Platinum Nanoparticles (PtNPs) With Poly (Tyramine) - Choline Oxidase (ChOx) Film for the Electrocatalysis Of Choline

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Platinum nanoparticles (PtNPs) are directly fabricated on glassy carbon electrode (GCE) and indium tin oxide electrode (ITO) by simple electrochemical deposition process. The Pt nanoparticles modified ITO electrode surface has been studied in detail using scanning electron microscopy (SEM) and atomic force microscopy (AFM). This PtNPs film modified GCE effectively exhibits the electro oxidation signals for the detection of H₂O₂. Poly Tyramine (pTy) provided –NH₃ group for Choline oxidase (ChOx) base on modified electrode. The Cyclic voltammetry (CVs), Linear Sweep voltammetry (LSV) and Differential pulse voltammetry (DPVs) has been used for the measurement of electroanalytical properties of analytes by means of modified electrodes. Especially, the proposed PtNPs/pTy/ChOx film modified GCE successfully showed oxidation for the detection of choline. This sensor shows excellent performance with a sensitivity of 69717 μA mM⁻¹ cm⁻², a linear range of 2.1×10⁻⁷ – 1.9×10⁻⁴ M, and a detection limit of 1.8×10⁻⁷ M. The proposed PtNPs/pTy/ChOx film modified electrode also retains the advantage of easy fabrication, high sensitivity and good repeatability. Future, this type of PtNPs/pTy/ChOx film modified electrode supports the selective detection of choline in clinical diagnosis.

Keywords: Platinum nanoparticles; Choline oxidase; Choline; Tyramine.

1. INTRODUCTION

Platinum (Pt) electrodes are widely used for the electrochemical oxidation of H₂O₂ [1-2]. A high overpotential (0.7V vs. Ag/AgCl) is required for oxidation of H₂O₂. To improve the performance of Pt-based electrode different approaches have been proposed [3-4]. Recently, platinum nanoparticles (PtNPs) have been attracting interest in the electrocatalytic properties towards oxidation/reduction of H₂O₂. It increases the surface areas with enhanced mass transport, decreases the overpotential for
oxidation of H$_2$O$_2$ [5-10]. Nanomaterials have attracted much attention for designing novel biosensing systems with enhanced performance of bioanalytical assay. Due to these reasons platinum nanoparticles modified electrodes are frequently used for the detection of target analyte H$_2$O$_2$ which is a product of oxidation of the substrates by oxidoreductase enzyme in the presence of oxygen. The PtNPs in combination with other metals such as gold, lead, tin, CNT and polymer showed better electrocatalytic properties than their counterparts [11-17]. Owing to these advantages of PtNPs it becomes significant to develop PtNPs-polymer based on electrochemical sensor with appropriate characteristics such as high sensitivity, fast response time, wide linear range, better selectivity and reproducibility.

Some investigators, however, have covalently attached the enzyme to the polymer [18-20], which can improve enzyme stability in addition to a reduction of leaching. Covalent attachment to the polymer requires the presence of appropriate moieties on the monomers, such as a primary amine, to allow a coupling reaction to proceed. Tyramine is an ideal monomer for the fabrication of enzyme layers as it can be both electropolymerised into an electrode surface [21-22] and can covalently attach the biorecognition molecule via the formation of a peptide linkage through a free amino group. There have been a few studies where polytyramine films have been investigated for biosensor applications [23-26].

Choline is distributed in central and peripheral nervous systems of mammals [27-29]. It is also an important component of phospholipids (lecithin and sphingomyelin), which is required for the synthesis of the neurotransmitter acetylcholine precursor [30]. Choline oxidase (ChOx), were reported for choline determination based on the detection of liberated hydrogen peroxide [31-34]. The choline is oxidized by ChOx in the presence of oxygen and H$_2$O$_2$ is produced [35-37]. This enzyme plays crucial role in the cholinergic neurons by regulating concentration of the neurotransmitter acetylcholine within the synaptic cleft. The nerve agents covalently block the active site serine residue of acetylcholineesterase (AChE) by the process of nucleophilic attack to produce a serine-phosphoester adduct. This irreversible inactivation leads to accumulation of acetylcholine causing cholinergic over stimulation, culminating in death caused by respiratory failure [38-40].

Enzymes could be immobilized by a variety of methods, such as absorption [41-42], covalent attachment [43], cross-linking [44], nanocomposite [45] and noncovalent [46-47]. Encapsulation, as an in situ immobilization method, has the advantage of providing enzyme with mild microenvironment, which would be favorable to maintain its activity and improve its stability [48]. In the process of encapsulating, the immobilization carrier is the crucial factor. Nafion, an ion-exchange polymer, is employed usually in encapsulate process due to its inherent properties, such as resistant to chemical attack. However, the rate of charge transfer in the pure Nafion film is quite low [49]. Nafion has excellent properties as an ion conductor, and it provides a biocompatible interface [50-51]. So many novel electron conductive materials, especially inorganic materials were introduced into Nafion to improve the stability of sensing interface.

Therefore, it is important to improve the sensitivity of H$_2$O$_2$ detection. To achieve our goal, we have utilized the unique properties of PtNPs-polytyramine (pTy), by using the ChOx approach for the determination of choline. This novel system has been developed by electropolymerization of the
tyramine on PtNPs modified glassy carbon electrode (GCE). Then ChOx was immobilized on the PtNPs/pTy modified electrode by stabilizing enzymes through Nafion. The key idea for using PtNPs/pTy modified GC electrode is to improve the electroactivity of H₂O₂.

2. EXPERIMENTAL

2.1. Materials

Choline oxidase (ChOx) (13 units/mg), Choline chloride (>98%), Tyramin (99%) and Hexachlorooplatinate (K₂[PtCl₆]) were purchased from Sigma–Aldrich (St. Louis, U.S.A.). All other chemicals used were of analytical grade and used without further purification. 0.1 M pH 7.0 phosphate buffer solutions (PBS) and pH 1.0 H₂SO₄ solutions were used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements.

2.2. Apparatus

Cyclic voltammetry (CVs), Linear Sweep voltammetry (LSV) and Differential pulse voltammetry (DPVs) were performed in an analytical system model CHI-1205A and CHI-410 potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm²). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by means of SEM (Hitachi S-3000H) and atomic force microscopy (AFM) (Being Nano-Instruments CSPM5000). Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroanch, Germany). All the solutions were purged with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. All the experiments were carried out at room temperature (≈ 25°C).

2.3. Preparation of modified electrode

2.3.1. Preparation of platinum nanoparticles(PtNPs)/pTy

Prior to modification, glassy carbon electrode was polished with 0.05 µm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. After that electrode was immersed in 0.1 M H₂SO₄ solution containing 1mM K₂[PtCl₆] from -0.4 to 0.4 V, scan rate 50 mVs⁻¹ (shows in Fig. 1A). The PtNPs modified electrodes were gently washed with distilled water and dried.
The electropolymerization of Tyramin was done by electrochemical oxidation of Tyramin (0.1 M) on the PtNPs modified glassy carbon electrode using 0.3 M NaOH in methanol. It was performed by consecutive CVs over a suitable potential range of -0.01 to 1.6 V; scan rate = 50 mV s⁻¹ (shows in Fig. 1B). The optimization of poly-Tyramin (pTy) growth potential has been determined by various studies with different electropolymerization potentials [52].

2.3.2. **Immobilization of choline oxidase on the modified electrodes**

The choline oxidase was immobilized onto the modified electrode surface by covalent attachment the enzyme with pTy. 2µL ChOx solution (10 mg/ml in pH 7.0 PBS) was pipetted onto the electrode and the surface was dried for approximately 1 h at room temperature. The PtNPs/pTy/ChOx modified GCE was coated with 2µL Nafion. The resulted biosensor was stored in a refrigerator at 4 °C for used. The film formed on the electrode surface can be expressed as the Scheme 1.
Scheme 1. Electrocatalytic reaction of choline by PtNPs/pTy/ChOx modified electrodes.

Figure 2: (A) Cyclic voltammograms in 0.1M PBS (pH = 7.0) for different electrodes: (a) only ChOx; (b) PtNPs/pTy; (c) PtNPs/pTy/ChOx; (d) PtNPs, Scan rate = 100mV s\(^{-1}\). (B) Electrochemical impedance spectra (EIS) of (a) only ChOx; (b) PtNPs/pTy; (c) PtNPs/pTy/ChOx; (d) PtNPs in pH 7.0 PBS containing 5 × 10\(^{-3}\) M [Fe(CN)\(_6\)]\(^{3-4}\) (Amplitude: 5 mV).
3. RESULTS AND DISCUSSIONS

3.1. Electrochemical characterizations of PtNPs/pTy/ChOx film

In the following experiments, each newly prepared film on GCE has been washed carefully in deionized water to remove the loosely bounded ChOx on the modified electrode. It was then transferred to pH 7.0 PBS for the other electrochemical characterizations. These optimized pH solutions have been chosen to maintain the higher stability (pH = 7.0). Fig. 2 (A) shows different types (a) only ChOx, (b) PtNPs/pTy, (c) PtNPs/pTy/ChOx and (d) PtNPs. The corresponding cyclic voltammetric have been measured at 100 mVs^-1 scan rate in the potential range of 0.2 to -1.0 V. The redox peak current at formal potential $E^0' = -0.52$ V represents the redox peak for PtNPs (curve d ). Curve (a) of figure shows cyclic voltammetric response of ChOx modified electrode. As can be seen, there is no obvious peak in the potential region studied. From this figure, comparison of curve (c) and curve (d), it is found that the presence of ChOx shows the currents decreased, which is similar to that of previous electrochemical impedance spectra (EIS) studies.

3.2. Electrochemical impedance spectra (EIS) of Analysis

The electrochemical activity of PtNPs/pTy/ChOx modified electrode has been examined using EIS technique. Impedance spectroscopy is an effective method to probe the features of surface modified electrodes. The interfacial changes originating from the biorecognition events at the electrode surfaces can be analyzed through impedance spectroscopy [53]. This study was employed to analyze detailed electrochemical activities of modified electrode with individual or mixed components. The complex impedance can be presented as a sum of the real, $Z'$ ($\omega$), and imaginary $Z''(\omega)$, components that originate mainly from the resistance and capacitance of the cell. From the shape of an impedance spectrum, the electron-transfer kinetics and diffusion characteristics can be extracted. The respective semicircle parameters correspond to the electron transfer resistance ($R_{et}$) and the double layer capacity ($C_{dl}$) nature of the modified electrode. Biological materials such as enzymes immobilized on conducting or semiconductor surfaces change the double layer capacitance and interfacial electron transfer resistance of those corresponding electrodes. The knowledge about these equivalent circuit components thus specify the presence of biomaterials and surfactants immobilized on the electrode surface. Fig. 2(B) shows different type films: only ChOx, PtNPs, PtNPs/pTy and PtNPs/pTy/ChOx on GCE using 5 mM Fe(CN)$_6^{3-/4-}$ in pH 7.0 PBS. The semicircle appeared in the Nyquist plot indicates the parallel combination of $R_{et}$ and $C_{dl}$ resulting from electrode impedance [54]. All the above said films exhibit semicircles with variable diameters in the frequency range 0.1 Hz to 100 kHz. The semicircles obtained at lower frequency correspond to a diffusion limited electron transfer process and those at higher frequency represent a charge transfer limited process.

Fig. 2(B) shows the PtNPs exhibits almost a straight curve (d) with a very small depressed semicircle arc ($R_{et}$ = 1646($Z'/\Omega$)) represents the characteristics of diffusion limited electron-transfer process on the electrode surface. At the same time, the PtNPs/pTy modified electrode shows like a depressed semicircle arc with an interfacial resistance due to the electrostatic repulsion between the
charged surface and probe molecule Fe(CN)$_6^{3/-4}$ (b). PtNPs/pTy/ChOx (curve c) modified electrode’s $R_{et}$ has been found as 28637 $(\Omega)$. The increase in the value of electron transfer resistance ($R_{et}$) due to the coating of enzyme as a barrier on electrode surface. Thus, the electron transfer process will become as a slow process on the GCE. Finally, these results clearly illustrate the electrochemical excellent activities of the PtNPs/pTy/ChOx modified GCE, respectively.

Immobilization of enzymes to solid electrode surface is a key step for the design, fabrication and performance of the biosensor, since it is well known that some enzymes retain their activity when they are immobilized [55]. In order to confirm stability of modified electrode, the cyclic voltammetric of the PtNPs/pTy/ChOx electrode using PBS (pH = 7.0) at different scan rates (30 to 2500 mV/s).

![Cyclic voltammograms](image)

**Figure 3.** (A) Cyclic voltammograms of 0.1 M PBS (pH = 7.0) at PtNPs/pTy/ChOx electrode at different scan rate from 30 mV s$^{-1}$ to 2500 mV s$^{-1}$, respectively. Calibration curve for data shows $E_{pa}$ & $E_{pc}$ vs. log(scan rate).

Fig. 3 have shown that the anodic and cathodic peak currents of both the film redox couples which have increased linearly with the increase of scan rates. Calibration curve for data in inset shows $E_{pa}$ & $E_{pc}$ vs. log(scan rate). The $\Delta E_p$ of each scan rate reveals that the peak separation of composite redox couple increases as the scan rate is increased.

### 3.3. Morphological characterization of PtNPs/pTy/ChOx film

In prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. Further, four different films; PtNPs, PtNPs/pTy, only ChOx and
PtNPs/pTy/ChOx have been prepared on the inoxide electrode (ITO) electrode were characterized using SEM.

![SEM images of PtNPs, PtNPs/pTy, ChOx, and PtNPs/pTy/ChOx on ITO electrode.](image)

**Figure 4.** SEM images of (A) PtNPs; (B) PtNPs/pTy; (C) only ChOx; (D) PtNPs/pTy/ChOx on ITO electrode.

From Fig. 4, it is significant that there are morphological differences between the films. It is a well known fact that the prolonged exposure to the electron beam will damage the ChOx films, so an at most care was taken to measure these images. The top views of nano structures Fig. 4(A) on the ITO electrode surface shows uniformly deposited homogeneously dispersed PtNPs on this electrode. The PtNPs/pTy film in Fig. 4(B) reveals that the pTy had covered the entire PtNPs. Comparison of (C) only ChOx and (D) PtNPs/pTy/ChOx reveals, these results in could be explained as the increase in deposition of ChOx over PtNPs, which creates plateaus instead of beads and covers over PtNPs. We can clearly see that the immersed PtNPs/pTy/ChOx have been gathered together and resemble like a circular one, respectively. The same modified ITO electrodes have been used to measure the AFM.
topography images of Fig. 5 (A) PtNPs, (B) PtNPs/pTy, (C) only ChOx and (D) PtNPs/pTy/ChOx electrode. In all these cases the observed morphological structure is similar to that of SEM.

![AFM images](image)

**Figure 5.** AFM images of (A) PtNPs; (B) PtNPs/pTy; (C) only ChOx; (D) PtNPs/pTy/ChOx on ITO electrode.

### 3.4. Electroanalytical response of choline at PtNPs/pTy/ChOx film

The PtNPs/pTy/ChOx film was synthesized on GCE at similar conditions as described in Materials and Methods. Then the PtNPs/pTy/ChOx modified electrode was washed carefully in deionized water and transferred to pH 7.0 PBS for the electrocatalysis of choline. All of the LSV were recorded at the constant time interval of 2 min with nitrogen purging before the start of each experiment. The enzymatic reaction in the use of ChOx as a receptor can be described as follows:

\[
\text{Choline} + 2\text{O}_2 + \text{H}_2\text{O}^{\text{ChOx}} \rightarrow \text{Betaine} + 2\text{H}_2\text{O}_2
\]  \hspace{1cm} (1)
\[
\text{PtNPs} \quad \ce{H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-} \quad (2)
\]

**Figure 6**: Linear Sweep voltammetry (LSV) of PtNPs/pTy/ChOx for the different concentrations of choline in pH 7.0 PBS (a) \(4 \times 10^{-5}\) M, (b) \(5 \times 10^{-5}\) M, (c) \(8 \times 10^{-5}\) M, (d) \(1 \times 10^{-4}\) M, (e) \(5 \times 10^{-4}\) M, (f) \(1 \times 10^{-3}\) M, (a') bare GCE in \(1 \times 10^{-3}\) M choline, scan rate = 100 mVs\(^{-1}\). Inset shows a current vs. concentration plot of choline.

**Figure 7**: Differential pulse voltammetry (DPVs) of PtNPs/pTy/ChOx for the different concentrations of choline in pH 7.0 PBS: (a) 0 M, (b) \(2 \times 10^{-7}\) M, (c) \(5 \times 10^{-7}\) M, (d) \(1 \times 10^{-6}\) M, (e) \(2 \times 10^{-6}\) M, (f) \(3 \times 10^{-6}\) M and (g) \(4 \times 10^{-6}\) M. Inset shows a current vs. concentration plot of Choline.
Fig. 6 shows the electrocatalytic oxidation of choline. Curve (a) to (a’) represent different concentrations of choline: (a) 4 × 10⁻⁵ M, (b) 5 × 10⁻⁵ M, (c) 8 × 10⁻⁵ M, (d) 1 × 10⁻⁴ M, (e) 5 × 10⁻⁴ M, (f) 1 × 10⁻³ M, (a’) bare GCE in 1 × 10⁻³ M choline at scan rate = 100 mV s⁻¹ in the potential range of -0.7 to 0.2 V. Inset shows a current vs. concentration plot of choline. Upon addition of choline a new growth in the oxidation peak at 342 mV of respective analytes have appeared at the current values. An increase in concentration of choline, simultaneously produced a linear increase in the oxidation peak currents of the analytes. The detection limit concentration range for each analyte almost covers the concentration range found in the physiological conditions. The choline is oxidized by ChOx in the presence of oxygen and H₂O₂ is produced. The observations at bare electrode (curve a’) clearly indicate that the fouling effect of the electrode surface with the oxidation products of H₂O₂ is the reason for obtaining the weak single peak for analytes. From all these above results it is clear that PtNPs/pTy/ChOx film is more efficient and exhibits enhanced functional properties comparing to that of bare alone.

3.5. Differential pulse voltammetry (DPVs) of the analytes at PtNPs/pTy/ChOx

Table. 1. Performance comparison of various choline biosensors based choline oxidase.

<table>
<thead>
<tr>
<th>Immobilization matrix</th>
<th>Transducer method</th>
<th>( E_{app} ) (V)</th>
<th>Detect limit (M)</th>
<th>Linearity (M)</th>
<th>Sensitivity (µA mM⁻¹ cm⁻²)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt/pmPD/pTy/ChOx</td>
<td>DPV</td>
<td>0.6</td>
<td>2.5 × 10⁻⁸</td>
<td>-</td>
<td>-</td>
<td>[56]</td>
</tr>
<tr>
<td>GC/MWCNT/(PVS/PAA)₃/ChOx</td>
<td>Amperometry</td>
<td>0.6</td>
<td>2 × 10⁻⁷</td>
<td>5 × 10⁻⁷ - 1 × 10⁻⁴</td>
<td>12.53</td>
<td>[57]</td>
</tr>
<tr>
<td>Pt/ChOx-F127M</td>
<td>Amperometry</td>
<td>0.6</td>
<td>5.0 µM</td>
<td>5.0-800 µM</td>
<td>-</td>
<td>[58]</td>
</tr>
<tr>
<td>GC/Ti-chitosan/ChOx</td>
<td>ECL</td>
<td>-</td>
<td>1 × 10⁻³</td>
<td>1.0 × 10⁻³ - 5 × 10⁻³</td>
<td>-</td>
<td>[4]</td>
</tr>
<tr>
<td>MnO₂/ChOx-chitosan</td>
<td>SWV</td>
<td>0.45</td>
<td>1.0 × 10⁻⁴</td>
<td>1.0 × 10⁻⁴ - 2.1 × 10⁻⁴</td>
<td>-</td>
<td>[59]</td>
</tr>
<tr>
<td>PEDOT/ChOx</td>
<td>UV</td>
<td>-</td>
<td>1.0 × 10⁻⁵ - 5.1 × 10⁻⁵</td>
<td>-</td>
<td>-</td>
<td>[60]</td>
</tr>
<tr>
<td>GC/RTIL/CNT/ChOx</td>
<td>Amperometry</td>
<td>0.45</td>
<td>1 × 10⁻⁴</td>
<td>1 × 10⁻⁴ - 8 × 10⁻⁴</td>
<td>9.069 × 10⁻⁰ (A/M m²)</td>
<td>[61]</td>
</tr>
<tr>
<td>GC/Ti-ChOx-Nafion</td>
<td>ECL</td>
<td>-</td>
<td>7.0 × 10⁻⁸</td>
<td>1.0 × 10⁻⁷ - 5 × 10⁻⁴</td>
<td>-</td>
<td>[62]</td>
</tr>
<tr>
<td>GC/AuPt/GA/ChOx</td>
<td>LSV</td>
<td>-</td>
<td>8 × 10⁻⁴</td>
<td>8 × 10⁻⁴ - 1.0 × 10⁻³</td>
<td>-</td>
<td>[63]</td>
</tr>
<tr>
<td>PtNPs/pTy/ChOx</td>
<td>DPV</td>
<td>0.48</td>
<td>2.1 × 10⁻⁷</td>
<td>2.0 × 10⁻⁷ - 1.9 × 10⁻⁷</td>
<td>69717.1</td>
<td>This work</td>
</tr>
</tbody>
</table>

Differential pulse voltammetry (DPVs); Cyclic voltammetry (CVs); electrochemiluminescent (ECL); Square wave voltammograms (SWV); Linear sweep voltammogram (LSV)

The Differential pulse voltammetry (DPVs) of have been obtained different concentrations of analyte at PtNPs/pTy/ChOx modified GCE, as shown in Fig. 7 shows the electrocatalytic oxidation of choline. The DPVs have been recorded at a constant time interval of 2 min with nitrogen purging before the start of each experiment. Interestingly, the peak currents for choline increases linearly with the increase of analyte concentration. They demonstrate the calibration curves for analyte, which are almost linear for a wide range of concentrations as shown in inset. The detection limit of
PtNPs/pTy/ChOx modified electrodes for choline was $1.8 \times 10^{-7}$ M, which covers the concentration range found in the physiological conditions. From the slopes of the linear calibration curves, the sensitivity of PtNPs/pTy/ChOx modified electrodes was 69717 $\mu$A mM$^{-1}$ cm$^{-2}$. Inset shows a current vs. concentration plot of choline. This sensor has competitive performance when compared with other ChOx based cholesterol biosensors in Table 1.

4. CONCLUSIONS

We have demonstrated application of PtNPs/pTy/ChOx modified electrode for determination of choline. The modified electrode showed stable response. This feature provides a favorable clinical diagnosis for the electrocatalytic oxidized of choline at PtNPs/pTy/ChOx electrode. High sensitivity and stability together with very easy preparation makes PtNPs/pTy/ChOx electrode as promising candidate for constructing simple electrochemical sensor for choline determination.

The SEM and AFM results have shown the difference between type films morphological data. Further, it has been found that the PtNPs/pTy/ChOx has an excellent functional property along with good electrocatalytic activity on choline. The experimental methods of CVs, LSV and DPVs with film biosensor integrated into the GCE and ITO which are presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. Therefore, this work establishes and illustrates, in principle and potential, a simple and novel approach for the development of a voltammetric or amperometric sensor which is based on the modified electrodes.

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