Electrochemical detection of cholesterol on screen printed electrode

- Synthesis and characterization of Fe$_3$O$_4$ and Fe$_3$O$_4$/Pd nanoparticle
- Direct electrochemistry of ChOx on (Fe$_3$O$_4$/Pd/PDDA/COO$^-$-MWCNTs)/SPE
- Effect of scan rate and pH at ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO$^-$-MWCNTs)/SPE
- Determination of cholesterol at enzyme modified electrode
Overview

A simple and facile microwave method was adopted to prepare Fe₃O₄ and Fe₃O₄/Pd nanoparticles, which possess the mean particle diameter of 10 nm and 90 nm, respectively. Formation of Fe₃O₄ and Fe₃O₄/Pd nanoparticles were confirmed from pXRD, TEM, EDX, and FT-IR spectroscopy techniques. Negatively charged multiwalled carbon nanotubes were wrapped with positively PDDA followed by coating with Fe₃O₄/Pd nanoparticle to get (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite. This composite was used for the determination of cholesterol by using ChOx enzyme on SPE. (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite provides biocompatible microenvironment for the ChOx to exhibit DET on electrode surface. The linear range of the enzyme modified SPE was found to be 10-80 μM (R=0.9972) with a detection limit of 1 μM of cholesterol. The sensitivity of the enzyme modified SPE was found to be 10.45 μA μM⁻¹ cm⁻².
5.1 Introduction

The magnetic nanoparticles in general and iron oxide (Fe₃O₄) in particular have attracted an increasing interest in the development of nanostructured materials for the use in biotechnology and medicine [1]. The main advantage of magnetic nanoparticles is that they can be easily separated from their matrix by an external magnetic field. Some of the common features associated with the Fe₃O₄ nanoparticles are good biocompatibility, strong super paramagnetic property, high surface area, low toxicity and ease of preparation [2, 3]. Fe₃O₄ nanoparticles have been used in a wide range of potential applications such as, electrochemical sensors/biosensors [4], catalysis [5], immunoassays [6], data storage [7] etc. Various methods have been used for the synthesis of magnetic nanoparticles which includes, hydrothermal synthesis [8], co-precipitation [9], sol-gel [10], microwave irradiation [11] etc. The later method has significant advantage with respect to higher reaction rates and product yields in a shorter period of time.

The importance of cholesterol and various enzyme immobilization methods for electrochemical determination of cholesterol has been given in the section 4.1 of chapter 4. Furthermore detailed literature survey on electrochemical determination of cholesterol is given in the section 2.1 of chapter 2.

In the present work, a simple and facile microwave method was adopted to prepare Fe₃O₄ and Fe₃O₄/Pd nanoparticles by following the reported procedure with slight modification [12]. Formation of both Fe₃O₄ and Fe₃O₄/Pd nanoparticles were confirmed by pXRD, TEM, EDX and FT-IR analysis. Negatively charged Fe₃O₄/Pd nanoparticles were mixed with positively charged PDDA wrapped MWCNTs to get negatively charged novel composite. This negatively charged novel composite was drop casted on the SPE.
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Positively charged ChOx (pH 4.0) was immobilized on the composite by drop casted method. Enzyme modified screen printed electrode showed electrocatalytic activity towards the detection of cholesterol. CV and EIS techniques were used to characterize the enzyme modified screen printed electrode. Above mentioned novel composite provides biocompatible microenvironment for the ChOx to exhibit DET on SPE.

5.2 Synthesis of Fe₃O₄ and Fe₃O₄/Pd nanoparticle, Enzyme solution preparation and Preparation of enzyme modified screen printed electrode

The detailed procedure for synthesis of Fe₃O₄ and Fe₃O₄/Pd nanoparticles are given in the section 3.4 of Chapter 3. Similarly, enzyme solution preparation (100 U/mL) and preparation of enzyme modified screen printed electrode are given in the section 3.3.2 and 3.8.2 of Chapter 3 respectively.

![Schematic representation for the fabrication of enzyme electrode based on the (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite.](image)

**Fig. 5.1** Schematic representation for the fabrication of enzyme electrode based on the (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite.
5.3 Characterization of Fe$_3$O$_4$ and Fe$_3$O$_4$/Pd nanoparticles using XRD, TEM, EDX and FT-IR techniques

Fig. 5.2a shows XRD patterns of the synthesized Fe$_3$O$_4$ nanoparticles. Diffraction peaks of Fe$_3$O$_4$ nanoparticles were obtained at 30.18°, 35.54°, 43.29°, 53.69°, 57.29° and 62.96° corresponding to the index planes (220), (311), (400), (422), (511) and (440) respectively. This is quite identical to pure Fe$_3$O$_4$ nanoparticles and matched well with that of JCPDS no. 82-1533. This revealed that the Fe$_3$O$_4$ nanoparticles have a cubic spinel structure [13, 14]. Also, no characteristic peaks of impurities were observed.

![XRD patterns and FT-IR spectrum](image)

**Fig. 5.2A (a)** XRD patterns of Fe$_3$O$_4$ (a) and Fe$_3$O$_4$/Pd (b) nanoparticles. **B.** FT-IR spectrum of Fe$_3$O$_4$ nanoparticles.

From the XRD data, the mean particle diameter of Fe$_3$O$_4$ nanoparticles was calculated from index planes (220), (311) and (400) by using Debye-Schererrer’s equation, which is given below.

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$  \hspace{1cm} (1)

Where, $\lambda$- wavelength of X-ray, $\beta$- full width at half maximum and $\theta$- Bragg’s diffraction angle. The mean particle diameter of Fe$_3$O$_4$ nanoparticles was found to be 10 nm. These
results infer that the Fe$_3$O$_4$ nanoparticles can be rapidly synthesized within 5-10 minutes. Usually most of the methods for the synthesis of Fe$_3$O$_4$ nanoparticles need more than an hour [15].

Formation of Fe$_3$O$_4$ nanoparticles by microwave irradiation, can be explained as follows

$$\text{Fe}^{2+} + 2\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2 + 2\text{NH}_4^+ \quad (2)$$

When ammonia is added to the FeSO$_4$ solution, Fe(OH)$_2$ is formed according to equation 2, which is oxidized to Fe$_3$O$_4$ nanoparticles under the influence of microwave irradiation as follows

$$3\text{Fe(OH)}_2 + \frac{1}{2}\text{O}_2 \xrightarrow{\text{Microwave Radiations}} \text{Fe}_3\text{O}_4 + 3\text{H}_2\text{O} \quad (3)$$

Similarly Fig. 5.2b shows XRD patterns of the Fe$_3$O$_4$ nanoparticles decorated on Pd. The addition diffraction peaks at 40.07°, 46.54° and 68.09° corresponding to the (111), (200) and (220) lattice planes were attributed to the formation of Pd nanoparticles [16, 17]. The mean particle diameter of Fe$_3$O$_4$/Pd nanoparticles was found to 90 nm according to equation 1. The possible mechanism for the formation of Fe$_3$O$_4$/Pd is shown below.

$$2 \text{Fe}^{2+} + \text{[Pd(NH}_3)_4]^+ + 2\text{OH}^- + 4\text{H}_2\text{O} \rightarrow 2 \text{Fe(OH)}_3 + \text{Pd} + 4\text{NH}_4^+ \quad (4)$$

$$\text{Fe}^{2+} + 2\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2 + 2\text{NH}_4^+ \quad (5)$$

$$2 \text{Fe(OH)}_3 + \text{Fe(OH)}_2 + \text{Pd} \xrightarrow{\text{Microwave Radiations}} \text{Fe}_3\text{O}_4/\text{Pd} + 4\text{H}_2\text{O} \quad (6)$$

FT-IR data of Fe$_3$O$_4$ nanoparticles is shown in Fig. 5.2B. It is noteworthy that in Fig. 5.2B, peak at 545 cm$^{-1}$ is attributed to the Fe-O bond vibration of Fe$_3$O$_4$ [18]. The broad peak at 3346 cm$^{-1}$ is due to stretching vibrations of -OH bond, which is absorbed by Fe$_3$O$_4$ nanoparticles. Also, the peak at ~1610 cm$^{-1}$ may be assigned to the deformation vibrations of water molecules trapped onto the magnetic nanoparticles [19]. These results confirm the formation of Fe$_3$O$_4$ nanoparticles. There was no major change in the FT-IR spectrum
of Fe$_3$O$_4$/Pd. Fig. 5.3A, B shows TEM images of Fe$_3$O$_4$ and Fe$_3$O$_4$/Pd nanoparticles respectively. Both Fe$_3$O$_4$ and Fe$_3$O$_4$/Pd nanoparticles appeared to be almost spherical in shape.

**Fig. 5.3A** TEM (a) HRTEM (b) images of Fe$_3$O$_4$ nanoparticles. **B.** TEM (a) HRTEM (b) images of Fe$_3$O$_4$/Pd nanoparticles. **C.** Bright field TEM image Fe$_3$O$_4$/Pd nanoparticles (a), the corresponding EDX maps depict the distribution of constituting individual elements within the structure as shown in (b-d). The images correspond to the (b) Fe, (c) Pd, and (d) O.
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TEM image of Fig. 5.3B (a) clearly depicts the presence of slightly bigger Pd nanoparticles (dark contrast) surrounded by Fe3O4 nanoparticles, which is evidenced in the difference in contrast. The average diameter of Fe3O4 nanoparticles was found to be 12.8 nm, whereas, the average particle size of Fe3O4/Pd nanoparticles was found to be 92.4 nm. These results are in well agreement with XRD results shown in Fig. 5.2A. The high resolution TEM (HRTEM) images of Fe3O4 and Fe3O4/Pd nanoparticles are shown in Fig. 5.3A (b) & 3B (b), respectively. The distance between two lattice planes of the Fe3O4 crystallite was 0.252 nm, which corresponds to the (311) plane of spinel Fe3O4. In the same way, HRTEM image of Fe3O4/Pd nanoparticle showed well resolved lattice fringes with a distance of 0.225 nm, corresponding to the (111) plane of cubic Pd. Furthermore, the presence of Pd nanoparticles in Fe3O4 was also confirmed using scanning transmission electron microscope (STEM) coupled with elemental mapping analysis (STEM-EDS). Fig. 5.3C (a) shows a bright field TEM image of Fe3O4/Pd nanoparticles. The corresponding elemental maps are given in Fig. 5.3C (b-d). The EDS mapping results suggest that metallic Pd core is surrounded by several Fe3O4 nanoparticles.

Fig. 5.4 displays the SEM images of unmodified SPE (a), (PDDA/COO−-MWCNTs)/SPE (b), (Fe3O4/Pd/PDDA/COO−-MWCNTs)/SPE (c) and ChOx-(Fe3O4/Pd/PDDA/COO−-MWCNTs)/SPE (d). The morphology of the unmodified SPE exhibits rough surface having different size grains of several microns. PDDA coated MWCNTs composite is uniformly deposited on SPE, which can be seen in image (b). Nanosized Fe3O4/Pd particles appear as small white grains which are incorporated into (PDDA/COO−-MWCNTs) composite clearly visible in image (c). Image (d) shows
uniform immobilization of ChOx enzyme on (Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs) composite. Inset shows the different size granules of ChOx enzyme.

![SEM images of unmodified SPE (a), (PDDA/COO'-MWCNTs)/SPE (b), (Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE (c) and ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE (d).](image)

**Fig. 5.4** SEM images of unmodified SPE (a), (PDDA/COO'-MWCNTs)/SPE (b), (Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE (c) and ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE (d).

### 5.4 Characterization of ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE using CV and EIS

Fe(CN)$_6^{3/-4}$ redox couple is widely used as an electrochemical probe to characterize the property of unmodified/modified electrodes. Fig. 5.5 illustrates, the cyclic voltammograms of SPE, (Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE and ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO'-MWCNTs)/SPE in 5 mM Fe(CN)$_6^{3/-4}$ containing PBS (pH 7.0) at scan rate of 50 mVs$^{-1}$. Irreversible voltammogram was observed at SPE (curve a, dotted line) with minimal cathodic peak current and anodic peak current. The cathodic peak potential...
and anodic peak potential were found at 521 mV and -256 mV respectively with peak to peak separation 777 mV. However, SPE modified with (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite, showed a well redox peak of Fe(CN)₆³⁻/⁴⁻ with $\Delta E_p$ 48 mV (curve b). These results depict that the over potential decreased by 729 mV and around 2 fold increase in current was observed for 5 mM Fe(CN)₆³⁻/⁴⁻ at (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE than with SPE. This shows the electrocatalytic activity of (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite. After immobilization of ChOx enzyme on (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE, decrease in peak current was observed (curve c). This could be attributed to macromolecular non conducting enzymatic structure, which impedes the electrochemical redox reaction of Fe(CN)₆³⁻/⁴⁻ at electrode surface. This also demonstrates that ChOx enzyme was successfully immobilized on (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite by means of electrostatic attraction.

![Cyclic voltammograms of unmodified SPE (a), Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (b) and ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (c) in 0.1 M PBS containing 5 mM Fe(CN)₆³⁻/⁴⁻ (pH 7.0); scan rate: 50 mVs⁻¹.](image)

**Fig. 5.5** Cyclic voltammograms of unmodified SPE (a), Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (b) and ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (c) in 0.1 M PBS containing 5 mM Fe(CN)₆³⁻/⁴⁻ (pH 7.0); scan rate: 50 mVs⁻¹.

EIS is a powerful and sensitive characterization tool for studying the charge transfer process at electrode/electrolyte interface [20]. Hence, characterization of SPE,
(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE and ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE was further investigated using EIS. EIS was carried out in the presence of 5 mM Fe(CN)₆⁴⁻/³⁻ as an electrochemical redox probe, in the frequency range of 100 kHz to 0.1 Hz with amplitude of 5 mV as shown in Fig. 5.6A. The equivalent circuit shown in the inset of Fig. 5.6B was used to fit experimental data. The simulated curve of experimental data and best fitting equivalent circuit are shown in the Fig. 5.6C. The obtained impedance data are shown in Table 5.1. The circuit includes the solution resistance, charge transfer resistance, double layer capacitance, Warburg impedance, Faradaic resistance, and Faradaic capacitance. At SPE, big semicircle having a $R_{ct}$ of 5714 Ω was observed for Fe(CN)$_6^{4-/3-}$ (curve a), which suggests that unmodified SPE exhibits sluggish and unfavorable Fe(CN)$_6^{4-/3-}$ electron transfer.

![Nyquist impedance plots](image)

**Fig. 5.6A** Nyquist impedance plots of unmodified SPE (a), Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (b) and ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE (c) in 0.1 M PBS containing 5 mM Fe(CN)$_6^{4-/3-}$ (pH 7.0). The frequency range is from 100 kHz to 0.1 Hz and
amplitude 5 mV. B. The equivalent circuit used to fit experimental data. C. Nyquist impedance plots of experimental data of ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE and best fitting by using equivalent.

However, SPE modified with (Fe₃O₄/Pd/PDDA/COO'-MWCNTs) composite, the big semicircle was replaced with straight line, which has an $R_c$ of 0.19 Ω as shown in Fig. 5.6A inset (curve b). This shows (Fe₃O₄/Pd/PDDA/COO'-MWCNTs) composite facilitates fast and favorable electron transfer towards electrode surface. Small semicircle was observed (curve c) with a $R_c$ of 557 Ω, when ChOx enzyme was immobilized on (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE, which illustrates that non conducting macromolecular ChOx was successfully immobilized on modified SPE and it opposes the electron transfer towards electrode surface.

Table 5.1 EIS data of unmodified SPE, (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE and ChOx- (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE in 5 mM Fe(CN)₆³⁻/⁴⁻.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>$R_s/\Omega$</th>
<th>$n$</th>
<th>$Q/\mu F$</th>
<th>$R_c/\Omega$</th>
<th>$W/\Omega$</th>
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</thead>
<tbody>
<tr>
<td>SPE</td>
<td>189</td>
<td>0.95</td>
<td>0.49</td>
<td>5714</td>
<td>0.0009</td>
</tr>
<tr>
<td>Fe₃O₄/Pd/PDDA/COO'-MWCNTs/SPE</td>
<td>198</td>
<td>0.97</td>
<td>0.23</td>
<td>0.19</td>
<td>0.006</td>
</tr>
<tr>
<td>ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE</td>
<td>196</td>
<td>0.78</td>
<td>14.5</td>
<td>557</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

5.5 Direct electrochemistry of ChOx on (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE

Fig.5.7 shows, cyclic voltammogram of ChOx on unmodified SPE (curve a) and (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE (curve b). When ChOx was immobilized on unmodified SPE, it does not facilitate DET. This illustrates that unmodified SPE does not provide micro environment for the immobilization of ChOx. However, ChOx immobilized on (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE exhibits a pair of well defined
redox peaks at -0.365 and -0.443 V. These peaks are assigned to FAD/FADH₂, which could be ascribed to electron transfer between ChOx and under laying electrode [21-23]. The potential difference between the two peaks ΔEₚ was 78 mV at a scan rate of 50 mVs⁻¹, which suggests that ChOx has undergone a quasi-reversible redox reaction (Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE. The surface area (I) of ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE was calculated using the following equation

\[ \tau = \frac{Q}{nFA} \] (7)

Where, Q is the charge, n is the number of electrons transferred, F is the Faraday constant. Therefore τ was found to be \(5.38 \times 10^{-9}\) mol cm⁻².

Fig. 5.7 Cyclic voltammograms of ChOx-unmodified SPE (a) and ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE in 0.1 M PBS (pH 7.0) scan rate:50mVs⁻¹.

5.6 Effect of scan rate and pH at ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE

To determine the kinetics of electrode reactions, the effect of scan rate on the voltammetric response of ChOx-(Fe₃O₄/Pd/PDDA/COO'-MWCNTs)/SPE in a 0.1 M PBS
of pH 7 was studied in the range of 5-75 mVs$^{-1}$ as shown in Fig. 5.8A. The linear regression equations are as given below

$$I_{pa} = -6.6628 \times 10^{-7} + 5.4642 \times 10^{-7} \text{v (Vs}^{-1}\text{);} R = 0.9981 \quad (8)$$

$$I_{pc} = -1.4345 \times 10^{-5} - 6.3799 \times 10^{-7} \text{v (Vs}^{-1}\text{);} R = 0.9932 \quad (9)$$

The redox peak current of ChOx increased linearly with increasing scan rate (Fig. 5.8B) and the peak to peak separation also increased, indicating that surface controlled quasi-reversible process is involved. In addition, the anodic peak potential shifted to a more positive potential value with increasing scan rate, whereas the cathodic peak potential shifted in a negative direction.

![Figure 5.8A](image)

**Fig. 5.8A** Cyclic voltammograms of ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO$^-$-MWCNTs)/SPE in PBS (pH 7.0) at different scan rates: (5-75 mV$^{-1}$). **B.** The plot of peak current vs. scan rate.

The pH of the electrolyte solution has a significant influence on the redox reaction of FAD/FADH$_2$ of ChOx with respect to peak current and peak potential. Fig. 5.9A. shows cyclic voltammograms of ChOx-(Fe$_3$O$_4$/Pd/PDDA/COO$^-$-MWCNTs)/SPE in response to effect of pH of electrolyte in the range 4 to 8. The electrochemical response of enzyme
immobilized on the electrode surface is due to redox reaction of its active site, i.e. FAD/FADH₂. Where, FAD is known to undergo redox reaction involving two electrons with two protons to form FADH₂. Due to protons involved in the reaction, the acidity of the solution has a significant effect on the redox potential of ChOx. Thus, the anodic and cathodic peak potentials of ChOx immobilized on the (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE should be pH dependent. It was observed that, redox peak potential of the enzyme shifted towards negative side with increase in pH as shown in Fig. 5.9B, indicating that protons are involved in the redox reaction. A good linear relationship was obtained between half wave potential ($E_{1/2}$) and the solution pH. The corresponding linear regression equation is given as

$$E_{1/2} = -0.025 - 0.058 \text{pH}; \quad R = 0.9908 \quad (10)$$

From the above equation, slope of $E_{1/2}$ is 58 mV, which is close to the theoretical value (59 mV pH⁻¹) for a classical Nernstian two electrons and protons process. Hence, ChOx redox system is a two proton, two electron redox process.

Fig. 5.9A. Cyclic voltammograms of ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE at various pHs of the solution (pH 4-8) scan rate: 50mVs⁻¹. B. Plots of potential vs. $E_{1/2}$ at pH (4-8).
5.7 Determination of cholesterol based on the direct electrochemistry of ChOx on the (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE

In this protocol, the direct electrochemistry of ChOx is based on the redox reaction of its active center, i.e. FAD, in the absence of oxygen; direct electron transfer of immobilized ChOx can be expressed as follows

\[
\text{ChOx-FAD} + 2\text{H}^+ + 2e^- \rightleftharpoons \text{ChOx-FADH}_2
\]  \hspace{1cm} (11)

In the presence of oxygen, the reduced enzyme is oxidized very quickly at the electrode surface. Electron transfer turnover rate of the molecular oxygen is about 700 s⁻¹ to accept electrons [24]. This is much faster than that of ChOx on the (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE. As a result, obvious electrocatalytic process towards the reduction of dissolved oxygen is given below

\[
\text{ChOx-FADH}_2 + \text{O}_2 \rightarrow \text{ChOx-FAD} + \text{H}_2\text{O}_2
\]  \hspace{1cm} (12)

Fig. 5.10 Cyclic voltammograms obtained at ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE in nitrogen saturated and oxygen saturated PBS (a and b), after addition of 50 μM cholesterol to oxygen saturated PBS (c). Scan rate: 50mVs⁻¹

The catalytic regeneration of the enzyme to its oxidized form causes the loss of reversibility, as a result increase in the size of the reduction peak is seen as shown in
Fig. 10 (curve b) [25]. By the addition of cholesterol, a competitive reaction take place at the vicinity of the enzyme modified electrode surface. Thus, leading to the decrease of reduction peak current (curve c) and as a result the sensitive determination of cholesterol. In other words, in the presence of oxygen, ChOx on modified electrode will catalyze the oxidation of cholesterol according to the following enzymatic reaction.

\[
\text{ChOx-FAD + Cholesterol} \rightarrow \text{ChOx-FADH}\text{H}_2 + \text{Choles-4-en-3-one} \quad (13)
\]

The reduction peak of the ChOx-(Fe\textsubscript{3}O\textsubscript{4}/Pd/PDDA/COO\textsuperscript{-}-MWCNTs)/SPE in oxygen saturated PBS (pH 7.0), decreased with addition of cholesterol, which suggests that the immobilized ChOx still retains its enzymatic activity. This could be due to the biocompatible, microenvironment provided by (Fe\textsubscript{3}O\textsubscript{4}/Pd/PDDA/COO\textsuperscript{-}-MWCNTs) composite. Thus, the addition of cholesterol restrains electrocatalytic reaction between the oxidized form of ChOx i.e. ChOx-FAD and cholesterol, which attenuates the concentration of the ChOx-FAD. This causes decrease in the reduction peak current of the enzyme [23]. Also, the dissolved oxygen mediates the enzymatic oxidation of cholesterol by ChOx. Therefore, the depletion of the oxygen proximal to the electrode surface makes the reduction of the oxidized form of ChOx less favorable, leading to the decrease of the reduction peak current of the enzyme [25]. Eventually, cholesterol is determined by measuring the decreased reduction peak current, by the addition of cholesterol in oxygen saturated PBS (pH 7.0). Fig.5.11A shows the differential pulse voltammograms of various cholesterol concentrations at ChOx-(Fe\textsubscript{3}O\textsubscript{4}/Pd/PDDA/COO\textsuperscript{-}-MWCNTs)/SPE in oxygen saturated PBS (pH 7.0). The reduction current decreased gradually upon increasing cholesterol concentration. Fig.5.11B shows the calibration current
corresponding to decrease of reduction current and concentration of cholesterol. The linear regression equation is given by:

\[ I_{pc} (\text{Cholesterol}) (\mu A) = -4.9712E-4 + 7.4202E-7 C(\text{Cholesterol}) (\mu A); \quad R = -0.9972 \quad (14) \]

Fig. 5.11A Differential pulse voltammetric measurements at ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE at oxygen saturated PBS (pH 7.0), without cholesterol (a) and (b-j) with cholesterol of 10, 20, 30, 40, 50, 60, 70, 80 and 90 µM. DPV parameters: scan rate: 20 mV s⁻¹, pulse height: 200 mV, pulse width: 0.05 s, step height: 10 mV and step width: 0.5 s. B. shows relationship between \( I_{pc} \) and concentrations of cholesterol.

Using slope of the above equation, the sensitivity of the enzyme modified SPE was calculated to be 0.742 µA µM⁻¹ or 10.45 µA µM⁻¹ cm⁻² (area of electrode surface is 0.071 cm²). Comparison of the enzyme modified SPE with other cholesterol determination based on SPEs are given in Table 5.2 [26-28]. Results shown in Table 5.2, depicts that the sensitivity of the ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE is much better when compared to other literature on cholesterol based on SPEs. Furthermore, applied potential and detection limit of the ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE is quite comparable with respect to other reports on cholesterol based on SPEs.
## Table 5.2 Comparison of the ChOx enzyme SPE with other ChOx based SPEs.

<table>
<thead>
<tr>
<th>Cholesterol Biosensor</th>
<th>Sensitivity (µA µM⁻¹)</th>
<th>Potential applied (mV)</th>
<th>Linear range (µM)</th>
<th>Detection limit (µM)</th>
<th>Reference</th>
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<td>GNS-nPt/SPE</td>
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<td>-400</td>
<td>10-70</td>
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<td>-600</td>
<td>50-300</td>
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<td>[28]</td>
</tr>
<tr>
<td>ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE</td>
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<td>-380</td>
<td>10-80</td>
<td>1</td>
<td>Present work</td>
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</table>

### 5.8 Stability and reproducibility of the ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE

Direct electron transfer of ChOx on (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE is very stable. When twenty consecutive CV curves obtained at ChOx-(Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE in 0.1 M PBS pH 7.0 at scan rate of 50 mVs⁻¹ were compared, there was no change in the peak to peak separation. However, peak current gradually decreased. The electrode retained 86.6% of its initial response after twenty consecutive cycles. These results show that ChOx binds strongly on (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite. To ascertain fabrication reproducibility, five sets of ChOx on (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs)/SPE were fabricated for the determination of cholesterol. The results show that the enzyme modified SPE had satisfying reproducibility with the relative standard deviation (RSD) of 9.5%.

### 5.9 Conclusion

Fe₃O₄ and Fe₃O₄/Pd nanoparticles were synthesized by simple and facile microwave method. Formation of Fe₃O₄ and Fe₃O₄/Pd nanoparticles were confirmed from pXRD and FT-IR techniques. Fe₃O₄/Pd nanoparticles were used for the preparation of biocompatible composite which comprised of negatively charged multiwalled carbon...
nanotubes (COO'-MWCNTs) wrapped with positively charged PDDA. This composite was successfully used for the determination of cholesterol by using cholesterol oxidase (ChOx) enzyme on screen printed electrode (SPE). DET of ChOx was observed on (Fe₃O₄/Pd/PDDA/COO'-MWCNTs) composite which shows that the composite provides biocompatible microenvironment for the ChOx. The linear range of the enzyme modified SPE was found to be 10-80 μM with detection limit of 1 μM. Common interferents such as ascorbic acid, uric acid and glucose did not cause any interference because of low operating potential.

5.10 References


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