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Electrodeposition of gold–platinum alloy nanoparticles on ionic liquid–chitosan composite film and its application in fabricating an amperometric cholesterol biosensor

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ABSTRACT

An electrodeposition method was applied to form gold–platinum (AuPt) alloy nanoparticles on the glassy carbon electrode (GCE) modified with a mixture of an ionic liquid (IL) and chitosan (Ch) (AuPt–Ch–IL/GCE). AuPt nanoparticles were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and electrochemical methods. AuPt–Ch–IL/GCE electrocatalyzed the reduction of H_2O_2 and thus was suitable for the preparation of biosensors. Cholesterol oxidase (ChOx) was then, immobilized on the surface of the electrode by cross-linking ChOx and chitosan through addition of glutaraldehyde (ChOx/AuPt–Ch–IL/GCE). The fabricated biosensor exhibited two wide linear ranges of responses to cholesterol in the concentration ranges of 0.05–6.2 mM and 6.2–11.2 mM. The sensitivity of the biosensor was 90.7 $\mu A\,mM^{-1}\,cm^{-2}$ and the limit of detection was 10 μM of cholesterol. The response time was less than 7 s. The Michaelis–Menten constant (K_m) was found as 0.24 mM. The effect of the addition of 1 mM ascorbic acid and glucose was tested on the amperometric response of 0.5 mM cholesterol and no change in response current of cholesterol was observed.

1. Introduction

Cholesterol has recently attracted much interest because its level in blood is an important parameter in the diagnosis and prevention of disease. Ideally, the total cholesterol concentration in a healthy person's blood should be less than 200 mg/dL (<5.17 mM). The borderline high is defined as 200–239 mg/dL (5.17–6.18 mM), and the high value is defined as above 240 mg/dL (≥6.21 mM) (Shen and Liu, 2007). The measurement of blood cholesterol concentration is a routine practice in medical screening or diagnosis. Enzymatic procedures for the determination of cholesterol have practically replaced the chemical methods because the traditional method of determination of cholesterol by spectrometry suffered from poor specificity, instability of color forming agent and standardization difficulties (Gopalana et al., 2009).

Biosensors using immobilized enzymes as the bio-recognition element are among the most widely investigated devices for both fundamental research and applications point of view (Weetall, 1974; Emr and Yacynych, 1995; Hall, 1991). Amperometric biosensors are usually chosen for biochemical analysis because of their good selectivity, rapid response, and low cost (Karube et al., 1982).

In the fabrication of a cholesterol biosensor, cholesterol oxidase (ChOx) is most commonly used as the biosensing element. Cholesterol oxidase catalyzes the oxidation of cholesterol to H_2O_2 and cholest-4-en-3-one in the presence of oxygen (Ahn and Sampson, 2004).

The enzymatic reaction in the use of cholesterol oxidase (ChOx) as a receptor can be described as follows:

$$\text{Cholesterol} + O_2 \overset{\text{ChOx}}{\rightarrow} \text{Cholest-4} - \text{en} - 3 - \text{one} + H_2O_2$$

The electrooxidation current of hydrogen peroxide is detected after application of a suitable potential to the system. The major problem for amperometric detection is the overestimation of the response current due to interferences such as ascorbic acid. This problem can be overcome by using a combination of two or three enzymes, which are more selective for the analyte of interest (Bongiovanni et al., 2001) or by devising techniques to eliminate or reduce the interference. Many researchers have reported the inclusion of metal nanoparticles with a catalytic effect in polymer modified electrodes to decrease the overpotential applied to the amperometric biosensors (Safavi et al., 2009; Hrapovic et al., 2004; Ren et al., 2005; Huang et al., 2004). Amperometric cholesterol biosensors based on carbon nanotube–chitosan–platinum–cholesterol oxidase nanobiocomposite was fabricated for cholesterol determination at an applied potential of 0.4 V (Tsai et al., 2008). To improve

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the selectivity of the biosensor, Gopalana et al. (2009) reported the construction of a cholesterol biosensor by monitoring the reduction current of H_2O_2 at −0.05 V.

Bimetallic alloys are widely used in catalysis and sensing fields. Owing to the interaction between two components in bimetallic alloys, they generally show many favorable properties in comparison with the corresponding monometallic counterparts, which include high catalytic activity, catalytic selectivity, and better resistance to deactivation. Among various bimetallic alloys, gold–platinum (AuPt) alloy is very attractive. It has excellent catalysis and resistance to deactivation due to the high synergistic action between gold and platinum (Xiao et al., 2009). Owing to these advantages of bimetallic nanoparticles, it becomes significant to develop AuPt nanoparticles for application in electrochemical sensors with appropriate characteristics such as high sensitivity, fast response time, wide linear range, better selectivity, and reproducibility.

Chitosan is an abundant natural biopolymer with excellent film forming ability, biocompatibility, nontoxicity, good water permeability and high mechanical strength (Zhang et al., 2004; Lu et al., 2006a; Lin et al., 2007). Chitosan can accumulate metal ions through various mechanisms, such as chelation, electrostatic attraction, and ion exchange, depending on the nature of the metal ion and pH of the solution. To improve the properties of chitosan, the combination of chitosan with other materials such as ethylene diamine (Katarina et al., 2006), thiourea (Mokhodoeva et al., 2007), carbon naotubes (Kang et al., 2007) and functionalized ionic liquids (Xiao et al., 2009) were reported. Kang et al. (2007) constructed a glucose biosensor on a glassy carbon electrode which was modified with gold–platinum alloy nanoparticles (AuPt NPs) by electrodeposition on multiwalled carbon nanotubes in chitosan film. Recently, AuPt NPs were fabricated on chitosan-ionic liquid (i.e., trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)imide, $[P(C_6)_3C_{14}][Tf_2N]$) film by using an ultrasonic electrodeposition method which displayed high catalytic activity towards the reduction of H_2O_2 (Xiao et al., 2009).

Ionic liquids (ILs), a new class of environmentally benign solvents which are composed entirely of ions, have been used in numerous applications. Because of their unique properties such as wide electrochemical window, high ionic conductivity, and good thermal stability, they are applied in different electrochemical applications such as in lithium batteries, capacitors, and solar cells (Howlett et al., 2004; McEwen et al., 1999; Wang et al., 2003). They are also widely applied as the binder in carbon ionic liquid electrodes (Maleki et al., 2006; Liu et al., 2005; Safavi et al., 2007) or as the electrode modifier especially for construction of different biosensors (Safavi and Farjami, 2010; Safavi et al., 2008; Zhu et al., 2009; Lu et al., 2006b). In this work, we have utilized the unique properties of AuPt bimetallic NPs for fabrication of a cholesterol biosensor. This system was developed by electrodeposition of AuPt nanoparticles on the GCE modified with chitosan and IL, 1-butyl-3-methylimidazolium chloride [bmim]Cl. Then cholesterol oxidase was immobilized on the surface of the electrode by cross-linking ChOx and chitosan through glutaraldehyde and the biosensor is designated as ChOx/AuPt–Ch–IL/GCE. The ChOx/AuPt–Ch–IL/GCE biosensor exhibited an excellent linear response to cholesterol for a wide concentration range with good selectivity and sensitivity.

2. Experimental

2.1. Reagents

1-Butyl-3-methyl-imidazolium chloride [bmim]Cl, potassium ferrocyanide, potassium ferriccyanide, tetrachloroauric acid, $HAuCl_4$ and hexachloroplatinic acid, H_2PtCl_6, were obtained from Merck. Glutaraldehyde (8%) and chitosan were purchased from Fluka. Cholesterol oxidase (ChOx) (EC232-842-1) and cholesterol were obtained from Sigma. A 30% H_2O_2 solution was purchased from Riedel–deHaen, and a fresh stock solution of H_2O_2 (0.1 M) was prepared daily. Solutions of 0.2% and 0.5% chitosan were made in 1.0% (v/v) acetic acid by ultrasonication for 30 min. Stock solution of cholesterol was prepared in isopropanol. Working solutions were prepared by diluting an appropriate amount of the stock solution in a volumetric flask containing 2% (v/v) isopropanol and 10% (m/v) TX-100 and stored at 4 °C. Cholesterol oxidase was dissolved in 0.05 M phosphate buffer (pH 7.0) and 0.9% NaCl.

2.2. Apparatus

Voltammetric and impedance measurements were performed using an Autolab electrochemical system (Eco-Chemie, Utrecht, The Netherlands) equipped with an Autolab PGSTAT-302N, GPES and FRA software (Eco-Chemie, Utrecht, The Netherlands). The electrochemical cell was assembled with a conventional three-electrode system. An Ag/AgCl/KCl (3 M) reference electrode (Metrohm) was used together with a platinum disk as a counter electrode for all experiments. The working electrode was a modified glassy carbon electrode (2 mm in diameter). Scanning electron micrographs (SEM) of the electrode surfaces were obtained by using scanning electron microscopy (Philips, model XL30) at an accelerating voltage of 25 kV. X-ray diffraction (XRD) data were recorded with a D8 ADVANCE diffractometer (BRUKER-Germany) using Cu Kα radiation (40 kV, 200 mA).

2.3. Preparation of the AuPt bimetallic NPs modified biosensor

Different amounts of IL, 1-butyl-3-methylimidazolium chloride, [bmim]Cl, were added to the chitosan solution (0.2% and 0.5%) and with the aid of ultrasonic agitation, a uniform solution was prepared. Then, 4 μL of Ch–IL solution was dropped on a cleaned GCE and the solvent was evaporated in air. Thus, a uniform film coated electrode was obtained. The electrochemical deposition of AuPt was performed on Ch–IL/GCE in 0.2 M Na_2SO_4 aqueous solution containing 0.5 mM $HAuCl_4$ and 0.5 mM H_2PtCl_6. The deposition time was 400 s and the potential was −0.2 V. After the preparation of AuPt–Ch–IL/GCE, 4 μL of Ch–IL was placed on it and dried. The electrode was then immersed in 0.25% glutaraldehyde solution for 2 h. Then, 5 μL of ChOx (50 U/mL) was dropped on the surface of the electrode and allowed to dry. The modified electrode was washed with PBS (50 mM, pH 7.0) solution, containing 0.9% NaCl and 1% (m/v) Triton X-100.

3. Results and discussion

3.1. Morphological analysis

Fig. 1 displays the SEM images of AuPt nanoparticles fabricated under the same conditions on (A) Ch/GCE and (B) Ch–IL/GCE. The positively charged chitosan can bind $AuCl_4^-$ and $PtCl_6^{2-}$, and the amine groups of chitosan can interact with the NPs, blocking the aggregation of NPs (Sorlier et al., 2001; Huang et al., 2004). As a result, dispersed AuPt NPs are obtained. The composite surface is well-coated with AuPt NPs; the diameters of the NPs are 50–200 nm. Comparing Fig. 1A and B reveals that the density of nanoparticles with diameters smaller than 80 nm is higher on Ch–IL/GCE and it is reasonable to deduce that IL played an important role here. As suggested by other researchers (Xiao et al., 2009), ILs have low interfacial tension and thus can enhance the nucleation rate, which is favorable to the formation of smaller AuPt NPs.

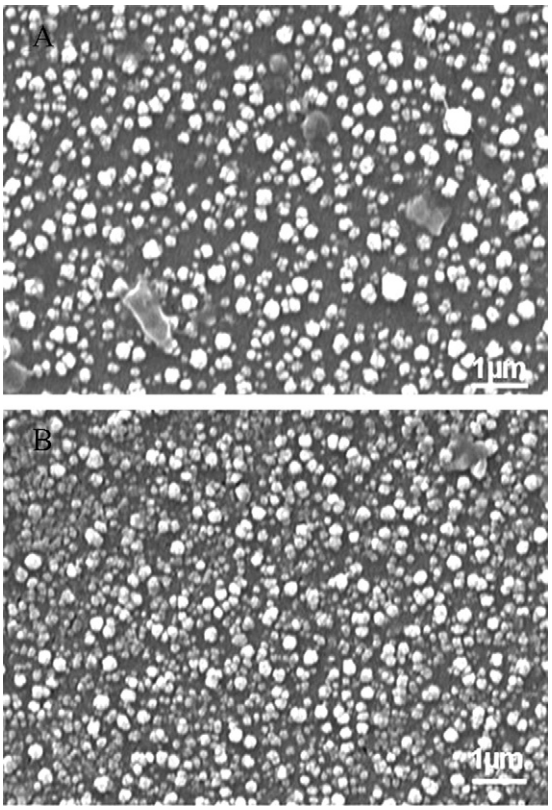


Fig. 1. The SEM image of (A) AuPt–Ch/GCE and (B) AuPt–Ch–IL/GCE.

Homogeneity of the particles indicates that they are consisted of a homogeneous material, such as an alloy in this case.

3.2. Structural analysis

To estimate the structure of AuPt alloy nanoparticles, they were characterized by XRD (Supporting Information, Fig. S1). The peaks are defined well enough to allow a definitive phase identification and structural characterization. The diffraction patterns of Au and Pt NPs on the Ch–IL film have been also shown in (Supporting Information, Fig. S1). The peaks of AuPt alloy fall well between the peaks of Au and Pt NPs suggesting that the AuPt NPs are, indeed, AuPt alloy (Xiao et al., 2009; Kang et al., 2007).

3.3. Electrochemical characterizations

Electrochemical impedance spectroscopy (EIS) is an effective method for probing the features of surface modified electrodes. The Nyquist plot of impedance spectra includes a semicircle portion and a linear portion, with the former at higher frequencies corresponding to the electron transfer limited process and the latter at lower frequencies corresponding to the diffusion process. The electron transfer resistance (R_{ct}) at the electrode surface is

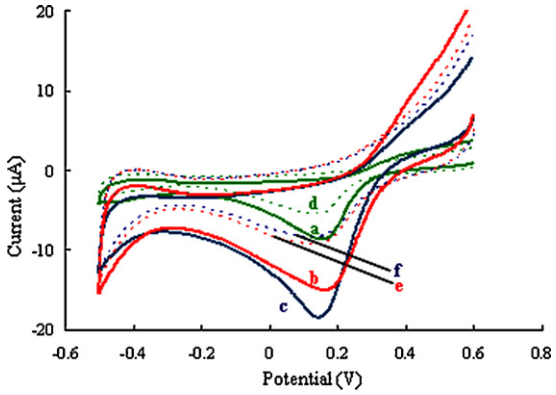


Fig. 2. Cyclic voltammograms of AuPt/GCE (a, d), AuPt–Ch/GCE (b, e), AuPt–Ch–IL/GCE (c, f), in the presence (a–c) and absence (d–f) of 0.5 mM H_2O_2 in 0.1 M PBS (pH 7.0), scan rate, 50 mV s^{-1}.

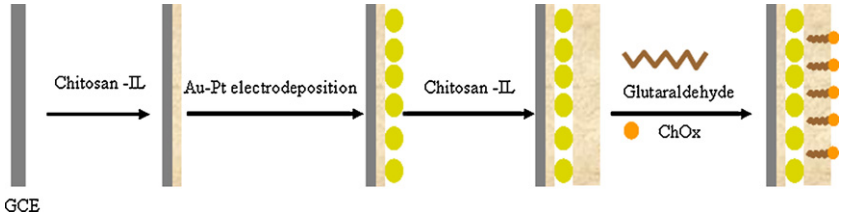
equal to the semicircle diameter, which can be used to describe the interface properties of the electrode. The Nyquist plots of Ch–IL/GCE (Supporting Information, Fig. S2, curve a) and AuPt-Ch-IL/GCE (Supporting Information, Fig. S2, curve b) were obtained in equimolar concentration of 2 mM of $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl. The semicircle was smaller for AuPt–Ch–IL/GCE. The estimated electron transfer resistance for Ch–IL/GCE and AuPt–Ch–IL/GCE are 826 Ω and 468 Ω, respectively. This result indicates that the presence of AuPt NPs is effective in enhancing the rate of electron transfer.

3.4. Fabrication of ChOx/AuPt–Ch–IL/GCE biosensor

The immobilization of proteins, enzymes and DNA on desired substrates plays an important role for rapid, sensitive and selective detection of desired analytes. In recent studies, chitosan is widely used as a biomaterial for immobilizing enzymes. It exhibits excellent membrane-forming ability, biocompatibility, nontoxicity, high mechanical strength, and hydrophilicity (Tsai et al., 2008; Ansari et al., 2009). In the present work, after the preparation of AuPt–Ch–IL/GCE, 4 μL of Ch-IL was placed on it and was left to dry. The electrode was then immersed in 0.25% glutaraldehyde solution for two hours. The amine groups in chitosan form covalent cross-links with free aldehyde groups in glutaraldehyde to form a 3D network for immobilization of enzyme. In the final stage of biosensor fabrication, ChOx was immobilized into AuPt–Ch–IL matrix to obtain ChOx/AuPt–Ch–IL/GCE. The preparation procedure is schematically illustrated in Scheme 1.

3.5. Performance of different modified electrodes toward hydrogen peroxide reduction

Fig. 2 presents the typical cyclic voltammograms of reduction of H_2O_2 at different electrodes. The reduction peaks observed in the absence of H_2O_2 at ca. 0.1 V are attributed to the reduction of platinum surface oxides and is a familiar feature of AuPt bimetallic nanoparticles. With the addition of H_2O_2, the reduction peak of H_2O_2 appeared at about the same potential. AuPt–Ch–IL/GCE



Scheme 1. Schematic illustration of preparation procedures of ChOx/AuPt–Ch–IL/GCE.

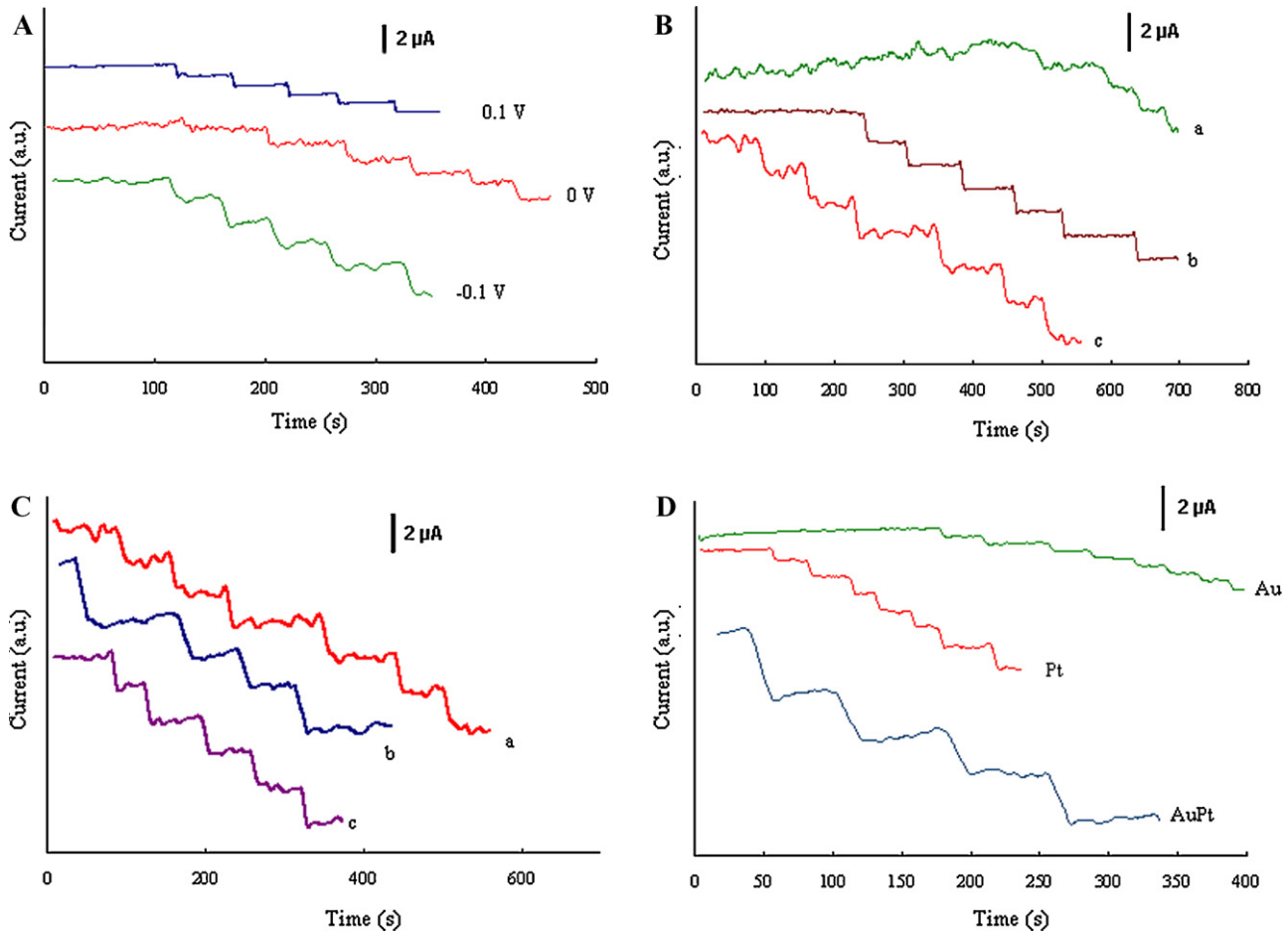


Fig. 3. Response current–time curves (A) of AuPt–Ch–IL/GCE at various potentials; (B) various modified electrodes, AuPt/GCE (a), AuPt–Ch (0.5%)/GCE (b), AuPt–Ch (0.2%)/GCE (c); (C) of the various modified electrodes, AuPt–Ch (0.2%)/GCE (a), AuPt–Ch (0.2%)-IL (0.02 g mL^{-1})/GCE (b), AuPt–Ch (0.2%)–IL (0.04 g mL^{-1})/GCE (c); (D) of the Ch–IL/GCE modified with different types of nanoparticles to the successive addition of 0.1 mM H_2O_2 in PBS (0.1 M, pH 7.0).

exhibits the most sensitive response when compared to AuPt/GCE and AuPt–Ch/GCE. Therefore, it can be deduced that the synergistic effect of the AuPt alloy NPs, chitosan and IL, can promote the electron transfer of H_2O_2 reduction. As described previously, this can be explained by the fact that the addition of IL to chitosan caused the particle density (the number of particles per unit area) to increase and particle size to decrease, which in turn results in a higher electrocatalytic effect.

3.6. Optimization of the modified electrodes

Fig. 3A shows the amperometric response currents of H_2O_2 at various potentials, indicating that determination can be carried out at a low overpotential with a rapid response time. As it can be seen from Fig. 3A, the current was increased with decreasing the reduction potential. So, −0.1 V was selected for further amperometric experiments.

The effects of the concentration of chitosan on the response currents of successive addition of 0.1 mM H_2O_2 were compared and the results are shown in Fig. 3B. The GCE that was not covered by chitosan before electrodeposition of AuPt NPs (AuPt/GCE, curve a) showed a worse response compared to the electrodes initially modified with 0.5% Ch (curve b) and 0.2% Ch (curve c). Response currents were greater for modified electrodes with 0.2% Ch. So the electrodes were modified with 0.2% Ch for further experiments prior to electrodeposition of AuPt NPs. The effects of addition of different concentrations of IL to the Ch solution and covering the GCE with a layer of 0.2% Ch and different concentrations of IL were also investigated and the typical results are shown in Fig. 3C. Since the response current of AuPt–Ch–IL/GCE (0.2% Ch, IL 0.02 g mL^{-1}, curve b) was better than in the absence of IL, this electrode was selected for further experiments. As it was stated before (Section 3.1), with the addition of IL, the size of particles was decreased and their density on the surface of the electrodes was increased. Therefore, the electrocatalytic current was increased. However, addition of more than 0.02 g mL^{-1} IL in 0.2% Ch, (typically 0.2% Ch, IL 0.04 g mL^{-1}, curve c), decreased the film forming ability of chitosan and caused a decrease in current.

As shown in Fig. 3D, AuPt alloy NPs exhibited a better catalytic property than did their monometallic counterparts of Au NPs and Pt NPs. This is attributed to the synergistic effect of the AuPt alloy.

To further study the catalytic property of AuPt–Ch–IL/GCE, the influence of the deposition time of AuPt NPs was investigated on the response current of H_2O_2 (not shown). With the increase in deposition time from 100 to 400 s, the response current enhanced but further increase in deposition time caused the current to slightly decrease, indicating that the electrochemical active surface area has been decreased. Thus, in this work, a deposition time of 400 s was chosen.

The effect of enzyme loading on the catalytic current for cholesterol oxidation was initially studied in the presence of 0.5 mM cholesterol. The response of the biosensor containing different amounts of the enzyme ChOx was recorded (Supporting Information, Fig. S3). The response current increases with increasing enzyme loading. This indicates that the catalytic currents are controlled by the enzyme activity in the hybrid film. The enzyme

loading higher than 0.25 U did not further enhance the response current. So, an enzyme loading of 0.25 U was used for further experiments to obtain the greatest sensitivity.

3.7. Electrochemical performances of cholesterol biosensor

The influences of pH, buffer concentration, and concentration of Triton X-100 on the performance of ChOx immobilized enzyme electrodes have been extensively investigated (Wang and Mu, 1999; Singh et al., 2006; Li et al., 2003; Arya et al., 2007; Vidal et al., 1999). In the present study, optimum conditions for the cholesterol detection were chosen based on the information available from earlier literature (Li et al., 2003). Thus, 50 mM PBS (pH 7.0) containing 1% (m/v) Triton X-100 and 2% (v/v) 2-propanol was employed through the study for cholesterol determination.

Fig. 4A displays the amperometric response of ChOx/AuPt–Ch–IL/GCE biosensor for the successive increase in cholesterol concentrations at an operating potential of −0.1 V. The calibration curve for the biosensor under the optimized experimental conditions is shown in Fig. 4B. The biosensor exhibited two wide linear ranges of response to cholesterol, in the concentration range of 0.05–6.2 mM and 6.2–11.2 mM with correlation coefficients of 0.9983 and 0.9992, respectively. The calibration curve slope was changed at cholesterol concentration of 6.2 mM and was decreased. Upon successive addition of cholesterol, the concentration of Triton X-100 was also increased in the solution which consequently caused a decrease in the biosensor response as it was suggested by other researchers (Vidal et al., 1999; Gopalana et al., 2009). The sensitivity of the biosensor was 90.7 μA mM^{-1} cm^{-2} and the limit of detection was 10 μM. The current at the modified electrode increased immediately after the addition of cholesterol and reached a steady value. The average time required to reach 90% of the saturation current value (response time, RT) was determined as less than 7 s. The RT at this biosensor is much lower than those reported for the cholesterol biosensors based on sol–gel/chitosan hybrid composite film (13 s) (Tana et al., 2005), polypyrrole–hydrogel (30 s) (Brahim et al., 2001) and ChOx in sol–gel film (51 s) (Yao and Takashima, 1998). This fast response is attributed to the synergetic influence of AuPt NPs, chitosan and IL. The reproducibility (RSD) of this sensor was 4.2% ($n = 5$) for the determination of 0.5 mM cholesterol.

Long-term stability of the biosensor was also evaluated by measuring its performance after every few days (Supporting Information, Fig. S4). The biosensor shows high stability for cholesterol detection, and retains about 90% of its original response to cholesterol after 30 days of storage. This shows that AuPt alloy NPs have good biocompatibility and maintain the biological activity of the immobilized enzyme.

The higher performance of this electrode in comparison with other cholesterol biosensor is obvious from Table 1. The apparent Michaelis–Menten constant (K_m), which gives an indication of the enzyme–substrate kinetics for the biosensors, can be calculated from the electrochemical version of the Lineweaver–Burk equation

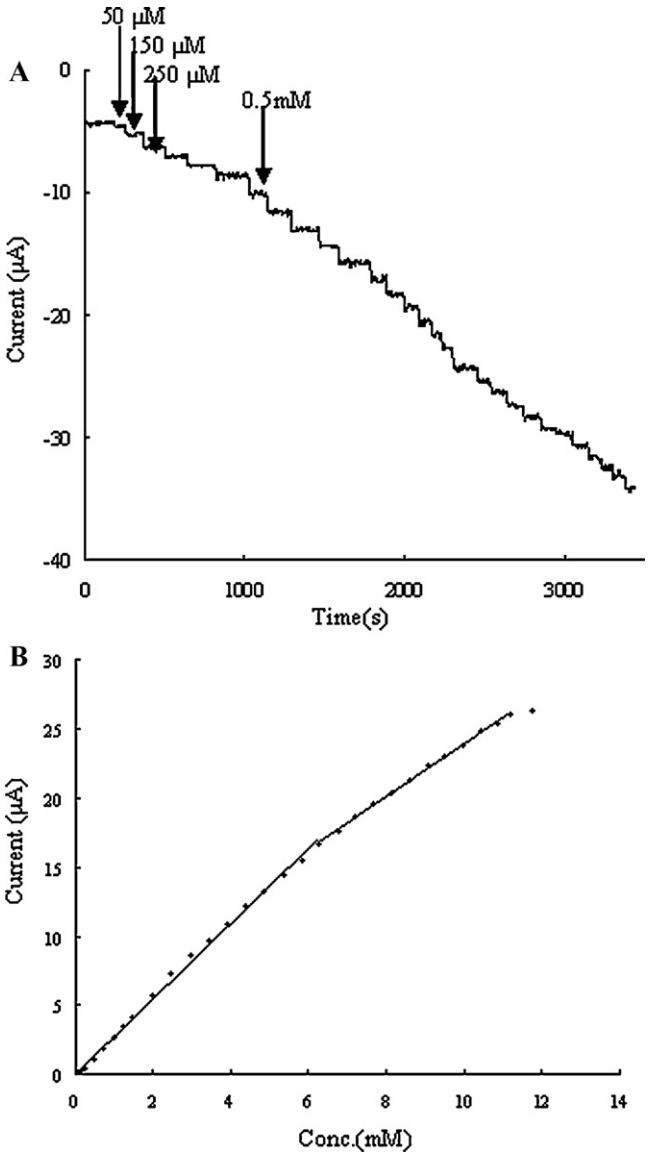


Fig. 4. (A) Amperometric responses for increasing cholesterol concentrations at ChOx/AuPt–Ch–IL/GCE in 50 mM PBS (pH 7.0) containing 1% Triton X-100 (m/v) and 2% (v/v) 2-propanol. Applied potential −0.1 V. (B) Calibration curve of ChOx/AuPt–Ch–IL/GCE with different concentrations of cholesterol.

$$\frac{1}{i_{ss}} = \frac{1}{i_{max}} + \frac{K_m}{i_{max}} C$$

where i_{ss} is the steady state current after the addition of substrate, C is the bulk concentration of the substrate, and i_{max} is the maximum current measured under saturated substrate condition.

Table 1
Characteristics of some cholesterol biosensors reported in recent years.

Electrode material	Linear range (mM)	Response time (s)	Applied potential (V)	Detection limit (μM)	Sensitivity (μA mM^{-1} cm^{-2})	Ref.
MWNT(SH)–Au/Chi–IL/ChOx	0.5–5	~7	−0.05	–	–	Gopalana et al. (2009)
MWCNT–chitosan–Pt–cholesterol	0.005–0.3	8	0.4	4.79	44	Tsai et al. (2008)
Poly(2-hydroxyethyl methacrylate)–polypyrrole	–	30	–	120	19.05×10^{-3}	Brahim et al. (2001)
Polypyrrole	0.025–0.3	0.7	0.7	5.7	43.99×10^{-3}	Vidal et al. (1999)
Polyaniline	–		0.45	–	2217.8×10^{-3}	Wang and Mu (1999)
Pt–ZnO	$(0.5–15) \times 10^{-3}$	<5	0.2	–	1886.4	Ahmad et al. (2010)
AuPt–Ch–IL/GCE	0.05–6.2, 6.2–11.2	<7	−0.1	10	90.7	This work

The K_m value of the cholesterol sensor was determined by plotting the steady state amperometric response ($1/i_{ss}$) vs. $1/C$ and was found to be 0.24 mM which was smaller than the reported results for ChOx immobilized in conducting polypyrrole film (9.8 mM) (Singh et al., 2004), polyaniline (2.72, 2.32 mM) (Wang and Mu, 1999), sol–gel (30.5 mM) (Yao and Takashima, 1998) and polymeric film (6 mM) (Bongiovanni et al., 2001), 0.41 mM for GC/PB/Ch–SiO_2–ChOx and the same as GC/PB/Ch–SiO_2–ChOx–MWCNTs (0.24 mM) (Tana et al., 2005). The smaller K_m value means that the immobilized ChOx possesses higher affinity to cholesterol. The immobilization of ChOx mentioned above appears to be beneficial and improves the biosensor performance.

3.8. Interference study

In the present work, the interference effects of 1 mM ascorbic acid (AA) and 1 mM glucose were tested on the amperometric response of 0.5 mM cholesterol (Supporting Information, Fig. S5). As stated before, generally, amperometric biosensors based on immobilized ChOx have been used to follow the current response of hydrogen peroxide generated by the enzyme catalyzed reaction. The amperometric detection of H_2O_2 is normally carried out by following the oxidation current responses at positive potentials (>0.5 V). However, current responses at these positive potentials are strongly influenced by oxidizable interferents such as glucose and ascorbic acid present in real samples. Hence, sensitive and selective detection of cholesterol based on monitoring the reduction current of H_2O_2 at a far negative potential (−0.1 V) caused these compounds not to interfere with the determination of cholesterol. No change in response current of cholesterol was observed in the presence of either 1 mM of AA or glucose.

3.9. Real sample analysis

To evaluate the ability of the sensor for routine analysis, the sensor was applied to the determination of cholesterol in blood serum samples. Most of the cholesterol in human blood is esterified with long chain fatty acids. Cholesterol esters do not function as substrates for ChOx biosensors. Hence, for the determination of total cholesterol in serum samples using the present biosensor, the samples were first pre-treated with cholesterol esterase. Cholesterol esterase catalyzes the hydrolysis of the esters to free cholesterol which can then become the substrate for ChOx oxidation. Here, 1 mL of treated serum sample was added to 10 mL of 50 mM PBS (pH 7.0) containing 1% Triton X-100 (m/v) and 2% (v/v) 2-propanol and the amperometric response of cholesterol was recorded at −0.1 V ($n = 3$). The results (Supporting Information: Table S1) show that the recoveries are in the range of 92–106%. Also, there is a very good agreement between the results obtained by the proposed biosensor and those obtained by the application of a routine enzymatic method (using cholesterol oxidase) in a local hospital.

4. Conclusions

A new amperometric cholesterol biosensor utilizing the synergetic influence of catalytic AuPt nanoparticles, the biocompatible chitosan and IL was fabricated. The biosensor electrode, ChOx/AuPt–Ch–IL/GCE, exhibited high selectivity, sensitivity, fast response time and good reproducibility. The selectivity of the biosensor is originated from the employed detection method based on reduction of hydrogen peroxide. The methodology employed in this study might be applied for a wide range of biosensors applications in relation to the detection of hydrogen peroxide.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.11.002.

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