Development and validation of biosensing method for acetaminophen drug monitoring

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ABSTRACT

A polyphenol oxidase (PPO) was immobilized by employing magnetic nanoparticles-zinc oxide/zinc hexacyanoferrate (Fe3O4NP-ZnO/ZnHCF) hybrid film electodeposited on the surface of Pt electrode. The surface functionalization of Fe3O4NP-ZnO/ZnHCF hybrid film was characterized by cyclic voltammetry (CV), scanning electron microscopy (SEM), atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS) before and after immobilization of PPO. The biosensor exhibited optimum response within 4s at pH 7.0 and 35°C and linearity in the range 0.04 to 10000 μM for acetaminophen with a detection limit of 0.04 μM (S/N=3). Accuracy of the proposed sensor was found to be 99%. The use of Fe3O4NP/ZnO/ZnHCF for construction of amperometric acetaminophen biosensor has resulted into relatively rapid response, higher sensitivity, broad linear range, lower detection limit, good reproducibility and long term stability of this biosensor. This sensing interface provides better avenue for the fabrication of various sensor. Copyright © 2015 VBRI press.

Keywords: Drug monitoring; acetaminophen; magnetic nanoparticles; zinc oxide/zinc hexacyanoferrate film; Pt electrode.

Jagriti Narang is currently working as an assistant professor at Amity University, Noida. She completed her PhD in Biochemistry and Genetics at Maharishi Dayanand University. She is running successfully DST young scientist project. Her research interest includes synthesis, characterization and applications of novel nanostructured materials with different dimensions and morphologies (such as: nanoparticles, nanowires, nanocubes, nanorods etc). Enzymatic and non-enzymatic electrochemical nanobiosensor biosensors based on nanostructured materials.

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Introduction

Acetaminophen (N-acetyl-p-aminophenol or Paracetamol) is an acetylated aromatic amide and is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin [1]. It is an effective and safe analgesic agent used worldwide for the relief from mild to moderate pain associated with headache, backache, for arthritis pain and postoperative pain [2]. Overdose and under dose of the drug induces adverse effects like renal imairement, hepatic insufficiency or in severe case it may cause death [1–4]. These point the call for the sensitive, accurate, fast, simple and rapid method for drug monitoring. The development of effortless, specific and accurate method for the analysis of this drug is very useful. Earlier techniques used for analysis of acetaminophen are colorimetric method, high pressure liquid chromatography (HPLC) and spectrometric methods. However most of these methods require time consuming pretreatment of sample, bulky apparatus and trained person to operate. Biosensors prove to be best alternative to overcome these problems due to their intrinsic specificity, low costs, fast analyses and minimal requirements for sample pretreatment [5]. Horseparishid peroxidase (HRP) [EC 1.11.1.7] catalyzes the oxidation of acetaminophen (paracetamol) into N-acetyl-p-benzoquinoneimine. Hence, the amperometric biosensors...
based on direct enzyme immobilization on the transducer surface are the main analytical strategies used for acetaminophen analysis [6].

Metal hexacyanoferrates have attracted considerable attention, as an excellent surface modifier, ever since reported by Neff [7] as deposition of a thin film of prussian blue (PB) on an electrode surface. The sensing electrodes have been modified with metal hexacyanoferrate through various techniques such as electrodeposition, adsorption, entrapping into a polymer matrix and mechanical attachment [8, 9]. Zinc hexacyanoferrate (ZnO/ZnHCF) has received much attention ever since its deposition on a carbon electrode reported by Joseph et al. [10]. Metal oxide nanoparticles exhibit higher ratios of surface area to volume than their bulk counterparts and thus provide larger electrochemically active areas for higher detection sensitivities for target molecules [11-16]. Nanostructured magnetic nanoparticles (Fe₃O₄NP) based electrochemical transducer surfaces promote the direct electron transfer reactions to amplify and orient the analytic signals of the biorecognition events [17].

In the present report, we first electrodeposited thin film of ZnO/ZnHCF on the surface of sensing electrode (Pt) and then magnetic nanoparticles were physically absorbed to the film modified electrode. Then purified enzyme PPO was immobilized on the surface of sensing electrode. Use of enzyme produces amplified signal as compared to non-enzymatic sensor. Our electrochemical measurements show that the Pt -modified ZnO/ZnHCF film provide excellent matrices for the immobilization of PPO and that the immobilized PPO exhibits high stability and retains high catalytical activity. The electrochemical response of the fabricated biosensor to acetaminophen shows broad range linearity. In addition, our study reveals that the presence of enzyme significantly enhances the sensitivity of the designed biosensor.

**Experimental**

**Materials**

Acetaminophen (Paracetamol) was purchased from SIGMA (St. Louis, MO). Zinc nitrate (Zn(NO₃)₂), potassium ferricyanide (K₃[Fe(CN)₆]), ferrous chloride tetrahydrate (FeCl₂.4H₂O) and ferric chloride hexahydrate (FeCl₃.6H₂O) from Sisco Research Laboratory Pvt. Ltd., Mumbai, India were used. All other chemicals were of analytic reagent grade. Double distilled water (DDW) was used in all experiments.

**Apparatus**

Cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) measurements were performed in a Potentiostat/Galvanostat (Autolab, Eco Chemie, The Netherlands. Model: AUT83785) with a three electrode system consisting of a Pt wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode and modified Pt wire as a working electrode. All the electrochemical experiments were performed at ambient temperature (25 °C).

**Synthesis of Fe₃O₄NP**

Fe₃O₄NP were prepared according to the method of Predoi [18]. The 0.5 M ferrous chloride tetrahydrate (FeCl₂.4H₂O) in 2M HCl and 0.5 M ferric chloride hexahydrate (FeCl₃.6H₂O) in DDW were mixed in equal volumes at room temperature. The mixture was dropped into 200 ml of 1.5 M NaOH solution under vigorous stirring for about 30 min. The resulting precipitates were isolated after centrifugation at 8000g and dried at 40 °C.

**Preparation of ZnO/ZnHCF film**

Prior to film deposition, the Pt was polished with alumina (Diameter 0.05 mm), then ultrasonically cleaned for about a min in DDW and finally washed thoroughly with DDW. The electrochemical deposition of ZnO/ZnHCF film was accomplished by potentiodynamic cycling of the Pt electrode between preset potential range in a suitable aqueous solution of H₂SO₄ (2 M, pH 2.0) containing 10⁻⁶M Zn(NO₃)₂ and 10⁻³ M K₃[Fe(CN)₆]. After 30 cycles, the electrode was taken out, rinsed thoroughly and used for further characterization. Cyclic voltammetric study of ZnO/ZnHCF film-modified electrode was recorded during electrodeposition of ZnO/ZnHCF hybrid film at a scan rate of +0.4 V to +1.6 V s⁻¹ at 50 mVs⁻¹ intervals.

**Construction of Fe₃O₄NP – ZnO/ZnHCF film modified Pt electrode**

Fe₃O₄NP were absorbed on the surface of ZnO/ZnHCF film by physical adsorption. Different durations of physiosorption of magnetic nanoparticles would result in different amounts of Fe₃O₄NP being deposited onto ZnO/ZnHCF film, which would eventually generate different catalytic activities towards acetaminophen. So, the optimal time duration was determined and found to be 24h for physiosorption of Fe₃O₄NP.

**Effect of applied voltage**

To optimize the applied potential for the acetaminophen determination, the effect of applied potential on the response current was investigated in the range -0.6 to +0.8 V vs Ag/AgCl. The optimal current was measured at +0.25 V vs Ag/AgCl and the same were used in subsequent electrochemical studies.

**Preparation of enzyme electrode**

The purified PPO enzyme was immobilized onto the surface of Fe₃O₄NP – ZnO/ZnHCF film modified Pt electrode by layering 10 µl of enzyme solution (40 mg ml⁻¹ protein) in acetate buffer (pH 5.0) and keeping it undisturbed for approximately 12 h at 4 °C. The electrode was finally washed with acetate buffer (pH 5.0) to remove unbound enzyme.

**Electrochemical characterization of PPO /Fe₃O₄NP-ZnO/ZnHCF/Pt electrode**

Cyclic voltammetry studies were carried out using a three electrode system composed of PPO/Fe₃O₄NP-ZnO/ZnHCF/Pt electrode as working electrode, Ag/AgCl as reference electrode and Pt wire as auxiliary electrode. To
discern the role of individual components, cyclic voltammograms of bare Pt electrode, Fe₃O₄NP-ZnO/ZnHCF/Pt electrode, and PPO/Fe₃O₄NP-ZnO/ZnHCF/Pt electrode was recorded in sodium phosphate buffer (0.1 M, pH 7.0) containing 0.1 mM H₂O₂ at a scan rate of 0.0 to +1 V s⁻¹ at an interval of 50 mV s⁻¹.

The principal of working of this biosensor included PPO as a biological component to convert acetaminophen to p-aminophenol, which was monitored amperometrically by oxidation at +0.25 V vs Ag/AgCl and the oxidation current was related to the concentration of acetaminophen. The chemical reactions are shown in scheme 1.

Scheme 1. Graphical representation of the stepwise amperometric sensor fabrication process.

**Preparation of acetaminophen solution**

Paracetamol (acetaminophen) was prepared in phosphate buffer solution (pH 7.0). Solutions of different concentrations of Paracetamol (acetaminophen) ranging from 0.04 to 10000 μM was prepared in 0.1M sodium phosphate buffer (pH 7.0) and stored at 4 °C until use.

**Amperometric determination of acetaminophen**

An accurate volume of 1.0 ml of each pharmaceutical product was stirred until complete dissolution and then diluted to 10, 20 and 50 ml with 0.1 mol l⁻¹ phosphate buffer solution (pH 7.0), respectively. Finally, an aliquot of 400 μl of each medicine solution was added to the cell containing 10 ml of 0.10 mol l⁻¹ phosphate buffer solution. The measurements were performed after successive additions of paracetamol or sample solutions. After each addition, cyclic voltammograms was recorded by cycling the potential between -0.6 and +0.8 V at a scan rate of 100 mV s⁻¹. Acetaminophen content in pharmaceutical product was determined by the present biosensor by replacing acetaminophen with pharmaceutical product and recording the current (mA) under its optimal working conditions. The amount of pharmaceutical product was extrapolated from standard curve between acetaminophen concentrations and current in mA.

**Storage stability of PPO/Fe₃O₄NP-ZnO/ZnHCF/Pt electrode hybrid film modified Pt electrode**

The long-term storage and stability of the working electrode its amperometric current response to 100 μM of paracetamol, was investigated over a period of 6 months at 4 °C.
Results and discussion

Surface morphological characterizations using SEM and AFM studies

The morphologies of (a) bare electrode, (b) Fe₃O₄NP-ZnO/ZnHCF hybrid film modified Pt electrode and (c) PPO immobilized onto Fe₃O₄NP-ZnO/ZnHCF hybrid film electrode were characterized by SEM studies and AFM studies. The SEM and AFM images of the Pt electrode showed a smooth and featureless morphology (Fig. 1 A (i) & B (ii)), whereas the granular morphology with heterogeneous roughness of Fe₃O₄NP showed the absorption of nanoparticles on ZnO/ZnHCF hybrid films (Fig. 1 A (ii)). Weak interactions and immobilization of PPO onto the Pt electrode showed a globular structure of enzyme (Fig. 1 A (iii)) and AFM image of Fe₃O₄NPs were uniformly distributed throughout the film surface (Fig. 1 B (ii)).

Electrochemical characterization of ZnO/ZnHCF hybrid film modified Pt electrode

Fig. 2 shows the cyclic voltammogram of Pt electrode during the electrodeposition process. The consecutively increasing currents for both anodic and cathodic peaks demonstrated that ZnO/ZnHCF hybrid film was deposited continuously on the electrode surface. Two redox couples representing ZnHCF and ZnO were noticed. By the reduction of nitrate, ZnO was deposited through the potentiodynamic cycling due to base generation.

Construction of PPO/Fe₃O₄NP-ZnO/ZnHCF hybrid film modified Pt electrode and cyclic voltammetric measurement

Fig. 3 summarizes reactions involved in fabrication of the acetaminophen biosensor based on immobilization of PPO onto Fe₃O₄NP-ZnO/ZnHCF/Pt electrode. In order to evaluate the charge-transfer properties on the surface of the modified electrodes, cyclic voltammetry technique was employed by using potassium ferrocyanide as redox probe. CV recorded in 2.5mM K₃Fe(CN)₆/K₄Fe(CN)₆ solution and sodium phosphate buffer 0.05 M (pH 7.2) are shown in Fig. 3. Small voltammetric response was observed for the unmodified electrode (Fig. 3 a). CV for the electropolymerization of Fe₃O₄NP- ZnO/ZnHCF hybrid film exhibits two oxidation peaks around -0.01 V to + 0.3 V (Fig. 3b). Voltammogram results were recorded after several preliminary scans. The preservation of a quasi reversible electrode process and the large increase in peak currents for the nanocomposite film modified electrode proved that Fe₃O₄NP- ZnO/ZnHCF hybrid film exerted an obvious improvement effect on electric conductivity of enzyme electrode. After immobilization of enzyme, there was slight decrease in current as slow redox process occurred at the electrode surface due to slow electron transfer from the surface showing the immobilization of enzyme (Fig. 3c).

Fig. 2. Cyclic voltammogram of electrochemical deposition of zinc oxide/zinc hexacyanoferrate films at pH 2 of H₂SO₄ solution at a scan rate of +0.2 V to +1.2 Vs⁻¹ with 50 mVs⁻¹.

Fig. 3. Cyclic voltammogram of (a) bare Pt electrode, (b) Fe₃O₄NP-ZnO/ZnHCF/Pt and (c) PPO/Fe₃O₄NP-ZnO/ZnHCF hybrid film modified Pt electrode in a 2.5mM K₃Fe(CN)₆/K₄Fe(CN)₆ solution and sodium phosphate buffer 0.05M (pH 7.2) at a scan rate of 50 mVs⁻¹.

Fig. 4. EIS of (i) bare Pt electrode, (ii) Fe₃O₄NP-ZnO/ZnHCF/Pt and (iii) PPO/Fe₃O₄NP-ZnO/ZnHCF hybrid film modified Pt electrode in a solution containing 1 mM Fe(CN)₆^3-/4+ with 0.1 M KCl at 0.20 mV s⁻¹ (frequency range of 0.01 Hz –10 kHz).

Electrochemical impedance studies of modified Pt electrode

Fig. 4 shows electrochemical impedance spectra (EIS) of (a) bare electrode (b) Fe₃O₄NP-ZnO/ZnHCF/Pt electrode and (c) PPO/Fe₃O₄NP-ZnO/ZnHCF hybrid film modified Pt electrode. The charge transfer process in PPO/Fe₃O₄NP-ZnO/ZnHCF hybrid film modified electrode has been studied by monitoring charge transfer resistance (Rct) at the
electrode and electrolyte interface. The value of the electron transfer resistance (semicircle diameter) \( R_{ct} \) depends on the dielectric and insulating features at the electrode/electrolyte interface. The decreased \( R_{ct} \) value of \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) modified Pt electrode compared to that of bare Pt electrode was due to the adsorption of \( \text{Fe}_2\text{O}_3\text{NP} \) onto \( \text{ZnO/ZnHCF hybrid film} \). This decrease in \( R_{ct} \) could be attributed to the fact that \( \text{Fe}_2\text{O}_3\text{NP} \) based electrochemical transducer surfaces promote the direct electron transfer reactions, amplify and orient the analytic signal of the recognition events. Upon immobilization of enzyme the electron transfer via redox couple was hindered by the presence of enzymes on electrode surface. The increased \( R_{ct} \) value of \( \text{PPO/Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) modified Pt electrode was due to the immobilization of enzymes onto \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) modified Pt electrode.

**Fig. 5.** Cyclic voltammograms (CV) of modified electrode (a) without and (b) with substrate at -0.6 to +0.8V with a scan rate of 50 mVs \(^{-1}\) in a 2.5 mM K\(_{3}\)Fe(CN)\(_6\)/K\(_4\)Fe(CN)\(_6\) solution and sodium phosphate buffer 0.05M (pH 7.2) at a scan rate of 50 mVs \(^{-1}\).

Electrocatalytic oxidation of paracetamol at the surface of \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) modified Pt electrode

It is expected that an electrocatalytic mechanism initiated by electrochemical oxidation of the reduced form of the complex exist at the surface of the electrode and then completed by chemical oxidation of paracetamol. To reveal the electrocatalytic activity of \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) towards the oxidation of paracetamol, the voltammetric behaviors of paracetamol were investigated at the surfaces of unmodified and modified electrode. **Fig. 5** shows the cyclic voltammograms of modified Pt electrode in \( \text{H}_2\text{SO}_4 \) solution (pH 2) in the presence of 100 \( \mu \)M paracetamol (a) at the surface of the modified electrode and (b) at the surface of the unmodified electrode. At unmodified electrode, the direct oxidation of paracetamol was not significant, and just a small anodic current was observed due to the oxidation of paracetamol (**Fig. 5a**). However if the electrode was modified with \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \), then a large anodic peak was observed with cathodic peak current decreasing accordingly (**Fig. 5b**) indicating the electrooxidation of paracetamol. **Fig 6** shows square wave voltammogram of enzyme electrode for different concentration of paracetamol (0.04 to 10000 \( \mu \)M). During response studies of the enzyme electrode, it was found that magnitude of the response current increased with increasing concentration of paracetamol. Calibration was also performed using cyclic voltammetry with increasing concentration of paracetamol. The peak current is linearly related to the various concentrations of deferiprone (**Fig. 7**). Detection limit of the biosensor was 0.04 \( \mu \)M (S/N=3). Calibration was also performed using cyclic voltammetry with increasing concentration of paracetamol. The peak current is linearly related to the various concentrations of deferiprone (**Fig. 7**).

**Fig. 6.** SWVs of modified electrode for different concentrations of substrate in 0.1 M phosphate buffer/0.1 M KCl (pH 7.0) at a potential range of -0.6 V to +0.8 V, vs. Ag/AgCl with scan rate 50 mVs \(^{-1}\).

**Fig. 7.** Cyclic voltammetric responses for different concentrations of substrate in 0.1 M phosphate buffer/0.1 M KCl (pH 7.0) at a potential range of -0.6 V to +0.8 V, vs. Ag/AgCl with scan rate 50 mVs \(^{-1}\).

Optimization of the biosensor

Effect of pH, response time, working potential and temperature on the biosensor was observed. The formation of \( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF hybrid film} \) on the Pt electrode surface was studied by dipping the modified electrode in \( \text{H}_2\text{SO}_4 \) supporting electrolyte of pH 1, 2, 3, 4 and 5. The cyclic voltammetric responses of \( \text{ZnO/ZnHCF hybrid film} \) modified electrodes in \( \text{H}_2\text{SO}_4 \) solution of pH 1, 2, 3, 4 and 5 respectively. There was a little increase in the current response at pH lower than 2, however, there was a decrease in the current response at pH above than 2. Thus pH 2.0 was selected for the formation of the film while the effect of pH of the buffer solution on the performance of the biosensor was also demonstrated. The effect of pH (ranging from 3 to 9) on PPO/\( \text{Fe}_2\text{O}_3\text{NP-ZnO/ZnHCF} \) bioelectrode has been investigated using CV technique in PBS (50mM) containing 5mM [Fe(CN)\(_6\)]\(^{3-4-}\) at scan rate of 50 mVs/s. The pH dependence of the sensor response was evaluated over the pH range from 3 to 9. The sensor response was found optimum at pH 7.0. (**Fig. 8 a**). The influence of
applied potential on paracetamol detection at hybrid nanocomposite ZnO-ZnHCF modified electrode was studied and an optimal potential of +0.1V vs. Ag/AgCl was found. The working potential was stepped from -0.6 to +0.8 V. This optimum applied potential of +0.25V make certain a minimizing of interference effects. The effect of incubation temperature on the biosensor was examined between 20 to 50 °C. (Fig. 8b) The current response of the biosensor increased with increasing temperature and reached a maximum at approximately 35 °C, and then went down as the temperature turned higher. In addition, the current response kept correspondingly steady between 20 to 50 °C, indicating that enzyme bioconjugate with modified electrode had good thermodynamic stability and life span.

The effect of time on the enzymes was also determined by recording the current response from 2 to 12 s at an interval of 2 s. The modified electrode showed maximum response at 4s (Fig. 8c).

![Graph](image)

**Fig. 8.** Effects of pH (a) temperature (b) and response time (c) on the electrochemical response of fabricated acetaminophen biosensor based on HRP/ZnO@ Fe₃O₄NP/CHIT in 0.1 M sodium phosphate buffer.

### Analytic recovery and precision study

The analytic recovery of added acetaminophen was 95-96%. The precision of the present method were determined over seven days. Each day ten replicate quality control samples were analyzed for acetaminophen content. The within and between batch precision were less than 2% and 3% respectively (data not shown). These results demonstrated that the described method was precise and reproducible.

### Table 1. Determination of acetaminophen by present sensor based on Fe₃O₄NPs/ZnO-ZnHCF/Pt electrode and standard enzymatic method.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Pharmacopoeia Method (µM)</th>
<th>Present Method (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>2500</td>
<td>2600</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>1000</td>
<td>1020</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>2000</td>
<td>1800</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>3000</td>
<td>3020</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>3600</td>
<td>3600</td>
</tr>
<tr>
<td>8.</td>
<td>H</td>
<td>5400</td>
<td>5450</td>
</tr>
<tr>
<td>9.</td>
<td>I</td>
<td>1450</td>
<td>1520</td>
</tr>
<tr>
<td>10.</td>
<td>J</td>
<td>4900</td>
<td>4900</td>
</tr>
</tbody>
</table>

### Table 2. Substrate specificity effect on Fe₃O₄NPs/ZnO-ZnHCF/Pt electrode.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative rate of oxidation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-DOPA</td>
<td>98.0</td>
</tr>
<tr>
<td>Catechol</td>
<td>90.2</td>
</tr>
<tr>
<td>Dopamine</td>
<td>98.0</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>5.2</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>10.3</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>10.3</td>
</tr>
</tbody>
</table>

### Interference study

Various interfering substances were tested which are used in acetaminophen pharmaceutical formulation such as citric acid, sodium benzoate, stearic acid, sodium metabisulphite and saccharin were evaluated using the proposed biosensor at their formulation concentration, none had practically any significant interference (data not shown). To study the substrate specificity on the electrochemical response of sensor, the following compounds such as L-DOPA, catechol, dopamine, hydroquinone, isoprenaline and terbutaline. All are phenolic compounds which were added in place of paracetamol in the reaction mixture individually at a final concentration of 1.0 mM and the electrochemical response was measured under the similar assay conditions. Results depicted that dopamine, catechol and L-DOPA was utilized as substrate, while hydroquinone and isoprenaline were practically unutilized. This shows the absolute substrate specificity of the developed sensor (Table 1).
Determination of acetaminophen in various commercial samples

The proposed procedure was applied to determine paracetamol in pharmaceutical formulations. Table 2 presents the results obtained for four commercial samples by replacing acetaminophen with samples. To study the accuracy of the present method, acetaminophen level in samples were determined by both the pharmacopoeial method (x) and the present method (y). The values obtained by both the methods matched with each other with a good correlation ($r^2 = 0.99$) (data not shown).

Stability of the enzyme electrode

The shelf life of the PPO/Fe$_3$O$_4$NP-ZnO/ZnHCF/PtE was investigated by measuring its electrochemical current response for 6 months at a regular interval of 1 week. The PPO/Fe$_3$O$_4$NP-ZnO/ZnHCF/Pt electrode retained about 50% of its initial activity even after 6 months, when stored in dry conditions at 4 °C. A comparison of analytic parameters of various nanoparticles based biosensors for detection of paracetamol with the present biosensor is summarized in Table 3.

Table 3. Comparison of present method with other biosensing methods.

<table>
<thead>
<tr>
<th>Matrix/method</th>
<th>Enzyme</th>
<th>Response time (s)</th>
<th>Detection limit (μM)</th>
<th>Linearity (μM)</th>
<th>Stability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Ni/GCE/DPV</td>
<td>-</td>
<td>-</td>
<td>7.8-110</td>
<td>-</td>
<td>-</td>
<td>[19]</td>
</tr>
<tr>
<td>Chronoamperometry MWNT film</td>
<td>-</td>
<td>0.04</td>
<td>0.1-20</td>
<td>-</td>
<td>-</td>
<td>[20]</td>
</tr>
<tr>
<td>Carbon nanoparticles (NP)/GCE</td>
<td>0.05</td>
<td>0.1-100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[21]</td>
</tr>
<tr>
<td>Cobalt hydroxyl nanoparticles/Cyclic voltammetry</td>
<td>-</td>
<td>10</td>
<td>2.5-1000</td>
<td>-</td>
<td>-</td>
<td>[22]</td>
</tr>
<tr>
<td>Nanogold/TTO/Cyclic voltammetry</td>
<td>-</td>
<td>0.18</td>
<td>0.2-1500</td>
<td>-</td>
<td>-</td>
<td>[23]</td>
</tr>
<tr>
<td>PPO/ZnO/ZnHCF/PtPPO</td>
<td>4s</td>
<td>0.04</td>
<td>0.04 to 10000</td>
<td>Present</td>
<td>-</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Conclusion

In the present work, we have described a promising electrochemical biosensor for the analysis of acetaminophen based on the sensing interface PPO/Fe$_3$O$_4$NP-ZnO/ZnHCF/PtE. The zinc oxide film was electrodeposited through potentiodynamic cycling. The decorated film possesses large surface area and good uniformity, ideal matrices for enzyme immobilization and also increases the electrical activity of the formed. Our electrochemical measurements reveal that the immobilized PPO exhibits high biological activity and stability and that the presence of magnetic nanoparticles further enhances the sensitivity of the designed electrochemical biosensor for analysis of acetaminophen. The developed biosensor exhibits fast amperometric response, high sensitivity, low detection limit (0.04 μM) and long-time stability.

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