International Journal of Biological Macromolecules 49 (2011) 707–715

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Construction of a triglyceride amperometric biosensor based on chitosan–ZnO nanocomposite film

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ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 23 June 2011
Accepted 1 July 2011
Available online 7 July 2011

Keywords:
Triglyceride
Amperometric biosensor
ZnONPs
Lipase
Glycerol
Kinase
Glycerol-3-phosphate oxidase

ABSTRACT

A method is described for construction of a novel amperometric triglyceride (TG) biosensor based on covalent co-immobilization of lipase, glycerol kinase (GK) and glycerol-3-phosphate oxidase (GPO) onto chitosan (CHIT) and zinc oxide nanoparticles (ZnONPs) composite film deposited on the surface of Pt electrode. The enzymes–ZnONPs–CHIT composite was characterized by X-ray diffraction (XRD), scanning electron microscope (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The sensor showed optimum response within 6 s at pH 7.5 and temperature of 35 °C. The sensor measures current due to electrons generated at 0.4 V against Ag/AgCl from H_2O_2, which is produced from triolein by co-immobilized enzymes. A linear relationship was obtained between a wide triolein concentration range (50–650 mg/dl) and current (mA) under optimum conditions. The biosensor showed high sensitivity, low detection limit (20 mg/dl) and good storage stability (half-life of 7 months at 4 °C). The biosensor was unaffected modified by a number of serum substances at their physiological concentrations. The biosensor was evaluated and employed for determination of TG in sera in apparently healthy subjects and persons suffering from hypertriglyceridemia.

1. Introduction

Triglyceride (TG), an ester derived from glycerol and three fatty acids molecules is a component of very-low-density lipoproteins (VLDLs) and chylomicrons, which play an important role in metabolism as energy source and transporters of dietary fat [1]. High levels of triglycerides in the bloodstream promote atherosclerosis, which indicates the risk of heart disease, coronary heart disease (CAD) and hypolipoprotenimia [2]. TG level in the serum of healthy person varies in the range 150–190 mg/dl. Level higher than 200 mg/dl or ranging between 200 and 499 mg/dl is an alarming situation, while level above >500 mg/dl is distressful. Existing analytical methods for determination of serum triglycerides are tedious, time consuming sample preparation, require expensive reagents, equipments and skilled person to operate [3–7]. Immobilized enzyme based biosensors are comparatively simpler, sensitive, rapid and does not require sample preparations. There are two types of enzyme based TG biosensors: (i) potentiometric biosensor using porous silicon [8] and (ii) amperometric biosensors based on artificial membranes such as collagen [9], cellulose acetate (CA) [10], polyvinylchloride (PVC) [11] and polyvinyl alcohol (PVA) [12]. Membranes had few obvious setbacks like time consuming preparation, long response time, fragility and low storage stability and above all a major problem of desorption or leaching of enzymes during washing.

Chemically modified electrodes have received extensive attention owing to their evident advantages such as high sensitivity, selectivity, stability, less prone to surface fouling and low over potential for smooth electron transfer [13]. Recently considerable attention has been paid on the use of nanoparticles for improving biosensor, e.g. Prussian blue modified screen printed electrode [14], iridium nano-particle modified carbon paste electrode [15] polyaniline (PANI)/single-walled carbon nanotubes (SWCNTs) ITO coated glass plate [16].

Nanostructured metal oxides have unique ability to promote kinetics for faster electron transfer between electrode and the active site of the desired enzymes [17–22]. Among the metal oxide nanoparticles, ZnO nanoparticles (ZnONPs) have been explored as a potential nanomaterial for biosensing due to their unusual but favorable properties such as high surface area, high catalytic efficiency, non-toxicity, chemical stability and strong adsorption ability (high isoelectric point pH 9.5). Nanoporous ZnONPs also enhances the active surface area for strong adsorption of biomolecules [20–22].

Chitosan (CHIT) is an interesting biopolymer for immobilization of biomolecules, because of excellent film-forming ability,

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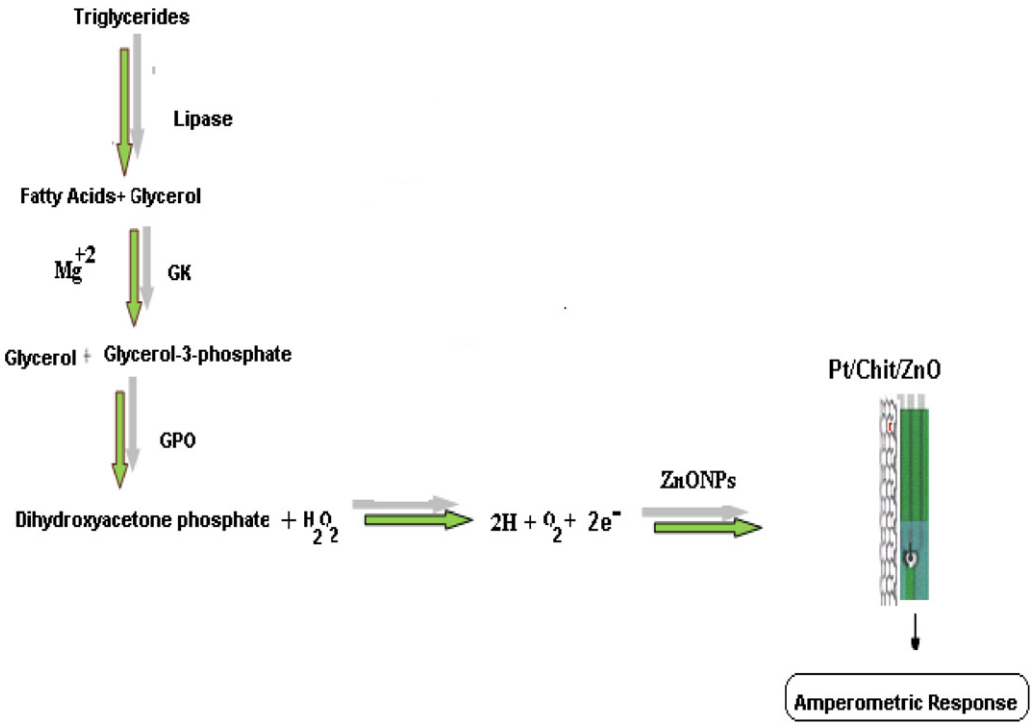
0141-8130/$ – see front matter.
doi:10.1016/j.ijbiomac.2011.07.001



Scheme 1. The enzymatic reaction scheme for measuring the TG levels using the present biosensor.

high permeability, mechanical strength, non-toxicity, biocompatibility, low cost and easy availability. Moreover, chemically modified amino groups of CHIT provides hydrophilic environment for the biomolecules [23,24]. ZnONPs dispersed in chitosan (CHIT) have been used as a support immobilization of enzymes for improvement of biosensors for detection of H_2O_2 and colorectal cancer DNA [25,26]. Present report describes the improvement of an amperometric TG biosensor employing ZnONPs dispersed in CHIT film on the surface of a Pt electrode.

1.1. Mechanism of TG sensing

The electrochemical reactions involved in measuring the TG by the present biosensor are given in Scheme 1. The enzymatically produced H_2O_2 from TG is splitted in to $2H^+ + O_2$ and $2e^-$, flow of these e^- under potential difference produces current which is sensed and monitored by an electrochemical sensor. Therefore, measured current determines the TG level, which is directly proportional to TG concentrations.

2. Experimental

2.1. Reagents and materials

Triton X-100 from Sigma Chemicals Co., St. Louis, USA; 3,5-dichloro-2-hydroxy benzene sulphonic acid (DHBS) from E-Merk Germany, triolein, ATP and zinc nitrate ($Zn(NO_3)_2{\cdot}4H_2O$) from SISCO Research Laboratory Pvt. Ltd., Mumbai, India and kit for enzymic colorimetric method (Enzo kit) for TG from Erba Transasia, Daman, India were used. All other chemicals were of analytic reagent (AR) grade.

2.2. Preparation of mixture of lipase, glycerol kinase (GK) and glycerol-3-phosphate oxidase (GPO)

To prepare mixture of lipase, GK and GPO, 3.0 mg of reagent 1 of Enzo kit for TG consisting of lipase (110 U), GK (80 U), GPO (500 U) and 4-aminoantipyrine was dissolved in 1 ml of 0.02 M sodium phosphate buffer pH 7.0 and loaded on a Sephadex G-100 gel column (24 cm × 1 cm) pre-equilibrated with 0.02 M sodium phosphate buffer pH 7.0. The column was run in the same buffer at a flow rate of 0.5 ml/min. The fractions (2 ml each) were collected and analyzed for proteins by Lowry method. The fractions showing high presence of proteins were pooled and treated as mixture of lipase, GK and GPO. The mixture was tested for combined activity of lipase, GK and GPO as given below.

2.3. Preparation of triolein solution

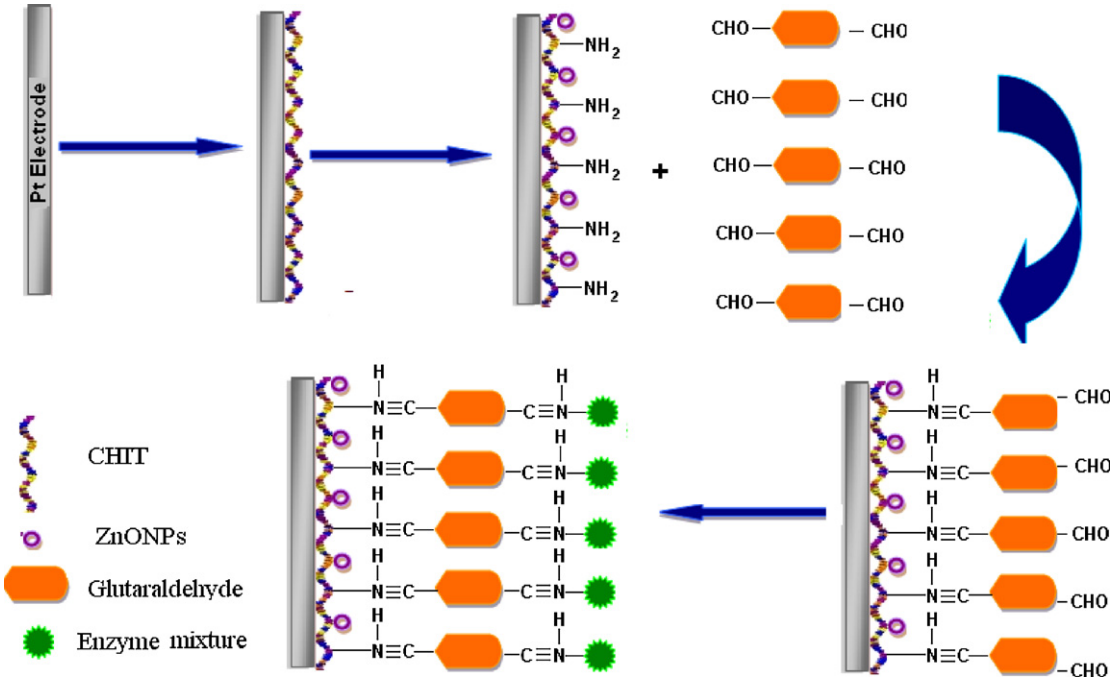
Triolein was used as a substrate for lipase, GK and GPO. Triolein emulsion was prepared as described [27]. Solutions of different concentrations of triolein ranging from 10 to 700 mg/dl were prepared in 0.1 M sodium phosphate buffer (pH 7.0) and stored at 4 °C until use.

2.4. Assay of mixture of free lipase, GK and GPO

The assay of mixture of free lipase, GK and GPO was carried out in a 15 ml conical flask wrapped in a black paper [3]. The reaction mixture consisted of 0.54 μmol $MgCl_2$, 0.63 μmol ATP, 1.0 μmol potassium ferrocyanide, 0.27 μmol 4-aminophenazone, 1.53 μmol DHBS and 1.0 μmol of Triton X-100/liter of 0.1 M sodium phosphate buffer, pH 7.0. To 900 μl reaction mixture, 50 μl of mixture of enzymes was added and pre-incubated at 37 °C for 5 min. The reaction was started by adding 50 μl of triolein (650 mg/dl). After incubation at 37 °C for 15 min, A_{510} was read. H_2O_2 generated in reaction was calculated from standard curve between A_{510} vs. H_2O_2 concentration. The unit activity of mixture of enzyme was expressed as 1 μmol H_2O_2/min/ml.

2.5. Synthesis of ZnO NPs–CHIT composite

The beaker containing NaOH solution (100 ml) was heated at 55 °C. Then previously prepared solution of 0.45 M zinc nitrate



Scheme 2. The mechanism for preparation of ZnONPs–CHIT electrode and immobilization of enzymes onto ZnONPs–CHIT nanocomposite.

$(Zn(NO_3)_2{\cdot}4H_2O)$ and 0.9 M sodium hydroxide (NaOH) in distilled water was added drop wise (slowly for 40 min) to the heated solution, under high speed stirring. The beaker was sealed at this condition for 2 h. The dried ZnO NPs were cleaned with deionized water and ethanol and then air dried at 60 °C. The precipitated ZnO nanoparticles (5 mg) were dispersed into transparent CHIT solution (0.5 g of CHIT dissolved in 10 ml of 0.05 M acetate buffer solution) and kept overnight at room temperature followed by its magnetic stirring for 30 min and then sonication for about 1 h. A hybrid nanocomposite of ZnONPs–CHIT was formed.

2.6. Construction of an amperometric TG biosensor and response measurement

The nanocomposite film was deposited onto Pt electrode (surface area of 0.25 cm^2) by dipping the electrode into solution of hybrid nanocomposite of ZnONPs–CHIT overnight. The nanocomposite film was activated by dipping the modified Pt electrode into 2.5% glutaraldehyde in 0.02 M phosphate buffer pH 7.0 and then keeping it at room temperature for 2 h. This activated electrode was removed and washed thoroughly in 0.02 M phosphate buffer pH 7.0. This activated nanocomposite film coated Pt electrode was immersed into mixture of enzyme solution and kept overnight for 4 °C for covalent immobilization of enzymes. The enzyme electrode (enzymes/ZnONPs–CHIT/Pt) was washed with deionized water followed by phosphate buffer (50 mM, pH 7.0) to remove unbound enzymes (Scheme 2). This working electrode along with Ag/AgCl as reference and Pt wire as auxiliary electrode were dipped into 4 ml reaction mixture [1.0 ml triolein and 3 ml 0.1 M sodium phosphate buffer pH 7.5], thereafter these electrodes were connected through potentiostat/galvanostat (Autolab, Eco Chemie, The Netherlands, Model: AUT83785). The electrodes were polarized at different potential (in volts) and the current was measured in mili amperes (mA).

2.7. Optimization of working conditions of TG biosensor

Various kinetic properties of enzymes/ZnONPs–CHIT/Pt electrode such as optimum pH, incubation temperature, time for maximum response and effect of substrate (triolein) concentration were studied amperometrically in order to optimize the working conditions of the biosensor.

2.8. Amperometric determination of TG in serum

Blood samples (1 ml each) centrifuged at 5000 rpm for 5 min and sera were collected from apparently healthy males, females (25 each) and persons suffering from hypertriglyceridemia of various categories such as peripheral vascular disease and pancreatitis from hospital Pt BDS PGIMS, University of Health Sciences Rohtak. TG content in serum was determined by the present biosensor in the similar manner as described above for its response measurement under its optimal working conditions except that triolein was replaced by serum. The current (mA) was measured and the amount of TG in serum was extrapolated from standard curve between triolein concentrations and current (in mA) prepared under optimal working conditions (Fig. 1).

2.9. Evaluation of TG biosensor

The biosensor was evaluated by studying analytic recovery, precision and correlation. The effect of various possible interfering substances found in blood such as urea, uric acid, glucose, fructose, ethanol, cholesterol, citric acid, ascorbic acid, lactic acid, malic acid, tartaric acid, alanine, leucine, bilirubin, creatinine and pyruvic acid was also studied at their physiological concentrations.

3. Results and discussions

3.1. Structural studies of ZnONPs

The ZnONPs obtained were characterized by XRD (Fig. 2a). All peaks were consistent with the peaks of wurtzite ZnO (JCPDS card No. 36-1451, $a = 3.249$ Å, $c = 5.206$ Å) with high crystallinity. Furthermore, no characteristic peaks were observed for other added impurities. Fig. 2b shows TEM image of the ZnONPs. The flower-like nanostructure was observed from image. The FTIR spectra of

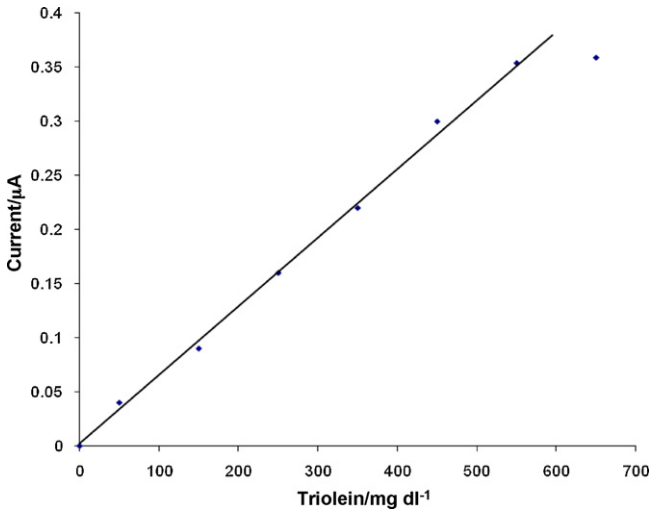


Fig. 1. The effects of triolein concentration (50–650 mg/dl) on the current response of the fabricated TG biosensor based on the modified enzymes/ZnONPs–CHIT/Pt electrode in 0.1 M sodium phosphate buffer at 0.4 V.

CHIT/Pt, ZnONPs–CHIT/Pt electrode and enzymes/ZnONPs–CHIT/Pt electrodes are given in Fig. 3. The CHIT exhibited characteristic absorption bands of amino saccharide at 3421 cm^{-1} (due to overlapping of –OH and $–NH_2$ stretching), 2811 cm^{-1} (due to $–CH_2$ stretching) and 1647 cm^{-1} (due to C–O stretching), while the ZnONPs–CHIT composite film showed additional peak at 492 cm^{-1} corresponding to the ZnONPs. The enzyme mixture was immobilized onto chitosan through covalent binding with glutaraldehyde. One –CHO group of glutaraldehyde was linked to $–NH_2$ group on surface of enzymes while other –CHO group was bound to $–NH_2$ group of chitosan on ZnONPs–CHIT composite film, which provided physically more stable complex. FTIR spectra of enzymes/ZnONPs–CHIT/Pt bioelectrode showed broadening of the peak at 3264 cm^{-1} and 1653 cm^{-1} due to the addition of carbonyl and amino groups confirming the binding of enzymes with the ZnONPs–CHIT matrix (Fig. 3).

3.2. Scanning electron microscopy and electrochemical impedance studies

The surface morphologies of bare electrode: (A) CHIT/Pt electrode, (B) ZnONPs–CHIT/Pt electrode (C) and enzymes/ZnONPs–CHIT/Pt (D) bioelectrode were investigated by SEM (Fig. 4). The bare electrode showed smooth surface (Fig. 4a) whereas CHIT/Pt electrode showed rough surface due to deposition of CHIT film (Fig. 4b). The granular morphology with heterogeneous roughness of ZnONPs–CHIT/Pt electrode showed the dispersed nanoparticles in CHIT matrix (Fig. 4c). Upon immobilization of enzymes, the granular morphology of ZnONPs–CHIT changed into the regular form, due to the interaction between ZnONPs–CHIT and enzymes (Fig. 4d). Fig. 5 shows electrochemical impedance spectra (EIS) of (a) CHIT/Pt electrode, (b) ZnONPs–CHIT/Pt electrode and (c) enzymes/ZnONPs–CHIT/Pt bioelectrode. The charge transfer process in enzymes/ZnONPs–CHIT/Pt bioelectrode has been studied by monitoring charge transfer resistance (*RCT*) at the electrode and

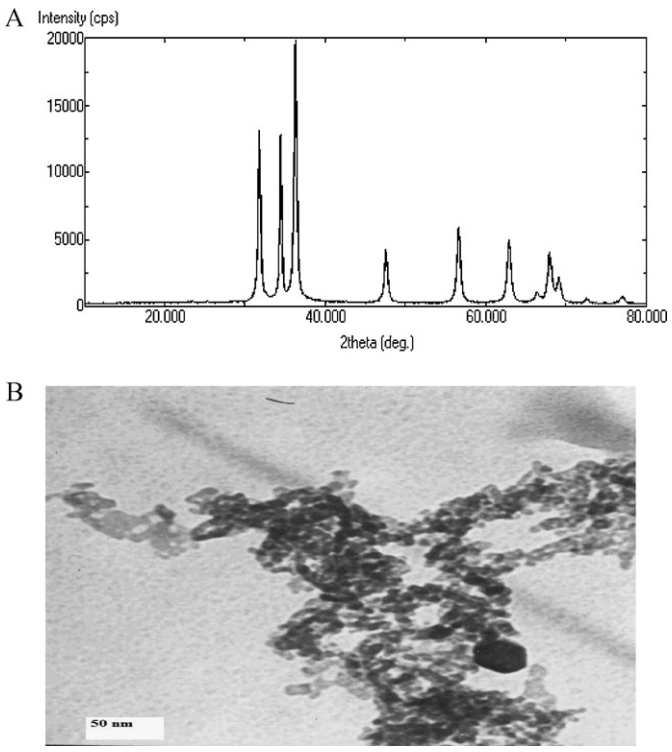


Fig. 2. X-ray diffraction pattern of ZnO nanoparticles (a) and transmission electron microscopic (TEM) images of ZnONPs (b).

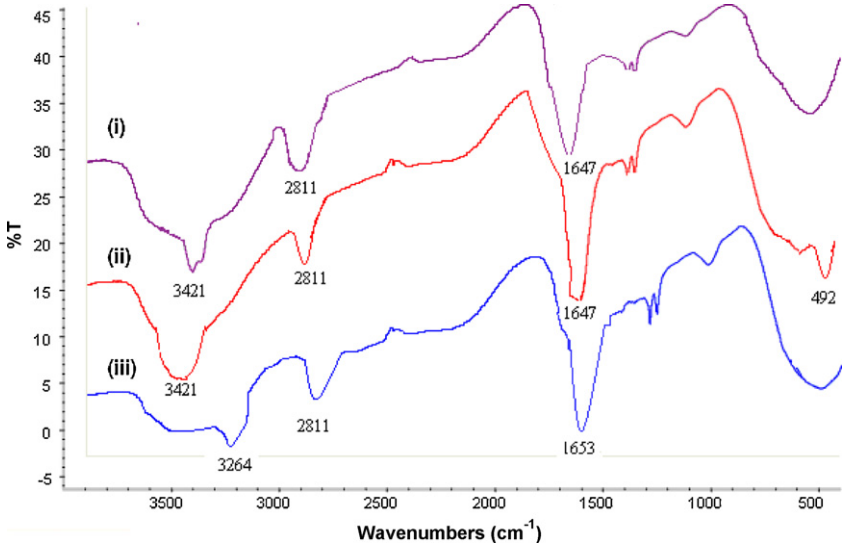


Fig. 3. FTIR spectra of (i) CHIT/Pt, (ii) ZnONPs–CHIT/Pt electrode and (iii) enzymes/ZnONPs–CHIT/Pt bioelectrode.

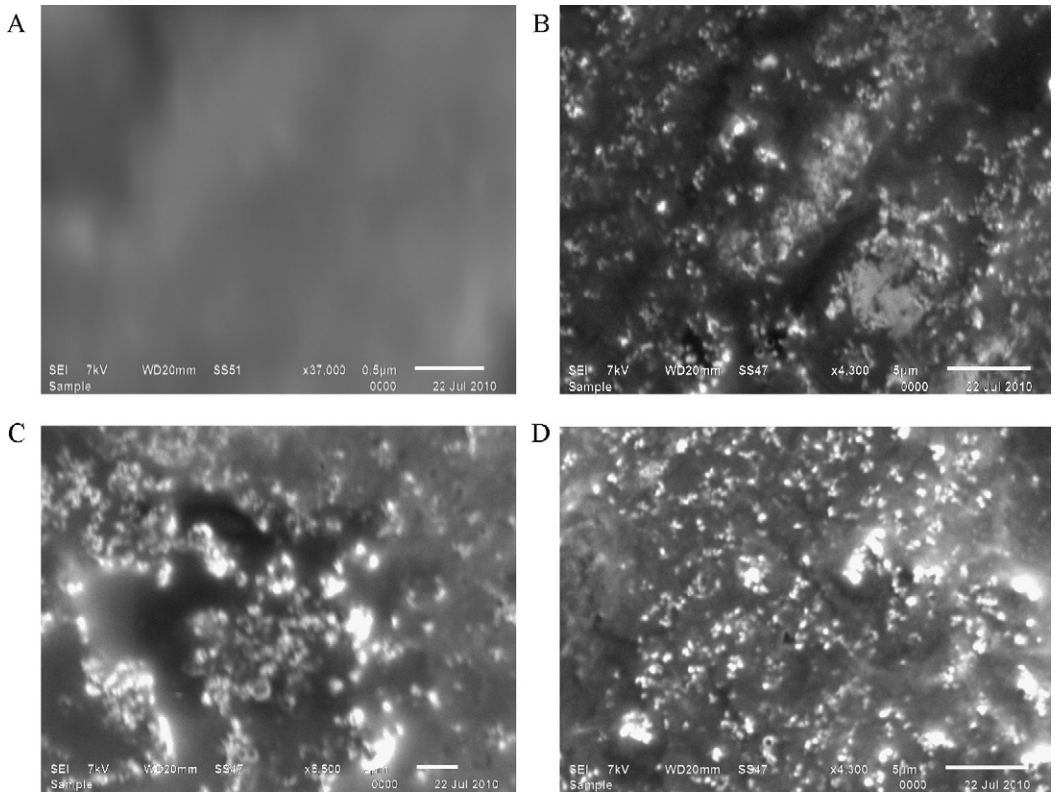


Fig. 4. Scanning electron microscopy of (a) bare electrode, (b) CHIT/electrode, (c) ZnONPs–CHIT electrode and (d) ZnONPs–CHIT/enzymes modified electrode.

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 *R*CT values for the CHIT/Pt, ZnONPs–CHIT/Pt and enzymes/ZnONPs–CHIT/Pt electrodes have been obtained as 6.68×10^2, 4.47×10^2 and $22.38 \times 10^2\ \Omega$, respectively. The electron transfer via redox couple is hindered by the presence of enzymes on electrode surface. The increased *R*CT value of enzymes/ZnONPs–CHIT/Pt electrode was due to the immobilization of enzymes onto ZnONPs–CHIT/Pt surface. This increase in *R*CT is attributed to the fact that most biological molecules, including enzymes, are poor electrical conductors at low frequencies (at least <10 kHz) and cause hindrance to the electron transfer.

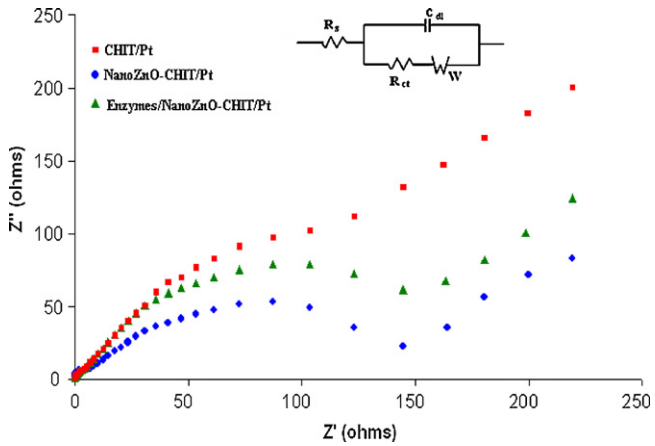


Fig. 5. Impedance spectroscopy study of (a) CHIT/Pt, (b) ZnONPs–CHIT/Pt and (c) enzymes/ZnONPs–CHIT/Pt in 0.1 M sodium phosphate buffer.

3.3. Cyclic voltammetry studies

Fig. 6a–c shows cyclic voltammogrammes for CHIT/Pt electrode, ZnONPs–CHIT/Pt electrode and enzymes/ZnONPs–CHIT/Pt bioelectrode in phosphate buffer saline (50 mM, pH 7.0, 0.9% NaCl) containing $[Fe(CN)_6]^{3}]^{4-}$ (5 mM). It was observed that the anodic potential shifted towards positive side with increase in current and the cathodic peak potential shifted in the reverse direction in presence of ZnO nanoparticles in the CHIT film. In other words the redox potential of ZnONPs–CHIT electrode shifted towards the higher side than that of pure CHIT film due to the incorporation of ZnO nanoparticles. It appeared that the ZnONPs–CHIT nanocomposite electrode provides a biocompatible environment to the enzymes, and ZnONPs act as an electron accelerator, resulting in a fast electron transfer between enzymes and electrode [28].

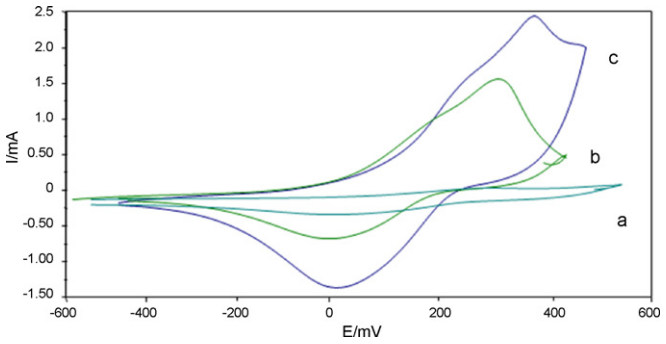


Fig. 6. Cyclic voltammogram of ZnONPs–CHIT/enzymes modified electrode (a) CHIT/Pt electrode, (b) ZnONPs–CHIT/enzymes and (c) ZnONPs–CHIT modified electrode.

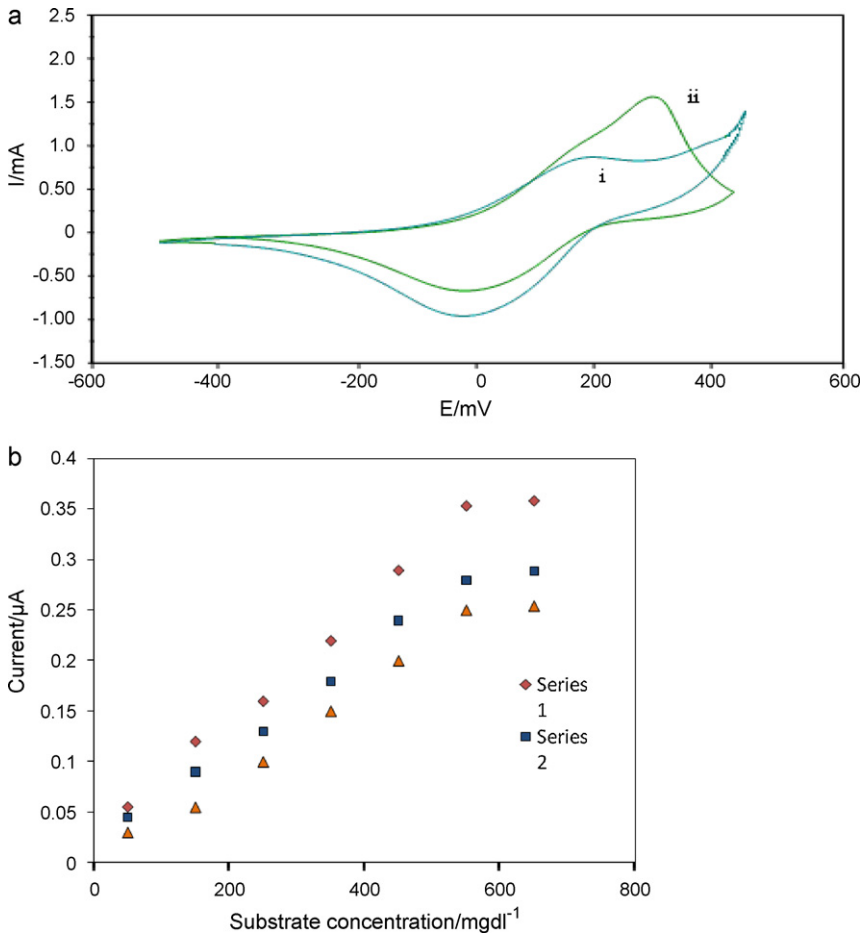


Fig. 7. (a) CV response of ZnO nanoparticles on the enzymes electrode current response (i) without ZnO nanoparticles; (ii) with ZnO NPs. (b) Calibration curves of the ZnONPs–CHIT/enzymes electrode with different concentrations of ZnO in 0.05 M phosphate buffer solution (pH 7.0). Operating potential: 200 mV vs. Ag/AgCl. The hybrid was prepared from a solution with different ratio of ZnO to chitosan. (a) 1:50; (b) 1:100; (c) 1:200; the calibration curves of enzymes Pt/CHIT/electrode.

3.4. Current response curves of enzyme electrode with ZnO nanoparticles

The effect of the ZnO nanoparticles on the sensitivities of the TG biosensor was studied. The current response curves of enzyme electrodes with and without ZnONPs are shown in Fig. 7a (i and ii). When TG concentration was 100 mM, the current response of the electrode without ZnONPs was 0.75 mA, and of the electrode with ZnONPs was 1.5 mA. The effect of various concentrations of ZnONPs in the stock ZnO/CHIT solution used for preparing immobilization films was studied. The effect of the concentrations of ZnO/CHIT solution used for preparing immobilization films was studied. Fig. 7b shows the calibration curves of the ZnO/CHIT electrode with different concentrations of ZnONPs in 0.067 M phosphate buffer solution (pH 7.5). These results clearly indicated that the amount of ZnONPs had an intense effect on the electrode response. Fig. 7b (i) showed that electrode response using the ZnONPs with the mass ratio of ZnO:CHIT = 1:50 showed the lowest response. As the amount of ZnONPs was decreased to the mass ratio of ZnO:CHIT = 1:100, the enzyme electrode response reached a maximum (Fig. 7b (ii)). However, further decrease of ZnONPs to the mass ratio 1:200 did not improve the response further (Fig. 7b (iii)). The varying sensitivities could be explained from one side as a consequence of the varying amount of enzymes immobilized in the hybrid film. The amount of enzymes immobilized is based on the absorption of nanoporous ZnONPs. When the ZnONPs concentration was low, the restricted amount of ZnONPs was not efficient for immobilizing sufficient enzymes. As the amount of ZnONPs was increased, the enzyme electrode showed an improved response. Nevertheless, too much ZnONPs in a ZnO/CHIT film was also not helpful for the electrode response as this would guide to a higher obstruction for diffusing species. It was seen that

Table 1
Analytical recovery of added triglyceride in serum samples determined by TG biosensor based on ZnO NPs.

Triglyceride added (mg/dl)	Triglyceride found (mg/dl)	Recovery (%)
–	180	–
20	199.2	96.00 ± 0.23
50	227.3	94.60 ± 0.4

Table 2
Within and between batch assay coefficient of variation (CV) for determination of serum triglycerides by TG biosensor based on ZnO NPs.

n	Triglycerides (mg/dl)	CV (%)
Within assay (5)		
110.89	109.61 ± 1.15	1.04
109		
107.99		
110.4		
109.8		
Between assay (5)		
114.4		2.75
115.5	117.1 ± 3.23	
120.9		
114.4		
120.3		

ZnONPs could significantly enhance the current response of the electrodes, which was mainly due to the large surface area of ZnO nanoparticles that make enzymes inevitably adsorbed on the surface of nanoparticles. Chen et al. [29] had proved that the surface of nanoparticles facilitates the immobilization of enzyme and improves its activity and stability. Results have shown that ZnO nanoparticles provided a conductive path from enzymes to the Pt electrode, so it could accelerate the electron transfer rate between enzymes and the Pt wire and make the current response improved markedly.

3.5. Effect of substrate concentration

Fig. 8a showed linear sweep voltammogram response of enzymes/CHIT–ZnONPs/Pt electrode for different concentration of triolein (50–650 mg/dl). Fig. 8b exhibited the typical electrochemical responses of modified ZnONPs–CHIT nanocomposite electrode at different concentration of substrate, which shows that as TG (triolein) concentration was increased, oxidation current was also increased. The detection limit was 20 mg/dl, a concentration that gave a signal equal to three times the standard deviation of the blank signal.

3.6. Optimization of the biosensor

Effects of working potential, pH, temperature and response time on the biosensor were observed. The results of the effect of working potential on biosensor have been presented in Fig. 9a. The working potential was stepped up from 0.2 to 0.6 V. The current response steadily increased with the corresponding rise in working potential from 0.2 to 0.4 V, and then reached a plateau from 0.4 to 0.6 V. Therefore, 0.4 V was optimally chosen as the working potential for amperometric detection of TG concentration. Fig. 9b

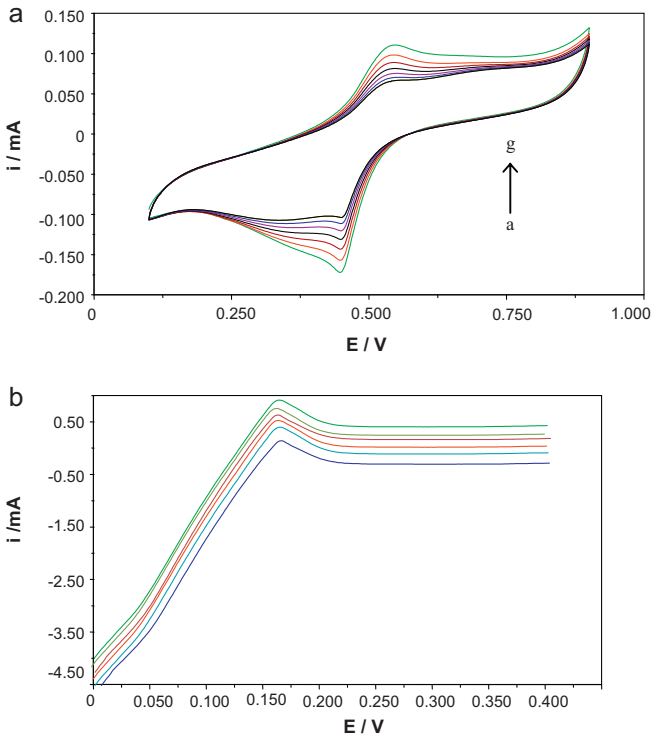


Fig. 8. (a) The effects of substrate concentration: (a) 50, (b) 150, (c) 250, (d) 350, (e) 450, (f) 550, (g) 650 mg/dl and (a) on the CV response (b) on the linear sweep voltammogram response of the fabricated TG biosensor based on the modified enzymes/ZnONPs-CHIT/Pt electrode in 0.1 M sodium phosphate buffer at 0–1.0 V at a scan rate of 100 mV/s.

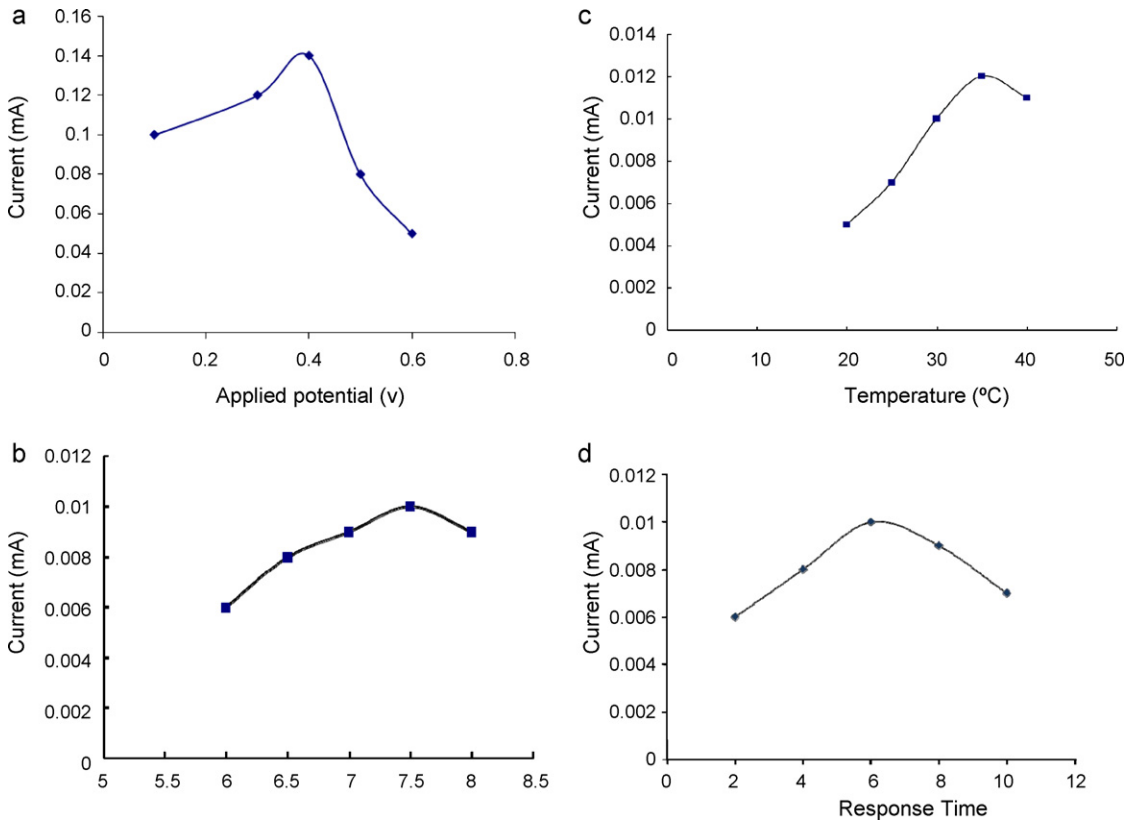


Fig. 9. The effects of various parameters on the current response of modified ZnONPs–CHIT/enzymes Pt/electrode. (a) Applied potential, (b) pH, (c) temperature and (d) time response.

Table 3
Interference effect of various compounds by TG biosensor based on ZnO NPs.

Interferents	Relative response (%)
Glucose	100
Fructose	100
Ethanol	100
Ascorbic acid	100
Citric acid	100
Lactic acid	100
Malic acid	97
Tartaric acid	100
Alanine	100
Leucine	100
Urea	98
Uric acid	95
Cholesterol	90
Bilirubin	100
Creatinine	100

shows the effect of pH of the reaction buffer on the response of the biosensor. The pH effect on the sensor response was evaluated in the pH range from 6.0 to 8.5. The optimum response was achieved at the pH 7.5. The effect of incubation temperature on the biosensor was examined between 25 and 45 °C. The current (mA) response of the biosensor increased with increasing temperature and reached a maximum at approximately 35 °C, and then started going down as the temperature turned higher (Fig. 9c). In addition, the current response kept correspondingly steady between 35 and 45 °C, indicating bioconjugate of enzymes and ZnONPs–CHIT had good thermodynamic stability and life span. However, the 35 °C was selected for this work. The time course study of the enzyme electrode carried out between time ranges of 2–12 s at a regular interval of 2 s, Maximum response was observed at 6 s as shown in Fig. 9d.

3.7. Precision and analytical recovery

The precision and accuracy of the present method for serum TG measurement were determined over seven days. Each day seven replicate serum samples were analyzed for TG content. The repeatability and inter-day precision were less than 3% (Table 1), also the recovery was observed within 94–96% (Table 2). This demonstrated that the present method was reproducible, precise and accurate for determination of TG.

3.8. Interference study

Among the various serum substances tested such as urea, uric acid, glucose, fructose, ethanol, cholesterol, citric acid, ascorbic acid, lactic acid, malic acid, tartaric acid, alanine, leucine, bilirubin, creatinine and pyruvic acid, at their physiological concentrations, none had practically any significant interference (Table 3).

3.9. Accuracy

To study the accuracy of the present method, TG level in 20 serum samples were determined by both the commercial enzymic colorimetric method (x) and the present method (y). The values obtained by both the methods matched with each other with a good correlation ($r = 0.985$).

3.10. Determination of serum TG

TG serum level in apparently healthy adults as measured by the present biosensor ranged from 50 to 180 mg/dl which is in established normal range (40–190 mg/dl). The TG level in the sera of persons suffering from hypertriglyceridemia was in the range 200–499 mg/dl.

Table 4
A comparison of analytical properties of recent enzymic TG biosensors with the present biosensor.

Type	Type of support for immobilization	Type of electrode	Method of immobilization	Optimum pH	Potential (V) for max. current	Detection limit	Linear range	Response time (s)	Storage stability (months)	Reference
Amperometric	Collagen membrane	Rotating platinum electrode (RPE)	NR	8.0	0.3	10 mg/dl	10–500 mg/dl	15 min	82% loss in 27 days	[8]
Amperometric	CA	Pt	BSA glutaraldehyde cross-linking	6.5	0.4	18 mg/dl	18–310 mg/dl	40 s	50% loss in 25 days	[9]
Amperometric	PVC	Pt	BSA glutaraldehyde cross linking	7.5	0.4	10 mg/dl	50–200 mg/dl	30 s	50% loss in 40 days	[10]
Amperometric	PVA	Pt	Glutaraldehyde cross linking	7.0	0.4	19 mg/dl	50–200 mg/dl	2 s	50% loss in 70 days	[11]
Potentiometric	Porous silicon	ND	ND	ND	ND	522 mg/dl	522–1860 mg/dl	15 min	6 months	[12]
Electrochemical	Iridium nanoparticles	Carbon	Physical	ND	+0.15 V	ND	0–855 mg/dl	ND	Disposable	[14]
Electrochemical	Polyaniline/single walled carbon nanotubes	ITO	Covalent cross-linking	7.4	NR	50 mg/dl	50–400 mg/dl	12 s	13 weeks	[15]
Amperometric	ZnONPs–CHIT nanocomposite	Pt	Covalent cross-linking	7.5	0.4 V	20 mg/dl	50–650 mg/dl	6 s	7 months	Present

3.11. Stability of the enzyme electrode

The shelf life of the enzymes/ZnONPs–CHIT/Pt electrode has been investigated for 7 months by measuring electrochemical current response at a regular interval of 1 week. The electrode retained 75% of its initial activity even after 6 months when stored in refrigerated conditions (4 °C) and thereafter the current response decreased to 50% after 7 months.

A comparison of various analytical properties of recent amperometric TG biosensors with the present one is summarized in Table 4.

4. Conclusion

The use of ZnONPs–CHIT composite film for preparation of an amperometric enzyme TG sensor has improved its performance in terms of response time (6 s), sensitivity ($0.89\,mg\,dl^{-1}\,\mu A^{-1}$) and stability (half-life of 7 months at 4 °C) compared to earlier biosensors. There was a good correlation between serum TG values as measured by the standard enzymic colorimetric method and present sensor. Unlike earlier biosensors, it is unaffected by a number of serum substances. Based on these observations this composite film could also be employed for improvement of other biosensors.

References

[1] IUPAC-IUB, Commission on Biochemical Nomenclature , Eur. J. Biochem. 2 (1967) 127–131.
[2] .D.L. Nelson, M.M. Cox, Lehninger, Principles of Biochemistry , 3rd ed., New York, Worth Publishing, 2000.
[3] .P. Fossati, L. Prencipe, Clin. Chem. 28 (1982) 2077–2080.
[4] .J.E. Voysey, D.C. Wilton, Clin. Chem. 40 (1994) 14–17.
[5] .M. Okazaki, N. Komoriya, H. Tomoike, N. Inowe, S. Itoh, S. Hoosaki, J. Chromatogr. B: Biomed. Sci. Appl. 709 (1998) 179–187.
[6] .E. Van Handel, D.B. Zilversmit, J. Lab. Clin. Med. 50 (1957) 152–157.
[7] .I. Bjorkhem, R. Blomstrand, L. Svensson, Clin. Chim. Acta 71 (1976) 191–198.
[8] .R. Reddy, R.C. Anju, B. Enakshi, Biosens. Bioelectron. 16 (2001) 313–317.
[9] .H. Winartasaputra, S.S. Kutan, G.C. Cuilbault, Anal. Chim. 54 (1982) 1987–1990.
[10] Minakshi, C.S. Pundir, Sens. Actuators B: Chem. 133 (2008) 251–255.
[11] J. Narang, M. Bhambi, Minakshi, C.S. Pundir, Anal. Lett. 40 (2010) 1–10.
[12] C.S. Pundir, B.S. Singh, J. Narang, Clin. Biochem. 43 (2010) 467–472.
[13] G. Premamoy, K.S. Samir, C. Amit, Eur. Polym. J. 35 (1999) 699–710.
[14] .B. Rejeb, F. Arduini, A. Amine, M. Gargouri, G. Palleschi, Anal. Chim. Acta 594 (2007) 1–8.
[15] .W.Y. Liao, C.C. Liu, T.C. Chou, Analyst 133 (2008) 1757–1763.
[16] .C. Dhand, P.R. Solanki, M. Datta, B.D. Malhotra, Electroanalysis 22 (2010) 2683–2693.
[17] .P. Pandey, M. Datta, B.D. Malhotra, Anal. Lett. 41 (2008) 157–207.
[18] .H. Zhou, X. Gan, J. Wang, X. Zhu, G. Li, Anal. Chem. 77 (2005) 6102–6104.
[19] .S.A. Kumar, S.M. Chen, Anal. Lett. 41 (2008) 141.
[20] .S.P. Singh, S.K. Arya, M.K. Pandey, B.D. Malhotra, S. Saha, K. Sreenivas, V. Gupta, Appl. Phys. Lett. 91 (2007) 063901.
[21] .A. Wei, X.W. Sun, J.X. Wang, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, W. Huang, Appl. Phys. Lett. 99 (2006) 123902.
[22] .J.X. Wang, X.W. Sun, A. Wei, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, Appl. Phys. Lett. 88 (2006) 233106.
[23] .H.L. Jiang, J.T. Kwon, Y.K. Kim, E.M. Kim, R. Arote, H.J. Jeong, J.W. Nah, Y.J. Choi, T. Akaike, M.H. Cho, C.S. Cho, Gene Ther. 14 (2007) 1389–1398.
[24] .J.D. Liao, S.P. Lin, Y.T. Wu, Biomacromolecules 6 (2005) 392–399.
[25] Y.Q. Miao, S.W. Tan, Analyst 125 (2000) 1591–1594.
[26] .K.J. Feng, Y.H. Yang, Z.J. Wang, J.H. Jiang, G.L. Shen, R.Q. Yu, Talanta 70 (2006) 561.
[27] N.P. Ehle, C.M. Schotz, J. Lipid Res. 17 (1976) 536–546.
[28] .H.L. Zhang, X.Z. Zou, G.S. Lai, D.Y. Han, F. Wang, Electroanalysis 19 (2007) 1869–1874.
[29] .Z.J. Chen, X.M. Ou, F.Q. Tang, L. Jiang, Colloids Surf. B 7 (1996) 173–179.