

*Full Paper*

## **Simultaneous Determination of Dopamine in Presence of Serotonin at a Graphite Pencil Electrode: a Voltammetric Study**

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**Abstract-** A graphite pencil electrode was used as a working electrode in the analysis of dopamine and serotonin by voltammetric techniques. The electrochemical investigations were carried out at a graphite pencil electrode at a scan rate of 0.05 Vs<sup>-1</sup> in a 0.2 M phosphate buffer solution. The electrochemical behavior of dopamine shows two redox pairs while the electrooxidation of serotonin shows irreversible behavior at a graphite pencil electrode at a scan rate of 0.05 Vs<sup>-1</sup>. A graphite pencil electrode shows excellent results for dopamine and serotonin when combined and acts as very good sensor for dopamine in the presence of serotonin.

**Keywords-** Graphite pencil electrode, Dopamine, Serotonin, Simultaneous, Sensor

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### **1. INTRODUCTION**

Dopamine and Serotonin are two important monoamine neurotransmitters and involved in a variety of central nervous system functions [1]. Dopamine (DA) is fascinated in a

divergence of physiological, behavioral, and endocrine functions [2,3]. These systems are believed subsequent critically significant in both the pathophysiology and the pharmacotherapeutics of a number of intellectual disorders, including Parkinson's epidemic, neurosis, drug abuse, schizophrenia, attention-deficit hyperactivity disorder (ADHD) and Tourette syndrome [2–4]. Restless legs sickness [5] and added heart rate, renal weakness and blood pressure have been discovered to involve elevated levels of dopamine response in the mesolimbic pathway and cut down levels of dopamine in the prefrontal cortex [6,7]. Likewise, serotonin is also a very important neurotransmitter involved with depression, anxiety, panic, aggression, dominance, obsessions, punishment, analgesia, behavioral inhibition, rhythmic motor activity, feeding, and more [8]. Less is known about how it works and what its function is than is known about dopamine, partly because there are at least 15 different serotonin receptors currently known [9] (there are only 5 DA receptors [10]). Many researchers hypothesize that an imbalance in serotonin may lead to depression in certain individuals [11]. Both DA and 5-HT are well oxidised, hence, electrochemical techniques have been extended for their analysis and design [12–18]. Modified carbon paste electrodes (MCPE), sensitive [19,20], pretreated carbon paste electrodes, polymer modified electrodes and fullerene-C<sub>60</sub> coated gold electrode have all been used for the detection of neurotransmitters. Such investigations have been conducted sensitively and simultaneously *in vitro* [21-23] for individual analysis of DA *in vitro* [20-22], for 5-HT at single-walled carbon nanotube modified glassy carbon electrode [24] and for sensitive determination of 5-HT both *in vivo* and *in vitro* [25-27]. Selective determination of DA and 5-HT has further been pertinent, for both emerge together in biological systems. Selective measurement of the neurotransmitters *in vivo* has been extremely unsuccessful discipline to the inability of the electrodes occupied to diverge the potential of these species significantly enough to allow for indisputable discrimination. Reports of the simultaneous detection of DA and 5-HT on graphite electrodes pasted on by carbon have furthermore been studied [17]. However, part of modifiers have been used for simultaneous measurement of DA and 5-HT levels which have tested and proved of greater worth because of their coexistence in biological systems. Some major problems have regularly been encountered in the determination of DA and 5-HT concentrations. One was the voltammetric responses of DA and 5-HT which interfere mutually each other. Therefore sensitivity is a key to the simultaneous detection of DA and 5-HT. These facts have assured electrochemists to materialize faster, simpler and more sensitive techniques to approach the distinct demands and many works have been published describing the measurement of serum DA and 5-HT concentrations via chemically modified electrodes [28-34]. The graphite pencil electrode (GPE) has been used effectively as a biosensor in the novel electroanalytical techniques [35-38]. GPE has a larger active electrode surface area and is therefore efficient to detect low concentrations and/or volume of the analyte [39]. The researcher concluded that the GPE, is commonly a carbon based electrode,

it is less expensive, more useful, and more easily renewable compared to the other electrodes as CPE or GCE [40,41]. Recently, DA in presence of 5-HT at various carbon based electrodes was modified with different modifiers were reported results showed low limit of detection and peak potential difference between DA and 5-HT was high [42,43]. This type of electrode has been effectively used in the study of various neurotransmitters and drugs [44-49]. The simultaneous determination of this neurotransmitter without modifier has been an important knock the chip off one shoulder in the work of electrochemical research.

In this research, a graphite pencil electrode was used to produce the DA and 5-HT yield of two well-defined oxidation peaks at 0.184 V and 0.360 V respectively. Moreover, the oxidation peak currents of DA and 5-HT increase significantly at the GPE when the scan rate is increased. A direct electrochemical approach for the simultaneous discretion of DA and 5-HT concentrations has been developed and the new procedure possesses more advantageous.

## **2. EXPERIMENTAL SECTION**

### **2.1. Materials**

The commercially available pencil-lead rods were HB 0.5 mm in diameter and 6cm length (Camlin Ltd, Mumbai) purchased from a local bookstore was used without purification. A 25 mM DA (Himedia) stock solution was prepared in 0.1 M of perchloric acid solution. A 25 mM stock solution of 5-HT (Sigma) was prepared in double distilled water. The buffer used was 0.2 M PBS of pH 7. The chemicals mentioned ahead were generally of analytical grade used without further purification. Water used in the preparation of solutions was double distilled water.

### **2.2. Apparatus**

The electrochemical experiments were carried out via a CH Instrument model 660c. The electrode system containing the working electrode consisted of a graphite pencil electrode (0.5 mm in diameter), a platinum counter electrode and a saturated calomel reference electrode.

### **2.3. Graphite pencil as working electrode**

The commercially available pencil-lead rods were purchased from the local book stores and used as electrode [50]. The length of the electrode surface was covered by cello tape and one end of the pencil electrode was connected to the copper wire. And other end of the electrode was made to dip in the analyte for analysis without any modification to the electrode.

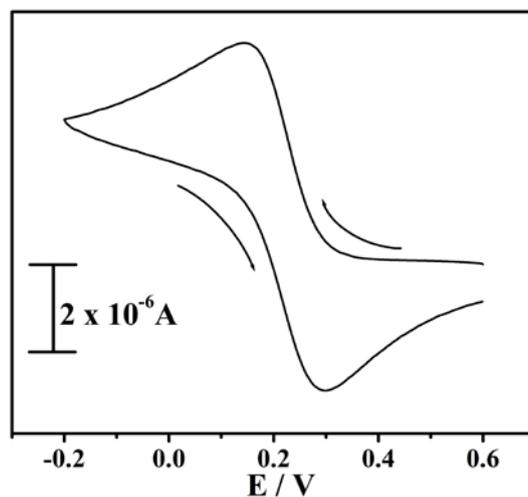
### 3. RESULTS AND DISCUSSION

#### 3.1. Electrochemical behavior of standard potassium ferricyanide system at GPE

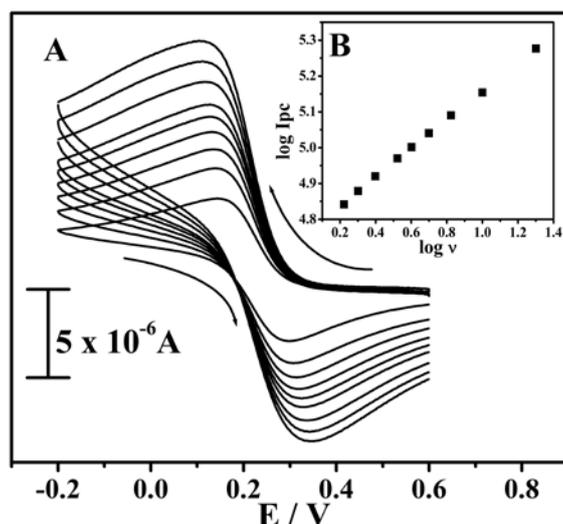
The freshly prepared 1 mM potassium ferricyanide and 1 M potassium chloride solutions were situated in the electrochemical cell. Fig. 1 shows the cyclic voltammogram recorded for the 1 mM of potassium ferricyanide at the GPE at a scan rate of  $0.1 \text{ Vs}^{-1}$ . The redox peak current signals were observed at the GPE. The scan rate was increased for potassium ferricyanide at the GPE in terms to suggest the surface area using the Randles-Sevcik equation (1) [22]. The surface area obtained for the GPE was  $2.2 \text{ mm}^2$ . The graph of the logarithmic cathodic peak current vs the logarithmic scan rate for the GPE was plotted (Fig. 2) and the slope obtained is 0.42, which shows conclude agreement with the theoretical value of 0.5 for a diffusion-controlled reaction [51], which summarize that the electrode process was controlled by the diffusion of the analyte.

$$I_p = 2.69 \times 10^5 \times n^{3/2} \times A \times D_0^{1/2} \times v^{1/2} \times C_0^* \quad (1)$$

Where,  $I_p$  - peak current,  $n$  - number of electrons involved in the electrochemical reaction,  $A$  - surface area,  $D_0$  - diffusion coefficient,  $v$  - scan rate,  $C_0^*$  - concentration of analyte.



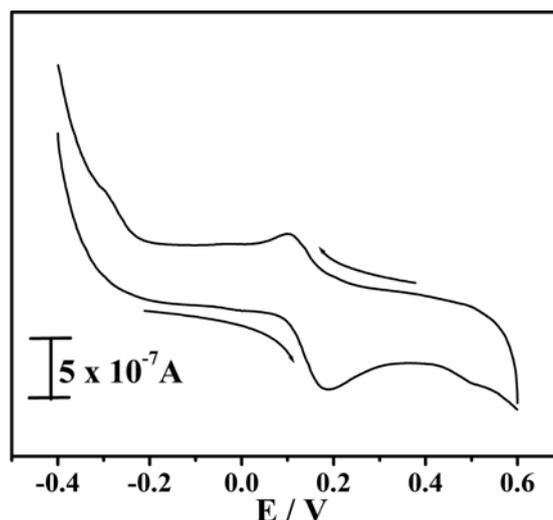
**Fig. 1.** Typical cyclic voltammogram for the determination of 1 mM Potassium ferricyanide in 0.1 M Potassium chloride solution at graphite pencil electrode with the scan rate of  $0.1 \text{ Vs}^{-1}$



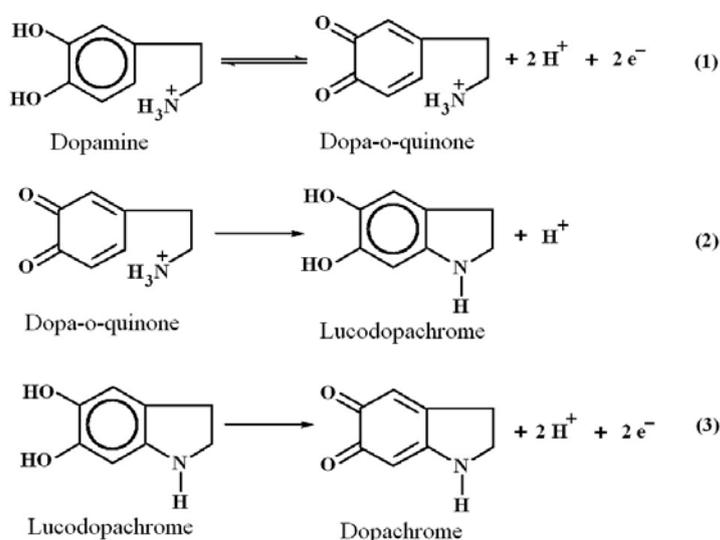
**Fig. 2.** (A) Cyclic voltammograms for the determination of 1 mM Potassium ferricyanide in 0.1 M Potassium chloride solution at graphite pencil electrode with different scan rates from 0.05 to 0.6  $\text{Vs}^{-1}$ ; (B) Graph of the  $\log I_{pc}$  vs  $\log v$

### 3.2. Electrochemical behavior of DA at GPE

Fig. 3 showed the cyclic voltammogram of 10  $\mu\text{M}$  of DA in 0.2 M PBS at pH 7.0 on the surface of the GPE at a scan rate of 0.05  $\text{Vs}^{-1}$ . Two pairs of redox peaks imply the DA. This shows that the graphite material in the pencil was acting as an electrode by transferring the electron through its accessible site. The DA at the GPE shows its anodic peak potential  $E_{pa1}$  at around 0.16 V and another peak potential  $E_{pa2}$  was found at 0.48 V. The cathodic peak potential  $E_{pc1}$  was at 0.096 V and another reduction peak  $E_{pc2}$  was found at -0.25 V. The electrochemical oxidation of DA at the bare carbon paste electrode shows one peak and one reduction [19-22] and at the GPE shows two oxidations and two reduction peaks; this could be due to slow electron transfer from DA to dopaquinone. The redox reaction was explained as follows: The DA oxidized and converted to dopa-o-quinone ( $E_{pa1}$  and  $E_{pa2}$ ) in the forward scan while, in the reverse scan, the DA remained unoxidised ( $E_{pc1}$  and  $E_{pc2}$ ). This is because the electron-rich nitrogen atom becomes deprotonated, leading to the formation of a cyclized product known as lucodopachrome (Scheme 1) [40,52-56].



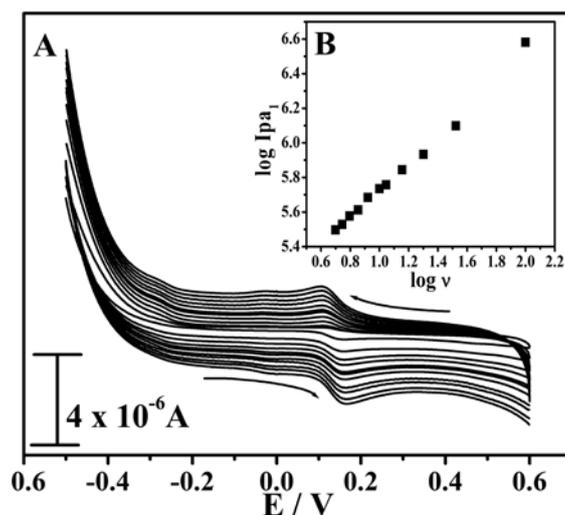
**Fig. 3.** Typical cyclic voltammogram for the determination of 10  $\mu\text{M}$  DA in 0.2 M PBS pH 7.0 at graphite pencil electrode with the scan rate of 0.05  $\text{Vs}^{-1}$



**Scheme 1.** Electrochemical oxidation mechanism of DA

### 3.3. Effect of scan rate towards the electro-oxidation of DA at GPE

To study the scan rate effect of DA at the GPE, voltammograms were recorded for different scan rates. Fig. 4A shows the cyclic voltammograms recorded for 10  $\mu\text{M}$  of DA in 0.2 M PBS at pH 7.0 at different scan rates at the GPE. The redox peak currents increased with the increase in the scan rate from 0.01 V to 0.2 V for the GPE. As the scan rate increased, the second oxidation peak disappeared and the graph of the logarithmic anodic peak current ( $\log I_{\text{pa1}}$ ) vs the logarithmic scan rate ( $\log \nu$ ) was plotted for the GPE as shown in Fig. 4B. The determined slope was 0.7745, which shows a close agreement with the theoretical value of 1 for an adsorption-controlled process [51].



**Fig. 4.** (A) Cyclic voltammograms of 10  $\mu\text{M}$  DA with different scan rates (0.01 to 0.2  $\text{Vs}^{-1}$ ) for the graphite pencil electrode in 0.2 M PBS at pH 7.0; (B) Graph of the  $\log I_{pa1}$  vs  $\log v$

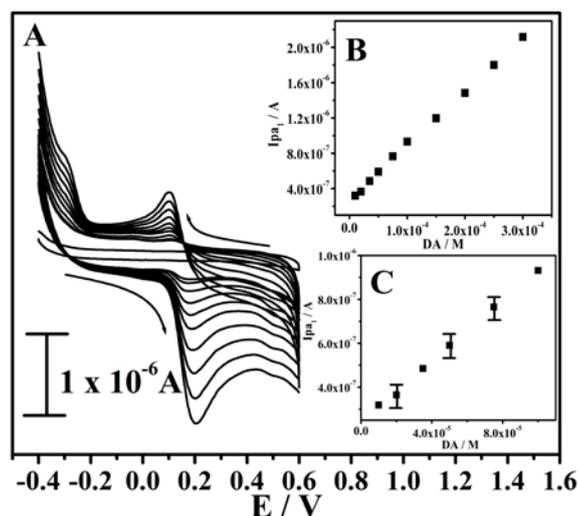
### 3.4. Effects of DA concentration on the surface of the GPE

The concentration effect of the DA was studied at the GPE in 0.2 M of PBS as supporting electrolyte at pH 7.0. Fig. 5A shows the voltammogram recorded for different concentrations of DA at the GPE. The oxidation and reduction peak currents increased with the increase in the concentration of DA. It was noticeable that the  $E_{pc2}$  was unable to form at lower concentrations of DA. At lower concentrations there is no second reduction peak for DA. At lower concentrations (5  $\mu\text{M}$ ), the amounts of oxidized molecules again reduced and may not be available for a ring-closing process. When the concentration was increased to 30  $\mu\text{M}$  the oxidized molecules reduced and unreduced molecules were further reduced to lucodopachrome. The calibration graph was plotted between the anodic peak current ( $I_{pa1}$ ) vs different concentrations of DA varying from  $10 \times 10^{-6}$  to  $3 \times 10^{-4}$  M (Fig. 5B). There was a dramatic enhancement in the anodic peak current ( $I_{pa1}$ ). The anodic peak current was proportional to the concentration of DA but the better linearity occurred in the range from  $10 \times 10^{-6}$  to  $10 \times 10^{-5}$  M (Fig. 5C). The graph shows good linearity with a correlation coefficient of 0.999. The linear regression equation for the DA anodic peak current is  $I_{pa1}$  ( $\mu\text{A}$ ) =  $0.0069$  ( $\text{C}\mu\text{M/L}$ ) +  $2.4 \times 10^{-7}$  (A). The limit of detection and limit of quantification were expected by using the equations given below (2) and (3) respectively [20,55]. The limits of detection and quantification were 3.9  $\mu\text{M}$  and 13  $\mu\text{M}$  respectively.

$$\text{LOD} = 3S/M \quad (2)$$

$$\text{LOQ} = 10S/M \quad (3)$$

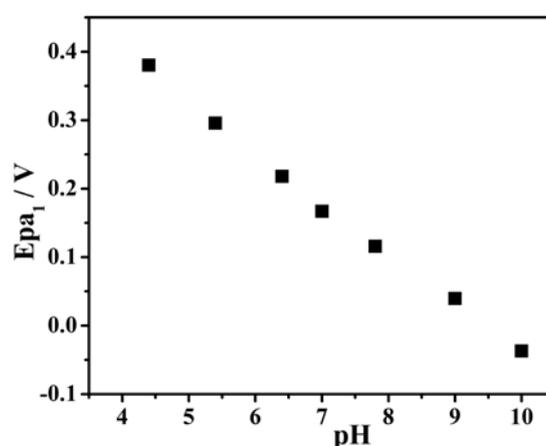
Where S is standard deviation and M is the slope of calibration plot.



**Fig. 5.** (A) Cyclic voltammograms for variation of DA concentration from 10 to 300  $\mu\text{M}$  at the graphite pencil electrode in 0.2 M PBS at pH 7.0. Scan rate  $0.05 \text{ Vs}^{-1}$ ; (B) Graph of the  $I_{pa1}$  vs. DA concentration in the range 10 to 300  $\mu\text{M}$ ; (C) Graph of the  $I_{pa1}$  vs. DA concentration in the range 10 to 100  $\mu\text{M}$

### 3.5. Effect of solution pH

The electrochemical response of DA was consistently pH dependent. The first oxidation peak potentials became less positive with an increase in the pH values at GPE. At GPE the anodic peak potential of DA shifted from 0.38 V to -0.037 V with respect to a pH of between 4 and 10. From Fig. 6 shows the diagram of the anodic peak potential vs pH of the solution. The graph has good linearity with a slope of  $0.061 \text{ V/pH}$ . This behavior essentially obeys the Nernst Equation for reactions involving an equal number of electron and proton transfers [56-59].

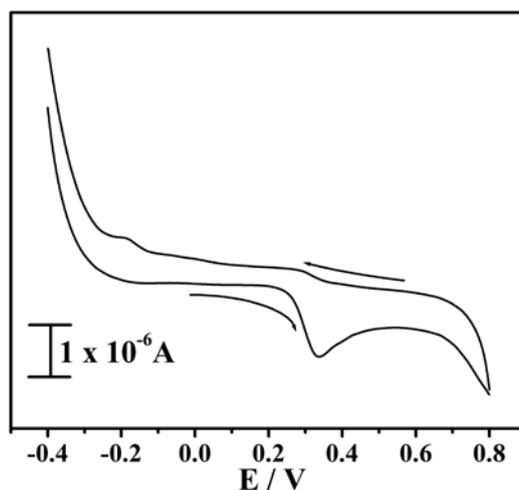


**Fig. 6.** Graph of  $E_{pa1}$  vs pH for 10  $\mu\text{M}$  DA at graphite pencil electrode from pH range 4 to 10 with scan rate  $0.05 \text{ Vs}^{-1}$

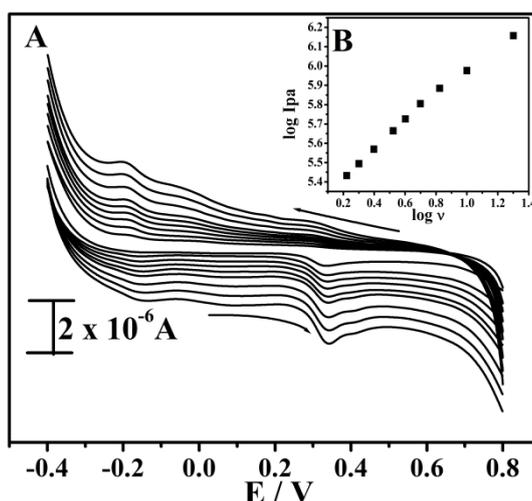
### 3.6. Electrochemical oxidation of 5-HT at a GPE

Fig. 7 reveals the oxidation of 5-HT at the GPE in a 0.2 M PBS of pH 7.0 at a scan rate of  $0.05 \text{ Vs}^{-1}$ . From Fig. 7 it can be seen that the anodic peak of 5-HT ( $10 \mu\text{M}$ ) at GPE with anodic peak potential was found at 0.34 V.

Furthermore, the scan rate effect of 5-HT was measured by varying the scan rate from  $0.05 - 0.6 \text{ Vs}^{-1}$  at the GPE as shown in Fig. 8A and Fig. 8B shows the graph logarithmic anodic peak current plotted against the logarithmic scan rate.



**Fig. 7.** Typical cyclic voltammogram for the determination of  $10 \mu\text{M}$  5-HT in 0.2 M PBS at graphite pencil electrode with the scan rate of  $0.05 \text{ Vs}^{-1}$



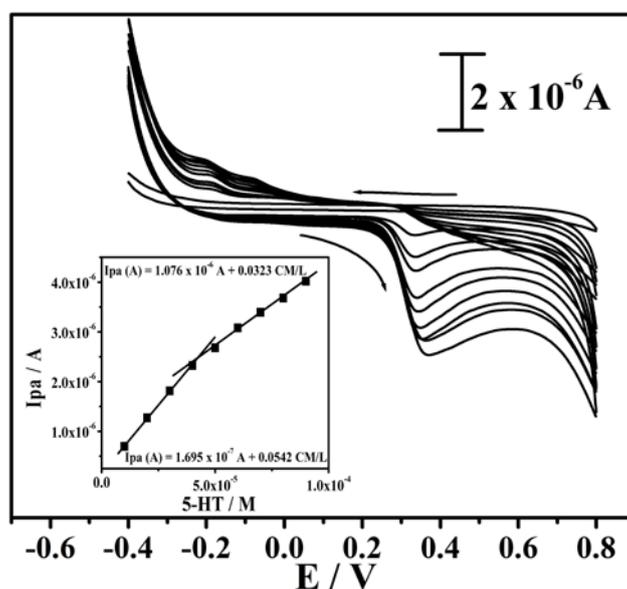
**Fig. 8.** (A) Cyclic voltammograms of  $10 \mu\text{M}$  5-HT with different scan rates ( $0.05$  to  $0.6 \text{ Vs}^{-1}$ ) for the graphite pencil electrode in 0.2 M PBS at pH 7.0; (B) Graph of the  $\log I_{pa}$  vs  $\log \nu$

The graph shows a good linear relationship between the logarithmic anodic peak current against the logarithmic scan rate and the slope obtained is 0.6 and this shows a good

agreement with the theoretical value of 0.5 for a diffusion-controlled reaction [51]. The correlation coefficients were found to be 0.9966 for the GPE. Hence the behavior of the electrode was controlled by diffusion at the GPE.

### 3.7. Effect of 5-HT concentration at GPE

The electrochemical oxidation of 5-HT was carried out by varying its concentration at the GPE. Fig. 9 shows that, for an increase in the concentration of 5-HT from  $10 \times 10^{-6}$  to  $90 \times 10^{-6}$  M, the electrochemical anodic peak currents went on increasing with an  $E_{pa}$  tending towards the positive, which indicates that the product was adsorbed onto the surface of the electrode.



**Fig. 9.** (A) Cyclic voltammograms for variation of 5-HT concentration from 10 to 90  $\mu\text{M}$  at the graphite pencil electrode in 0.2 M PBS at pH 7.0. Scan rate  $0.05 \text{ Vs}^{-1}$ . Inset Graph of the  $I_{pa}$  vs 5-HT concentration.

**Table 1.** Comparison of the detection limit for different electrodes

Electrode	Detection limit	Techniques	Ref.
Gold nanoparticles modified indium tin oxide electrode	3.0 nM	SWV	[31]
Screen-Printed Electrode Modified with MWNTs-SiO <sub>2</sub> -chitosan Composites	0.01 $\mu\text{M}$	DPV	[43]
Glassy Carbon Electrode Modified with Carbon-Spheres	0.7 $\mu\text{M}$	DPV	[60]
CNF electrode	0.25 $\mu\text{M}$	DPV	[61]

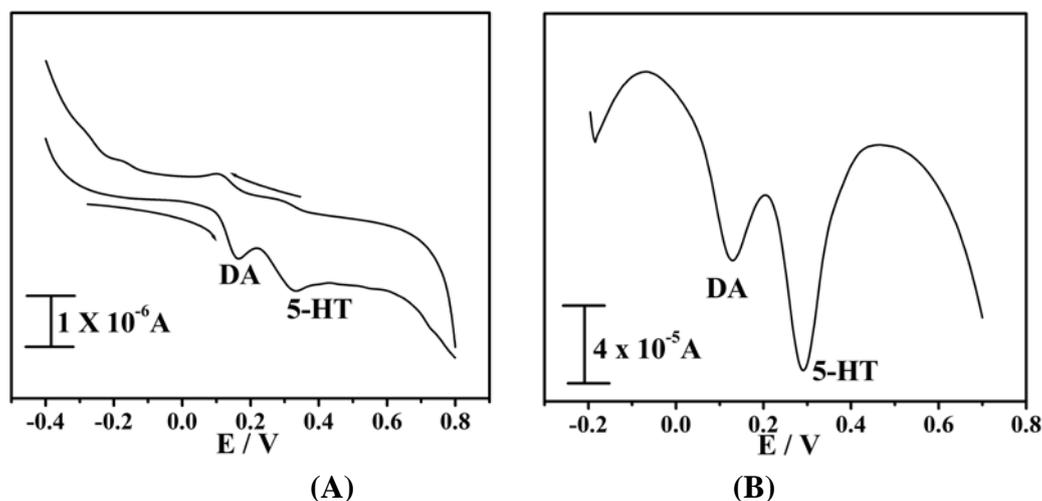
Graphite Electrode	Pencil	4.0 $\mu\text{M}$	CV	This Paper
Graphite Electrode	Pencil	0.678 $\mu\text{M}$	DPV	This Paper

The inset Fig. 9 represents the graph of the anodic peak current vs the concentration of 5-HT, showing two linear relationships ranging between  $10 \times 10^{-6}$  to  $40 \times 10^{-6}$  and  $40 \times 10^{-6}$  to  $90 \times 10^{-6}$  M with linear regression equations of  $I_{pa}(A) = 1.695 \times 10^{-7} + 0.0542 \text{ CM/L}$  and  $I_{pa}(A) = 1.076 \times 10^{-6} + 0.0323 \text{ CM/L}$ , respectively. The correlation coefficient for the first linearity was 0.9999 and for the second it was found to be 0.9979. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation [59]. The detection limit for 5-HT in the lower range was found to be  $4 \times 10^{-6}$  M and the quantification limit was  $1.33 \times 10^{-5}$  M. The detection limit and quantification limits were proposed by using the formulae (2) and (3) [20,55], where S is the standard deviation and M is the slope obtained from the three calibration plots and obtained results were compared with found literature [31,43,60,61] showed in Table 1.

### 3.8. Interference investigation

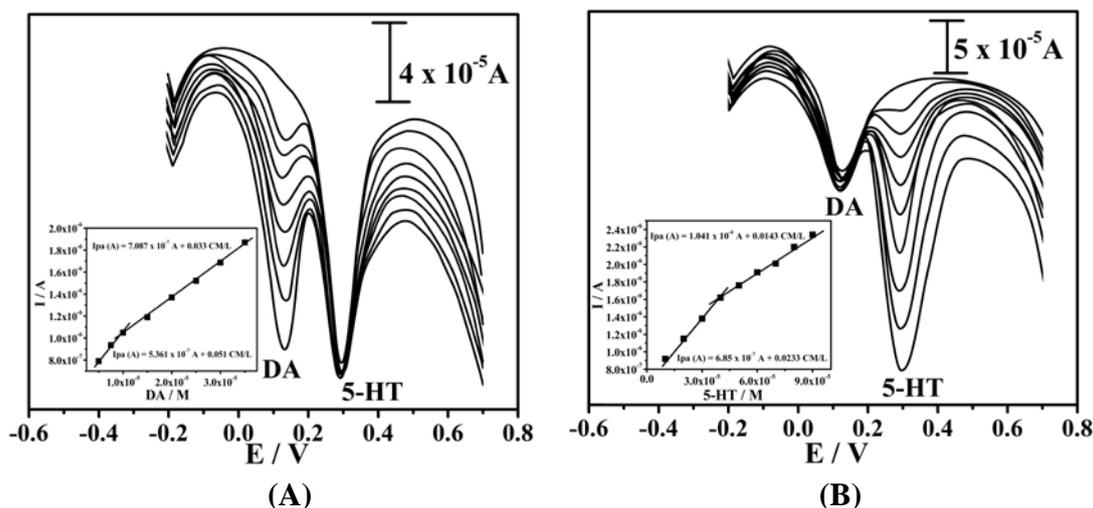
In term to notice the sensitivity and selectivity of the GPE, the electrochemical fashion of a mixture of  $10 \times 10^{-6}$  M DA and  $30 \times 10^{-6}$  M 5-HT was investigated via a cyclic voltammetric technique. Fig. 10A shows the cyclic voltammogram obtained for the DA and 5-HT. The GPE resolved the voltammetric signal into two well-defined voltammetric peaks at -0.051 V and 0.26 V indistinguishable to the DA and 5-HT respectively. The separation between the oxidative peaks of DA and 5-HT was approximately  $0.19 < 0.2$  V. The differential pulse voltammetric technique (DPV) was used on the GPE for analysis. The GPE resolved the voltammetric signal into two well-defined voltammetric peaks at -0.051 V and 0.257 V indistinguishable to DA and 5-HT respectively as shown in Fig. 10B. In the DPV no second peak was observed. DPV was furthermore used for the determination in their mixture was furthermore performed when the concentration of one species remained constant at GPE. As can be seen in Fig. 11A the concentration of DA increased from  $5 \times 10^{-6}$  to  $40 \times 10^{-6}$  M when the concentration of 5-HT was maintained at  $30 \times 10^{-6}$  M. The anodic peak current was proportional to the concentration of DA and there was no change in the peak current and peak potential for 5-HT, which shows two linear relationships ranging between  $5 \times 10^{-6}$  to  $15 \times 10^{-6}$  M and  $15 \times 10^{-6}$  to  $40 \times 10^{-6}$  M with linear regression equations of  $I_{pa}(A) = 5.361 \times 10^{-7} A + 0.051 \text{ CM/L}$  ( $r^2 = 0.999$   $n = 3$ ) and  $I_{pa}(A) = 7.087 \times 10^{-7} A + 0.033 \text{ CM/L}$  ( $r^2 = 0.998$   $n = 6$ ) respectively (see inset Fig. 11A). The limit of lower detection (LOD) was  $1.08 \times 10^{-6}$  M for the GCE. The LOD was predetermined according to the equation  $\text{LOD} = K S^0 / S$ , where K was a constant familiar to the confidence level. In accordance with the instruction of the IUPAC, the

value of  $K$  was fixed at 3 at the 99% confidence level.  $S^0$  was the standard deviation of six blank-solution measurements (no added DA), and  $S$  was the slope of the calibration graph [21,22].



**Fig. 10.** (A) Typical cyclic voltammogram for simultaneous determination of 10  $\mu\text{M}$  DA and 30  $\mu\text{M}$  5-HT at the graphite pencil electrode in 0.2 M PBS at pH 7.0 with scan rate 0.05  $\text{Vs}^{-1}$ ; (B) Typical differential pulse voltammogram for simultaneous determination of 10  $\mu\text{M}$  DA and 30  $\mu\text{M}$  5-HT at the graphite pencil electrode in 0.2 M PBS at pH 7.0 with scan rate 0.05  $\text{Vs}^{-1}$

Similarly Fig.11B shows the concentration effect of 5-HT from  $10 \times 10^{-6}$  to  $90 \times 10^{-6}$  M at a constant  $30 \times 10^{-6}$  M DA.



**Fig. 11.** (A) Differential pulse voltammograms from 5  $\mu\text{M}$  to 40  $\mu\text{M}$ , DA in 0.2 M phosphate buffer solution at pH 7.0 in the presence of 30  $\mu\text{M}$  5-HT and inset the graph of the anodic peak current vs the concentration of DA in the presence of 5-HT; (B) Differential pulse voltammograms from 10  $\mu\text{M}$  to 90  $\mu\text{M}$ , 5-HT in 0.2 M phosphate buffer solution at pH 7.0 in

the presence of 30  $\mu\text{M}$  DA and inset the graph of the anodic peak current vs the concentration of 5-HT in the presence of DA

The anodic peak current was proportional to the concentration of 5-HT and there was no change in the peak current and peak potential for DA. 5-HT further shows two linear relationships ranging between  $10 \times 10^{-6}$  to  $40 \times 10^{-6}$  M and  $40 \times 10^{-6}$  to  $90 \times 10^{-6}$  M with the linear regression equations of  $I_{pa}(A) = 6.85 \times 10^{-7} A + 0.0233$  CM/L ( $r^2 = 0.999$   $n = 4$ ) and  $I_{pa}(A) = 1.041 \times 10^{-6} A + 0.0143$  CM/L ( $r^2 = 0.998$   $n = 6$ ) respectively as shown in inset Fig.11B. The limit of lower detection (LOD) was  $6.78 \times 10^{-7}$  M for 5-HT at the GPE and obtained results for 5-HT was compared with previous found literature [31,43,60,61] showed in Table.1. These results showed that DA and 5-HT existed independently in the sample mixture at pH 7.

#### 4. CONCLUSION

Simultaneous determination of DA was analyzed electrochemically at physiological pH, containing a large excess of 5-HT at GPE. The GPE underwent adsorption behavior for DA and diffusion behavior for 5-HT. The GPE showed a lower detection limit of  $1.08 \times 10^{-6}$  M for DA and further a lower detection limit of  $6.78 \times 10^{-7}$  M for 5-HT. It has excellent sensitivity and selectivity. This GPE acts as indeed good sensor for dopamine in the presence of serotonin. This approach can be direct the development of electrochemical sensors for dopamine and devoted neurotransmitters.

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