ELECTROCATALYTIC OXIDATION OF DOPAMINE AT MUREXIDE AND TX-100 MODIFIED CARBON PASTE ELECTRODE: A CYCLIC VOLTAMMETRIC STUDY

3.1. Introduction

In this chapter, electrochemical oxidation of dopamine at murexide modified carbon paste electrode was studied by cyclic voltammetric technique in 0.2 M phosphate buffer solution at pH 7.4. The modified electrode exhibited strong promoting effect and stability towards the detection of dopamine. From the studies of scan rate effect the overall electrode process was found to be diffusion controlled. The concentration effect reveals that the detection limit and quantification limit of dopamine were $1.496 \times 10^{-7}$ M and $4.988 \times 10^{-7}$ M respectively. The effect of pH suggested that equal number of protons and electrons were involved in the electrochemical oxidation of dopamine. The presence of Triton X-100 on the Murexide modified carbon paste electrode showed excellent electrocatalytic effect towards the detection of dopamine.

3.2. Chemistry of Dopamine

Dopamine (DA), also known as "4-(2-aminoethyl) benzene-1, 2-diol" belongs to a member of the catecholamine family. Since the discovery of DA as a neurotransmitter in the late 1950s, DA has become the most widely studied catecholamine [1]. DA plays an important role in the functions of the central nervous system, renal, hormonal, and cardiovascular systems. DA was first synthesized by George Barger and James Ewens in 1910 however; it was not until 1958 when Arvid Carlsson discovered that DA was not just a precursor of norepinephrine and epinephrine that DA began to gain considerable interest in studies related to its role in the central nervous system and neurological disorders. In the brain, DA functions as a neurotransmitter activating DA receptors and is produced in various areas of the brain such as the substantial nigra and the ventral segmental area. DA is also a neurohormone released by the hypothalamus. Its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the pituitary. DA can be supplied as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood pressure. However, since DA cannot cross the blood-brain barrier, DA given as a drug does not directly affect the central nervous system. To increase the amount of DA in the brains of patients with diseases such as Parkinson's disease and Dopa-Responsive
Dystonia, a synthetic precursor to DA such as L-DOPA (levodopa) can be given, since this will cross the blood-brain barrier. DA undergoes oxidation to form dopaquinone as shown in scheme 3.1.

![Scheme 3.1. Oxidation mechanism for Dopamine](image)

3.2.1. Biosynthesis of Dopamine

DA is synthesized by the body and is involved in physical and cognitive functions. The synthesis process of DA is shown in Scheme 3.2. DA is biosynthesized in the body (mainly by nervous tissue and the medulla of the adrenal glands) first by the hydroxylation of the amino acid L-tyrosine to L-DOPA via the enzyme tyrosine 3-monooxygenase, also known as tyrosine hydroxylase, and then by the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase (which is often referred to as dopa decarboxylase). In some neurons, DA is further processed into norepinephrine by DA beta-hydroxylase. Dopaminergic neurons located in the midbrain are the main sources of DA in the central nervous system. A basic definition of a neuron is a nerve cell in the nervous system that is responsible in processing and transmitting messages by electrochemical signaling. The structure of a typical neuron contains a central cell body called the soma that is surrounded by dendrites that receives signals from other neurons and is attached to a long thin axon that carries messages away from the cell body. The incoming messages from the dendrites are passed to the end terminals of the axons and the neurotransmitters (Dopamine) are released into the synapse. The DA molecules diffuse across the synapse and interacts/attaches to its specialized protein called DA receptors which send the message on to other neurons. After the message is passed on, the receptors release the DA particles back into the synapse where the excess DA is recycled for reuse. Therefore the imbalance or dysfunction of the dopaminergic neuron process can result into various neurological diseases, sleeping and eating disorders, additive behaviors associated with drug abuse [2, 3].
3.2.2. Dysfunction of Dopamine

The developments of methods for measuring DA in biological systems are of importance for the analysis and diagnosis of neurological disorders such as Parkinson’s disease. There are over 1.2 million people in the U.S. who suffer from Parkinson’s disease with 50,000 new cases reported annually and are one of the most common neurological diseases in North America [4]. Parkinson’s disease is defined as a neurological or degenerative disorder of the central nervous system. Patients who are diagnosed with Parkinson’s disease have a 60 to 80 percent loss of these DA producing neurons when symptoms appear. Diagnosis is normally based on medical history and a neurological examination which consists of observations using the Unified Parkinson’s Disease Rating Scale and an interview. Therefore early signs and symptoms of the disorder can be dismissed as just the effects of normal ageing. Numerous studies have shown that DA affects the brain processes that control movement, emotional response, and the ability to experience pleasure and pain. Also an imbalance of DA can lead to eating and sleeping disorders and additive behaviors associated with drug abuse. Therefore, there is an immediate need to develop simple and rapid methods for selectively determining DA in routine analysis.
3.2.3. Biological Relevance of Dopamine

DA has many functions in the brain. DA affects the basal ganglia motor loop which in turn affects the way the brain controls movements. Shortage of DA, particularly the death of DA neurons in the nigrostriatal pathway, causes Parkinson's disease, in which a person loses the ability to execute smooth, controlled movements. Degeneration of DA, decline of cognitive function, motor symptoms, and other problems lead to decreased efficiency and function of the brain and body. This leads to a downward spiral of further decrease in efficiency and function, which results in degeneration, aging, breakdown, and death. Neurotransmitter release is initiated by an electrical impulse called an action potential. Each neuron has a
resting membrane. When an appropriate neurotransmitter binds to receptors on the dendrites or cell bodies, ion channels open, allowing an influx of Na\(^+\) that changes the membrane potential and initiates an action potential or firing. It then propagates down the axon to the terminal at a rate of 0.5 m/s [5]. This firing causes voltage-gated Ca\(^{2+}\) channels to open in the terminals. The resultant Ca\(^{2+}\) influx triggers the vesicles to fuse with the cell membrane and release their contents, a process termed as exocytosis. Because some vesicles are docked adjacent to the membrane, exocytosis occurs on a millisecond timescale [6]. Neurotransmission involves the conversion of an electrical impulse to a chemical event and then to another electrical event, is extremely rapid. Action potentials and neurotransmitters represents the bricks with which the internal representation of the external world is build.

3.3. Chemistry of Murexide

Murexide is the ammonium salt of purpuric acid (Scheme 3.3), which in its dry state has the appearance of a reddish purple powder slightly soluble in water and is used in analytical chemistry as a complexometric indicator for complexometric titrations, most often of calcium ions, but also for Cu, Ni, Co, Th and rare earth metals. The murexide (5,5'-nitrilodebarbituric acid, mono ammonium salt) in addition to its classical use as a metallochromic indicator has been employed as an efficient scavenger for superoxide and hydroxyl radicals [7,8] and recently as a chromogenic agent [8,9].

![Scheme 3.3 - Structure of Murexide](image-url)
3.4. Review of Electrochemistry of Dopamine

Electroanalytical methods have been used during the last three decades to investigate the role of neurotransmitters in the brain due to their electroactive natures [10-12]. This area of analytical chemistry, so-called “brain chemistry,” was introduced originally by Ralph Adams in the 1970s [13]. In vivo detection of neurotransmitters in mammalian brain has been the subject of considerable interest by using modified electrodes and microelectrodes [11, 13, 14]. DA [10] was discovered to be an important neurotransmitter in mammalian central nervous system in the late 1950s and it is found in high amounts (50 nmol/g) in a region of the brain known as the “caudate nucleus” [11]. The very low concentration of DA in the “extracellular fluid” of the caudate nucleus provides a large challenge for the detection of DA. It was also found that patients with schizophrenia, Parkinson’s disease [16-20] and HIV [11, 15, 16, 19, 20] infection shows an almost complete depletion of DA in this region. Thus, there is a continuing interest in the development of a simple, sensitive and reliable method for the determination of DA. The fact that DA and other catecholamines are easily oxidized makes their detection possible by electrochemical methods [21]. However, the direct electroanalysis of DA at a bare electrode often suffers from the interferences of other commonly coexisting electroactive substances. The oxidation peak potential of AA is close to that of DA and the working electrode is often fouled owing to the accumulation of oxidized product [22, 23]. In order to overcome these problems, different kinds of modifications were done to carbon paste electrode for the determination of DA. Because of the simple preparation and easy renewal of the surface, carbon has been used extensively as a working electrode for a variety of electrochemical applications. It has also been shown that carbon tends to be more compatible with biological tissues than other commonly used electrode materials [11, 24]. Among the carbon electrodes, the carbon paste electrode (CPE) is of particular importance. The ease and speed of preparation and of obtaining a new reproducible surface, the low residual current, porous surface and low cost of carbon paste are some advantages of CPEs over all other carbon electrodes. Therefore, the CPE can provide a suitable electrode substrate for preparation of modified electrodes.
Triton X-100 (TX-100) is a non ionic surfactant which has a hydrophilic head on one side and hydrophobic tail on another side is commonly used as detergents in laboratories. Apart from laboratory use, TX-100 can be found in several types of cleaning compound, ranging from heavy-duty industrial products to gentle detergents. Some less soluble surfactants were employed in the immobilization of macro molecules or other functional materials. Wu et al [25] developed a stable multi-wall carbon nanotube (MWNT) modified electrode based on the immobilization of MWNT in the film of insoluble dihexadecyl phosphate (DHP) on a glassy carbon electrode. This electrode exhibited an electrocatalytic activity towards biomolecules and has been used as a sensor for the determination of these species [26, 27]. The electroanalytical techniques have been excellently applied for the determination of various electroactive species [28 – 65].

In continuation of our studies concerning the preparation of modified electrodes [66–74], in the present work, Murexide /Triton X-100 modified carbon paste electrode (Mu-MCPE/TX-100) possesses high stability and good electrocatalytic activity toward the electrocatalytic oxidation of DA. Cyclic voltammetry was used to characterize the electrochemical properties of the Mu-MCPE and to investigate its electrocatalytic effect on DA oxidation. The modified electrode has good electrocatalytic activity such as sensitivity, low detection limit and also Quantification limit when compared to traditional carbon paste electrode.

3.5. Experimental Part

3.5.1. Materials

Murexide (Mu) and Dopamine (DA) were obtained from Himedia chemical company and of analytical grade used without further purification. 25 mM DA stock solution was prepared in 0.1 M perchloric acid. Graphite powder of 50mm size was purchased from Loba and silicon oil was purchased from Himedia. Chemicals for preparation of buffer solution was purchased from Merck. Phosphate buffer (0.2 M pH 7.4) was used as supporting electrolyte. Sodium hydroxide and acetic acid were used for increasing and decreasing the pH of the buffer. Water used in all the measurements was double distilled.
3.5.2. Apparatus

Cyclic voltammetry (CV) was performed in a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional electrochemical cell. The electrode system contained a carbon paste working electrode (3.0 mm in diameter), a platinum wire as counter electrode and saturated calomel as reference electrode.

3.5.3. Preparation of Bare Carbon Paste Electrode (BCPE) and Murexide Modified Carbon Paste Electrode (Mu-MCPE)

The carbon paste electrode was prepared as follows, 70% graphite powder and 30% silicone oil were mixed by hand about 45 minutes to produce a homogeneous bare carbon paste electrode (BCPE). The paste was then packed into the cavity of a homemade carbon paste electrode and smoothed on a weighing paper. The murexide modified carbon paste electrode (Mu-MCPE) was prepared by grinding different amount of Mu in milligrams along with 70% graphite powder and 30% silicone oil.

3.6. Results and Discussion

3.6.1. Electrochemical Response of DA at Mu-MCPE

Fig. 3.1 shows the cyclic voltamogramms of 20 μM DA at BCPE and Mu-MCPE in 0.2 M phosphate buffer solution at pH 7.4 and scan rate 100 mV/s. At the BCPE the cyclic voltammogram of DA (dashed line) shows an oxidation peak potential at 0.130 V and reduction peak potential at 0.087 V with low current signals. The electrochemical response of DA at Mu-MCPE showed well defined redox waves of DA with strong increase of the redox peak currents (solid line). The oxidation peak potential occurs at 0.145 V and reduction peak potential at 0.087 V respectively, with the peak potential separation (ΔEp) 0.058 V. The value of i_p/ /i_c was about 1.15, and negligible shift in the redox peak potentials. The modified electrode exhibited strong promoting effect and high stability towards the electrochemical oxidation of DA. It was observed that the peak currents enhanced at the Mu-MCPE, which provides more evidence for asserting that the Mu in the CPE possessed high electrocatalytic activity towards the DA detection.
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3.6.2. Effect of Scan Rate

According to Randles-Sevick’s equation, the scan rate is directly proportional to peak current. Fig. 3.2a reveals the cyclic voltammogram of DA at Mu-MCPE for 50 μM DA in 0.2 M phosphate buffer solution and scan rates from 100 to 500 mV s⁻¹ at pH 7.4. The effect of scan rate on the anodic peak current of DA was studied by cyclic voltammetry and increase in the scan rate increase is anodic peak current ($i_{pa}$). The graph of anodic peak current ($i_{pa}$) vs. scan rate ($v$) and square root of scan rate ($v^{1/2}$) was plotted. The graph obtained were nearly straight lines shown in Fig. 3.2b and Fig. 3.2c. A good linearity of the anodic peak currents were proportional to the scan rate ($v$) and also the to the square root of scan rate($v^{1/2}$) with correlation coefficient 0.9951 and 0.9995 for $i_{pa}$ vs. $v$ and $i_{pa}$ vs. $v^{1/2}$ respectively. In the range of 100 to 500 mVs⁻¹, the anodic peak currents indicate that the electrode transfer reaction is diffusion-controlled process on the modified electrode surface.

3.6.3. Effect of Concentration of Dopamine

The electrocatalytic oxidation of DA was carried out by varying its concentration at Mu-MCPE. Fig. 3.3a shows that by increasing the concentration of DA from $1.0 \times 10^{-6}$ to $1.0 \times 10^{-4}$ M the electrochemical anodic and cathodic peak currents goes on increasing with shifting $E_{pa}$ towards positive and $E_{pc}$ towards the negative direction slightly. Fig. 3.3b shows that the graph of anodic peak current vs concentration of DA shows two linear relationships ranges $1 \times 10^{-6} - 6 \times 10^{-6}$ M and $10 \times 10^{-6} - 100 \times 10^{-6}$ M with the linear regression equations as $i_{pa}(A) = 6.119 \times 10^{-7} + 0.57528$ C M/L and $i_{pa}(A) = 4.250 \times 10^{-6} + 0.101$ C M/L, respectively. The correlation coefficient for the first linearity was 0.9967 and for the second it was found to be 0.9710. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation [69, 75]. The detection limit for DA in the lower range region was found to be $1.496 \times 10^{-7}$ M and quantification limit was $4.988 \times 10^{-7}$ M. The detection limit and quantification limit was calculated by using the formulas (1) and (2) [76, 68], where S is the standard deviation and M is the slope obtained from the three calibration plots. The detection limit of various electroanalytical methods proposed for determination of DA is compared with our analytical data in Table. 3.1.
From the data shown, a lower limit of detection (LOD) can be achieved using the proposed method [23, 77–80].

\[
\text{LOD} = 3S/M \quad (1)
\]

\[
\text{LOQ} = 10S/M \quad (2)
\]

3.6.4. Effect of pH on Electrocatalytic Oxidation of DA

The effect of pH on the formal potential and anodic peak current was investigated by cyclic voltammetry in the solution containing \(20 \times 10^{-6}\) M DA. The values of \(E_0\), which was dependent on the pH value of the buffer solution, show that the redox couple of the DA includes some proton transfer in the redox processes. According to the Nernst equation, the slope of 66 mV/pH reveals that the proportion of the electron and proton involved in the reaction is 1:1. The effect of pH on the electrode signal and oxidation potential was investigated by cyclic voltammetry in the solution containing \(20 \times 10^{-6}\) M DA. The \(E_0\) vs. pH graph clearly indicates that the catalytic peak shifts to a more negative potential with increasing pH. From Fig. 3.4, the better shape of the voltammogram were observed at pH 7.4 suggested it as optimal pH value.

3.6.5. Effect of TX-100 Surfactant

There are three types of surfactants viz [sodium dodecyl sulphate (SDS) (anionic), cetyltrimethyl ammonium bromide (CTAB) (cationic) and Triton x-100 (non ionic)] were used as both mobilized and immobilized method to know the electrocatalytic effects of DA. Among these the Triton X-100 (TX- 100) was showed excellent electrocatalytic activity in both methods for investigation of DA (Fig. 3.5). Cyclic voltammogrames were recorded for TX-100/Mu-MCPE in a solution containing DA (20\(\mu\)M) in 0.2M phosphate buffer solution at pH 7.4. The TX-100/ Mu-MCPE was calibrated by varying the immobilization time interval from 5 min to 20 min. The graph of \(i_{pa}\) vs different time in minutes was plotted (Fig. 3.6a). The higher current signal was obtained at 10min time intervals. Hence, the 10 min time gap was fixed for further investigation. The effect of immobilization study was done by varying the concentration of TX-100 in \(\mu\)L (5 to 20\(\mu\)L) on to the surface of Mu-MCPE with the immobilization time interval 10 min. The graph of
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The experimental results revealed that the $1 \times 10^{-6}$M TX-100 with 10μL concentration with 10 min time gap showed good electrocatalytic activity towards the detection of DA. The Fig. 3.6c shows the comparison of bare CPE (dotted line), Mu-MCPE (dashed line) and TX-100/ Mu-MCPE (solid line) towards the detection of DA. Among all the TX-100/ Mu-MCPE showed the excellent electrocatalytic activity for detection of DA. The mobilization effect of TX-100 towards the electrocatalytic oxidation of DA was studied by addition of different concentration of TX-100 in μL ($5 \mu L - 20 \mu L$) directly in to the solution containing the DA (20μM) in 0.2M phosphate buffer solution as supporting electrolyte. However, the better redox current signal was obtained at 15μL addition of TX-100 which could be seen in Fig. 3.6d. The results showed that, the effect of surfactant concentration in mobilization method also catalyze the oxidation of DA. When the surfactant concentration is below 15μL, CMC of surfactant at room temperature, both $i_{pa}$ and $i_{pc}$ increases rapidly with the increase of surfactant concentration. The concentration of the surfactant was increased from 5 to 25μL in both mobilization and immobilization method was shown in Fig. 3.6d and Fig. 3.6b respectively. The redox currents signal for DA was superior in immobilization when compared to mobilization method. This may be due to the oxygen group present in the murexide molecule could form the hydrogen bond with the hydrogen atom present in the hydroxyl group of DA. After immobilization of TX-100 on the surface of Mu-MCPE strongly facilitate the movement of DA ions by electrostatic attraction. Therefore immobilization is more favorable than mobilization method [81].

3.7. Conclusions

This study has indicated that TX-100/Murexide modified carbon paste electrode exhibits highly electrocatalytic activity towards detection of DA oxidation. The redox response of the modified electrode is that it anticipated for a surface-immobilized redox couple. The electrochemical behavior of the modified electrode is strongly dependent on the solution pH. From the results of comparative study of BCPE, Mu-MCPE, TX-100/ Mu-MCPE suggests that TX-100/ Mu-modified carbon paste electrode showed the significant electrocatalytic activity for detection of DA.
From the comparative results the mobilization and immobilization methods, immobilization is more favorable than mobilization method for the detection of DA at carbon paste electrode. The experimental results revealed that the $1 \times 10^{-6}$ M TX-100 with 10μL concentration with 10min time gap showed significant electrocatalytic activity towards the detection of DA. The detection limit of DA was found to be $1.496 \times 10^{-7}$ M and quantification limit of DA was found to be $4.988 \times 10^{-7}$ M. With its low cost, high sensitivity, very easy preparation of the modified electrode and the reproducibility of the voltammetric response makes the prepared modified system very useful in the construction of simple devices for the determination of DA in clinical and pharmaceutical preparations. Hence this modified electrode can be applied for the detection of other neurotransmitters. TX-100/Mu-MCPE was acting as a good sensor for the detection of DA.
<table>
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<th>Electrode</th>
<th>Detection limit (mol/L)</th>
<th>Method</th>
<th>Reference</th>
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<tr>
<td>Metallothioneins self-assembled gold electrode</td>
<td>$6.0 \times 10^{-6}$</td>
<td>CV</td>
<td>[78]</td>
</tr>
<tr>
<td>Ionic liquid modified carbon paste electrode</td>
<td>$7.0 \times 10^{-7}$</td>
<td>CV</td>
<td>[23]</td>
</tr>
<tr>
<td>Poly (caffeic acid)/GCE</td>
<td>$2.0 \times 10^{-7}$</td>
<td>CV</td>
<td>[79]</td>
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<tr>
<td>α-CD/CNT/PGE</td>
<td>$1.0 \times 10^{-6}$</td>
<td>DPV</td>
<td>[80]</td>
</tr>
<tr>
<td>Poly (p-toluene sulfonic acid) modified glassy carbon electrode</td>
<td>$6.0 \times 10^{-7}$</td>
<td>DPV</td>
<td>[81]</td>
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<td>$1.496 \times 10^{-7}$</td>
<td>CV</td>
<td>This work</td>
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</tbody>
</table>

Table 3.1 - Comparison of different modified electrodes for DA determination.
Fig. 3.1 - Cyclic voltammogram of BCPE (dotted line) and Mu-MCPE (Solid line)
in the presence of 20 μM DA and 0.2 M phosphate buffer, in pH 7.4
Scan rate: 100mVs⁻¹.

Fig. 3.2a - Cyclic voltammograms of different scan rate in the presence of 20 μM DA and 0.2 M phosphate buffer, in pH 7.4 Scan rate: 100 mVs⁻¹-500 mVs⁻¹.
Fig. 3.2b - Effect of variation of square root of scan rate on the anodic peak current of 20 µM DA in 0.2M phosphate buffer, at pH 7.4, Scan rate: 100 mVs⁻¹-500 mVs⁻¹.

Fig. 3.2c - Effect of variation of scan rate on the anodic peak current of 20 µM DA in 0.2M phosphate buffer, at pH 7.4.
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Fig. 3.3a - Cyclic voltammogram of variation of concentration of DA from 2 µM-100 µM in presence of phosphate buffer solution at pH 7.4 at Scan rate: 100 mVs⁻¹.

Fig. 3.3b - Effect of variation of concentration of DA on the anodic peak current of 20 µM DA in phosphate buffer solution at pH 7.4, Scan rate: 100 mVs⁻¹.
Fig. 3.4 - Effect of variation of pH on the anodic peak potential of 20 μM DA phosphate buffer solution at Mu-MCPE at Scan rate: 100 mVs⁻¹.

Fig. 3.5 - Cyclic voltammogram of 20×10⁻⁶ M DA at a) 5μL SDS (dotted line) b) 5μL CTAB (dashed line) and c) 5μL TX-100 (solid line) immobilized carbon paste electrode in 0.2 M phosphate buffer solution (pH 7.4) at scan rate 100 mVs⁻¹.
Fig. 3.6a - Graph of $i_{pa}$ vs. time in min at $10 \, \mu$L of $1 \times 10^{-6}$ M TX-100 on to the surface of Mu-MCPE in presence of 20 \mu M DA and 0.2M phosphate buffer at scan rate: $100 \, \text{mVs}^{-1}$.

Fig. 3.6b - Graph of $i_{pa}$ vs. different concentration of $1 \times 10^{-6}$ M TX-100 in \mu L on to the surface of Mu-MCPE in presence of 20 \mu M DA and 0.2M phosphate buffer at pH- 7.4 Scan rate: $100 \, \text{mVs}^{-1}$.
Fig. 3.6c - Cyclic voltammogram of 20μM DA at a) BCPE (dotted line) b) Mu-MCPE (dashed line) c) 10μL TX-100 (solid line) immobilized Mu-MCPE in 0.2M phosphate buffer solution (pH 7.4) at scan rate: 100mVs⁻¹.

Fig. 3.6d - Graph of $i_p$ vs. different concentration of $1 \times 10^{-6}$ M TX-100 into the solution of Mu-MCPE in presence of 20 μM DA and 0.2 M phosphate buffer at pH 7.4. Scan rate: 100mVs⁻¹.
3.8. References


