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Voltammetric resolution of dopamine in the presence of ascorbic acid and uric acid at poly (calmagite) film coated carbon paste electrode

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ABSTRACT

The poly (calmagite) film was synthesized on the surface of carbon paste electrode by electrochemical method. The synthesized polymer film coated electrode exhibits excellent electrocatalytic activity towards the detection of dopamine at neutral pH. The scan rate effect was found to be adsorption controlled electrode process. The concentration effect of dopamine was studied. The redox peak potentials of dopamine were depend on pH. This polymer film coated electrode was very good at simultaneous study of dopamine in the presence of high concentrated ascorbic acid and uric acid. The incorporation study was done by varying the concentration of one species while other two are kept constant. The proposed method was applied to the detection of dopamine in injection samples.

1. Introduction

In recent days the electropolymer modified carbon paste electrodes are showed very much excellent effort in acting as a sensor to investigate the one of the neurotransmitters like dopamine [1–3]. Especially, the electropolymer film coated electrodes with dyes have good stability, reproducibility, more active sites, homogeneity in electrochemical deposition [4,5]. The electropolymerisation generally results in polymer film which is uniform and strongly adherent to the electrode surface. In addition the polymer film can be deposited onto the small area with high degree of geometrical conformity and controllable thickness. Several redox dyes are known to artificial electron donors [6]. Such dyes are able to undergo electropolymerisation from aqueous solution producing stable redox active layer [7,8].

Dopamine (DA) is a well-known biogenic amine acting as a neurotransmitter in the brain. It has received considerable attention because of its suspected role in a variety of neuropsychiatric disorders such as Parkinson's disease and Schizophrenia [9–12]. It has been found that the dopamine possesses very strong electrochemical activity by giving dopamine-o-quinone as oxidation product. However the determination remains a challenge because of the presence of large excess of ascorbic acid (AA) and uric acid (UA). It is generally believed that direct redox reactions of these species at bare electrode are irreversible and therefore requires high over potential [13]. Moreover the direct redox reactions of these species at bare electrodes take place at very similar potential and often suffer from a fouling effect, which results in rather poor selectivity and reproducibility. The ability to determine DA, UA and AA selectively has been a major goal of electroanalytical research [14]. Development of both sensitivity and selectivity are of equal importance in voltammetric procedure.

The aim of our work was to fabricate stable electrode by electropolymerizing calmagite (the oxidation mechanism of calmagite was shown in Scheme 1) on the surface of carbon paste electrode to achieve the challenge of simultaneous determination of DA in the presence of AA and UA in physiological pH. Although no examination of the detection of dopamine in the presence of both ascorbic acid and uric acid in physiological pH at poly (calmagite) film coated carbon paste electrode has been reported. This novel work discussed about sensitivity, selectivity and reproducibility of neurotransmitter at poly (calmagite) film coated carbon paste electrode at physiological pH.

2. Experimental

2.1. Reagents

The graphite fine powder (Loba Chemie) and silicon oil (Himedia) were used to prepare carbon paste electrode. 0.5 mM calmagite

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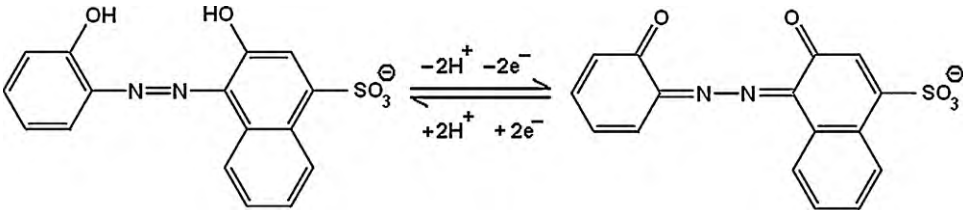


Scheme 1. Oxidation mechanism of calmagite.

in aqueous solution was used to synthesis electropolymer on the surface of carbon paste electrode. DA was prepared 1×10^{-5} M stock solution by dissolving in 0.1 M perchloric acid solution. UA was prepared 1×10^{-4} M stock solution by dissolving in 0.1 M sodium hydroxide solution and that of 1×10^{-4} M AA in double distilled water. Phosphate buffer solution was prepared by mixing the appropriate quantity of 0.2 M aqueous sodium dihydrogen phosphate monohydrate and 0.2 M aqueous disodium hydrogen phosphate. Chemicals mentioned above were all of analytical grade. The water used in the preparation of solutions was double distilled water.

2.2. Apparatus

The electrochemical experiments were carried out using a model-201 electroanalyser (EA-201 chemilink system). All the experiments were carried out in a conventional three electrochemical cell. The electrode system contained a working electrode was bare CPE and poly (calmagite) film modified CPE (3 mm in diameter), a platinum wire as counter electrode and saturated calomel electrode as reference electrode.

2.3. Preparation of poly (calmagite) modified carbon paste electrode

The carbon paste electrode was prepared by hand mixing 70% graphite powder and 30% silicon oil in an agate mortar. The 0.5 mM aqueous calmagite was placed in the electrochemical cell along with 0.2 M phosphate buffer solution of pH 9.0. The CPE was scanned 10 multiple cycles between the potential ranges from −200 to 2000 mV at 100 mV s^{-1}. After this process, the electrode was immersed in 0.2 M phosphate buffer solution of pH 7.0 until use.

3. Results and discussion

3.1. Electrochemical polymerization of calmagite on carbon paste electrode

Calmagite is a redox indicator which is undergoing electropolymerisation between the ranges of −200 and 2000 mV on the surface of carbon paste electrode at the sweep rate of 100 mV s^{-1} for 10 cycles. Fig. 1 shows the growth of polymer film of 0.5 mM aqueous calmagite solution on the surface of carbon paste electrode in 0.2 M phosphate buffer solution of pH 9.0 as supporting electrolyte. During the process of multiple cycles the voltammogram has gradually descended with increase of cyclic time increasing. This indicates that the poly (calmagite) film was formed and deposited on the surface of carbon paste electrode [15].

3.2. Electrochemical characterization of poly (calmagite) film coated CPE

Fig. 2 exhibits the typical voltammogram of the poly (calmagite) film coated CPE in the range of −200–600 mV at various sweep rates in the 0.1 M HCl. A pair of redox broad peaks was obtained. On increasing the scan rate the redox peak current also increased. The anodic peak currents were linearly dependent of scan rate and the correlation coefficient was found to be 0.999. The ratio of anodic peak current to cathodic peak current was almost equal to unity. The separation of peak potentials (ΔEp) was 50 mV. With increasing scan rate ΔEp also increased. The above results suggest that the electrode reaction was a quasi-reversible electron transfer process [15].

3.3. SEM analysis of poly (calmagite) modified carbon paste electrode

Fig. 3 explains the surface morphology of bare carbon paste electrode and poly (calmagite) modified carbon paste electrode using scanning electron microscopy. The surface of bare carbon paste electrode was irregularly shaped micrometer sized flakes of graphite. However, the poly (calmagite) film coated carbon paste electrode has typical uniform arrangement of poly (calmagite) molecules on the surface of carbon paste electrode. This confirms the carbon paste electrode was coated by poly (calmagite) film.

3.4. Electrocatalytic response of DA at poly (calmagite) modified carbon paste electrode

Fig. 4 demonstrates the cyclic voltammogram of DA (1×10^{-5} M) at bare carbon paste electrode and poly (calmagite) modified carbon paste electrode at sweep rate of 50 mV s^{-1}. It is easily oxidizable catecholamine, showed poor sensitivity and reproducibility at bare carbon paste electrode (dotted line). The cyclic voltammogram of DA in phosphate buffer solution of pH 7.0 as supporting electrolyte at sweep rate of 50 mV s^{-1}, showed its anodic (Epa) and cathodic peak potentials (Epc) at 229 and 130 mV respectively. The peak

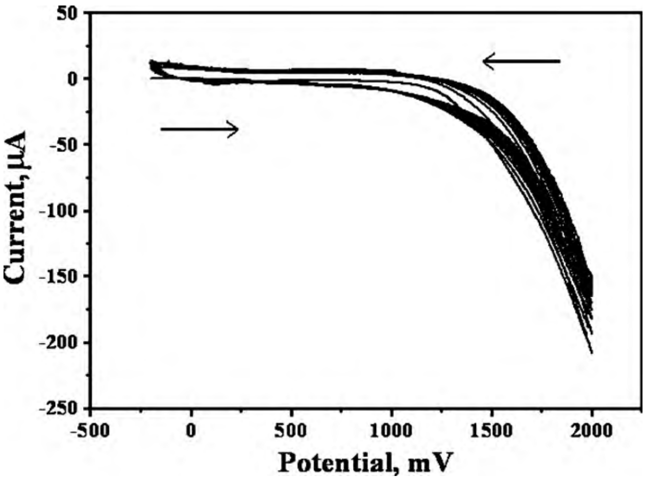
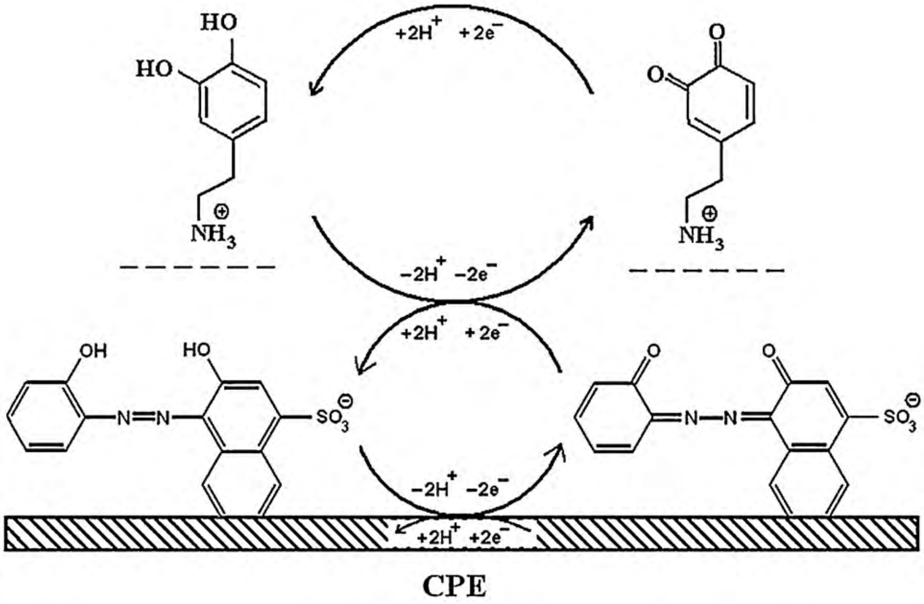


Fig. 1. Cyclic voltammogram of preparation of poly (calmagite) film coated CPE. 0.5 mM aqueous calmagite in 0.2 M phosphate buffer solution of pH 9.0 at 10 cycles with sweep rate of 100 mV s^{-1}.



Scheme 2. Electrocatalytic oxidation of DA at the surface of poly (calmagite) film coated CPE.

potentials difference (ΔEp) was found to be 99 mV. The anodic and cathodic peak current ratio (Ipa/Ipc) was about 1.5. However, in the same condition, our polymer coated electrode (solid line) exhibited excellent sensitivity for DA. Both anodic and cathodic peak current signals were got 19-fold increments when comparing the bare

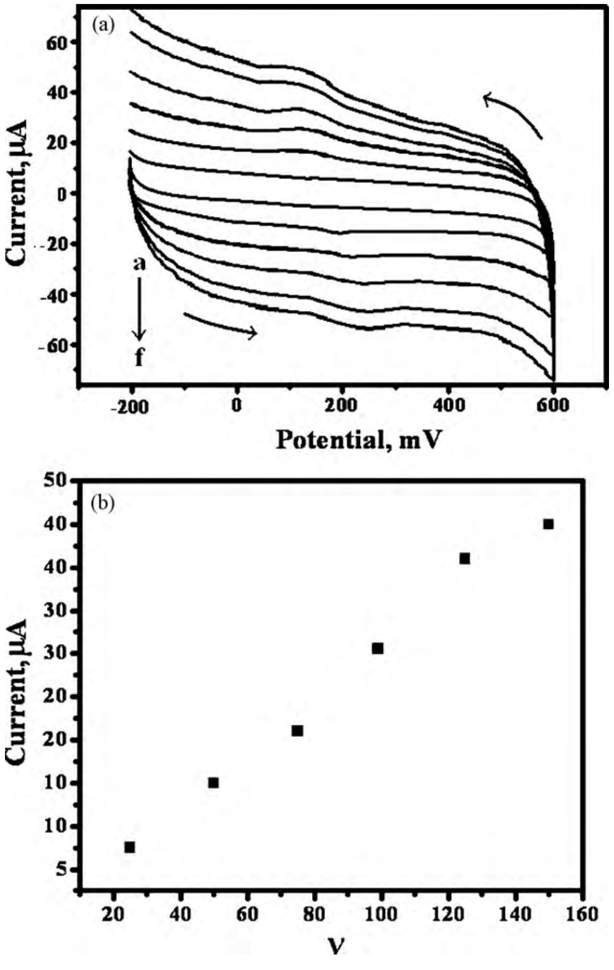


Fig. 2. (a) Cyclic voltammogram of poly (calmagite) film coated CPE in 0.1 M HCl at different scan rate (a–f; 25, 50, 75, 100, 125 and 150 mV s^{-1}). (b) Graph of anodic peak current vs scan rate.

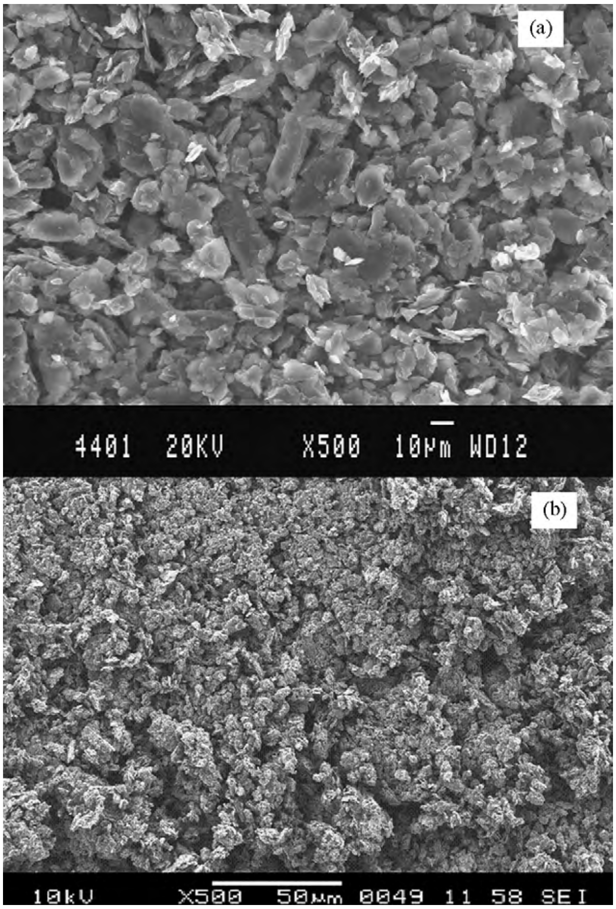


Fig. 3. SEM image of (a) bare carbon paste electrode and (b) poly (calmagite) film coated CPE.

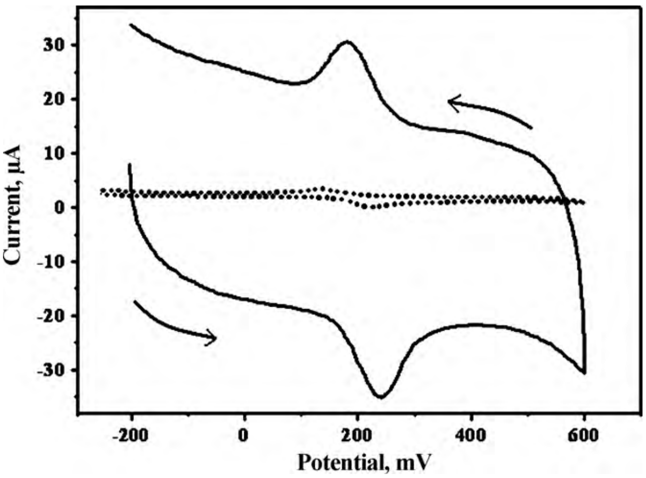


Fig. 4. Cyclic voltammogram of 1×10^{-5} M DA in 0.2 M phosphate buffer solution of pH 7.0 at bare CPE (dotted line) and poly (calmagite) film coated CPE (solid line).

electrode. The Epa and Epc were located at 238 and 181 mV. The ΔEp was found to be 57 mV. The Ipa/Ipc was 1.07. This high electrocatalytic activity of poly (calmagite) modified CPE because of formation of high concentration of negative charged $–SO_3^-$ group and electron rich oxygen atom on the electrode surface at neutral pH. These electron rich groups result in that the poly (calmagite) modified electrode shows a well affinity towards the DA positive ions by exchanging the electrons and enhanced the oxidation of DA. This process was represented in Scheme 2.

Scheme 2 shows the oxidation mechanism of DA at poly (calmagite) film coated carbon paste electrode surface. The calmagite was scanned in positive direction in sufficient potential window at carbon paste electrode in phosphate buffer solution at pH 9.0, the electrode gains the electrode gains the 2 electron from the calmagite and gives the respective oxidation product. Depending on applied the multiple scan, the oxidation product of calmagite forms a uniform layer which was rich in negatively charged $–SO_3^-$ ions. The negatively charged ions drag the cation containing DA towards itself by means of electrostatic attraction. This poly (calmagite) layer already lost its 2 electron, hence it eager to take the 2 electrons from the DA by oxidizing it to dopa-o-quinone and could get back to its original calmagite structure in forward scan. The electrochemical behavior of DA shows reversible nature, hence while in reverse scan DA takes back its 2 electron and it retains its original structure [15,16].

3.5. *The effect of scan rate*

The effect of scan rate for DA was studied by CV at poly (calmagite) modified CPE. Poly (calmagite) modified CPE showed increase in the redox peak current with increase in scan rate (50–400 mV s^{-1}) (Fig. 5a). The graph of current (Ipa) vs square root of scan rate (ν) was plotted. The graph obtained was good linearity between the scan rate and Ipa (Fig. 5b). In the range from 50 to 400 mV s^{-1} the anodic peak currents were proportional to the scan rate (ν). The correlation coefficient was 0.999, which indicate the electrode reaction was adsorption controlled.

3.6. *Effect of pH*

The electrochemical response of DA at poly (calmagite) modified CPE was generally pH dependent. The voltammograms of DA were recorded at 0.2 M phosphate buffer solution of different pH by cyclic voltammetric method. Fig. 6a demonstrates the pH dependence of DA at poly (calmagite) modified CPE at sweep rate of 50 mV s^{-1}.

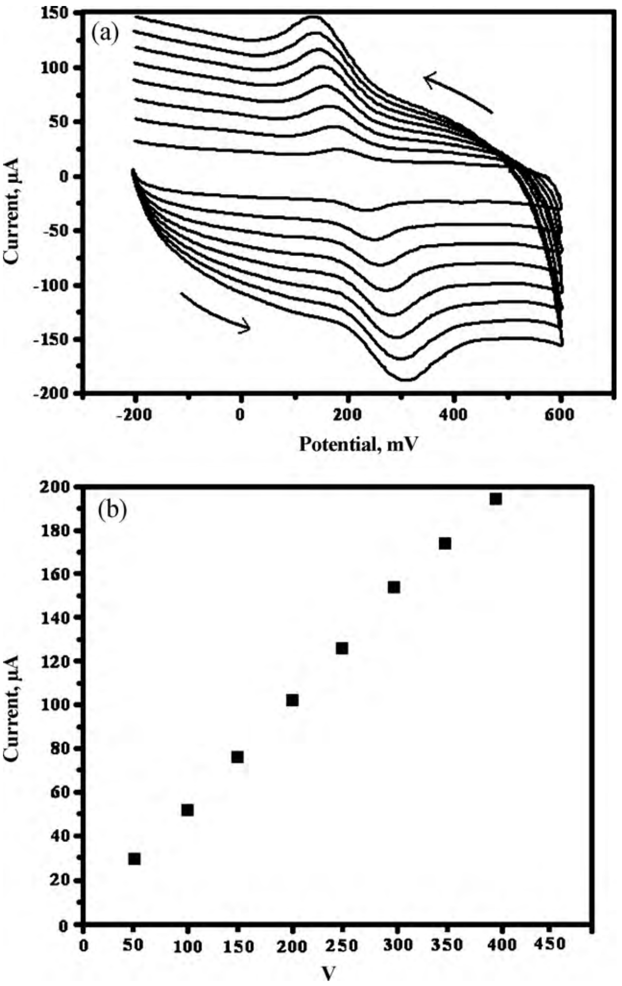


Fig. 5. (a) Variation of scan rate for DA at poly (calmagite) film coated CPE (a–h; 50–400 mV s^{-1}). (b) Graph of Ipa vs scan rate of DA.

The both anodic and cathodic peak potentials were shifted to less positive side with increasing in the pH values. The anodic peak potential of DA shifted from 331 to 249 mV with respect the pH from 2 to 9. The potential diagram was constructed by plotting the graph of calculated E^0 vs pH of the solution (Fig. 6b). The graph has good linearity with a slope of 52 mV/pH this behavior is nearly obeyed the Nernst Equation for equal number of electron and proton transfer reaction [17,18]. From the graph of Ipa vs pH, it can be seen that the current was maximum at pH 3.5. This is because the pK_a value of $R\text{-}SO_3H$ (R: Aryl or Alkyl moiety) is between 3 and 4 [15,16]. Thus, in this pH, the poly (calmagite) film dissociates favorably into negative charged $–SO_3^-$ group hence high current signal. But we are interested in investigate the electro-oxidation of DA at neutral condition, hence we selected pH 7.0 (Fig. 7).

3.7. *Electrocatalytic oxidation of AA and UA at poly (calmagite) modified CPE*

Fig. 8a reveals the oxidation of AA at bare carbon paste electrode and poly (calmagite) modified CPE in 0.2 M phosphate buffer solution of pH 7.0 at sweep rate of 50 mV s^{-1}. From Fig. 8a it can be notice that the oxidation peak of AA (1×10^{-4} M) at bare carbon paste electrode was broad and poor in sensitivity (dotted line). The anodic potential was found around at 160 mV. However, at poly (calmagite) modified CPE the oxidation peak for AA was shifted towards negative direction with high current enhancement (solid line). The anodic peak potential was located at 110 mV. This shift-

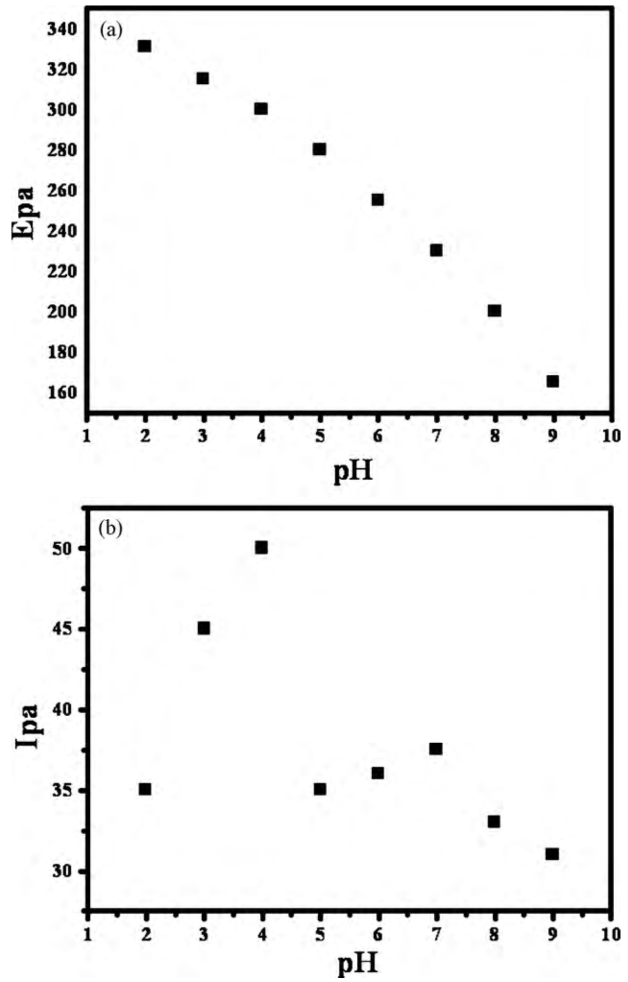


Fig. 6. (a) Cyclic voltammogram of 1×10^{-5} M DA for different pH (from 3.6 to 9 pH) at poly (calmagite) film coated CPE. (b) Graph of Epa vs different pH.

ing of peak potential and enhancing the current signal confirms the electrocatalytic activity of poly (calmagite) modified CPE towards AA. Further the scan rate effect was done by varying the scan rate from 50 to 400 mV s^{-1}. The graph of anodic peak current vs scan rate shows linear relationship and correlation coefficient was found to be 0.9988 (Fig. 8b). The concentration of AA was varied from the 0.5×10^{-4} to 5×10^{-4} M. The graph of anodic peak current vs concentration of AA was shown very good linearity (data not shown).

Fig. 9a the UA (0.5×10^{-4} M) shows its anodic peak at 304 mV at bare CPE at 50 mV s^{-1} sweep rate in 0.2 M phosphate buffer solution of pH 7.0. The obtained voltammogram at bare CPE was less sensible. However, the voltammogram obtained at poly (calmagite) modified CPE for UA in the same condition was with high current signal comparing to bare CPE. The anodic peak potential was located at 330 mV. This result of enhancement in current signal showed very good sensor activity of poly (calmagite) modified CPE for UA. The scan rate study was done by varying the scan rate from 50 to 400 mV s^{-1}. The anodic peak current was increased with increase in the scan rate. The graph of anodic peak current vs scan rate results in linear relationship and correlation coefficient was found to be 0.9992 (Fig. 9b). The concentration of UA was varied from the 0.1×10^{-4} to 3×10^{-4} M. The graph of anodic peak current vs concentration of UA was results very good linearity (data not shown).

The obtained experimental results from Figs. 8b and 9b were used to calculated the number of electron (n) and the heterogeneous rate constant (k^0) as in the literature [19]. The experimentally

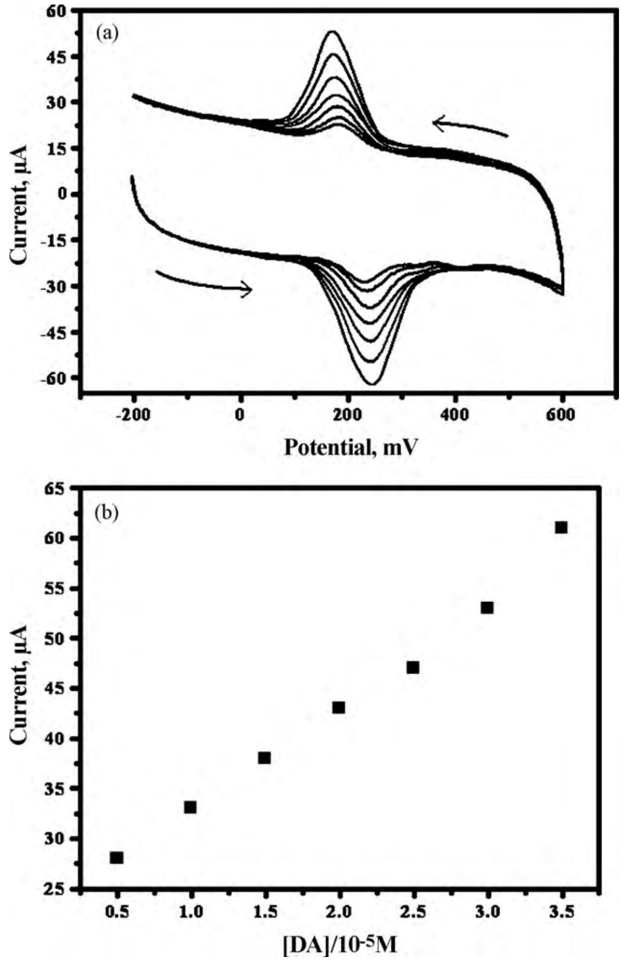


Fig. 7. (a) Cyclic voltammogram for different concentration of DA (a) 1×10^{-5} M DA, (b) 1.5×10^{-5} M DA, (c) 2×10^{-5} M DA, (d) 2.5×10^{-5} M DA and (e) 3×10^{-5} M DA at poly (calmagite) film coated CPE with scan rate 50 mV s^{-1}. (b) Graph of Ipa vs concentration of DA.

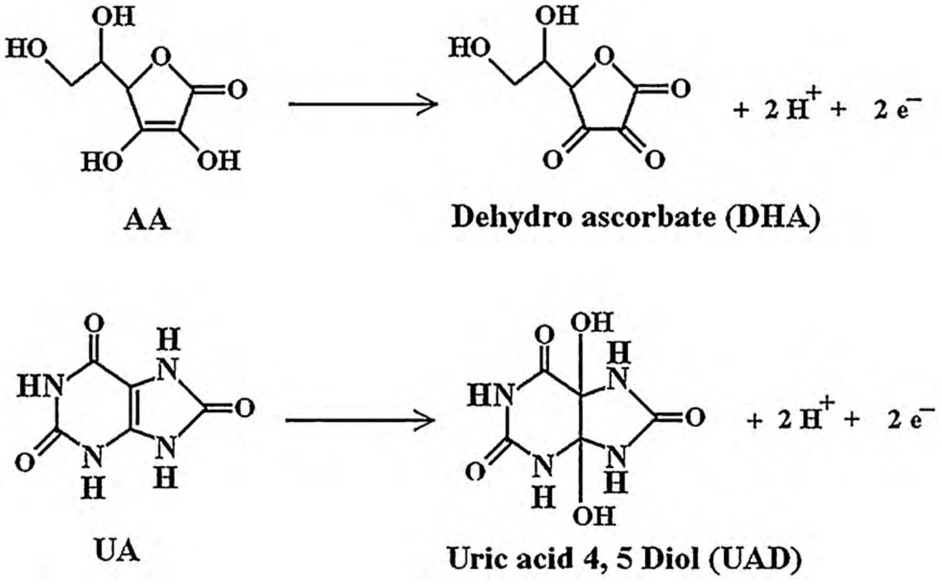
calculated Epa/2, n and k^0 for AA and UA were tabulated in Tables 1 and 2. In each case the number of electrons (n) was nearly equal to 2 for electrocatalytic oxidations. This shows that both AA and UA follows 2 electron change at the surface of poly (calmagite) film coated carbon paste electrode and this oxidation mechanism was shown in Scheme 3.

3.8. Simultaneous determination of DA, AA and UA

AA and UA were present along with DA in mammalian brain and the concentrations of these were much higher than that of DA. Since, the oxidation potential of both AA and UA was nearly same as that of DA result in an overlapped voltammetric response at bare CPE. Fig. 10a showed the cyclic voltammetric response

Table 1
Electrochemical parameters of AA at different scan rates.

Scan rate (mV s^{-1})	Epa (mV)	Epa/2 (mV)	n	k^0/s^{-1}
50	110	7.5	1.8	0.0479
100	125	65	1.76	0.0864
150	137	110	1.92	0.1500
200	150	135	1.88	0.2347
250	163	152	1.71	0.3262
300	170	155	1.74	0.3660
350	175	165	1.83	0.4019
400	189	181	1.77	0.4728



Scheme 3. Mechanism of electrocatalytic oxidation of AA and UA at poly (calmagite) film coated carbon paste electrode.

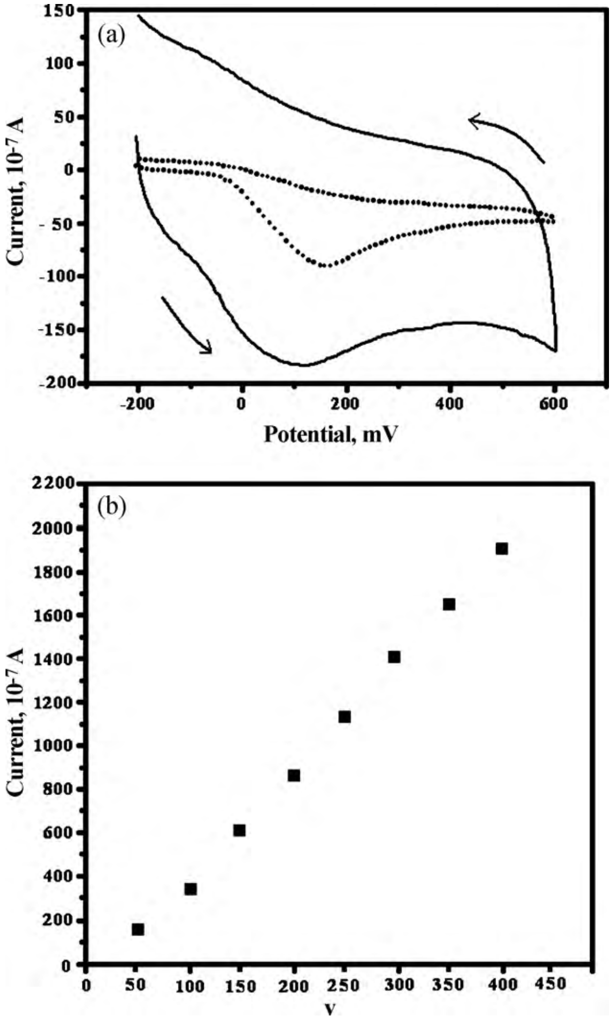


Fig. 8. (a) Cyclic voltammogram of 1×10^{-4} M AA at bare CPE (dotted line) and poly (calmagite) film coated CPE (solid line) with scan rate of $50\,\mathrm{mV\,s^{-1}}$. (b) Graph of anodic peak current vs scan rate.

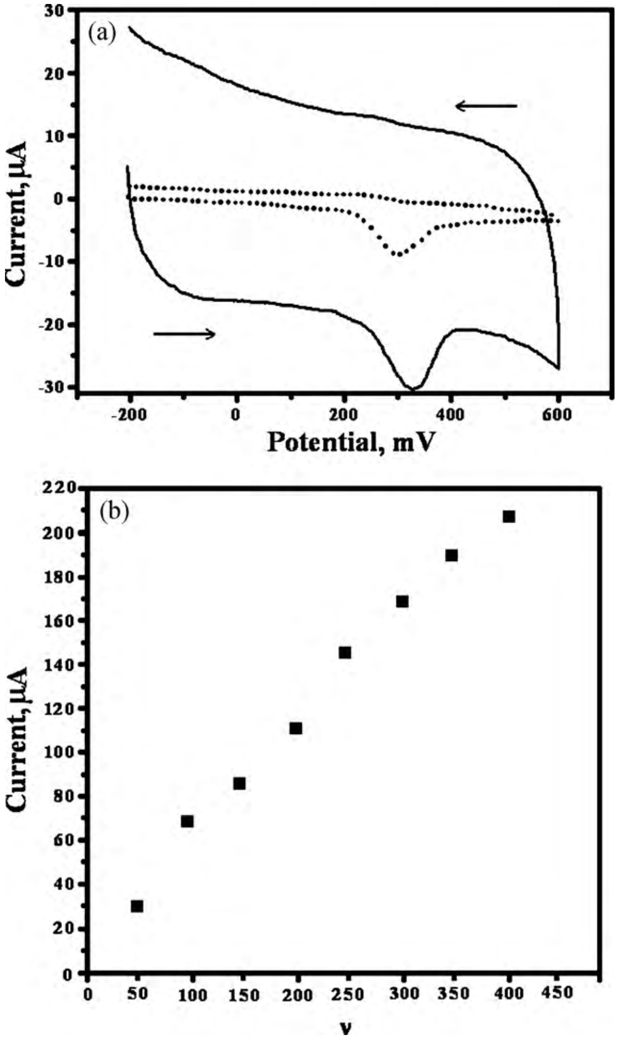


Fig. 9. (a) Cyclic voltammogram of 0.5×10^{-4} M UA at bare CPE (dotted line) and poly (calmagite) film coated CPE (solid line) with scan rate of $50\,\mathrm{mV\,s^{-1}}$. (b) Graph of anodic peak current vs scan rate.

Table 2
Electrochemical parameters of UA at different scan rates.

Scan rate (mV s^{-1})	Epa (mV)	Epa/2 (mV)	n	k^0/s^{-1}
50	330	210	1.91	0.0478
100	350	299	1.88	0.105
150	375	335	1.75	0.139
200	382	360	1.72	0.194
250	390	380	1.76	0.2472
300	412	395	1.83	0.2827
350	430	415	1.77	0.3352
400	451	435	1.89	0.3381

of DA (1×10^{-5} M) in the presence of UA (0.5×10^{-4} M) and AA (1×10^{-4} M) in phosphate buffer solution of pH 7.0 at both bare CPE and poly (calmagite) modified CPE. The voltammogram obtained for mixture of sample at bare CPE was broad, less sensible and overlapped wave at the potential of 220 mV (dotted line). However, our poly (calmagite) modified CPE faced this challenge and achieved the separation. The resulted voltammogram at poly (calmagite) modified CPE had three well defined peaks for DA, AA and UA at different potentials. The oxidation peak potentials of DA, AA and UA were at 235, 80 and 380 mV respectively. The peak to peak separation of DA–AA was 155 mV and that of DA–UA was 145 mV. This results were large sufficient to identify DA in the presence of AA and UA at poly (calmagite) modified CPE.

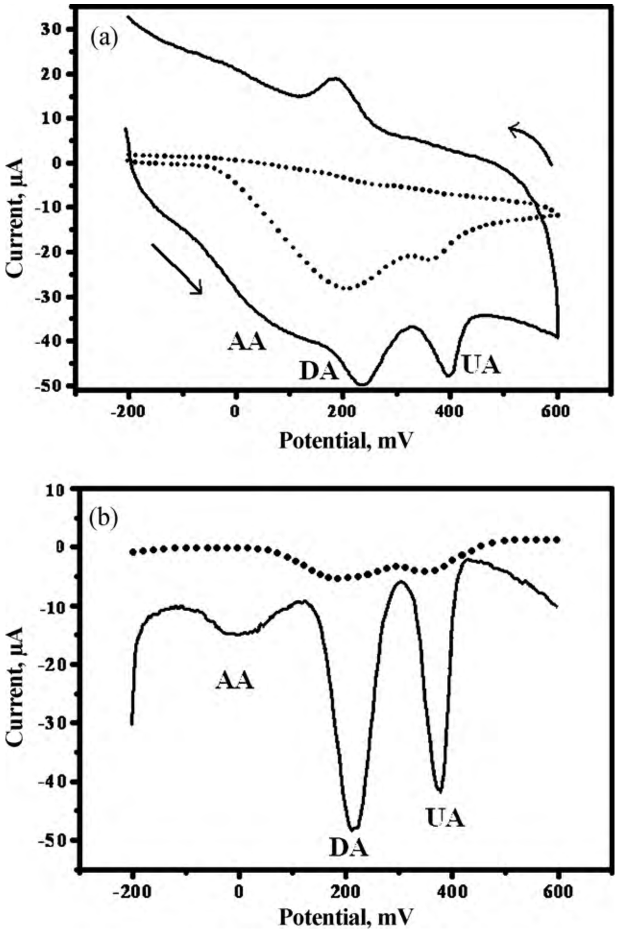


Fig. 10. (a) Cyclic voltammogram for simultaneous determination of 1×10^{-5} M DA, 1×10^{-4} M AA and 0.5×10^{-4} M UA at bare CPE (dashed line) and poly (calmagite) film coated CPE (solid line) with the scan rate of 50 mV s^{-1}. (b) Differential pulse voltammogram for simultaneous detection of 1×10^{-5} M DA, 1×10^{-4} M AA and 5×10^{-5} M UA at poly (calmagite) film coated CPE (solid line) and at bare CPE (dotted line) with the scan rate of 50 mV s^{-1}.

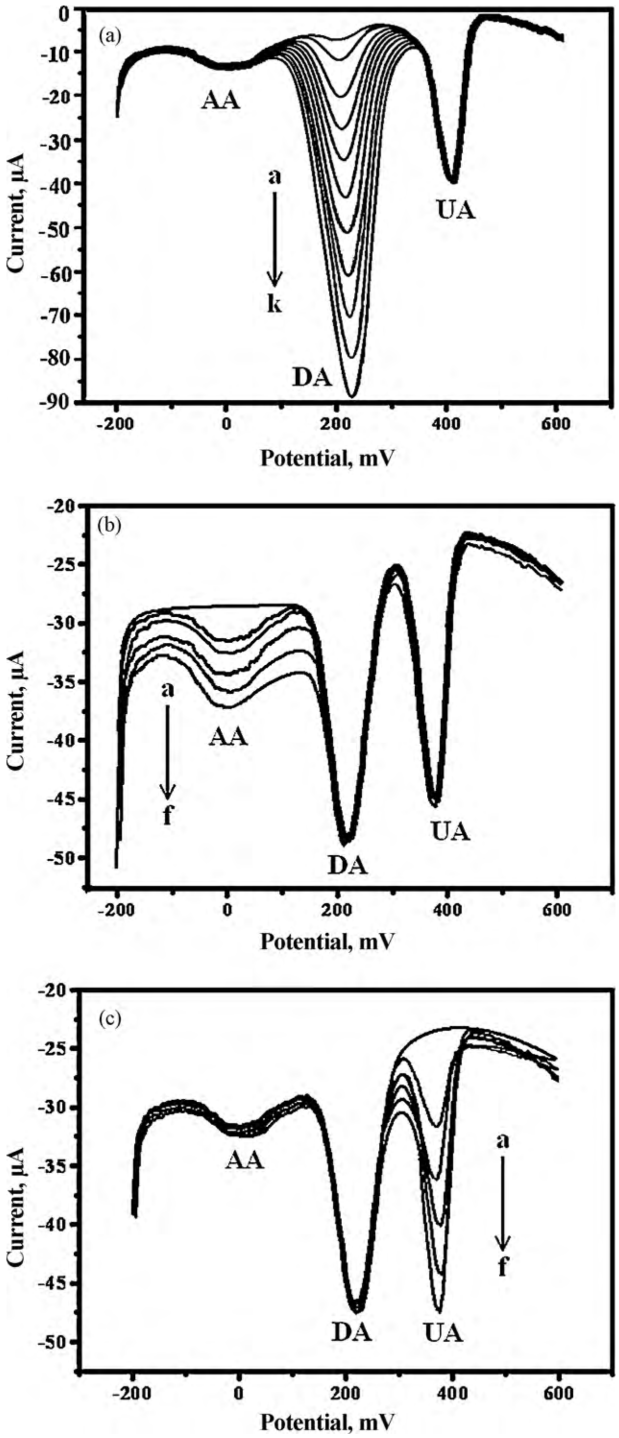


Fig. 11. (a) Differential pulse voltammogram of (a) 9.0×10^{-6} M, (b) 9.2×10^{-6} M, (c) 9.4×10^{-6} M, (d) 9.6×10^{-6} M, (e) 9.8×10^{-6} M, (f) 1.0×10^{-5} M, (g) 1.5×10^{-5} M, (h) 2×10^{-5} M, (i) 2.5×10^{-5} M, (j) 3.0×10^{-5} M and (k) 3.5×10^{-5} M DA in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 1.0×10^{-4} M AA and 0.5×10^{-4} M UA at poly (calmagite) film coated CPE. (b) Differential pulse voltammogram of (a) 0 M, (b) 2×10^{-4} M, (c) 4×10^{-4} M, (d) 6×10^{-4} M, (e) 8×10^{-4} M and (f) 10×10^{-4} M AA in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 1×10^{-5} M DA and 0.5×10^{-4} M UA at poly (calmagite) film coated CPE. (c) Differential pulse voltammogram of (a) 0 M, (b) 0.2×10^{-4} M, (c) 0.4×10^{-4} M, (d) 0.6×10^{-4} M, (e) 0.8×10^{-4} M and (f) 1×10^{-4} M UA in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 1×10^{-5} M DA and 1×10^{-4} M AA at poly (calmagite) film coated CPE.

Table 3
Comparison of different modified electrodes for DA determination.

Electrode	Detection limit (mol/L)	Method	Reference
Metallothioneins self-assembled gold electrode	6.0×10^{-6}	CV	[25]
Ionic liquid modified carbon paste electrode	7.0×10^{-7}	CV	[26]
Poly (caffeic acid)/GCE	2.0×10^{-7}	CV	[27]
α-CD/CNT/PGE	1.0×10^{-6}	DPV	[28]
Poly (p-toluene sulfonic acid) modified glassy carbon electrode	6.0×10^{-7}	DPV	[29]
Poly (calmagite) film coated carbon paste electrode	1.0×10^{-8}	DPV	This work

DPV was used for the determination of DA, AA and UA at poly (calmagite) modified CPE because to get higher current sensitivity and better resolution. The simultaneous study was carried out in the potential range from −200 to 600 mV (Fig. 10b). The DPV showed the simultaneous determination of DA, AA and UA with well separated three anodic peaks corresponding to their oxidation could be possible at poly (calmagite) modified CPE. The 1×10^{-5} M DA showed its Epa at 211 mV, 1×10^{-4} M AA was at −21 mV and 0.5×10^{-4} M UA was at 385 mV. The peak separation between DA and AA was 232 mV and for DA and UA the peak separation was 174 mV which were greater when comparing to peak separation occurred by CV.

3.9. *Interference study*

The simultaneous determination of DA, AA and UA in the mixture was carried out at poly (calmagite) modified CPE when concentration of one species changed, whereas the others remained kept constant. From Fig. 11a, it can be seen that the peak current of DA was proportional to its concentration, which was increased from 0 to 3×10^{-5} M when keeping the concentration of UA 0.5×10^{-4} M and AA 1×10^{-4} M. There were no change in the peak current and peak potential occurred for AA and UA. Similarly in Fig. 11b and c self explains the concentration effect of AA from 0 to 1×10^{-3} M and UA from 0 to 1×10^{-4} M respectively. These results show that the DA, AA and UA were exist independently in their mixtures of samples.

To study the effect of concentration of DA, differential pulse voltammetric technique was used [20–22]. The concentration of DA was varied from 9×10^{-6} to 35×10^{-6} M. Fig. 12 shows that the graph of anodic peak current vs concentration of DA shows two linear relationships ranges 9×10^{-6}–1×10^{-5} and 1×10^{-5}–3.5×10^{-5} M with the linear regression equations as Ipa (μA) = 40.02 C μM/L − 3.6×10^{-4} and Ipa (μA) = 1.99 C μM/L + 2.05×10^{-5}, respectively. The correlation coefficient for the first linearity was 0.99997 and for the second it was found to be 0.99966. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation [23]. The detection limit for DA in the lower range region was found to be 1×10^{-8} M and quantification limit was 3.3×10^{-8} M. The detection limit and quantification limit was calculated by using the formulas, LOD = $3S/M$ and LOQ = $10S/M$ [24]. Where S is the standard deviation and M is the slope obtained from the three calibration plots. The detection limit of various electroanalytic methods proposed for determination of DA is compared with our analytical data in Table 3. From the data shown, a lower limit of detection (LOD) can be achieved using the proposed method [25–29].

3.10. *Analytical application*

The modified electrode was applied to the determination of dopamine hydrochloride injection. The DA injection sample purchased from sterile specialities India Private Ltd with a specified content of DA of 40.0 mg/mL. The sample was used after suitable dilution. 0.2 M phosphate buffer was used for diluting the injection samples. The results were shown in Table 4. The recovery and RSD were acceptable, showing that the proposed methods could be efficiently used for the determination of DA in injections with recovery in the range 99.25–100.5% (Fig. 12).

Table 4
Detection of DA in injection samples (n = 5).

Sample	Content (mg/mL)	Found (mg/mL)	RSD (%)	Recovery (%)
1	4.0	3.97	2.4	99.25
2	4.0	4.02	2.1	100.5
3	4.0	4.02	2.5	100.5

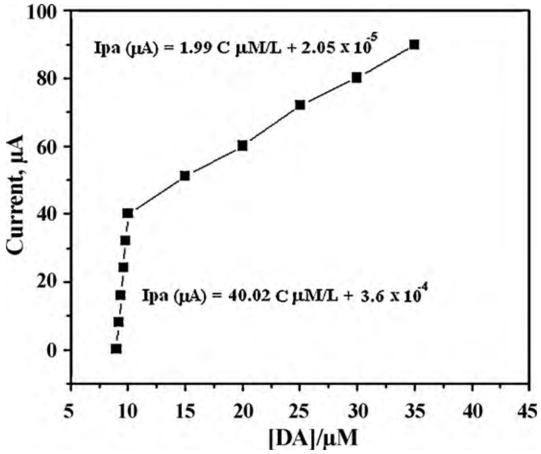


Fig. 12. Calibration plot of DA concentration.

4. Conclusion

In conclusion the synthesized poly (calmagite) modified CPE can detect DA electrochemically in mixture of solution, at physiological pH, containing large excess of AA and UA both by cyclic voltammetry and differential pulse voltammetry techniques. The detection limit of DA was 1×10^{-8} M. The modified electrode showed excellent sensitivity, selectivity and anti-fouling properties. The proposed methods can be applied to the detection of DA in injection. We believe that this approach can readily be applied to the development of electrochemical sensors for dopamine and related neurotransmitters.

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