE had a special electrocatalytic effect toward DA and ST, rather than AA or $[Fe(CN)^6]^{3-/4-}$ molecular probe, which had significance in selectively detecting DA and ST, thus avoiding interference from other ions or molecules.

3.5. Effect of concentration on DPV response

The results of the concentration (c) effect on the differential DPV response can be seen in Fig.5. The increase in DA concentrations enhanced its respective peak currents, without affecting significantly the peak current of ST whose concentration was held constant (Fig.5A). This indicated that the oxidation reaction of DA was not interfered by the presence of the other species. Similarly, from Fig. 5B, the oxidation reaction of ST was not interfered by DA. With increasing ST concentration, its peak current was enhanced without affecting DA peak current. The same result was also found for both DA and ST. While the two analytes increased in concentration by a constant ratio, just as shown in Fig. 5C, their corresponding peaks proportionally increased in intensity. This suggests that no competitive adsorption occurred on the CA film. In pH 7.4 solution, the amine groups of DA (pK_a = 8.9) and ST (pK_a = 9.8) are positively charged. In addition, no interference was observed from common ions such as 1000-fold Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-}, BO_3^{3-}, SO_4^{2-} and CH_3COO^-.

3.6. Amperometric determination of DA and ST

Amperometric responses of the CA/ACPE to the concentration changes of DA and ST are presented in Fig. 6. The determination was made at an applied potential (E_{app}), by injecting different volumes of analyte to a stirred 0.1 M PBS solution (20 mL, pH 7.4) with the concentration of 50 μM. After each addition, the current response reached a stead state within 5 s, which indicated the sensor had a fast response. Calibration plots were acquired from the amperometric responses within the successive addition of DA or ST to buffer solution in the range of 10-780 nM or 30-2340 nM(Fig. 6A and C), the linear equations of DA and ST were displayed respectively as follows: i/μA = 0.0298 + 0.504 c/μM (R = 0.9944), i/μA = 0.0005344 + 0.2354 c/μM (R = 0.9996), and the amperometric sensitivities were shown as 10.29 $\mu A \cdot \mu M^{-1} \cdot cm^{-2}$ for DA and 4.8 $\mu A \cdot \mu M^{-1} \cdot cm^{-2}$ for ST, The LODs were shown to be 0.5 and 3 nM for DA and ST, respectively (S/N = 3).

Selective detection of DA as well as avoidance of interference from ST was achieved through setting the E_{app} of 0.2 V. When we applied the CA/ACPE to analyze the concentration of DA and ST in a mixed sample, E_{app} can be set from 0.20 V to 0.40 V by potential step; at each step, current response corresponded to the concentration of DA or ST in a mixed sample.

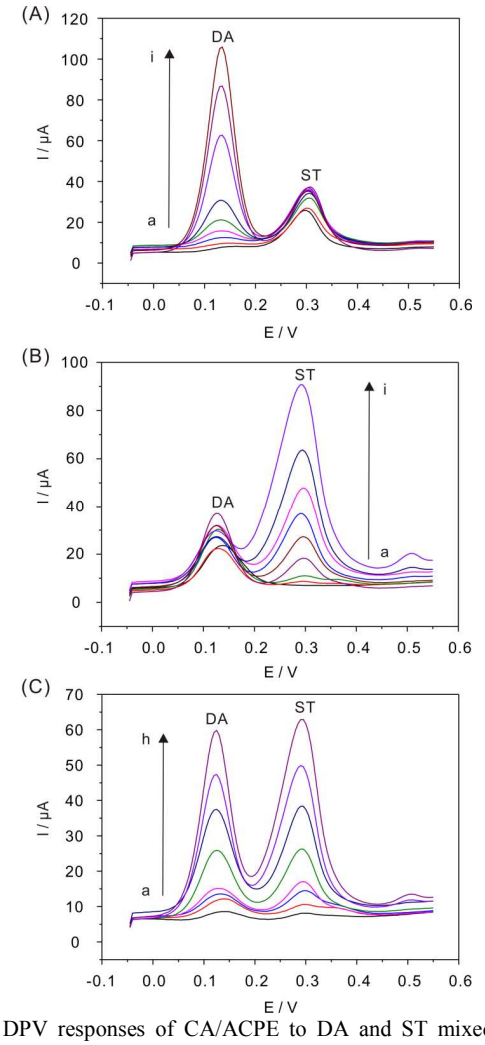


Fig. 5 DPV responses of CA/ACPE to DA and ST mixed in the buffer of 0.1 M PBS (pH 7.4). (A) c(ST) = 12 μM; c(DA) (a→i): 0, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 μM; (B) c(DA) = 3 μM; c(ST) (a→i): 0, 1, 2, 4, 8, 12, 16, 24, and 32 μM; and (C) c(DA) and c(ST) (a→h): 5, 10, 15, 20, 25, 30, 35, and 40 μM; Pulse amplitude = 50 mV, pulse width = 50 ms, sample width = 40 ms and pulse period = 100 ms.

At the end of each experiment, the modified electrode was taken out from solution and washed with doubly-distilled water, cleaned by 75 cycles in 0.1 M $NaHCO_3$ between 0-2.5 V. Then the ACPE was modified again in 1.0 mM CA solution (pH 7.4) by fifteen cyclic potential scans. When the electrode was cleaned and used to detect analyte at the same concentration successively, the current response was almost unchanged, indicating that we acquired a clean and stable electrode. The clean electrode was kept in a nitrogen-filled bottle for further use.

Table 1 Successive determination of DA and ST in pharmaceuticals using CA/ACPE.

Sample	Labeled ($mg \cdot mL^{-1}$)[a]	Added ($mg \cdot mL^{-1}$)	Found ($mg \cdot mL^{-1}$)	Recovery (%)
DA 1	10	–	9.89	–
		9.482 (n = 5)	19.50, 19.41, 19.34, 19.39, 19.34	100.35± 0.65(n = 5)
DA 2	10	–	9.92	–
		9.482 (n = 5)	19.66, 19.42, 19.44, 19.38, 19.34	101.11 ± 1.69(n = 5)
DA 3	10	–	9.81	–
		9.482 (n = 5)	19.35, 19.35, 19.35, 19.24, 19.22	99.93 ± 0.73(n = 5)
	Calculated (μM)[b]	Added (μM)	Found (μM)	Recovery (%)
Mixture	DA: 0.1055 ST: 0.1000	–	DA: 0.1060	–
		DA: 0.1 (n = 5)	DA:0.1000, 0.0981, 0.1003, 0.1009, 0.0999	DA:99.49±1.41(n= 5)

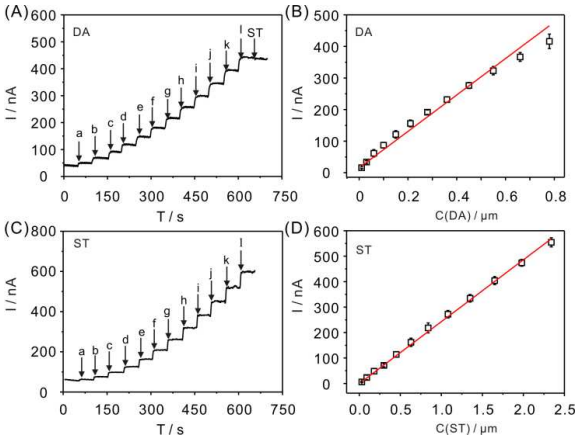


Fig. 6 Amperometric responses of CA/ACPE to successive addition of DA and ST to a stirred 0.1 M PBS solution (20 mL, pH 7.4), and the resulting calibration plots (insets). (A) E_{app} = 0.20 V, c(DA) =0.01, 0.03, 0.06, 0.1, 0.15, 0.21, 0.28, 0.36, 0.45, 0.55, 0.60, and 0.78 μM (a→l); (C) E_{app} = 0.40 V, c(ST) = 0.03, 0.09, 0.18, 0.30, 0.45, 0.63, 0.84, 1.08, 1.35, 1.65, 1.98, and 2.34 μM (a→l); the linear relationships shown in panels (B) and (D) are obtained from the data in panels (A) and (C), respectively.

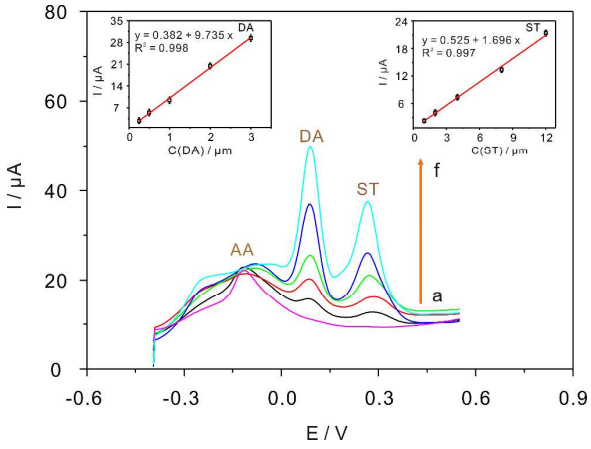


Fig. 7 DPV responses of PCA/ACPE to AA, DA and ST mixed in the buffer of 0.1 M PBS (pH 7.4). c(AA) = 50 μM; c(DA) (a→f): 0, 0.25, 0.5, 1, 2 and 3μM; c(ST) (a→f): 0, 1, 2, 4, 8 and 12 μM; Pulse amplitude = 50 mV, pulse width = 50 ms, sample width = 40 ms and pulse period = 100 ms.

3.7. Interferences study

As is known that AA showed the major interference in the electrochemical analysis of DA and ST.[38] The interference of AA toward the CA/ACPE was studied. DPV measurements were performed for the detection of various concentrations from 0.25 to 3μM of DA, 1 to 12μM of ST at the CA/ACPE in the presence of 50 μM of AA, respectively (Fig 7). The experimental results showed that presence of AA didn't affect the simultaneous detection of DA and ST. The linear equations of DA and ST were displayed respectively as follows: i/μA = 0.382 + 9.735 c/μM (R = 0.998), i/μA = 0.525 + 1.696 c/μM (R = 0.997).

3.8. Analytical applications

The CA/ACPE was applied to the amperometric detection of DA in dopamine hydrochloride injection (labeled as 10 $mg \cdot mL^{-1}$), respectively, by standard addition method. The injection solutions were diluted with doubly-distilled water by 50 times for DA, and then 20 μL of the diluted solutions or standard DA solutions was successively injected into to the stirred PBS (20 mL, pH 7.4). The applied potential was 0.20 V for DA.

The same CA/ACPE was then used to determine DA and ST in a mixed sample, which was 50 mL distilled water spiked with 1.00 mL dopamine hydrochloride injection and 10.63 mg ST. Four 2 μL of the mixed sample was injected into the stirred PBS (20 mL, pH 7.4), and then the potential was stepped from 0.20 to 0.4 V at a time interval of 50 s. The resulting two steps of current response

corresponded to the concentrations of DA and ST in the mixture. With that, the standard solutions of DA and ST were severally added to the buffer solutions for the measurement of their original concentrations and recovery.

Results of the above assaying were listed in Table 1. The data indicated that DA and ST could be reliably determined from their pharmaceutical formulations, thus demonstrating the suitability of the proposed CA/ACPE as a sensor. The successive addition of the samples did not worsen the sensing performance, due to the good antifouling property of the CA film.

4. Conclusions

A novel CA film modified carbon paste electrode was fabricated by electrodeposition of CA at physiological pH. The resulting film displayed a good electrocatalytic activity also at physiological pH to the oxidation of DA and ST, but not to AA or $[Fe(CN)^6]^{3-/4-}$ systems. These results may suggest a special interaction and biocompatibility between CA and the neurotransmitters. In addition, CA/ACPE can be applied to selectively detect DA and ST, avoiding the interference of amperometric determination, and this biosensor achieved amperometric sensitivities of 10.29 $\mu A \cdot \mu M^{-1} \cdot cm^{-2}$ in the range of 10-780 nM for DA and 4.8 $\mu A \cdot \mu M^{-1} \cdot cm^{-2}$ in the range of 30–2340 nM for ST. The lowest limit of detection of DA was 0.5 nM, 3 nM for ST. We hope our work can provide a basic platform for fabricating novel micro-electrodes which can be applied in-vivo to explore the real-time interactions between CA and neurotransmitters.

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