

*Full Paper*

## **Simultaneous Determination of Dopamine, Serotonin and Folic acid at Torasemide Modified Carbon Paste Electrode: A Cyclic Voltammetric Study**

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*Received: 10 May 2016 / Received in revised form: 25 June 2016 /*

*Accepted: 4 July 2016 / Published online: 15 August 2016*

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**Abstract-** A carbon paste electrode modified by torasemide was used for determination of dopamine (DA). The modified electrode exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine at pH 7.0 in phosphate buffer solution (PBS). The parameters which influence the electrode response like paste composition; effects of scan rate, concentration, pH, surfactants and interferences have been studied. The linear range of DA 0.9990 and the detection limit for DA was found to be  $2.4 \times 10^{-6}$  M. Anionic surfactant Sodium Dodecyl Sulphate (SDS) showed very good electrocatalytic effect on the modified carbon paste electrode. The preparation of the modified electrode was easy and renewed by simple polishing gives very good reproducibility, high stability in its voltammetric response and low detection limit for DA.

**Keywords-** Dopamine, Serotonin, Folic acid, Torasemide Modified electrode, Surfactants, Cyclic voltammetry, Simultaneous

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### **1. INTRODUCTION**

Voltammetric detection provides a highly sensitive approach to the electroanalysis of a wide range of analytes [1–3]. However, this approach can sometimes be restricted by limitations of selectivity due to the interference from the other redox active molecules which may undergo electrolysis at similar potentials to the target species in the medium [4–6]. The most important strategy to overcome such problems is to modify the surface of the electrode

to produce a chemically modified electrode that aims to alter the electrode kinetics of both target species and the interfering species. The potential under which the target species undergo oxidation becomes shifted from that required to electrolyse the interfering species [7–12].

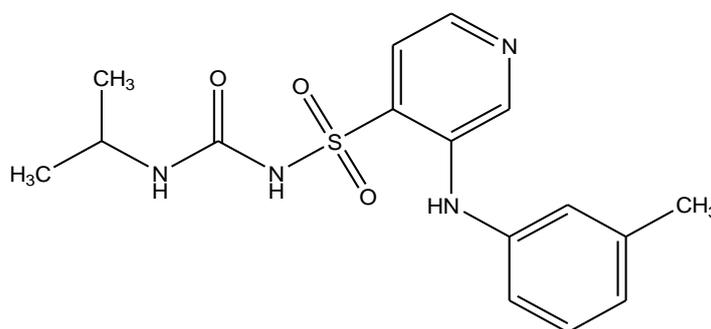
Dopamine is one of the well-known prevalent biogenic amine acting as a neurotransmitter present in mammals plays a significant role in physiological actions, occupying in functioning of nervous, and renal, hormonal and cardiovascular systems. Anomalous level of DA in the body fluids causes diseases such as Alzheimer's, Parkinson's and Schizophrenia [13-15] as a consequence of these innovations, catecholamine drugs are extensively used in the treatment of those diseases. DA is electrochemically active that oxidized to form dopamine-o-quinone (DOQ). There are different techniques have been employed for the detection of DA including fluorimetry, UV-visible spectroscopy, chemiluminescence, capillary electrophoresis and electrochemical methods. As a result, electrochemical techniques for the detection of neurotransmitter have received considerable interest because of its fast speed; low cost, high accuracy and low detection limit [16-19].

There is considerable interest in developing electrochemical techniques for measurement of neurotransmitters such as dopamine (DA) and serotonin (5-hydroxytryptamine (5-HT)). Dopamine (DA), 3, 4-dihydroxyphenylethylamine, is an important neurotransmitter of the catecholamine group that exists in the mammalian central nervous system and is characterized by presenting very strong electrochemical activity [20]. In recent years, there has been considerable interest in developing new methods to measure this neurotransmitter in biological samples such as brain tissues [21,22]. It is therefore very important to take into account both selectivity and sensitivity in the development of new voltammetric sensors for the determination of DA. Several works in the literature describes the development of new methodologies that employ chemically modified electrodes for the determination of DA in the presence of AA [23–29]. The intensive use of chemically modified electrodes has gained much attention now a days because of their interesting features for analytical use, such as good sensitivity, high selectivity, reproducibility, better stability and anti-fouling behavior [30-31]. 5-HT is widely distributed in the brain, and together with other neurotransmitters, makes an important contribution to brain function. For example, 5-HT has been implicated in control and regulation of various physiological functions such as sleep, thermoregulation, food intake, and sexual activity, as well as in psychopathological states such as depression, anxiety, alcoholism, and drug dependency [32].

Folic acid (FA) is a water-soluble vitamin B that helps to build up healthy cells. The deficiency of FA is a common cause of anemia and is thought to increase the shells of heart attack and stroke. Many studies suggest that diminished folate status is associated with enhanced carcinogenesis, as FA, along with vitamin B12, participates in nucleotide synthesis, cell division, and gene expression [33]. Per conceptual supplementation of FA has been

demonstrated to significantly reduce the incidence and reoccurrence of neural tube defects, such as spina bifida of women [34]. A survey of the literature reveals that there are various methods available for the determination of FA, which include liquid chromatography, [35] high performance liquid chromatography [36], flow-injection Chemiluminometry [37], Isotope Dilution-Liquid Chromatography/Tandem Mass Spectrometry [38], and spectrophotometric method [39]. As FA is an electroactive component, some electrochemical methods have been reported for its determination [40]. Compared with other techniques, the electrochemical method is more desirable because of its convenience and low cost.

Surfactant belongs to a class of molecules with surface active properties. This behaviour is due to their amphiphilic structure, which contains both a polar or hydrophilic head and non-polar or hydrophobic tail [41]. Surfactants are normally classified according to the head group type [42] Ionic (anionic and cationic), non-ionic and amphoteric (Zwitter ions). Adsorption of ionic surfactants onto the surface generates charge. Cationic surfactants will lead to a positively charged surface and anionic surfactants will give a negative charged surface. A non-ionic surfactant molecule has no charge in aqueous media but normally consists of a highly polar region such as polyoxyethylene groups. Amphoteric surfactants develop a negative or positive charge depending on the pH of the solution. At low concentration, surfactant molecules are unassociated monomers. As the concentration of surfactant is increased, the attractive and repulsive forces between the molecules cause self-aggregation to occur resulting in the formation of monolayer or micelles. The concentration at which these micelles form is called the Critical Micelles Concentration (CMC). The characteristics of micelles can be controlled by small changes in the chemical structure of the surfactant molecules or by varying the conditions of the disperse phase. Changes in the pH, ionic strength and temperature are all known to influence the size and shape of surfactant micelles. Related works have been done by our research group [43-47].



**Scheme 1.** Structure of Torasemide

Torasemide (TOR), 1-isopropyl-3-(4-m-toluidinepyridine-3-sulphonyl) urea, is the leader of sulphonylurea class of high ceiling loop diuretics, used mainly in the treatment of hypertension and edema associated with congestive heart failure. One adverse effect of loop

diuretics is the induction of kaliuresis resulting from increased potassium excretion rates. TOR is a potent natri-uretic and potassium-sparing more than the most often used loop diuretics 'furosemide'; consequently it is the most favorable one to be used [48-50].

Now a days, the use of diuretics is not limited to therapeutic aims, owing to their features that make them attractive in the world of sport and fitness, for some different purposes, including fast weight reduction through water loss (a) masking the presence of other drugs through 'faster excretion, urine dilution and urine pH variation (b) and emphasizing muscles where it is essential for body building(c). Due to these reasons, diuretics have been banned by the International Olympic Committee (IOC) since 1988 and are currently included in the list of substances prohibited in- and out-of-competition [51]. Because of its high potency, low therapeutic doses are required, where in once daily dose; TOR is effective in the treatment of hypertension without either a significant hypokalemia, elevation of blood sugar or lipid disorders if compared with those of thiazides and indapamide. TOR is well absorbed, yields bioavailability of about 80-90% and highly bound to plasma proteins 99%. It undergoes extensive hepatic metabolism 'including hydroxylation at various positions, oxidation and reduction' and only 20-25% of the parent drug is excreted unchanged in urine [52-55].

The aim of the work is to establish a simple and sensitive electrochemical method for the determination of dopamine in the presence of Torasemide and surfactant; the oxidation peak current of dopamine remarkably increases at the CPE suggesting significant improvement of determining sensitivity and which might be able to explain the enhancement effects of surfactant in electroanalytical chemistry for Torasemide. There has been increased interest in the development of modified electrodes, especially for the use as biosensors and more recent development involves the direct electron transfer from the modified electrodes to the analytes. The advantages of include the high sensitivity, extreme simplicity, rapid response and low cost. In future these electrocatalytic modified electrodes which act as sensors can be used in the medicine and biotechnology field and also the development of sensors that holds great promise for green chemistry applications [56-57].

## **2. EXPERIMENTAL PART**

### **2.1. Apparatus and reagents**

The electrochemical experiments were carried out using a CHI-660c (CH Instrument-660 electrochemical workstation) connected to a personal computer for control and data storage. All electrochemical experiments were performed in a standard three-electrode cell. The bare or modified CPE was used as a working electrode, platinum electrode as counter electrode and saturated calomel electrode (SCE) as reference electrode. All potentials reported were versus the SCE.

Torasemide, Dopamine, Uric acid and Folic acid were obtained from Himedia and Serotonin, sodium dodecyl sulphate, cetyl trimethyl ammonium bromide and perchloric acid used from Sigma-Aldrich chemicals. The DA sample was prepared in 0.1 M perchloric acid. The water used was a double distilled and Phosphate Buffer [0.2 M] was prepared by 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate. Freshly prepared solutions were used in each experiment. All chemicals were of analytical grade quality and were used without further purification.

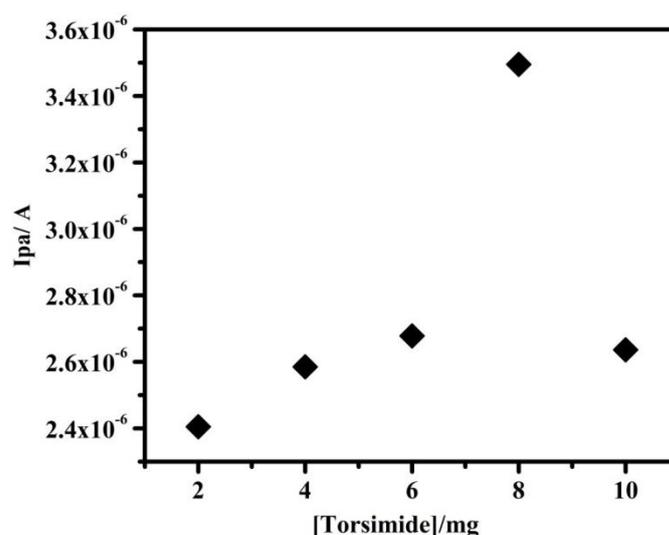
## 2.2. Preparation of bare and modified carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of graphite powder and silicon oil at a ratio of 70:30 (w/w) in an agate mortar until a homogenous paste was obtained. The prepared carbon paste was tightly packed into a PVC tube (3 mm internal diameter) and the electrical contact was provided by a copper wire connected to the paste in the end of the tube. Similarly the modified carbon paste electrode was prepared by grinding different concentration of Torasemide along with graphite powder.

## 3. RESULTS AND DISCUSSIONS

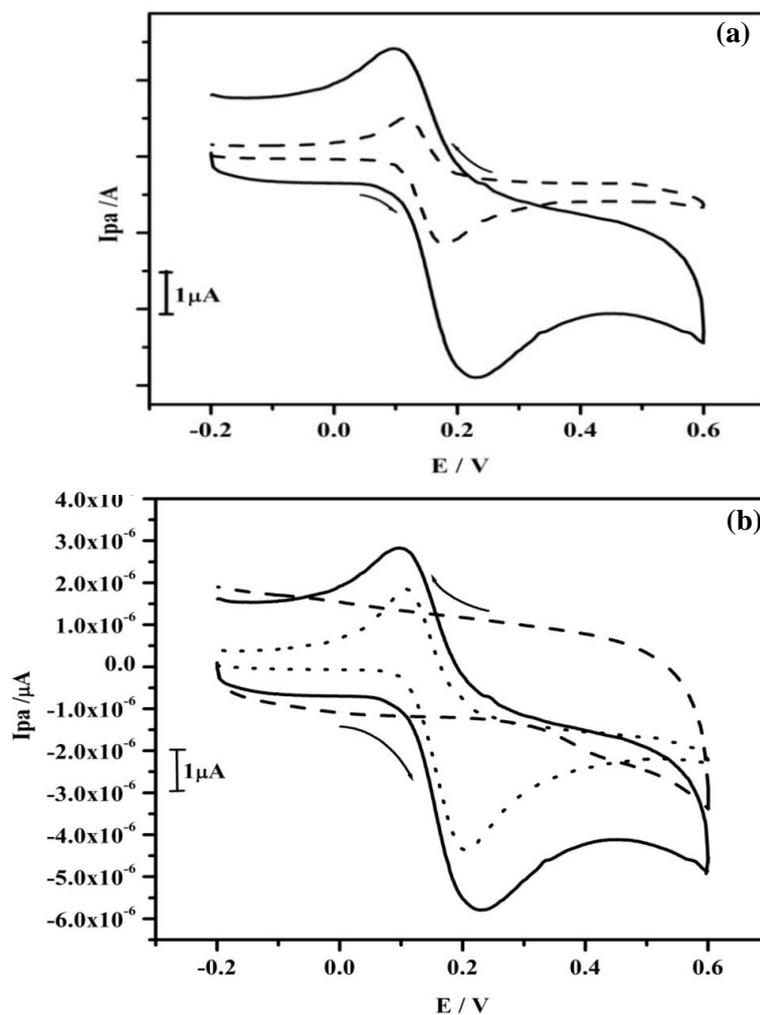
### 3.1. Influence of Torasemide concentration

Torasemide (TOR) used as a modifier in the preparation of Torasemide Modified Carbon Paste Electrode (TORMCPE). The characterization of TORMCPE was investigated by using cyclic voltammetric technique.



**Fig. 1.** Plot of anodic peak current of DA vs. concentration of Torasemide in 0.1 mM DA pH 7.0 phosphate buffer solution

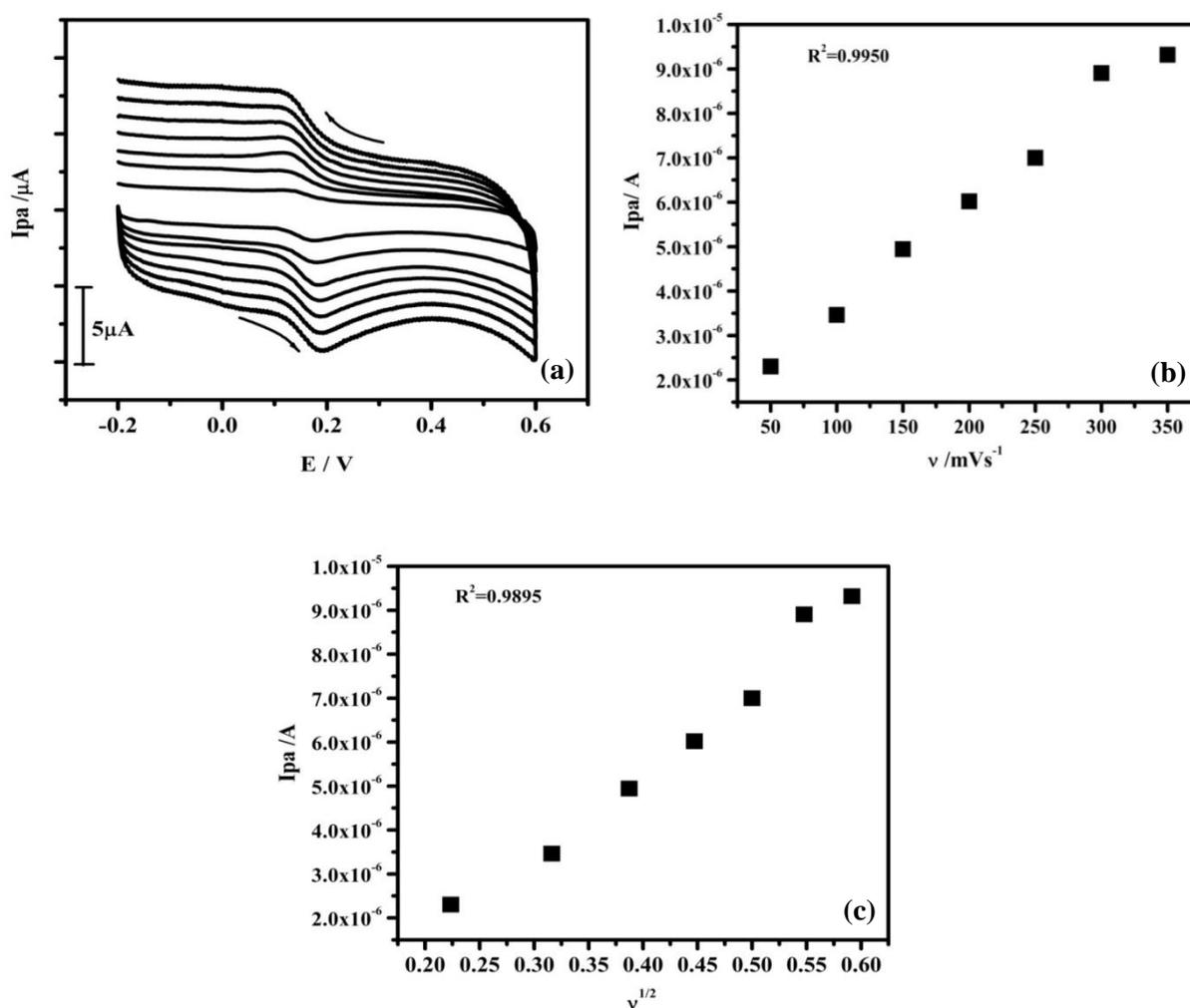
Torasemide Modified Carbon Paste Electrode (TORMCPE) was prepared of different ratios by adding different amount of Torasemide. The effect of the modifier on the development of sensors for DA has been studied [17-22]. In our study Torasemide was varied from 2 mg to 10 mg for investigation of 0.1 mM DA in 0.2 M phosphate buffer solution pH 7.0. This is shown more distinctively in Fig. 1, the plot of anodic peak current verses concentration of Torasemide. As the figure illustrates DA oxidation peak is enhanced at 8 mg of the modifier and then decreases when the amount of modifier was increased further. This may be due to a decrease in oxidation sites in the paste and consequent reduction of the actual electrode area. Therefore the role of modifier is to enhance the peak current and also decreases the over potential for the oxidation and reduction of DA.



**Fig. 2.** (a) Cyclic voltammograms obtained for the electrochemical response of DA at torasemide MCPE (solid line) and bare carbon paste electrode (dashed line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1 mM DA scan rate 100 mV/s; (b) Cyclic voltammograms obtained for the electrochemical response of DA at torasemide MCPE (solid line), torasemide MCPE without DA (dashed line) and bare carbon paste electrode (dotted line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1 mM DA scan rate 100 mV/s

### 3.2. Electrocatalytic Oxidation of Dopamine at Torasemide Modified Carbon Paste Electrode

Fig. 2. Shows the cyclic voltammograms obtained for the electrochemical response of DA at Torasemide Modified Carbon Paste Electrode (TORMCPE) solid line and bare carbon paste electrode (BCPE) dashed line in 0.2 M phosphate buffer solution pH 7.0. At BCPE, the oxidation and reduction peak potentials of DA occurs at 113.7 and 178 mV respectively. Under the identical conditions, TORMCPE produces significantly increased peak current and more reversible electron process of DA with the oxidation and reduction peak potentials at 227.8 and 98 mV respectively. The remarkable enhancement of peak currents provides clear evidence of electrocatalytic effect of TORMCPE.



**Fig. 3.** (a) Cyclic voltammogram of different scan rate in the presence of 0.1 mM dopamine and 0.2 M phosphate buffer solution at pH 7.0. scan rate 50 mV/s-350 mV/s; (b) Plot of anodic peak current versus scan rate; (c) Plot of anodic peak current versus square root of scan rate

### 3.3. Effect of scan rate

According to Randles-Sevick's equation increase in the scan rate increases the peak current. The TORMCPE showed increase in the peak current with increase in scan rate (50 mV/s to 350 mV/s) in 0.1 mM dopamine and 0.2 M phosphate buffer solution at pH 7.0. Cyclic voltammogram for dopamine at TORMCPE is shown in Fig. 3. The graph of current  $I_{pa}$  versus scan rate and square root of scan rate were plotted. The graph obtained were nearly straight line as shown in Fig. 3a. and Fig. 3b. In the range from 50 mV/s to 350 mV/s the anodic peak currents were proportional to the scan rate and also the to the square root of scan rate with correlation coefficient 0.9950 and 0.9895. This indicates that, the electrode transfer reaction is adsorption controlled.

### 3.4. Effect of concentration of DA

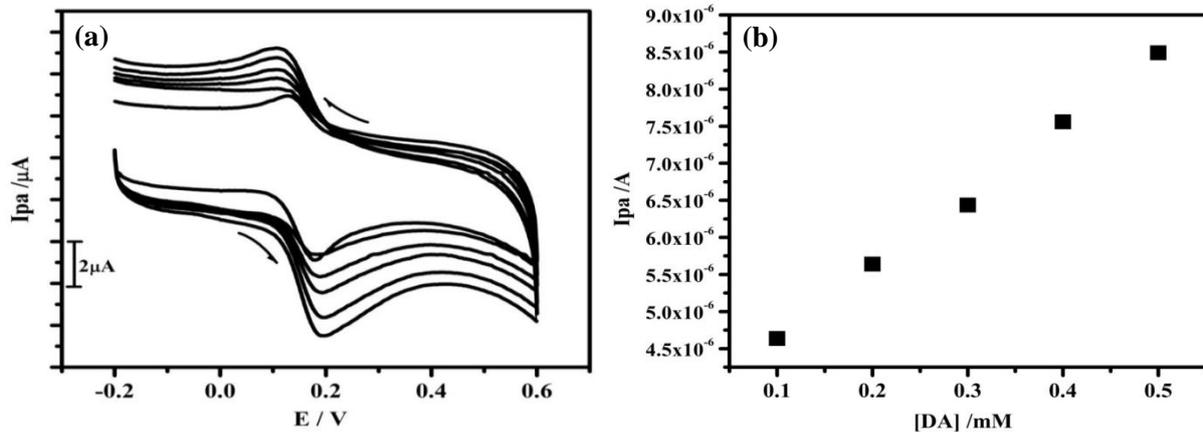
The electrocatalytic oxidation of DA was carried out by varying its concentration at TORMCPE in Fig. 4a. By increasing the concentration of DA from 0.1 mM to 0.6 mM, the  $I_{pa}$  and  $I_{pc}$  was found to be increasing with shifting of  $E_{pa}$  towards positive potential and  $E_{pc}$  towards slightly negative potential. The concentration curve of DA shows increase in electrochemical peak current in Fig. 4b which indicates that  $I_{pa}$  was proportional to concentration of DA. The plot of  $I_{pa}$  vs. concentration of DA shows a linear relation with correlation coefficient of 0.9990 and the detection limit for DA was found to be  $2.4 \times 10^{-6}$  M (Table 1).

**Table 1.** Comparison of different modified electrodes for DA determination

Sl. No.	Electrode	Detection limit ( $\mu\text{mol/L}$ )	Techniques	Ref.
1	Triazole self-assembled monolayer modified gold electrode	0.5	DPV	[63]
2	Ferrocene modified carbon paste electrode (Fc-MCPE)	9.4	CV	[64]
3	$\text{Bi}_2\text{O}_3/\text{GCE}$	0.2	CV	[65]
4	Poly[L-methionine] modified electrode	0.42	CV	[66]
5	Ionic liquid carbon paste electrode (IL CPE)	0.7	CV	[67]
6	$\text{CoFe}_2\text{O}_4$ nanoparticle modified glassy carbon electrode (CF/GCE)	0.036	CV	[68]
7	Torasemide Modified Carbon Paste Electrode	0.24	CV	This work

The detection limit was calculated by using the formulas (1) [58,59], where  $S$  is the standard deviation and  $M$  is the slope obtained from the three calibration plots. From the data, a lower limit of detection (LOD) can be achieved using the proposed method [60-62].

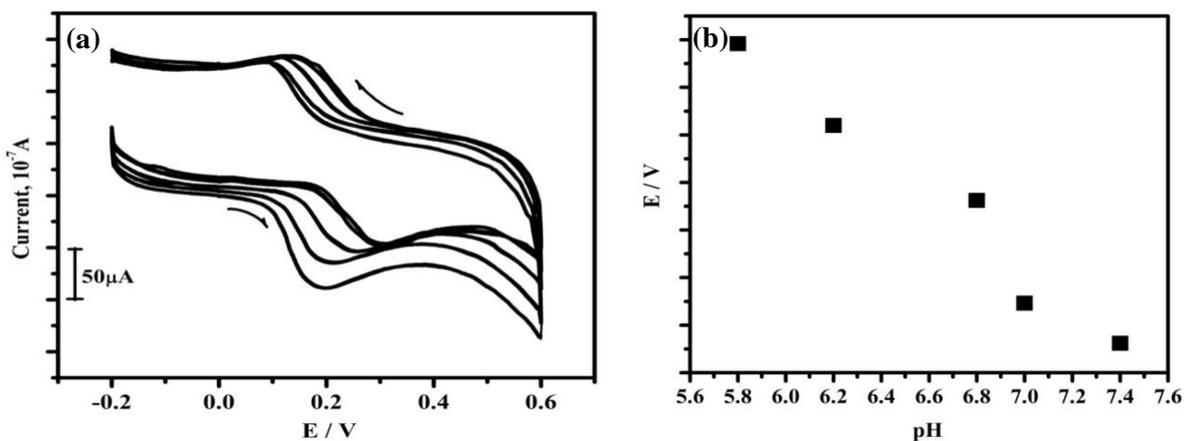
$$\text{LOD} = 3S/m \quad (1)$$



**Fig. 4.** (a) Cyclic voltammogram of variation of concentration of dopamine from 0.1 mM to 0.6 mM in presence of phosphate buffer solution at pH 7.0; (b) Plot of anodic peak current versus the concentration of DA

### 3.5. Effect of pH

To optimize the electrochemical response of modified carbon paste electrode for the oxidation and reduction of DA, the effect of pH on the electrode response was studied. The voltammogram of dopamine for pH is shown in the Fig. 5a.

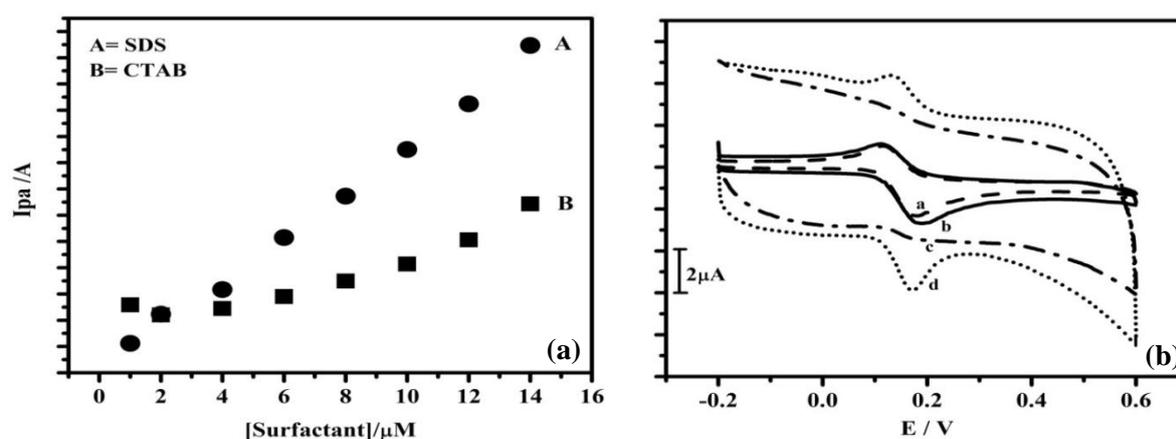


**Fig. 5.** (a) Cyclic voltammograms of 0.1 mM DA for different pH (from 5.8 to 7.4 pH) at TORMCPE; (b) Plot of anodic peak potential versus pH.

As the pH increases from 5.8 to 7.4, the anodic peak current shifted towards the negative side and the well redox peak is arrived at the neutral pH=7.0. The dependence of  $E_{pa}$  vs pH (Fig. 5b) was also investigated. From the plot it was found that the anodic peak potential decreases with the increase in pH, with slope of 71 mV / pH which indicates that equal number of protons take part in the reaction.

### 3.6. Effect of Surfactant

To study the effect of addition of surfactants the experiments were carried out using anionic surfactant sodium dodecyl sulphate (SDS) and cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB).



**Fig. 6a.** Effect of variation of concentration of surfactants mobilization for 0.1 mM Dopamine at TORMCPE in 0.2 M PBS pH 7.0, scan rate 100 mV/s; **(b)** Cyclic voltammogram of 0.1 mM DA at BCPE (dashed line), TORMCPE (solid line), 14  $\mu\text{M}$  CTAB (solid dashed line) and 14  $\mu\text{M}$  SDS on the modified CPE (dotted line) in 0.2 M phosphate buffer solution pH 7.0 scan rate 100 mV/s

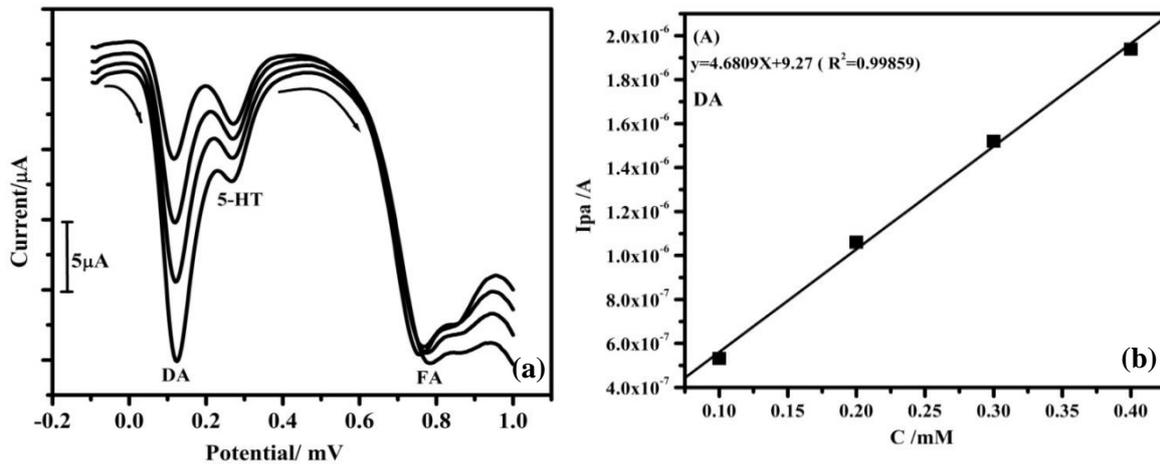
Initially, cyclic voltammogram were recorded for TORMCPE a solution containing Dopamine (0.1 mM) in phosphate buffer solution pH 7.0. Keeping the concentration of Dopamine constant, the concentration of the surfactant was increased from 1  $\mu\text{M}$  to 14  $\mu\text{M}$  by mobilization method. Fig. 6a shows the effects of surfactant concentration in mobilization when the surfactant concentration is lower than 4  $\mu\text{M}$  the critical micelle concentration (CMC) of surfactant at room temperature, both  $I_{pa}$  and  $I_{pc}$  increases rapidly with the increase of surfactant concentration. From these results SDS shows good enhancement in the peak current compared to CTAB.

Fig. 6b shows comparison of two surfactants SDS and CTAB on mobilization method at TORMCPE in the presence of 0.1 mM Dopamine in Phosphate Buffer Solution pH 7.0. The more anodic peak current enhancement in SDS compared to CTAB. In this work, the SDS at

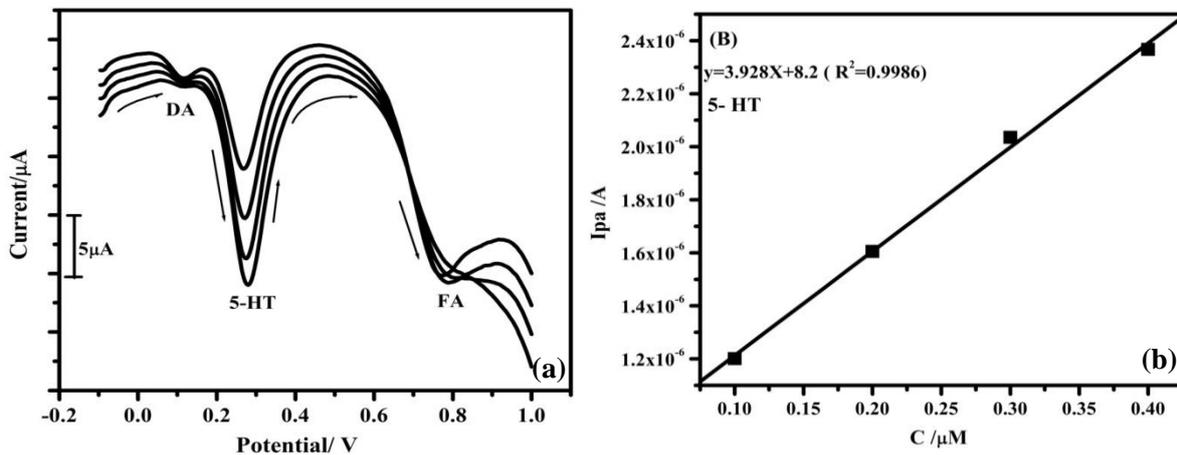
TORMPCE and Dopamine was explored by cyclic voltammetry, which might be able to explain the enhancement effect of surfactant in electroanalytical chemistry.

### 3.7. Interference study

The simultaneous determination of DA, 5-HT and FA in the mixture was carried out at TORMCPE when concentration of one species changed, whereas the others kept constant.



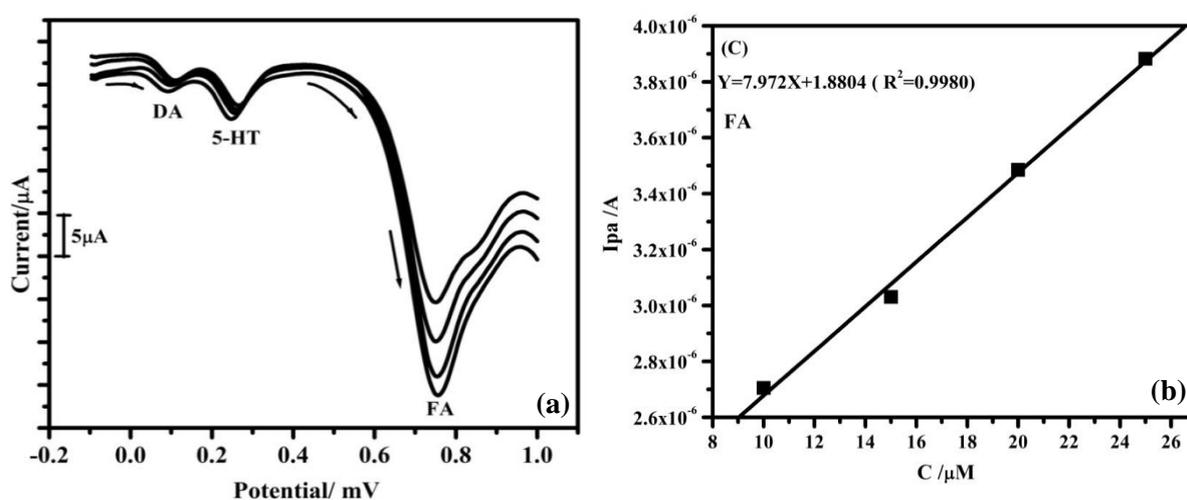
**Fig. 7.** (a) Differential pulse voltammograms of DA (0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 10 μM 5-HT and 10 μM FA at TORMCPE with the scan rate of 100 mV/s; (b) The plot shows anodic peak current ( $I_{pa}$ )



**Fig. 8.** (a) Differential pulse voltammograms of 5-HT (10 μM, 20 μM, 30 μM, 40 μM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA and 10 μM FA at TORMCPE with the scan rate of 100 mV/s; (b) The plot shows anodic peak current ( $I_{pa}$ ) versus 5-HT concentration.

From the Fig. 7a it can be seen that the peak current of DA was proportional to its concentration, which was increased from 0.1 to 0.4 mM when keeping the concentration of

5-HT 10  $\mu\text{M}$  and FA 10  $\mu\text{M}$ . There were no change in the peak current and peak potential occurred for 5-HT and FA. Similarly in Figs. 8a and 9a self explains the concentration effect of 5-HT from 10 to 40  $\mu\text{M}$  and FA from 10 to 25  $\mu\text{M}$  respectively. These results shows that the DA, 5-HT and FA were exist independently in their mixtures of samples. The corresponding graphs of anodic peak current versus various concentrations of DA(0.1-0.4 mM), 5-HT(10-40  $\mu\text{M}$ ), FA(10-25  $\mu\text{M}$ ) showed linear relationships with linear regressions for A (DA)  $I_{pa} (\mu\text{A})=3.928 \text{ Cm M L}^{-1}+8.2$ , B (5-HT)  $I_{pa} (\mu\text{A})=4.6809 \text{ Cm M L}^{-1}+9.27$ , C (FA)  $I_{pa} (\mu\text{A})=7.972 \text{ Cm M L}^{-1}+1.88$ , the correlation coefficient for these linear graphs was 0.9986, 0.9985 and 0.9980 respectively for this TORMCPE and the detection limit for DA was found to be  $0.025 \times 10^{-6} \text{ M}$ . which were shown in Figs.7b, 8b, 9b respectively.



**Fig. 9.** (a) Differential pulse voltammograms of FA (10  $\mu\text{M}$ , 15  $\mu\text{M}$ , 20  $\mu\text{M}$ , 25  $\mu\text{M}$ ) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA and 10  $\mu\text{M}$  5-HT at TORMCPE with the scan rate of 100  $\text{mV/s}$ ; (b) The plot shows anodic peak current ( $I_{pa}$ ) versus FA concentration

#### 4. CONCLUSIONS

In this work, chemically modified Torasemide carbon paste electrode acts as a good sensor, exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine in presence of 5-HT and FA. The scan rate effect was found to be adsorption controlled electrode process. The concentration effect, pH effect and surfactant effect was well investigated by using cyclic voltammetric technique. Anionic surfactant SDS showed very good electrocatalytic effect on the Torasemide modified carbon paste electrode. The Torasemide modified electrode acts as a good sensor for DA and it can be further applied for the investigation of other neurotransmitter.

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