CHAPTER 4

Pristine multi-walled carbon nanotubes/SDS
MCPE as an electrochemical sensor for Epinephrine

Electrochemical sensor: MCPE/pMWCNTs/SDS

Methods of modification: Bulk modification followed by casting
4.1 Introduction

Chapter 4 documents the chemistry of EP, and its biological and pharmacological significance. A brief literature survey on the electrochemical sensors developed for the detection of EP by different modified electrodes with a special emphasis on modification of CPE with various modifiers and modification techniques have been presented. Research gaps were identified after a thorough literature survey in order to develop MCPEs as electrochemical sensors with improved performance for the determination of EP. This work involves the construction of an amperometric sensor (MCPE/pMWCNTs/SDS) which could be used under physiological conditions. The results of electrochemical investigation of EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS have been presented. The results of surface analysis and EIS of all the electrodes used are discussed. A comparative study of the adsorption of SDS on MCPE/pMWCNTs and MCPE/MWCNTs has been carried out. The effects of variation of concentration of SDS, scan rate and pH on the electrochemical response of EP is discussed. LOD, LDR and interference studies for EP at MCPE/pMWCNTs/SDS are presented. Analytical applications of MCPE/pMWCNTs/SDS have been demonstrated by estimating EP in pharmaceuticals and in body fluids by recovery studies.

4.2 Chemistry of Epinephrine

Epinephrine (Adrenaline, EP) is one of the catecholamine neurotransmitters and hormone in mammals.\(^1\) IUPAC name of EP is 4-[(1R)-1-Hydroxy-2-(methylamino)ethyl]-1,2-benzenediol. The chemical structure of EP is shown in Fig. 4.1. William Bates reported the discovery of extract produced by adrenal glands in 1886. But these extracts were first obtained by N. Cybulski\(^2\) in 1895. Takamine successfully isolated and purified the hormone from the adrenal glands of sheep.\(^2\) EP was the first hormone to be discovered.\(^2\) EP was first synthesized in laboratory by F. Stolz and H.D. Dakin\(^2\) in 1904. EP is chemically synthesized by the reaction of catechol with chloroacetyl chloride, followed by reaction with methylamine to give the ketone which is then reduced to the EP. It is produced in

![Figure 4.1: Structure of epinephrine.](image-url)
some neurons of the CNS, and in the chromaffin cells of adrenal medulla from amino acids phenylalanine and tyrosine.\(^3\) EP is stored in the vesicles of adrenal medullary cells at a concentration \(~360\) mM. It exists as a large organic cation in nervous tissue and body fluids.\(^4\) The biosynthetic pathway of EP is shown in chapter 3, scheme 3.1.

### 4.3 Biological significance of EP

EP secreted by the adrenal glands into the blood stream in response to the stimuli of the sympathetic nervous system.\(^1\) It prepares the body for an action in perceived emergency situations by boosting the supply of oxygen and glucose to brain and muscles. Hence, it is also known as fight or flight hormone. It simultaneously elevates the blood sugar level by inducing the conversion of glycogen to glucose in liver and lipolysis in adipose tissue.\(^5\) Low levels of EP are found in patients of Parkinson's disease and orthostatic hypotension.\(^6\) High levels of EP are associated with stress and thyroid hormone deficiency.\(^7\) It serves as a chemical mediator for transmitting the nerve impulse to efferent organs. DA is the metabolic precursor of EP in biosynthetic pathway hence alterations in DA synthesis have a direct effect on EP.\(^8\)

### 4.4 Pharmacological significance of EP

Clinically, EP is a common emergency health care medicine.\(^9\) EP is used in the treatment of severe allergic reactions, anaphylaxis, sepsis, emphysema and myocardial infarction. It acts as bronchodilator and vasoconstrictor.\(^10\) It is also used in the treatment of glaucoma.\(^11\) EP is added to injectable forms of a number of local anesthetics as vasoconstrictor. It can be used as a potent doping agent to improve the performance of athletes in sports and its use is banned in competitive games by World Anti Doping Agency\(^12\) (WADA). EP is used on the skin or mucous membranes during surgeries to control bleeding of wounds because it constricts blood vessels.

### 4.5 Literature survey on MCPEs being used as electrochemical sensors for EP

Adams and coworkers have investigated electrochemical oxidation pathways of EP.\(^13\) Voltammetric analysis of EP at CPE is complicated by following factors:

- EP coexists with other electroactive biological molecules such as AA and UA in biological matrices and at CPE these molecules have redox potentials similar to that of EP. Concentrations of AA and UA in biological fluids are \(10^2–10^4\) times higher than concentrations of EP.\(^14\) Hence, voltammetric response of EP is usually
overlapped by the responses of AA and UA.

- Adsorption of EP and its oxidized products at CPE causes the passivation of the electrode.\textsuperscript{15}
- The large anodic overpotential and irreversible nature of EP restrict the voltammetric investigations at bare CPE.\textsuperscript{16}

The most common route to overcome these difficulties is by the modification of electrodes. Thus many modifiers have been taken up for the same purpose. Various strategies have been employed for the selective and sensitive voltammetric determination of EP using MCPEs.

The electrochemical sensors for EP are prepared using MCPEs incorporating modifiers of biological origin. The modifiers include crude extract of cara root\textsuperscript{17} and plant tissues\textsuperscript{18} which act as source of polyphenol oxidase and laccase.\textsuperscript{19} These enzyme can catalyze electrochemical oxidation of EP. The anionic surfactant SDS\textsuperscript{20} and cationic surfactant CTAB\textsuperscript{21} have been used to prepare MCPEs for the detection of EP. Charge transfer mediators iron phthalocyanine,\textsuperscript{22} and molybdenum (VI) complex\textsuperscript{23} and polymers like anion-exchange resin,\textsuperscript{24} poly(tannic acid),\textsuperscript{25} and poly(isonicotinic acid)\textsuperscript{26} have been utilized for modification of CPE. Electrostatic interaction enables selectivity for these electrodes. Organic modifiers such as 2-(4-Oxo-3-phenyl-3,4-dihydro-quinazolinyl)-N'-phenyl-hydrazinecarbothioamide,\textsuperscript{27} 2,2'-[1,2-butanediylbis(nitriiloethyldiyne)]-bishydroquinone,\textsuperscript{28} hydroquinone,\textsuperscript{29} and have been used for the preparation of the MCPEs for the detection of EP. Nanomaterials such as CNTs,\textsuperscript{30} Pt,\textsuperscript{31} Au,\textsuperscript{32} C,\textsuperscript{33} TiO\textsubscript{2},\textsuperscript{34} and ZrO\textsubscript{2}\textsuperscript{35} nanoparticles have also been used in the preparation of MCPEs for the sensitive detection of EP. Bulk modification method was employed whenever these modifiers were used. Al-incorporated SiO\textsubscript{2} modified CPE\textsuperscript{36} and pre-anodized CPE\textsuperscript{37} have also been used as electrochemical sensor for EP. Recent trend shows that MCPEs with ionic liquid exhibit electrocatalytic activity towards EP oxidation.\textsuperscript{38} Ionic liquids are usually mixed with carbon paste during the modification. The combination of different modifiers also used for the development of electrochemical sensor for EP.\textsuperscript{30-38}

Literature survey also reveals the electroanalysis of EP using electrodes other than CPE. GCE modified with MWCNTs,\textsuperscript{39} Cobalt(II) tetrasulfophthalocyanine,\textsuperscript{39} Au nanoparticles,\textsuperscript{40} poly(o-Aminobenzoic acid),\textsuperscript{41} polyaniline,\textsuperscript{40} poly(L-methionine),\textsuperscript{42} poly(p-aminophenol)\textsuperscript{43} and poly(Adizol Black B)\textsuperscript{44} have been used as electrochemical
sensors for EP. Gold electrodes modified with mercaptoacetic acid self-assembled monolayer (SAM),\textsuperscript{45} Penicillamine SAM,\textsuperscript{46} Cysteine SAM on Au nanoparticles,\textsuperscript{47} overoxidised dopamine film\textsuperscript{48} and poly(4-methoxyphenol)\textsuperscript{49} are shown to be useful in the electroanalysis of EP. Boron doped diamond film electrode,\textsuperscript{50} poly(neural red) modified carbon fiber microelectrodes,\textsuperscript{51} chitosan modified ITO\textsuperscript{52} and MWCNTs modified pyrolytic graphite electrode\textsuperscript{53} were constructed to be used as electrochemical sensors for EP.

4.6 Research gaps to develop MCPEs as electrochemical sensors for EP

- The CPE modification for the electrochemical determination of EP is not exploited completely as compared to other electrodes such as MGCE, gold, graphite, diamond and ITO electrodes. The use of pMWCNTs for such applications is not reported.
- The reports on the preparation of MCPEs using pMWCNTs are comparatively less.
- Adsorption of SDS at MWCNT_{SOX} and pMWCNTs modified CPEs were not compared in any of the previous reports.
- Casting of sodium dodecyl sulphate (SDS) on the surface of pMWCNTs bulk modified CPE was not previously reported for the amperometric determination of EP.

4.7 Sodium dodecyl sulphate as a modifier

SDS is an anionic surfactant. It is having a hydrophobic tail and a hydrophilic head giving it amphiphilic properties. It is derived from coconut and palm oils. It is a common component in all domestic cleaning products. These have been widely used in electroanalysis because of their ability to improve the properties of the electrode-solution interface.\textsuperscript{54} Surfactant modified electrodes have been used as an electrochemical sensor for catecholamines.\textsuperscript{55–57} The ability of a surfactant to get adsorbed at CPE, MCPEs, etc., and to initiate electrostatic interaction with analytes inspired us to use SDS as modifier. The structure of SDS is shown in Fig. 4.2.

\[
\text{Figure 4.2: Structure of SDS.}
\]

4.8 Preparation of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS

Preparation of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS
are described in chapter 2, sections 2.6.3, 2.7.1.2 and 2.7.3.2 respectively.

4.9 Results and discussions

4.9.1 Surface morphology of electrodes

Figure 4.3: FE-SEM images of (a) CPE, (b) MCPE/pMWCNTs, (c) MCPE/SDS and (d) MCPE/pMWCNTs/SDS.

The morphological characteristics of all the electrodes were compared by recording the FE-SEM images. Fig. 4.3a–d, show the FE-SEM images of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS, respectively. It was understood from Fig. 4.3a that a smoother surface was obtained for CPE compared to MCPE/pMWCNTs. Fig. 4.3b reveals the uniform dispersion of pMWCNTs in CPE matrix. Modification with SDS does not drastically alter the surface morphologies of the above electrodes (Fig. 4.3c–d). Fig. 4.4a–d, show the EDX analysis of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS, respectively. EDX analysis of the electrodes indicates the presence of C, Si and O. The presence of S both at MCPE/SDS and

Table 4.1: Elemental analyses of electrodes using EDS.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Elements in Wt %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>CPE</td>
<td>96.08</td>
</tr>
<tr>
<td>MCPE/pMWCNTs</td>
<td>93.98</td>
</tr>
<tr>
<td>MCPE/SDS</td>
<td>95.31</td>
</tr>
<tr>
<td>MCPE/pMWCNTs/SDS</td>
<td>94.39</td>
</tr>
</tbody>
</table>
MCPE/pMWCNTs/SDS (Fig. 4.4c-d) convincingly confirm the adsorption of SDS at CPE and MCPE/pMWCNTs. The wt % of elements at the surface of the electrodes are displayed in Table 4.1.

**Figure 4.4:** EDX analyses of (a) CPE, (b) MCPE/pMWCNTs, (c) MCPE/SDS and (d) MCPE/pMWCNTs/SDS.

### 4.9.2 Characterization of electrodes by electrochemical impedance spectroscopy

EIS spectra of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS are shown in the form of Nyquist plot in Fig. 4.5. EIS data of electrodes were recorded in ac frequency range varying from 0.1 Hz to 100.0 kHz at an oxidation peak potential (Epa, 215.0 mV) of $5.0 \times 10^{-3}$ M EP in 0.1 M KPBS of pH 7.0. $R_{ct}$ values at different electrodes were obtained by fitting the obtained impedance data to an appropriate circuit (Fig. 4.6). The $R_{ct}$ values of $5.0 \times 10^{-4}$ M EP at CPE, MCPE/pMWCNTs, MCPE/SDS and
MCPE/pMWCNTs/SDS are displayed in Table 4.2.

The semicircle part of Nyquist plot at higher frequency region corresponds to the electron transfer limited process. The presence of pMWCNTs in carbon paste matrix and adsorption of SDS at surface results in a least charge transfer resistance and improves the charge transfer kinetics at MCPE/pMWCNTs/SDS. Hence, MCPE/pMWCNTs/SDS was employed for the development of amperometric sensor for EP.

Table 4.2: Electrochemical and kinetic parameters for the electrochemical oxidation of EP at different electrodes.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>$R_c$ (Ω)</th>
<th>D (cm$^2$s$^{-1}$)</th>
<th>$k^*$ (cm s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPE</td>
<td>1000.7</td>
<td>$3.13 \times 10^{-6}$</td>
<td>$1.47 \times 10^{-3}$</td>
</tr>
<tr>
<td>MCPE/pMWCNTs</td>
<td>752.4</td>
<td>$4.11 \times 10^{-6}$</td>
<td>$2.13 \times 10^{-3}$</td>
</tr>
<tr>
<td>MCPE/SDS</td>
<td>234.2</td>
<td>$1.89 \times 10^{-5}$</td>
<td>$4.51 \times 10^{-3}$</td>
</tr>
<tr>
<td>MCPE/pMWCNTs/SDS</td>
<td>198.9</td>
<td>$4.10 \times 10^{-5}$</td>
<td>$6.38 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

4.9.3 Electrochemical behavior of EP

The electrochemical behavior of EP was investigated using the CV technique. The resulting CVs for $5.0 \times 10^{-4}$ M EP in 0.1 M KPBS of pH 7.0 at CPE (dotted line), MCPE/pMWCNTs (short dashed line), MCPE/SDS (dash dotted line) and
Figure 4.7: CVs of 5.0 × 10^{-4} M EP in 0.1 M KPBS of pH 7.0 at CPE (dotted line), MCPE/pMWCNTs (short dashed line), MCPE/SDS (dash dotted line), MCPE/pMWCNTs/SDS (solid) and blank (long dashed line). Scan rate: 50 mV s^{-1}.

MCPE/pMWCNTs/SDS (solid line) are shown in Fig. 4.7. The absence of voltammetric peaks for the blank (Fig. 4.7, long dashed line) at MCPE/pMWCNTs/SDS indicates its electrochemical inertness in the potential window of interest. EP shows an irreversible electrochemical behavior at all the electrodes. Epa of EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS are 248.0 ± 2.4, 200.0 ± 4.8, 223.0 ± 4.1 and 199.0 ± 3.9 mV respectively. The corresponding Ipa of EP at the respective electrodes are −20.8 ± 1.8, −23.7 ± 3.2, −53.3 ± 4.1 and −88.4 ± 4.3 µA, respectively. A negative shift 49.0 mV in Epa and 4.4 times enhancement in Ipa clearly demonstrates the electrocatalytic activity of MCPE/pMWCNTs/SDS towards the oxidation of EP. The negative shift is quite evident at MCPE/pMWCNTs and MCPE/pMWCNTs/SDS compared to CPE and MCPE/SDS. Hence, it can be concluded that the presence of pMWCNTs in the CPE matrix is responsible for the shift in potential on account of the favorable electronic properties of pMWCNTs. The edge plane or edge plane like defects on the surface of pMWCNTs also facilitates the electron transfer. The adsorption of the anionic surfactant SDS at the surface of the electrodes takes place in such a way that the hydrophobic alkyl chain will get adsorbed spontaneously at the surface of hydrophobic CPE and MCPE/pMWCNTs through Van der Waals interactions, keeping the sulphate group orienting outwards. This creates

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high negative charge density at the surface of these electrodes and promotes the electrostatic attraction of EP, which is a cation at pH 7.0 (pKa = 8.8). The hydroxyl group of EP can form hydrogen bond with the electronegative oxygen atoms of the sulphate group in SDS. This weakens the bond energy and makes the oxidation more viable. Also, modification with pMWCNTs provides more surface area for SDS molecules to get adsorbed. This causes maximum current sensitivity at MCPE/pMWCNTs/SDS.

In order to ascertain that the pMWCNTs are the best base electrode material for the study of EP, the electrochemical behaviour of 5.0 × 10⁻⁴ M EP was investigated at MCPE/MWCNTsOX (dotted line) and MCPE/MWCNTsOX/SDS (dash dotted line) in 0.1 M KPBS of pH 7.0 under the same conditions which are displayed in Fig. 4.8. Epa of EP at MCPE/MWCNTsOX and MCPE/MWCNTsOX/SDS is 215.0 ± 6.8 and 248.0 ± 7.9 mV, respectively. The electrochemical oxidation of EP at MCPE/MWCNTsOX/SDS involves higher energy. The corresponding Ipa for EP at the respective electrodes are –48.5 ± 6.3 and –62.8 ± 5.4 µA. It is evident from Fig. 4.8, that the electrodes modified with MWCNTsOX offer a higher background current as compared to the ones modified with pMWCNTs (Fig. 4.8, short dashed line, solid). The MWCNTsOX carries oxygen functionalities which are possibly responsible for high background current. The adsorption of SDS molecules at the negatively charged surface of MCPE/MWCNTsOX may not be uniform due to the
repulsion. Moreover, it was also observed that the hydrophilicity of MCPE/MWCNTs_{SOX} surface due to the presence of MWCNTs_{SOX} caused surface rupture during the eight minute adsorption time of the surfactant and this was subsequently reflected in the measurement. A 49.0 mV positive shift in Epa and 25.6 µA decrease in Ipa of EP were observed at MCPE/MWCNTs_{SOX}/SDS as compared to MCPE/pMWCNTs/SDS which proves the poor redox kinetics at MCPE/MWCNTs_{SOX}/SDS. The pMWCNTs modified electrode suppresses the background current to a greater extent, provides better redox kinetics and a maximum current sensitivity for EP. These being the desired qualities for an electrochemical sensor, MCPE/pMWCNTs/SDS is more suitable for this purpose.

4.9.4 Effect of variation of scan rate on peak parameters of EP

The influence of potential scan rate on the electrochemical oxidation of 5.0 × 10^{-4} M EP at MCPE/pMWCNTs/SDS was investigated using CV. Ipa was observed to vary linearly with square root of scan rate in the range 10 – 150 mV s^{-1} as depicted in Fig. 4.9. Such a behavior suggests that the electrochemical oxidation of EP at MCPE/pMWCNTs/SDS is a diffusion controlled process. The linear regression equation for the potential range studied is Ipa (µA) = 2.23 – 11.9 √ν (mV s^{-1})^{1/2} with R^2 = 0.9906. Epa of EP shifts in the positive direction with increase in scan rate.

![Figure 4.9: Plot of variation of Ipa with square root of scan rate for 5.0 × 10^{-4} M EP at MCPE/pMWCNTs/SDS.](image)
4.9.5 Effect variation of pH on peak parameters of EP

Figure 4.10: Plot of variation of (a) Ipa and (b) Epa of $5.0 \times 10^{-4}$ M EP in 0.1 M KPBS of different pH at MCPE/pMWCNTs/SDS. Scan rate: 50 mV s$^{-1}$.

The effect of pH on Epa and Ipa of $5.0 \times 10^{-4}$ M EP was investigated in 0.1 M KPBS of different pH ranging from 3.0 – 8.0 using CV technique. As represented in Fig. 4.10a, the Ipa attains maximum value at pH 7.0. It was observed that the Ipa decreased with increase in pH between 3.0 and 6.0. Hence, it could be concluded that the protons could catalyze the electrochemical oxidation of EP. Moreover, the sulphate groups of SDS will remain protonated in acidic pH and the electrostatic attraction of EP cation will not prevail. At neural pH, the electrostatic attraction of EP cation and SDS reaches a maximum value and a sharp increase in the value of Ipa is observed. As expected, there was a large decline in current in basic pH. Since the maximum current sensitivity was at pH 7.0 which was closer to the physiological pH, we maintained the same pH in all our experiments. Furthermore, it could be observed that Epa shifts linearly in negative direction with increase in pH as depicted in Fig. 4.10b, which indicates the involvement of protons in the electrochemical oxidation of EP at MCPE/pMWCNTs/SDS. The linear regression equation of Epa versus pH plot is $Epa$ (mV) = 672.5 – 65.3 pH with $R^2 = 0.9875$. Equation (4.1) represents the Nernst equation which describes the relationship between $Epa$ and pH, where $n$ and $m$ represent the number of electrons and protons involved in the reaction, $a$ and $b$ are coefficients of oxidant and reductant in the reaction.\[63]

$$Epa = E^o + \left(\frac{0.0591}{n}\right) \log \left[\frac{(OX)^a}{(R)^b}\right] - \left(0.0591 \frac{m}{n}\right) pH$$  \hspace{1cm} (4.1)
A slope of $-65.9$ pH / mV obtained closely matches with the Nernstian slope for the electrode reaction which involves an equal number of electrons and protons ($m = n$). Two protons and two electrons are involved in electrochemical oxidation of EP at MCPE/pMWCNTs/SDS, which is in accordance with the mechanism of EP already reported elsewhere. Scheme 4.1 represents the electrochemical oxidation mechanism of EP at MCPE/pMWCNTs/SDS.

\[
\text{HO}-\begin{array}{c}
  \text{CH}_2 \\
  \text{N}^- \\
  \text{CH}_3
\end{array} \text{CH}_3 \xrightarrow{-(2H^+ + 2e^-)} \text{HO}-\begin{array}{c}
  \text{CH}_2 \\
  \text{N}^- \\
  \text{CH}_3
\end{array}
\]

Scheme 4.1: Electrochemical oxidation mechanism of EP at MCPE/pMWCNTs/SDS.

4.9.6 Determination of diffusion coefficient and heterogeneous electron transfer rate constant

Diffusion coefficient of EP ($D_{EP}$) in 0.1 M KPBS of pH 7.0 at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS were determined using the procedure described in chapter 2, section 2.11. The chronoamperograms and the related plots are displayed in Fig. 4.11a–c. $D_{EP}$ calculated from these slopes and Cottrell equation is displayed in Table 4.2. It is clear from Table 4.2 that the values of $D_{EP}$ were enhanced upon
modification with pMWCNTs and SDS. The maximum value for $D_{EP}$ was observed at MCPE/pMWCNTs/SDS. The electrostatic attraction of EP molecules and SDS film at the electrode surface causes the $D_{EP}$ to increase.

The $k^*$ for the irreversible electrochemical oxidation of EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS were calculated using the procedure described in chapter 2, section 2.12. The $k^*$ values at different electrodes are displayed in Table 4.2. The increase in $k^*$ on modification indicates a better charge transfer kinetics at the modified electrodes. The highest value for $k^*$ was observed at MCPE/pMWCNTs/SDS which confirms the electrocatalytic activity of the electrode towards EP. Therefore, it can be concluded from Table 4.2 that the $R_{ct}$ values decrease on modification while $D_{EP}$ and $k^*$ values increase. The observations made during the CV investigation of EP were consistent with these results.

4.9.7 Effect of loading pMWCNTs in the carbon paste matrix

![Plot of variation of Ipa of 5.0 × 10⁻⁴ M EP with different quantities of pMWCNTs.](image)

Figure 4.12: Plot of variation of Ipa of 5.0 × 10⁻⁴ M EP with different quantities of pMWCNTs.

The amount of pMWCNTs in CPE matrix plays an important role in the performance of MCPE/pMWCNTs/SDS. Moreover, the presence of pMWCNTs affects the adsorption of SDS at MCPE/pMWCNTs. Hence, MCPE/pMWCNTs/SDS was prepared by mixing different quantities of pMWCNTs (1.0 – 5.0 mg). Ipa attains the highest value at an
electrode containing 2.0 mg of pMWCNTs as shown in Fig. 4.12. The higher loading of pMWCNTs induced higher background current and resulted in the reduction of Ipa. Furthermore, the adsorption of SDS at electrodes diminishes as the amount of pMWCNTs in CPE matrix increases because of the decrease in hydrophobicity of electrode surface. These two factors largely determine the sensitivity of MCPE/pMWCNTs/SDS. It was further supported by the observation that the adsorption of SDS at CPE surface (in the absence of pMWCNTs) under identical conditions presented high background currents (Fig. 4.7). Thus, MCPE/pMWCNTs/SDS was prepared using 2.0 mg of pMWCNTs and the same was maintained throughout our studies.

4.9.8 Effect of variation of concentration of SDS

![Plot of variation of Ipa of 5.0 x 10^-4 M EP with different concentrations of SDS.](image)

Figure 4.13: Plot of variation of Ipa of 5.0 x 10^-4 M EP with different concentrations of SDS.

The effect of variation of concentration of SDS on the voltammetric response of EP and hence on the performance of the sensor was investigated by varying the concentrations of SDS between 1.0 and 9.0 mM. The variation of Ipa with the concentration of SDS is depicted in Fig. 4.13. It was found that the Ipa attained its highest value at 3.0 mM, after which it decreased and then remained almost a constant till 7.0 mM and again decreased thereafter. Hence, it could be concluded that the degree of SDS adsorption reaches a maximum value at 3.0 mM and further increase in concentration does not improve the adsorption of SDS. Therefore, Ipa remained constant in the concentration range 3.0 to
7.0 mM. When concentration of SDS approaches its critical micelle concentration (CMC, 8.0 mM), there is a corresponding decrease in the number of free SDS molecules available for adsorption. Consequently, the Ipa of EP started to decrease beyond the concentration of 7.0 mM. Therefore, 3.0 mM SDS was used in the preparation of MCPE/pMWCNTs/SDS.

4.9.9 Analytical characterization of MCPE/pMWCNTs/SDS

Trace level detection of EP at MCPE/pMWCNTs/SDS was carried out under hydrodynamic conditions using amperometry. Fig. 4.14a shows the amperometric response of EP in 0.1 M KPBS of pH 7.0 at MCPE/pMWCNTs/SDS whose potential was set at 215.0 mV versus SCE. MCPE/pMWCNTs/SDS provided quick response on the addition of aliquots of EP with an interval of 30.0 s and response attained stability in 5.0 s. Fig. 4.14b displays a linear increase in current response with increase in the concentration of EP. The calibration plot presents two linear dynamic ranges, $1.0 \times 10^{-7} - 1.0 \times 10^{-6}$ M and $1.0 \times 10^{-6} - 1.0 \times 10^{-4}$ M respectively for EP. The linear regression equations corresponding to these ranges are $I_{pa} (\mu A) = 0.02 - 0.61 \ C_{EP} (\mu M)$, with $R^2 = 0.9991$ and $I_{pa} (\mu A) = -0.69 - 0.12 \ C_{EP} (\mu M)$, with $R^2 = 0.9958$ respectively. The detection limit of EP at MCPE/pMWCNTs/SDS is $(4.5 \pm 0.18) \times 10^{-8}$ M.
4.9.10 Advantages of the sensor with other sensors reported in the literature

Table 4.3: Comparison of the present work at MCPE/pMWCNTs/SDS with the performance of other electrodes reported in the literature.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>pH</th>
<th>Linear dynamic range (M)</th>
<th>Detection limit (M)</th>
<th>Technique used</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt/P. chrysosporium</td>
<td>4.5</td>
<td>$5.0 \times 10^{-6} - 1.0 \times 10^{-4}$</td>
<td>$1.04 \times 10^{-6}$</td>
<td>CV</td>
<td>66</td>
</tr>
<tr>
<td>Ru/WGE</td>
<td>4.0</td>
<td>$3.0 \times 10^{-6} - 9.0 \times 10^{-5}$</td>
<td>$8.0 \times 10^{-7}$</td>
<td>DPV</td>
<td>67</td>
</tr>
<tr>
<td>Au–Cys–SWCNT–CoTAPc</td>
<td>7.0</td>
<td>$1.22 \times 10^{-5} - 1.3 \times 10^{-4}$</td>
<td>$6.0 \times 10^{-6}$</td>
<td>SWV</td>
<td>68</td>
</tr>
<tr>
<td>MDWCNTPE</td>
<td>7.0</td>
<td>$7.0 \times 10^{-7} - 1.2 \times 10^{-3}$</td>
<td>$2.16 \times 10^{-7}$</td>
<td>DPV</td>
<td>69</td>
</tr>
<tr>
<td>GCE/poly(taurine)</td>
<td>7.4</td>
<td>$2.0 \times 10^{-6} - 6.0 \times 10^{-4}$</td>
<td>$3.0 \times 10^{-7}$</td>
<td>DPV</td>
<td>70</td>
</tr>
<tr>
<td>Au/4α–NiII-TAPc SAMF</td>
<td>5.0</td>
<td>$5.0 \times 10^{-8} - 5.0 \times 10^{-7}$</td>
<td>$5.0 \times 10^{-8}$</td>
<td>Amperometry</td>
<td>71</td>
</tr>
<tr>
<td>DMSA/Au</td>
<td>7.7</td>
<td>$4.0 \times 10^{-4} - 4.0 \times 10^{-3}$</td>
<td>$5.8 \times 10^{-8}$</td>
<td>DPV</td>
<td>72</td>
</tr>
<tr>
<td>MZ–CPE</td>
<td>7.0</td>
<td>$1.0 \times 10^{-6} - 2.5 \times 10^{-3}$</td>
<td>$5.0 \times 10^{-7}$</td>
<td>DPV</td>
<td>73</td>
</tr>
<tr>
<td>OMC/Nafion/GCE</td>
<td>7.0</td>
<td>$5.0 \times 10^{-7} - 2.0 \times 10^{-4}$</td>
<td>$2.0 \times 10^{-7}$</td>
<td>DPV</td>
<td>74</td>
</tr>
<tr>
<td>MIP/PIL–MWNT/ITO</td>
<td>7.4</td>
<td>$2.0 \times 10^{-7} - 6.7 \times 10^{-4}$</td>
<td>$6.0 \times 10^{-8}$</td>
<td>Amperometry</td>
<td>75</td>
</tr>
<tr>
<td><strong>MCPE/pMWCNTs/SDS</strong></td>
<td>7.0</td>
<td>$1.0 \times 10^{-7} - 1.0 \times 10^{-6}$ &amp; $1.0 \times 10^{-6} - 1.0 \times 10^{-4}$</td>
<td>$(4.5 \pm 0.18) \times 10^{-8}$</td>
<td>Amperometry</td>
<td>Present work</td>
</tr>
</tbody>
</table>

The analytical performance of the MCPE/pMWCNTs/SDS was compared with other modified electrodes and the results are displayed in Table 4.3. The preparation of Pt/P. chrysosporium involves the pretreatment of Pt electrode and the immobilization of P. chrysosporium which is a complex procedure. Also, it requires the presence of a mediator such as $K_3[Fe(CN)_6]$ for the proper functioning of Pt/P. chrysosporium, with an optimum response at pH 4.5, which restricts its applications for biological samples. The preparation of MCPE/pMWCNTs/SDS is relatively simpler and eliminates the need for exhaustive pretreatment and processing steps. Analytical characterization of Ru/WGE was carried out at pH 4.0 and the real sample analysis hasn't been carried out. Au–Cys–SWCNT–CoTAPc involves a tedious preparation procedure and the use of Au electrode and the modifier, SWCNT makes it an expensive sensor, apart from its requiring a chemical modification of
SWCNT in its preparation. The D and k values for electrochemical oxidation of EP are much higher at MCPE/pMWCNTs/SDS as compared to MDWCNTPE. GCE has to undergo a rigorous pretreatment prior to the preparation in the case of GCE/poly(taurine) and OMC/Nafion/GCE. The utility of the sensor for the estimation of EP in biological samples hasn't been demonstrated for GCE/poly(taurine). Preparations of Au/4α−NiII TAPc SAMF and DMSA/Au suffer from a long time of 48.0 and 30.0 h respectively spent in their preparation and are expensive too. Moreover, the electrochemical determination of EP at Au/4α−NiII TAPc SAMF has been carried out in an acidic medium. The Au electrode employed for modification, requires pretreatment prior to its modification. A higher oxidation overpotential has been reported at MZ−CPE for the determination of EP compared to MCPE/pMWCNTs/SDS with an additional advantage of a twofold higher value of D for MCPE/pMWCNTs/SDS. A higher energy is required for the oxidation EP at MIP/PIL−MWNT/ITO as compared to MCPE/pMWCNTs/SDS apart from a cumbersome two stages involved in its preparation. The sensor suffers from a set back as it has not been tried for any analytical applications. Looked at from various angles, MCPE/pMWCNTs/SDS has the much desired properties for a modified electrode to be used as a sensor. The properties - a relatively easy method of modification, inexpensiveness, freedom from pre-treatment, good reproducibility, sensitivity, stability, better linear dynamic range, amenability to be used at physiological pH and better detection limits - makes it superior and more convenient for use as a sensor as compared to many modified electrodes reported in the literature for the detection of EP.

4.9.11 Interference studies

EP co-exists with many biomolecules in biological matrix. Many of such molecules are electroactive and have redox potential similar to EP. In order to rule out the possibility of interference, effect of these molecules on the amperometric determination of EP was investigated. Amperometric response of interfering molecules was investigated at MCPE/pMWCNTs/SDS after fixing its potential at 215.0 mV versus SCE. It is evident from Fig. 4.15 that 1.0 × 10⁻⁵ M 5-HT, AAP, FA, UA, Trp, Tyr and Cys have not given any amperometric signal at MCPE/pMWCNTs/SDS. It was also observed that concentration of DA less than 3.0 × 10⁻⁷ M and AA at concentrations lower than 1.0 × 10⁻⁶ M have not been shown to be detected at MCPE/pMWCNTs/SDS. The individual amperometric analysis of DA and AA at MCPE/pMWCNTs/SDS reveals that the lowest concentration of DA and AA
that can be detected at this electrode at step potential of 215.0 mV is $3.0 \times 10^{-7}$ M and $1.0 \times 10^{-6}$ M, respectively. The DA and AA interfere with amperometric detection of EP above these concentrations at MCPE/pMWCNTs/SDS. The CV studies of DA and AA at MCPE/pMWCNTs/SDS indicate that Epa of these molecules are lower than the step potential of EP. Hence, it co-oxidizes with EP at higher concentrations. AA exists as anion while DA exists as a cation at physiological pH. AA was repelled at MCPE/pMWCNTs/SDS while DA got attracted. This leads to an increased sensitivity for DA while the sensitivity for AA is decreased at MCPE/pMWCNTs/SDS. Since the MCPE/pMWCNTs/SDS is completely free from the interference of all the above mentioned molecules at their significantly high concentrations, it can be used as potential electrochemical sensor for EP.

4.9.12 Reproducibility and stability of the electrode

A series of six modified electrodes were prepared in the same manner and amperometric response of $5.0 \times 10^{-6}$ M EP was recorded consecutively. A RSD of 2.7% observed for these measurements indicate an excellent reproducibility of MCPE/pMWCNTs/SDS.

It was also found that properly stored MCPE/pMWCNTs/SDS could retain 93.6%
of initial amperometric response for $5.0 \times 10^{-6}$ M EP even after two weeks. This confirms the long term stability of MCPE/pMWCNTs/SDS. The amperometric response of $1.0 \times 10^{-4}$ M EP at MCPE/pMWCNTs/SDS over a period of half an hour is displayed in Fig. 4.16. The amperometric response remains constant throughout the experiment. Hence, it can be concluded that MCPE/pMWCNTs/SDS is stable and does not undergo any surface fouling.

![Graph of Current vs Time](image)

Figure 4.16: Amperometric response of $1.0 \times 10^{-4}$ M EP at MCPE/pMWCNTs/SDS for 30.0 minutes in 0.1 M KPBS of pH 7.0. Applied potential: 215.0 mV.

4.9.13 Analytical applications of MCPE/pMWCNTs/SDS

The ultimate use of any sensor depends on how effectively it could be applied to the detection of an analyte in real samples. In this respect, the sensor was put to test for its analytical utility. The MCPE/pMWCNTs/SDS was employed to determine the EP content in adrenaline tartrate injection and human blood serum by using a procedure described in chapter 2, section 2.14. Good quantitative recoveries were obtained for EP; both in adrenaline tartrate injection and the blood serum at MCPE/pMWCNTs/SDS. The results are listed in Table 4.4. This procedure was repeated five times ($n = 5$) for each sample. The values of relative standard deviation (RSD) for all analysis were less than 3.0 % which is an indication of good precision of MCPE/pMWCNTs/SDS in EP analysis. These observations ascertain the utility of MCPE/pMWCNTs/SDS as a reliable probe for the analysis of EP, both in pharmaceuticals and biological fluids.
Table 4.4: Results of real sample analysis using MCPE/pMWCNTs/SDS.

<table>
<thead>
<tr>
<th>Real samples</th>
<th>EP added (µM)</th>
<th>EP found (µM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood serum</td>
<td>1.0</td>
<td>1.03 ± 0.0014</td>
<td>103.0</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.93 ± 0.0018</td>
<td>97.7</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.95 ± 0.0012</td>
<td>99.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Adrenaline injection</td>
<td>0.2</td>
<td>0.19 ± 0.0011</td>
<td>95.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.41 ± 0.0008</td>
<td>102.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.58 ± 0.0012</td>
<td>97.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

4.10 Conclusions

The present work demonstrates that MCPE/pMWCNTs/SDS with adequate sensitivity, selectivity and stability can be used successfully as an amperometric sensor for the determination of EP. The MCPE/pMWCNTs had significant influence as a supporting material for the modification using SDS in comparison with MCPE/MWCNTsOX in the performance of the sensor in terms of better current sensitivity, oxidation overpotential and background current. The modified electrode exhibited excellent electrocatalytic activity towards the oxidation of EP. 100-fold excess of 5-HT, AAP, FA, UA, Trp, Tyr and Cys did not interfere in the determination of EP at MCPE/pMWCNTs/SDS. 10-fold excess of AA and twofold excess of DA do not alter the amperometric signal of EP. Small amounts of the modifiers used were sufficient to bring about an impressive electrocatalytic response. The pMWCNTs used for the modification can be used directly without subjecting them to any kind of processing. Practical applications of MCPE/pMWCNTs/SDS have been demonstrated by estimating EP in pharmaceuticals and in body fluids. A wide linear dynamic range, low detection limit, simple fabrication procedure and quick response proved that MCPE/pMWCNTs/SDS is an efficient electrochemical sensor for the determination of EP in various matrices.