

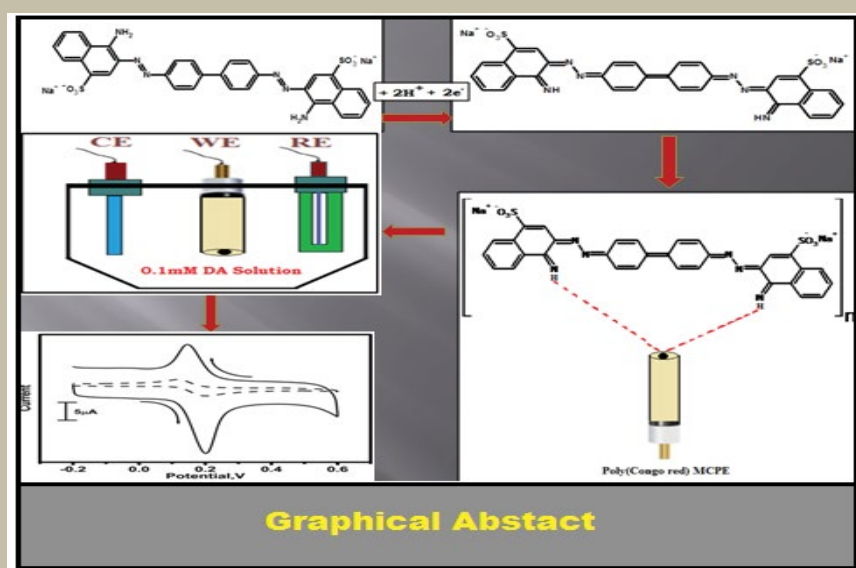
Electropolymerized Congo Red Film based Sensor for Dopamine: A Voltammetric Study

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Abstract

The polymerization film of Congo red was prepared on the surface of carbon paste electrode by electropolymerization using cyclic voltammetric method. The higher catalytic activity was obtained for electrocatalytic oxidation of Dopamine, with drastic enhancement of the reversibility and peak current in 0.2 phosphate buffer solution of pH 7.0 at the sweep rate 100 mV/s. The variation of sweep rate and pH were investigated. The limit of detection of Dopamine was found to be 0.06 μ M. The effect of interference studies was done by differential pulse voltammetric technique. In the simultaneous look at, Dopamine and Uric acid were well separated by cyclic voltammetric technique. The proposed method showed good sensitivity, selectivity, and reproducibility.

Keywords: Dopamine; Uric acid; Congo red; Cyclic voltammetry; Electropolymerization; Carbon paste electrode



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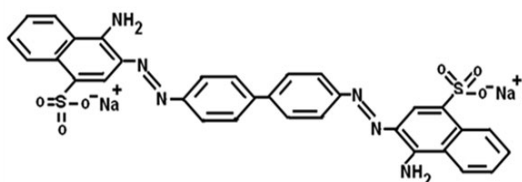
Introduction

Dopamine, which belongs to the catecholamine family of neurotransmitters, is synthesized in a sequential reaction in which tyrosine hydroxylase and amino acid decarboxylase convert tyrosine to L-dihydroxyphenylalanine (L-dopa), followed by decarboxylation of L-dopa to dopamine. Dopamine plays a vital role in the control of movements and has been associated with the motor symptoms experienced in patients with

Parkinson's disease [1]. Other studies show that the dopamine oxidation products can inhibit the function of specific proteins [2] and correlate formation of cysteine l-dopamine conjugates with dopamine-induced neurotoxicity. As a potent neurotransmitter, changes in the level of dopamine in adrenal glands impact many aspects of brain circuitry. For example, Parkinsonism is associated with a reduced level of dopamine; while schizophrenia is related to increased dopamine activity [3].

In vivo concentrations of dopamine are in the nanomolar range. Given the wide range of physiological and path physiological implications, the development of analytical assays for precise, low level, and selective measurement of dopamine are highly desirable [4]. DA is produced by substantial nigra neurons and found in large quantities (50 m mol/g) in the caudate nucleus, a region of the brain [5,6]. In the human body, DA is typically broken down by the oxidation that is catalyzed by enzyme monoamine oxidase. Conversely, DA is also able to undergo autoxidation i.e., it reacts with oxygen, yielding free radicals along with quinones as products [7,8]. Hence, it is essential to develop a simple and rapid quantification method for DA in routine analysis for diagnostic, neurological and pharmaceutical applications.

On the other hand, Uric acid is the primary end product of purine metabolism in the human body [9]. In a healthy human being, the typical concentration of UA in urine is around 2 mM and in the blood, is in between 120 μ M to 450 μ M ranges [10,11]. Extreme abnormalities of UA levels are Symptomatic of several diseases, such as cardiovascular disease [12], hyperuricemia, uric acid stones [13], gout and Le-seh-Nyhan syndrome [14]. Increased rate level also leads to pneumonia and leukaemia [15,16]. Dopamine and uric acids usually coexist in physiological samples, dopamine normally present at low concentration along with uric acid which is at higher concentration. The simultaneous determination of these compounds is a special interest in the development of electrochemical sensors [17]. The successful route to overcome the problems of selectivity is to modify the carbon paste electrode surface because the modified electrode could decrease the over voltage, improve the velocity of mass transfer efficiency and enhances the selectivity of the analyte [18]. The modification can be done by using organic and inorganic substances and biomolecules [19-21]. In the present day's electropolymerization technique was used to prepare the polymer-modified electrodes have received wide interest in the detection of analytes because of its high selectivity, and homogeneity in electrochemical deposition, strong adherence to the electrode surface and chemical stability of the films [22-24]. Shahrokhian et al. [25] has reported the Synthesis of Polypyrrole in the Presence of Congo Red; Application to Selective Voltammetric Determination of Dopamine in the Presence of Ascorbic Acid. Until now, different methodologies have been used to prepare polymeric film modified electrodes. Among them, electropolymerization yields a modified electrode with a three-dimensional distribution of mediators. This type of electrodes enhances the sensitivity and improves the catalytic activity than monolayers and few reports have been reported [26-29]. Congo red is the sodium salt of 3,3'-([1,1'-biphenyl]-4,4'-diyl) bis (4-aminonaphthalene-1-sulfonic acid) is an azoic compound synthesized by Paul Böttiger is used to stain microscopic prepares, especially as a cytoplasm and erythrocyte stain (**Scheme 1**) [30].



Scheme 1 Structure of Congo red.

In this study, the modification of stable working electrode by electro polymerizing Congo red on the bare carbon paste electrode surface by CV method and used for the voltammetric determination of DA and UA. The poly (Congo red) MCPE shows very good enhancement when compared to BCPE and also it shows good sensitivity, selectivity, and stability for the determination of the two neurotransmitters like DA and UA.

Materials and Methods

Cyclic voltammetric experiments were performed on a model CH660c (CH instrument). All the electrochemical experiments were carried out in a three-electrode cell system, which contained a bare carbon paste electrode (BCPE)/ Poly (Congo red) film coated MCPE as the working electrode, a platinum wire and saturated calomel electrode as counter and reference electrode.

Reagents and chemicals

Graphite powder of 50 mm size was purchased from Loba and silicon oil was purchased from Himedia. Congo red, Dopamine hydrochloride (DA) and Uric acid (UA) were obtained from Himedia. All the chemicals are of analytical grade quality and were used as supplied without further purification. 25×10^{-4} M Congo red was prepared in double distilled water, 25×10^{-4} M DA was prepared in 0.1 M Perchloric acid (HClO₄), 25×10^{-4} M UA was prepared in 0.1 M sodium hydroxide, and Phosphate buffer solution (PBS) of same ionic strength was prepared (0.2 M) by mixing appropriate ratio of sodium dihydrogen phosphate (NaH₂PO₄·H₂O) disodium hydrogen phosphate (Na₂HPO₄).

Preparation of bare carbon paste electrode

Bare carbon paste electrodes (BCPE) were made with silicon oil (30%), and graphite powder (70%). The two components were thoroughly mixed in an agate mortar for about 30 minutes. The BCPE was packed into a homemade Teflon cavity having a current collector and was polished on a weighing paper.

Results and Discussion

Electropolymerization of poly (Congo red) MCPE at CPE surface

The modified carbon paste electrode was fabricated by electrochemical polymerization process over the potential range from -0.2 to 1.5 V at a sweep rate of 100 mV/s for 20 cycles. The poly (Congo red) modified carbon paste electrode (MCPE) was prepared by placing 1 mM Congo red with 0.1 M NaOH in an electrochemical cell. **Figure 1** suggests that during first cycle a small anodic peak was observed corresponding to the oxidation of Congo red monomer [31]. During the polymerized process, with increasing the number of cyclic time corresponding voltammogram was slowly decreased. It shows that the poly (Congo red) film was produced and deposited at the surface of BCPE. Once electropolymerization process complete; the MCPE was rinsed carefully with double distilled water.

Effect of different cycles of poly (Congo red) MCPE

From the above experimental result shows, the thickness of the

film has a major role on the electrocatalytic property of the poly (Congo red) MCPE. The formation of the layer can be adjusted by controlling the cyclic number of voltammetric scans on the BCPE (from 5 to 30) and corresponding anodic peak current at 0.1 mM DA in presence 0.2 M PBS of pH 7.0. The graphs of anodic peak current of DA versus number of cycles were plotted as shown in **Figure 2**. However, by considering the peak nature of the cyclic voltammogram the increment in the anodic peak current, the twenty cycles polymerized MCPE was optimized for the further electrochemical analysis. The probable mechanism of formation of the polymer film as reported in the literature [30].

Voltammetric behaviour of DA at Poly (Congo red) MCPE

Figure 3 showed the CV recorded for the oxidation 0.1 mM DA at BCPE (dashed line) and Poly (Congo red) MCPE (solid line) in pH 7.0 of 0.2 M PBS with the sweep rate 100 mV/s. In BCPE, the oxidation and reduction of DA were less sensible due slow electron transfer and the peak potentials were observed at 202 and 116 mV respectively. [$\Delta E_p=96$ mV]. Under the same condition at poly (Congo red) MCPE shows significantly enhanced in the redox peak current, the oxidation and reduction peak potentials were observed at 202 and 146 mV [$\Delta E_p=56$]. It above result shows, the oxidation peak of dopamine was enhanced at MCPE, because of formation of high concentration of negatively charged $-\text{SO}_3^-$ group and electron rich oxygen atom on the surface of the electrode at neutral pH. These electron rich groups result in that the poly (Congo red) MCPE shows a good affinity towards the DA positive ions by exchanging the electrons and enhanced the oxidation of DA [25,30,32].

Effect of sweep rate

The **Figure 4a** shows the effect of applied sweep rate for the oxidation of 0.1 mM DA recorded at different sweep rates using poly (Congo red) MCPE in 0.2 M PBS of pH 7.0. From the above figure shows an increase in the redox peak current with increases the sweep rates from 50 to 400 mV/s. To evaluate the electrode process, the graph of I_p versus sweep rates (ν) was plotted (**Figure 4b**). The obtained graph was the good linearity almost nearly straight line having a correlation coefficient value 0.9997 and 0.9989 respectively but in the same time the graph of I_p versus square root of sweep rates ($\nu^{1/2}$) as shown in the **Figure 4c**, having correlation coefficient value 0.9880 and 0.9927 respectively. From the above scrutiny, overall electrode process was controlled by adsorption process at poly (Congo red) MCPE.

The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, **Equation (1)** was used for such voltammograms whose ΔE_p values are greater than 10 mV [33].

$$\Delta E_p = 201.39 \log (\nu / k^0) - 301.78 \quad (1)$$

From the experimental ΔE_p values as shown in **Table 1** and **Equation (1)**; the values of the k^0 for the PA oxidation was determined. All the parameters are tabulated in **Tables 1 and 2**.

Concentration effect of DA

Figure 5a depicts cyclic voltammograms of poly (Congo red)

MCPE with different concentration from 0.1 to 0.5 mM in 0.2 M PBS of pH 7.0 at the sweep rate 100 mV/s. It can be seen that, redox peak current goes increase by increasing DA concentration. The graph of anodic peak current versus concentration of DA was

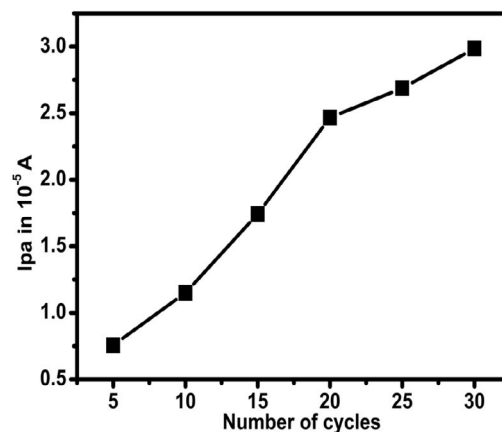


Figure 1 Electropolymerization of 1 mM of Congo red at 0.1 M NaOH as supporting electrolyte for 10 cycles with the sweep rate of 100 mV/s.

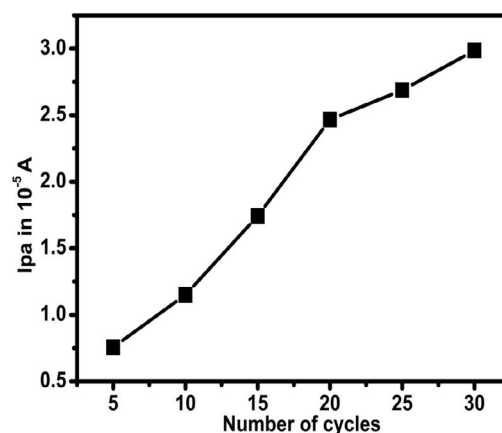


Figure 2 The graph of anodic peak current versus number of cycles.

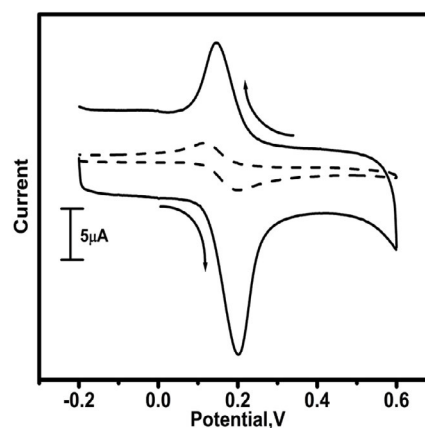


Figure 3 Cyclic voltammograms of 0.1 mM DA in 0.2 M PBS solution of pH 7.0 at BCPE (dashed line) and poly (Congo red) MCPE (Solid line) at the sweep rate of 100 mV/s.

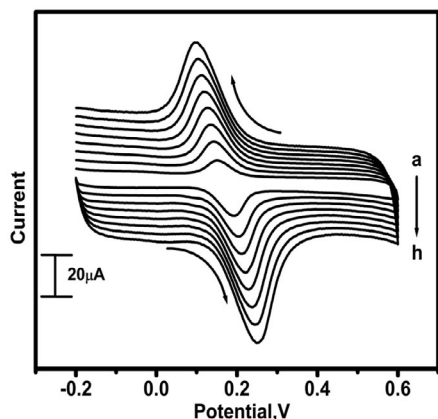


Figure 4a Cyclic voltammograms of 0.1 mM DA in 0.2 M PBS solution of pH 7.0 at poly (Congo red) MCPE at different sweep rates (a-h; 50 to 400 mV/s).

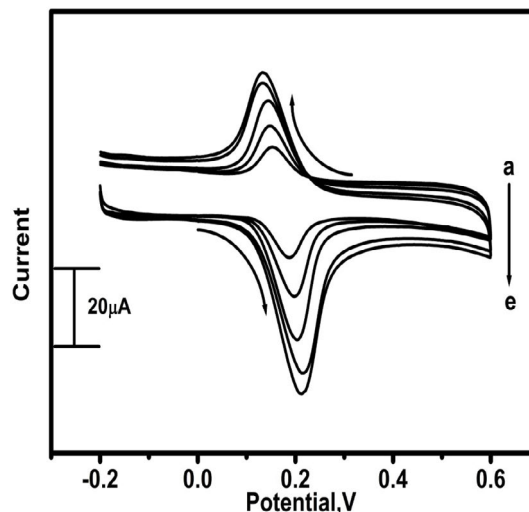


Figure 5a Cyclic voltammograms of DA in 0.2 M PBS solution of pH 7.0 at poly (Congo red) MCPE at sweep rate of 100 mV/s with different concentrations (a-e: 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM and 0.5 mM).

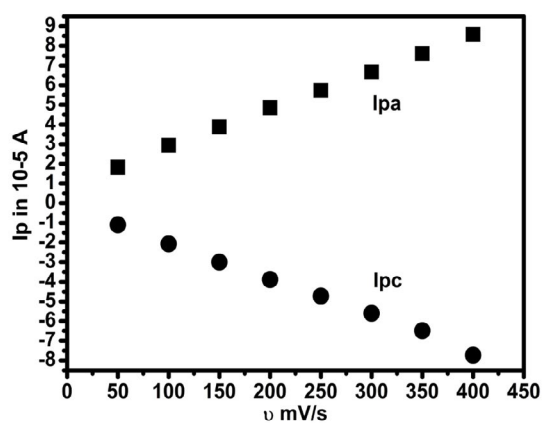


Figure 4b The graph of anodic peak current versus sweep rates.

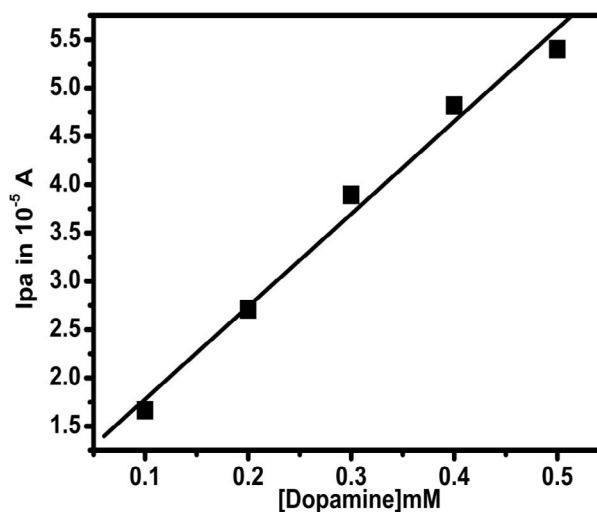


Figure 5b The graph of anodic peak current versus different concentration of DA.

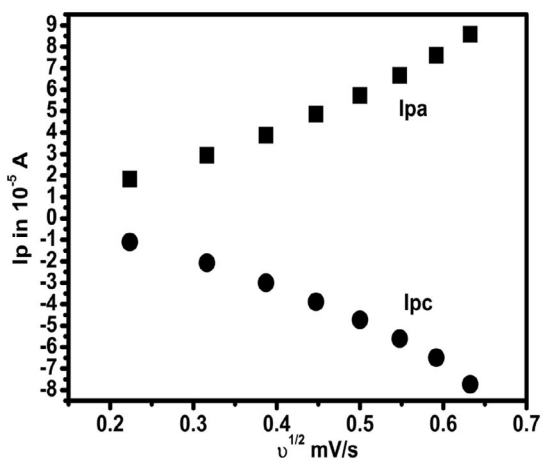


Figure 4c The graph of anodic peak current versus square root of sweep rates.

Table 1 Electrochemical parameters of DA at different sweep rates.

ν mV/s	ΔE_p (mV)	k^0 (s^{-1})
50	44	0.9574
100	74	1.3614
150	80	1.4996
200	96	2.1134
250	112	2.2029
300	127	2.2284
350	143	2.1627
400	158	2.0844

plotted and it was good linearity as shown in the **Figure 5b**. The correlation coefficient (r^2) was found to be 0.9933. The limit of detection of the lower concentration range for DA was 0.06 μ M for the poly (Congo red) MCPE. The limit of detection (LOD) was

calculated by using with equation (2). Where S is the standard deviation and M is the slope of obtained from the graph.

$$\text{LOD} = 3S/M$$

(2)

Table 2 Comparison of some modified electrodes for the determination of DA.

S No	Electrode	Detection limit (μM)	Techniques	Reference
1	Banana/MWCNTs/MCPE	2.09	DPV	[35]
2	Bicopper complex modified GCE	1.4×10^{-6}	DPV	[36]
3	Self-assembled gold nanoparticle modified Gold electrode	9.0×10^{-5}	DPV	[37]
4	Poly (p-toluene sulfonic acid) modified glassy carbon electrode	6.0×10^{-7}	DPV	[38]
5	Ionic liquid modified carbon paste electrode	7.0×10^{-7}	CV	[39]
6	Poly (caffeic acid)/GCE	2.0×10^{-7}	CV	[40]
7	Metallothioneins self-assembled gold electrode	6.0×10^{-6}	CV	[41]
8	Poly (Congo red) MCPE	0.06	CV	In this work

The effect pH on DA at Poly (Congo red) MCPE

The supporting electrolyte of pH has a major role in the electrochemical behaviour of DA at poly (Congo red) MCPE. The **Figure 6a** depicts, cyclic voltammograms recorded in the solution containing 0.1 mM DA. The graph of anodic peak potential versus different pH was plotted. The graph shows good linear relationship. The linear regression can be expressed by the equation: $E_{pa}=0.061(\text{pH})-0.633$, having correlation coefficient value 0.9969, with the slope of 61 mV/pH. This behaviour is nearly obeyed the Nernst Equation for a same number of proton and electron were involved in redox mechanism [34,42].

Electrochemical determination of UA at Poly (Congo red) MCPE

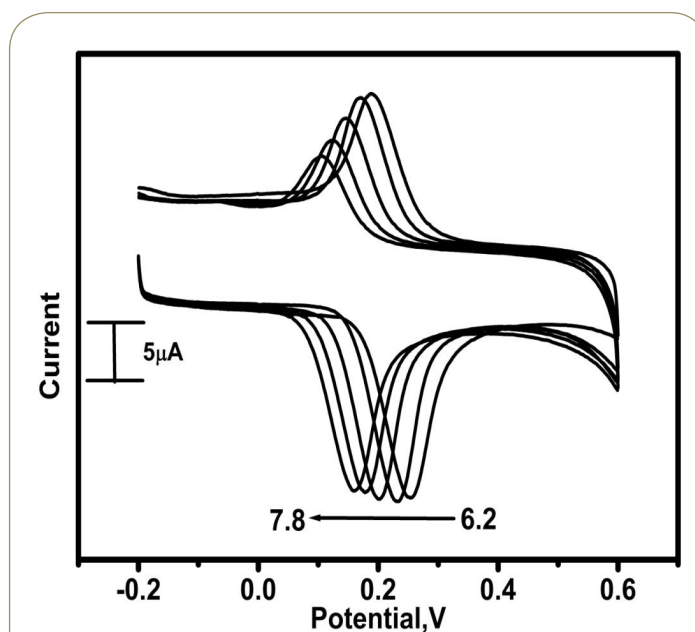
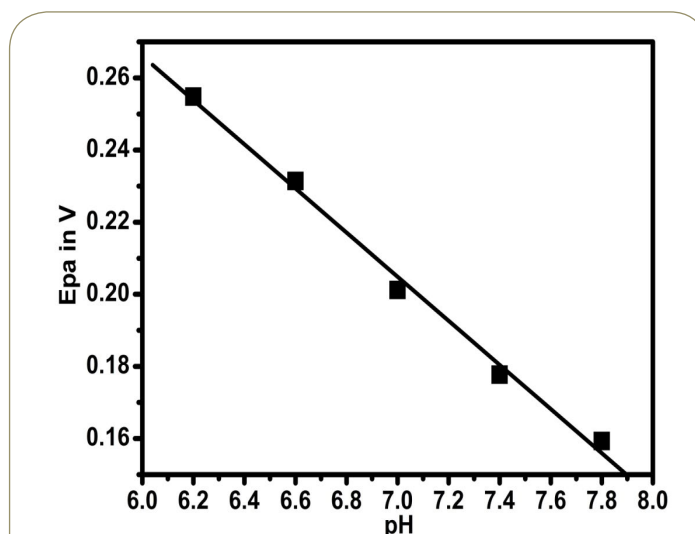
Figure 7 depicts electrochemical responses of 0.1 mM UA at both BCPE (dashed line) and poly (Congo red) MCPE (Solid line) in 0.2 M PBS pH 7.0 at the sweep rate 100 mV/s. From the above result shows at BCPE, a small broad oxidation peak potential at 326 mV with less sensitivity and slow electron transfer kinetic. While in the same condition, poly (Congo red) MCPE for UA shows significantly enhanced peak current corresponding oxidation peak potential was located at 352 mV. It is certified that poly (Congo red) MCPE shows very good sensor activity for UA.

DA and UA electrochemical determination by simultaneous method

Figure 8 depicts the cyclic voltammograms obtained from the mixture containing 0.1 mM DA and 0.1 mM UA at pH 7.0 PBS with the sweep rate 100 mV/s. In bare carbon paste electrode (dashed line) DA and UA shows poor current response, the oxidation peak potential located at 198 and 353 mV. In poly (Congo red) MCPE (solid line) exhibits huge enhancement in the peak current of the binary mixture when compared to bare carbon paste electrode. The obtained DA and UA oxidation peak potential were observed at 173 and 324 mV respectively. The peak to peak separation was 151 mV. This potential difference was enough to recognize DA and UA at poly (Congo red) MCPE.

Interference study

The interference investigation was done by DPV technique and the binary mixture of sample contains 0.1 mM DA and 0.1 mM UA in 0.2 M PBS of pH 7.0. In which, the concentration of DA


Figure 6a Cyclic voltammograms of different pH (a-e: 6.2 to 7.8) of poly (Congo red) MCPE.

Figure 6b The graph of anodic peak potential versus different pH of DA.

was varied from 0.1 to 0.9 mM, while keeping the concentration of UA was constant (**Figure 9**). The obtained voltammogram shows current will be linearly increased with the increase in

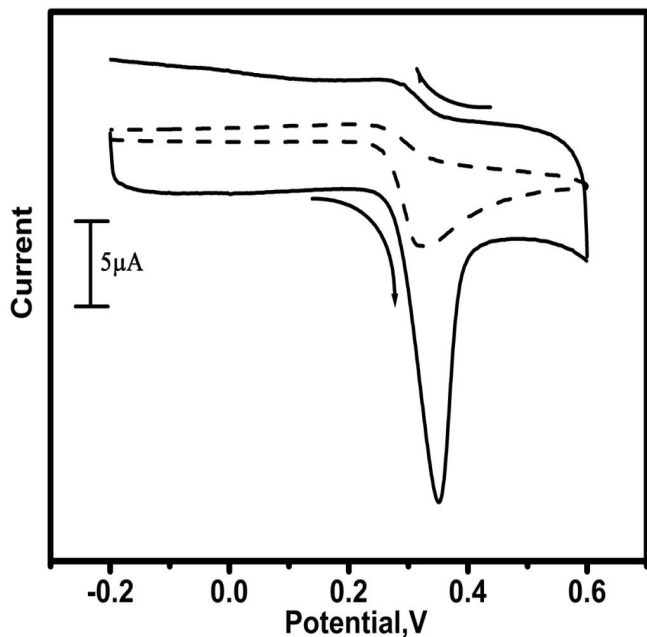


Figure 7 Cyclic voltammograms of 0.1 mM UA in 0.2 M PBS solution of pH 7.0 at BCPE (dashed line) and poly (Congo red) MCPE (Solid line) at the sweep rate of 100 mV/s.

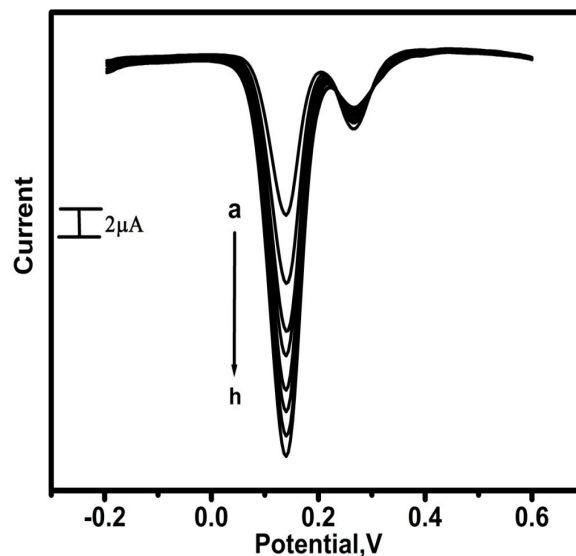


Figure 9 Differential pulse voltammograms (a) 0.1 mM (b) 0.2 mM, (c) 0.3 mM, (d) 0.4 mM, (e) 0.5 mM (f) 0.6 mM (g) 0.7 mM and (h) 0.8 mM DA in 0.2 M PBS of pH 7.0 in the presence of 0.2 mM UA at poly (Congo red) MCPE.

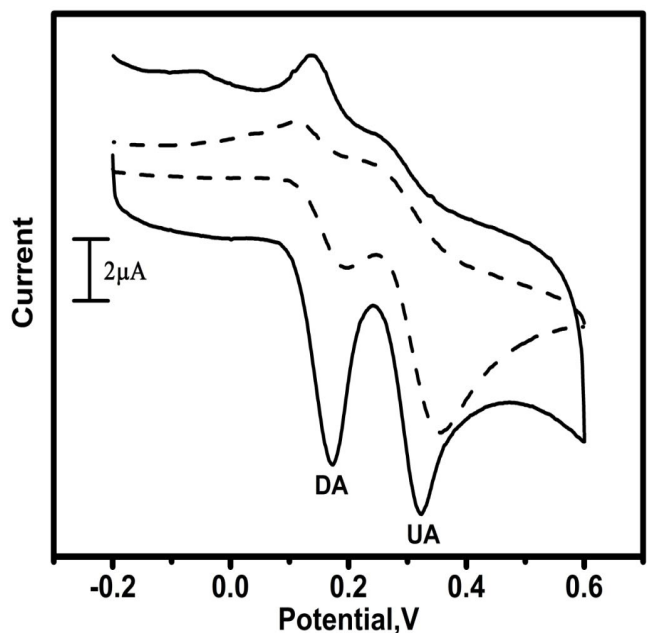


Figure 8 Simultaneous determination for 0.1 mM DA and 0.1 mM UA at BCPE (dashed line) and poly (Congo red) MCPE (solid line) at sweep rate of 100 mV/s.

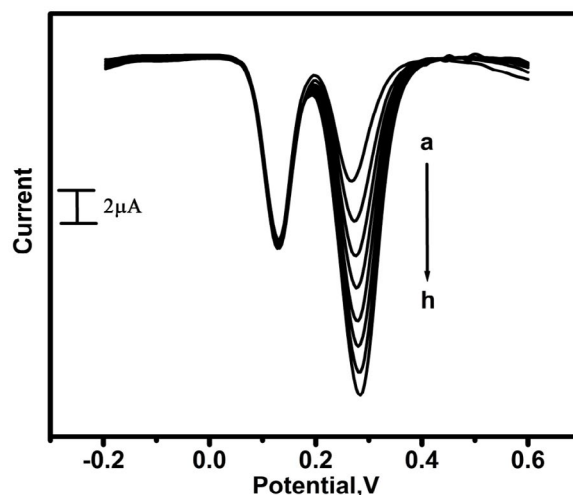


Figure 10 Differential pulse voltammograms of (a) 0.1 mM (b) 0.2 mM, (c) 0.3 mM, (d) 0.4 mM, (e) 0.5 mM (f) 0.6 mM (g) 0.7 mM and (h) 0.8 mM UA in 0.2 M PBS of pH 7.0 in the presence of 0.2 mM DA at poly (Congo red) MCPE.

concentration and no shift in the peak potential of UA. Similarly, by varying the concentration of UA (0.1 to 0.9 mM) only peak current of UA increased but no change in the peak potential of DA (**Figure 10**). From the above result, the developed electrode shows the oxidation of DA and UA were independent of each other at poly (Congo red) MCPE

Conclusion

Congo red has been directly electropolymerized on the carbon paste electrode surface by CV method. The prepared poly (Congo red) MCPE shown good electrocatalytic activity towards the oxidation of DA and UA and also shows the significant increment in the oxidation of DA and UA individually and simultaneously. The pH effect indicates the participation of equal number of protons and electrons in the catalytic oxidation. The modified electrode was very suitable and effective for simultaneous determination

of DA and UA was possible at poly (Congo red) MCPE with peak to peak separation of 151 mV by CV techniques. Over all the poly (Congo red) MCPE shows stability, selectivity, sensitivity, reproducibility for the determination of neurotransmitter. It is

expected that with its high electrocatalytic behaviour the poly (Congo red) MCPE could hold great application in the fields of electro analytical chemistry and biosensors.

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