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An electrochemical biosensor for fructosyl valine for glycosylated hemoglobin detection based on core–shell magnetic bionanoparticles modified gold electrode

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ABSTRACT

A high-performance amperometric fructosyl valine (FV) biosensor was developed, based on immobilization of fructosyl amino-acid oxidase (FAO) on core–shell magnetic bionanoparticles modified gold electrode. Chitosan was used to introduce amino groups onto the surface of core–shell magnetic bionanoparticles (MNPs). With FAO as an enzyme model, a new fructosyl valine biosensor was fabricated. The biosensor showed optimum response, when operated at 50 mV s^{-1} in 0.1 M potassium phosphate buffer, pH 7.5 and 35 °C. The biosensor exhibited excellent sensitivity [the detection limit is down to 0.1 mM for FV], fast response time (less than 4 s), wide linear range (from 0 to 2 mM). Analytical recovery of added FV was 95.00–98.50%. Within batch and between batch coefficients of variation were <2.58% and <5.63%, respectively. The enzyme electrode was used 250 times over 3 months, when stored at 4 °C.

1. Introduction

Over the past several decades, diabetes mellitus has become a major health problem worldwide, reaching epidemic proportions in many developing countries as well as in minority groups in the developed world (Bennett et al., 1971; King and Rewers, 1993). Diabetes can be associated with serious complications and premature death. Glycosylated hemoglobin (HbA_{1c}) is the major glycohemoglobin species in human blood. The HbA_{1c} level, defined as the ratio between HbA_{1c} concentration and total hemoglobin concentration, is considered to be a very useful diagnostic marker for diabetic patient in addition to the measurement of the glucose level (John, 2003). Glycation of hemoglobin has been associated with cardiovascular disease, nephropathy and retinopathy in diabetes mellitus. Since the lifetime of hemoglobin in blood is approximately 2–3 months, the HbA_{1c} level provides a good indication of glucose level over this period of time. The determination of HbA_{1c} in clinical practice is now accepted as the gold standard of glycemic assessment, providing an objective and retrospective view of diabetes control within 2 months preceding blood collection (Krishnamurti and Steffes, 2001). Therefore, a cost-effective measurement of HbA_{1c} level is essential for management of diabetic patient (Goldstein et al., 2004). Many methods have been proposed for the routine measurement of the HbA_{1c}. These methods are based on different analytical principle such as ion-exchange chromatography, electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC) and immunoassay (Hageman and Kuehn, 1977; Goldstein et al., 1986; John, 1997; Turner et al., 1999). However, these suffer from certain drawbacks such as time-consuming, labor-intensive, expert handling, pretreatment of sample etc. Electrochemical methods for clinical diagnosis have advantages such as good selectivity, relatively low cost and the potential for miniaturization and automation (Markovarga et al., 1995; Wang, 1999). Also, electrochemical measurement of HbA_{1c} level is a convenient method for the point-of care application and can be integrated with the current glucose meter for the diabetic patient management. Hence, there is great interest to develop an electrochemical enzyme sensor for HbA_{1c}. The enzymatic method for HbA_{1c} assay consists three steps: (1) proteolysis of the HbA_{1c} β-subunits to release fructosyl amino acid [*viz.* fructosyl valine (FV)], (2) the oxidative deglycation of the produced FV and (3) the detection of the enzymatically produced hydrogen peroxide. A fructosyl amino-acid oxidase (FAO) catalyzes the oxidative deglycation of fructosyl amino acids to produce the corresponding amino acids, glucosone and hydrogen peroxide (Horiuchi et al., 1989) as follows:

$$\underset{\text{(N-terminal residue of β-chain in } HbA_{1c})}{\text{Fru-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-ser}\ldots} \xrightarrow{\text{Protease}} \underset{\text{(FV)}}{\text{Fructosyl valine}} + \underset{\text{(His, Leu, Thr)}}{(\text{amino acid})_{n-1}} \quad (1)$$

$$\text{FV} + O_2 + H_2O \xrightarrow{\text{FAO}} \text{Valine} + \text{D-glucosone} + H_2O_2 \quad (2)$$

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As shown in reaction (2), the last reaction step in producing and detecting the hydrogen peroxide from FV can be used as the base for a FV biosensor. Based on this approach, the development of a FV biosensor is relatively simpler and expected to demonstrate the feasibility of the development of an HbA_{1c} biosensor. Hence the electrochemical enzymatic assay shown in reactions (1) and (2) for FV detection has been used for present work. Earlier a dehydrogenase-based biosensor for amperometric detection of FV has been reported (Sode et al., 2001). Stöllner et al. (2002) developed an amperometric immunosensor for glycated hemoglobin using membrane immobilized haptoglobin as affinity matrix. In this work, haptoglobin was immobilized on a carbonyldiimidazole activated cellulose membrane for binding of Hb. A flow-injection analysis (FIA) enzyme sensor for FV was also developed (Ogawa et al., 2002). This FIA was claimed to be the first sensor towards fructosyl peptide, though with insufficient sensitivity. A single-use, disposable iridium-modified electrochemical biosensor has also been reported (Fang et al., 2008). However, some of these methods lead to sensors of insufficient sensitivity and repeatability. Recently, magnetic nanoparticles as special biomolecule immobilizing carriers have aroused great interest in research (Tsang et al., 2000; Khin and Si-Shen, 2005). In the present report, we synthesized Fe_3O_4 magnetic bionanoparticles (MNPs) and silanized to form core–shell (Fe_3O_4–SiO_2) structure. After modification with amino groups from chitosan, the nanoparticles were cross-linked to FAO by glutaraldehyde. Then, the bio-nanoparticles were immobilized on the surface of electrode by electrodeposition. The biosensor was successfully used for the detection of glycosylated hemoglobin. Compared with earlier amperometric biosensors, the sensor prepared by immobilizing FAO on the modified core–shell MNPs showed advantages of high sensitivity and wide determining range.

2. Materials and methods

2.1. Reagents and apparatus

Fructosyl-amino acid oxidase (FAO, EC 1.5.3, 0.45 U mg^{-1} from recombinant *Escherichia coli*) was purchased from Sigma–Aldrich. Fructosyl valine (FV) was synthesized and purified as reported (Keil et al., 1985; Rajkumar et al., 2007). Tetraethylorthosilicate (TEOS) was from Fluka. Chitosan (MW $\sim 1 \times 10^6$; 75–85% deacetylation), l-valine, pyridine and polyethylene glycol (PEG) were from SRL, Mumbai. All other chemicals were of analytical reagent (AR) grade. Fresh serum samples of healthy individuals and diabetic patients were collected from hospital of Pt. BDS University of Health & Medical Science, Rohtak and stored at −20 °C until use.

2.2. Apparatus

All electrochemical experiments were performed on an Autolab PGSTAT 30 electrochemical workstation (Eco Chemie BV, The Netherlands) with the GPES 4.9 software. A core shell MNPs modified Au electrode of 1.0 mm diameter, a KCl-saturated Ag/AgCl electrode and a Pt electrode served as the working, reference and counter electrodes respectively were used in all electrochemical experiments. All potentials were measured with respect to the reference electrode. Scanning electron microscopic (SEM) images were taken at Dept. of Chemistry, M.D.U. Rohtak. Fourier transform infrared (FTIR) spectroscopy was performed on FTIR spectrometer (Model iS10, Thermoelectron, USA).

2.3. Preparation of MNPs

The synthesis of Fe_3O_4 MNPs was carried out following a typical procedure. Before mixing, 0.4 M hydrochloric acid and 0.7 M ammonia solution were bubbled by N_2 for 10 min. 8.5 g $FeCl_3 \cdot 6H_2O$ and 3 g $FeCl_2 \cdot 4H_2O$ were dissolved in 38 ml of 0.4 M hydrochloric acid, then added quickly to 375 ml ammonia solution under vigorous stirring (non-magnetic) at room temperature. After half-an-hour stirring, the precipitates were isolated by magnetic force. The precipitates were washed three times with distilled water then diluted with 150 ml doubly distilled water. Next, the silica was coated on the magnetic Fe_3O_4 core (Kuang et al., 2002). Fe_3O_4 suspension (20 ml) was added to 200 ml of 2-propanol and sonicated for 20 min. PEG (5.36 g), 20 ml water, 10 ml ammonia solution (28 wt.%) and 1.2 ml TEOS were added respectively into the suspension and allowed to react for 24 h under continuous stirring at room temperature (Sun et al., 2004). After the reaction was completed, the products were collected through centrifugation at 4000 rpm for 5 min, sonicated with ethanol and distilled water twice and then lyophilized to obtain the core–shell MNPs. To modify the surface of core–shell MNPs with amino groups, 0.5 g core–shell MNPs were suspended in 10 ml methanol, and sonicated for 3 min. Then 0.5 ml of 5% chitosan (in 2% acetic acid) was added, and the mixture was agitated for 12 h at room temperature. Then the precipitates were respectively washed with methanol and water in an ultrasonic bath, centrifugated and washed with PBS several times and suspended in 5 ml PBS. Thus, MNPs were obtained and stored at 4 °C until use.

2.4. Preparation of MNPs/Au electrode

Prior to the surface modification, the Au electrode was cleaned with piranha solution [H_2SO_4:H_2O_2 in ratio 3:1 (v/v)] for 20 min and then rinsed thoroughly with distilled water. Then the electrode was polished with alumina slurry. MNPs were electrochemically deposited onto Au electrode through cyclic voltammetry (CV) technique by applying 20 polymerization cycles at −0.6 to +0.6 V as described (Zhang et al., 2007) at a scan rate of 50 mV s^{-1} by immersing Au electrode in a solution (25 ml) containing 22 ml electrolyte [2.5 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ (1:1)] and 3 ml bionanoparticles (in 0.1 M KCl). The resulting MNPs/Au modified electrode was washed thoroughly with distilled water to remove unbound matter and kept in dry petri plate at 4 °C.

2.5. Preparation of enzyme electrode (FAO/MNPs/Au)

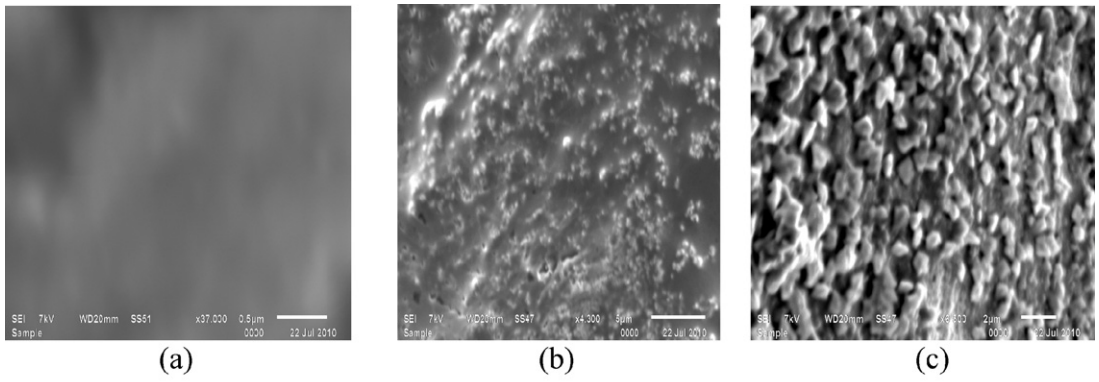
FAO was immobilized onto the MNPs modified Au electrode surface by glutaraldehyde coupling. The FV biosensor was constructed by mixing 100 μl of FAO with 1.0 ml of 2.5% glutaraldehyde, and then dropping this solution onto MNPs modified gold electrode surface. The resulting enzyme electrode was dried and then stored in the refrigerator at 4 °C.

2.6. FV synthesis

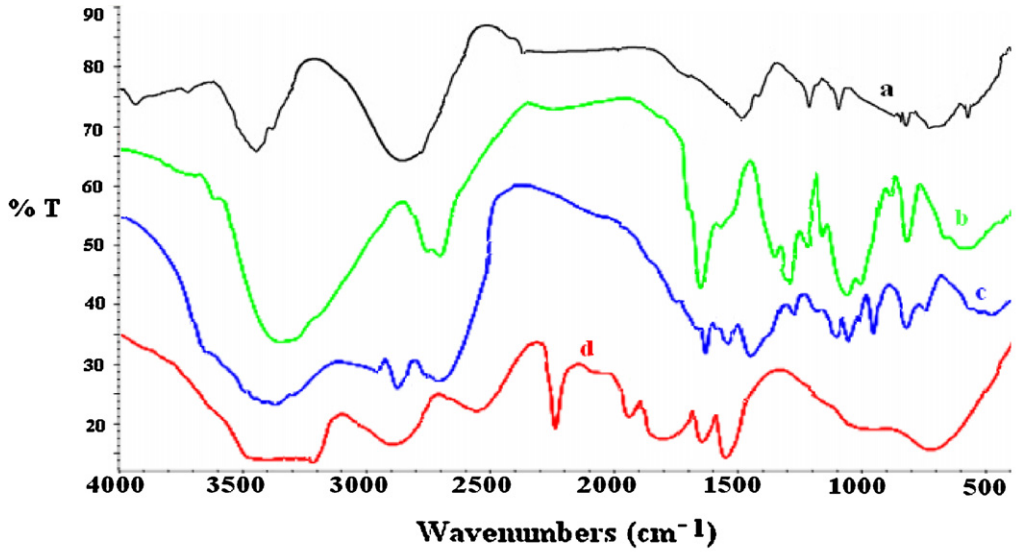
Since FV is not easily available from commercial sources, it was synthesized in our laboratory as described (Keil et al., 1985; Rajkumar et al., 2007). L-valine (0.16 mol, 18.8 g) in 400 ml pyridine and 400 ml acetic acid were mixed and stirred for 30 min at room temperature. Then (0.22 mol, 40 g) of glucose (Sigma–Aldrich) was added, purged with N_2 for 5 min and stirred for 4 days at room temperature. The dark colored mixture was filtered and the solvent was evaporated. A slightly yellowish amorphous product was obtained from re-crystallization in methanol and lyophilized to a colorless powder.

2.7. Optimization of FAO/MNPs/Au electrode

CV voltammetric peak height of the modified electrode consisting immobilized enzyme depends strongly on the type of supporting electrolyte. Among different electrolytes tested were



(A)



(B)

Fig. 1. (A) Scanning electron micrographs of (a) bare gold electrode, (b) magnetic bionanoparticles/Au electrode, (c) FAO/magnetic bionanoparticles/Au electrode. (B) FTIR transmission spectra (a) pure silica nanoparticles, (b) pure Fe_3O_4 nanoparticles, (c) chitosan-magnetic bionanoparticles and (d) FAO/magnetic bionanoparticles.

$K_3Fe(CN)_6/K_4Fe(CN)_6$ (2.5 mM) and KCl (1 N). To determine the optimum working conditions of the enzyme electrode, the pH of reaction buffer (0.1 M) was varied from pH 5.0 to 9.0 at an interval of pH 0.5 using potassium phosphate buffer. Similarly, to determine optimum temperature, the reaction mixture was incubated at 20–50 °C at an interval of 5 °C. To determine the optimum response time, the current response was studied from 5 s to 25 s. To study the effect of substrate concentration, different FV concentration, ranging from 0.1 mM to 2 mM corresponding to the physiological concentration of HbA_{1c} (Ogawa et al., 2002) were used.

2.8. Detection of HbA_{1c} in whole blood sample by FAO/MNPs/Au electrode

The procedure for HbA_{1c} detection from whole blood was same as that described for response measurement of the electrode, except that FV was replaced by whole blood sample. As FV concentration corresponds to HbA_{1c}, thus HbA_{1c} concentration was determined from the standard plot, extrapolated between different FV concentrations (mM) versus respective current (μA). The level of HbA_{1c} is defined as the ratio between glycosylated hemoglobin (mM) and total hemoglobin (mM).

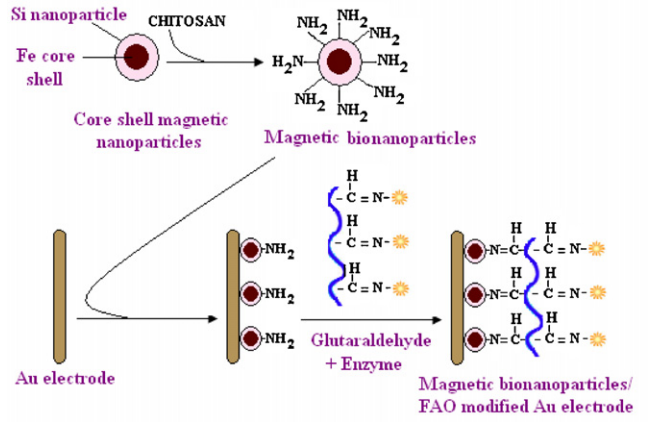
$$\%\,HbA_{1c} = \frac{\text{glycosylated hemoglobin (mM)}}{\text{total hemoglobin (mM)}} \times 100 \quad (3)$$

Results were evaluated using different statistical parameters and correlation with standard autoanalyser method. Total Hb value, measured using the standard routine method, were obtained from Pt BDS PGIMS Hospital, from where the samples were collected.

3. Results and discussion

3.1. Electrode surface characterization by SEM and FTIR

The morphology of bare Au electrode, MNPs/Au electrode and FAO immobilized onto MNPs/Au electrode was characterized by SEM studies (Fig. 1A). The SEM image of the bare Au electrode (image a) showed a smooth and featureless morphology, whereas nanocomposite (image b and c) displayed a stepwise layering of magnetic bio-nanoparticles and immobilization of FAO onto the



Scheme 1. Chemical reactions and sequence of electropolymerization of magnetic bionanoparticles onto gold electrode and immobilization of FAO onto modified Au electrode.

gold electrode, respectively. Fig. 1B displays the FTIR spectra of pure silica nanoparticles (curve a) which showed presence of prominent IR bands at 540, 800, 1080 and 1200 cm^{-1} band, attributed to excitation of Si–O–Si stretching mode of vibration, in accord with the literature (Bruni et al., 1999). These bands are very intense and correspond to the formation of the SiO_2 network. The peak observed at 3419 cm^{-1} in pure silica nanoparticles, indicated the presence of free Si–OH groups. On the low frequency side of this broad band, there appeared a band at 2891 cm^{-1}, which indicated the presence of methanol. The spectra of Fe_3O_4 nanoparticles (curve b) exhibit strong bands in the low frequency region 800 cm^{-1} due to the iron nanoparticle skeleton. Another prominent band at 582 cm^{-1} belongs to the stretching vibration mode and the torsional vibration mode of Fe–O bonds in the tetrahedral sites and in the octahedral sites of Fe_3O_4 nanoparticles. The FTIR spectrum of chitosan-MNPs (curve c) showed a new peak at around 1370 cm^{-1} associated with C–N stretching due to COO^- group in carboxylic acid salt of chitosan (Tian et al., 2003). A broad band in the region of 3200–3500 cm^{-1} was assigned to the H-bonded N–H and O–H stretching vibrations of chitosan (Bhattarai et al., 2005). A well-resolved band due to C–O stretching of saccharine moiety at 1075 cm^{-1} was also observed (Wyrna and Beyer, 2002; Daniel and Astruc, 2004). The characteristic bands of chitosan, amide I (1656 cm^{-1}), amide II (1593 cm^{-1}), and amide III (1373 cm^{-1}) were shifted to 1625, 1527, and 1450 cm^{-1} on the interaction with MNPs. Shifting of such amide bands to either higher or lower energies indicates the attachment of MNPs with chitosan through the amide bond region. FTIR of FAO/MNPs (curve d) showed characteristic peak at 1550 and 1640 cm^{-1} attributed to the primary and secondary amide linkages of enzyme molecules, peak at 1615–1700 cm^{-1} confirmed the presence of C=N bond and hence immobilization of FAO via amine group, introduced by chitosan on MNPs surface. However, the shape of the absorption peaks becomes broader due to the overlapping of the functional group of the FAO and MNPs indicating the immobilization of FAO onto nanocomposite.

The fabrication of the FV biosensor based on electrodeposition of MNPs onto Au electrode is summarized in Scheme 1. Firstly, MNPs were deposited electrochemically on the surface of Au electrode as this method is easy and the layer thickness could be controlled. Our results showed that MNPs provided a remarkable synergistic effect towards the oxidation of FV. Enzyme, when treated with glutaraldehyde solution leads to formation of covalent bond (C=N bond) between $-NH_2$ of enzyme and –CHO group of glutaraldehyde.

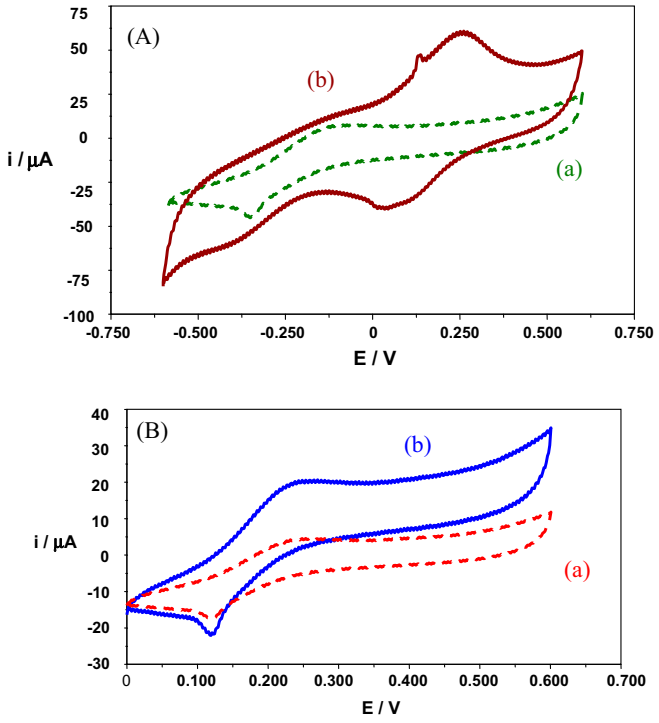


Fig. 2. (A) Cyclic voltammograms (CVs) of (a) bare gold electrode, (b) magnetic bionanoparticles modified electrode. (B) CVs of modified electrode (a) with and (b) without substrate at 50 mV s^{-1}.

3.2. Cyclic voltammetric studies

In order to evaluate the charge-transfer properties on the surface of the modified electrodes, cyclic voltammetry technique was employed, using $K_3Fe(CN)_6/K_4Fe(CN)_6$ as redox probe. Cyclic voltamogramms recorded in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution and potassium phosphate buffer 0.1 M (pH 7.5) are shown in Fig. 2A. For bare Au electrode, the well defined oxidation and reduction peaks caused by Fe^{3+}/Fe^{2+} redox couples were noticed in forward and reverse scan [curve (a)]. After the electrode was modified by MNPs current response of CVs was increased [curve (b)]. The values of the peak separation (difference between anodic peak potential and cathodic peak potential, $\triangle E_p$) were also determined. The obtained values on bare Au electrode and MNPs modified Au electrode were 150 mV and 250 mV, respectively. The electrodeposition of MNPs on gold electrode surface led to vast increase in current intensity as a result of the increase in electro active area. The preservation of a quasi reversible electrode process and the large increase in peak currents for the nanocomposite film modified electrode proved that MNPs exerted an obvious improvement effect on electric conductivity of enzyme electrode.

3.3. Response towards FV at the MNPs/Au electrode

To evaluate the catalytic activity of FAO at the MNPs/Au electrode, the modified electrode was characterized by a cyclic voltammogram in presence of FV in the potential range from 0 V to +0.6 V. Fig. 2B shows cyclic voltammograms of the MNPs/Au electrode in an unstirred 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution and 0.1 M potassium phosphate buffer (pH 7.5) with [curve (a)] and without [curve (b)] FV solution at a scan rate 50 mV s^{-1}. The reduction as well as oxidation current obviously increased with the addition of 2 mM FV, showing the catalytic properties of modified electrode to the oxidation of FV.

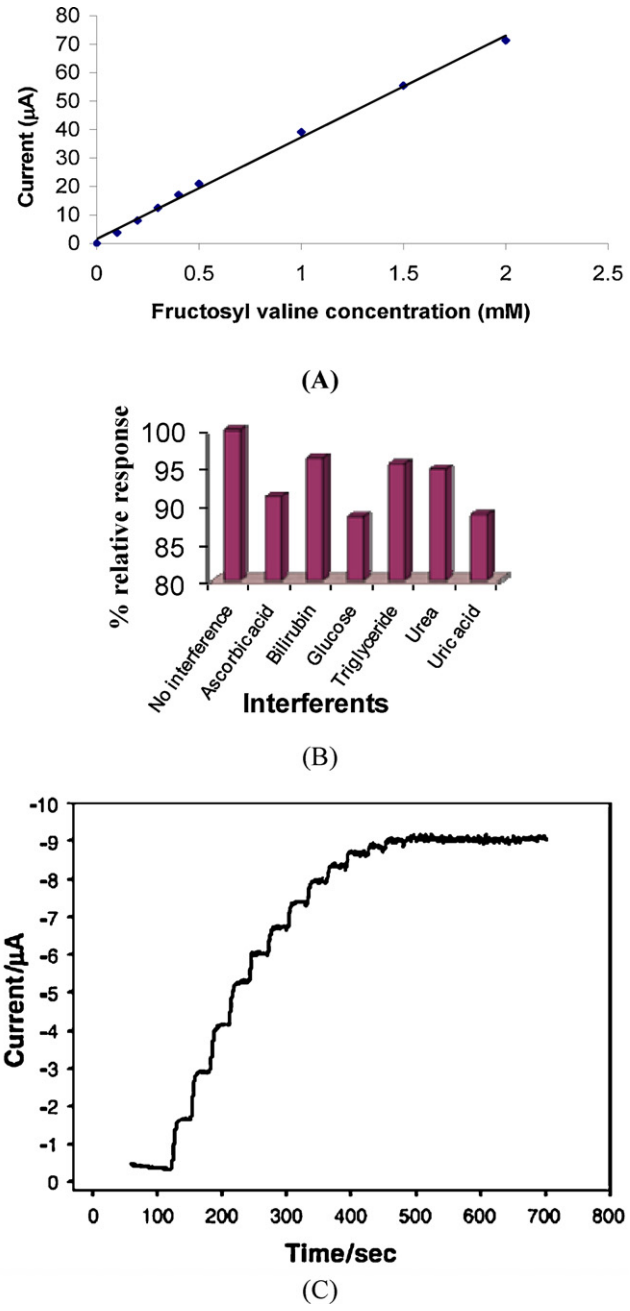


Fig. 3. (A) Effect of substrate concentration on response of magnetic bionanoparticles modified Au electrode. (B) Effect of potential interferents on biosensor response. (C) Current–time responses curve of the magnetic bionanoparticles modified Au electrode with successive addition of 2 mM FV into 0.1 M potassium phosphate buffer solution (pH 7.5).

3.4. Optimization of biosensor

To study optimum response conditions for the biosensor (MNPs/Au electrode) pH, temperature and scan rate were studied. The effect of pH from 5.0 to 9.0 for 2 mM FV solution was investigated. The current response resulting from the enzyme-catalyzed reaction achieved a maximum value at pH 7.5. Therefore, a pH of 7.5 was used in further experiments. The optimum temperature of biosensor was 35 °C. The effect of the scan rate from 25 to 200 mV s^{-1} on the biosensor response using 2 mM FV in 0.1 M potassium phosphate buffer solution (pH 7.5) was also investigated. A potential scan rate of 50 mV s^{-1} was selected, since with these experimental conditions, the highest analytical signal and very

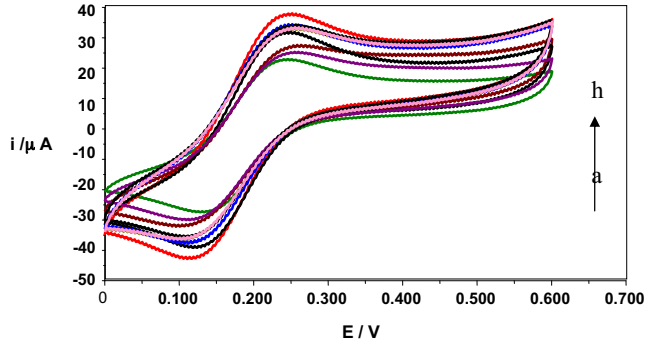


Fig. 4. Cyclic voltammograms recorded at different concentration of fructosyl valine (FV) from (a) 0.1, (b) 0.2, (c) 0.3, (d) 0.4, (e) 0.5, (f) 1, (g) 1.5, and (h) 2 mM. Scan rate: 50 mV s^{-1}.

good cyclic voltammogram profiles were obtained. The response of the present electrode system in relation to the change in FV concentration was found linear in the range of 0.1–2.0 mM (Fig. 3A).

The effect of several possible interfering substances such as ascorbic acid, bilirubin, urea, uric acid, triglycerides and glucose on the present biosensor was investigated at 0.6 V versus Ag/AgCl in phosphate buffer (pH 7.5). The decrease in the current was calculated as FV concentrations equivalence (i.e. the concentration of FV that can produce the same decrease as the interferents). Negligible interference was observed with glucose, urea, ascorbic acid and bilirubin (Fig. 3B).

3.5. Chronoamperometric determination of FV

Fig. 3C illustrates current-time plots for magnetic bionanoparticles modified Au electrode with successive addition of 2 mM FV. As FV is injected into the phosphate buffer solution, the reduction current rose steeply to reach a stable value. The time required to attain the 95% of the steady state response was within 4 s, which indicated a very fast diffusion process.

3.6. Evaluation of biosensor

There was a linear relationship between current and FV concentration ranging from 0.1 to 2 mM in reaction mixture (Fig. 4). The detection limit (S/N = 3) of the present electrode system was 0.1 mM. To study analytical recovery, exogenous FV of known concentration was added to the serum sample. The concentration of HbA_{1c} was measured in serum before and after addition of exogenous FV. The % recovery of added FV in serum was calculated. Analytical recovery of added FV in serum (1 mM and 2 mM) in % was 95.0% and 98.5%, respectively, indicating good efficiency of the biosensor. The within and between batch coefficient of variation (CV) for FV determination in serum by the present biosensor were 2.58% and 5.63%, respectively, revealing the reproducibility and reliability of the method. The accuracy of the present method was tested by comparing HbA_{1c} values (in %) in 20 serum samples by the present method (y) with those obtained by standard auto analyzer method (x). The values obtained by both methods showed a very good correlation with $r = 99.19$ with regression equation, $y = 1.0281x - 0.4658$ (Fig. 5). These results indicate a very good analytical performance of the present biosensor in biological samples.

3.7. Stability and reusability of biosensor

The electrode lost 25% of its initial activity after its 250 uses during the span of 3 months when stored at 4 °C which is much better than earlier electrodes (Fang et al., 2008).

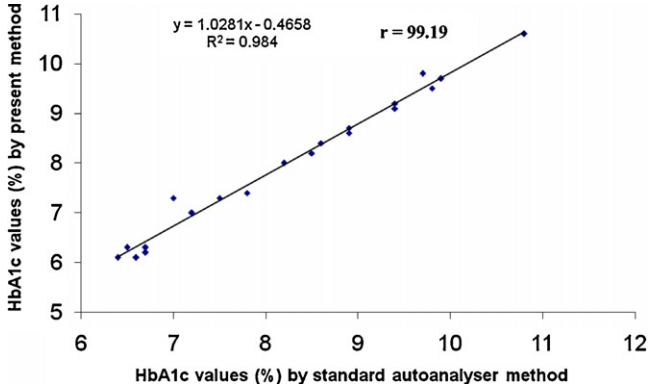


Fig. 5. Correlation between serum FV values determined by standard autoanalyser method (*x*-axis) and the present method by biosensor employing FAO/magnetic bionanoparticles/Au electrode (*y*-axis).

The good stability, repeatability and reproducibility observed for the present biosensor could be attributed to the immobilization of FAO onto MNPs electrodeposited on Au electrode.

4. Conclusion

A novel amperometric biosensor for determination of FV was fabricated, based on immobilization of FAO onto MNPs/Au nanocomposite. This biosensor was used for the detection of fructosyl valine, a model compound for the HbA_{1c} detection. The sensor showed high sensitivity, linearity, reproducibility and stability and fast response time. This biosensor prototype could be used effectively as the basis for HbA_{1c} detection for diabetic patient's medical management.

References

Bennett, P.H., Burch, T.A., Miller, M.A., 1971. Lancet 2, 125–128.
Bhattarai, N., Edmondson, D., Veiseh, O., Matsen, A.F., Zhang, M., 2005. Biomaterials 26, 6176–6184.
Bruni, S., Cariati, F., Casu, M., Lai, A., Musinu, A., Piccaluga, G., Solinbas, S., 1999. Nanostruct. Mater. 5, 573–579.
Daniel, M.C., Astruc, D., 2004. Chem. Rev. 104, 293–346.
Fang, L., Li, W., Zhou, Y., Liu, C.C., 2008. Sensor Actuator B 137, 235–238.
Goldstein, D.E., Little, R.R., Lorenz, R.A., Malone, J.I., Nathan, D., Peterson, C.M., Sacks, D.B., 2004. Diabetes Care 27, 1761–1773.
Goldstein, D.E., Little, R.R., Wiedmeyer, H.M., England, J.D., McKenzie, E.M., 1986. Clin. Chem. 32, 64–70.
Hageman, J., Kuehn, G., 1977. Anal. Biochem. 80, 547–554.
Horiuchi, T., Kurokawa, T., Saito, N., 1989. Agric. Biol. Chem. 53, 103.
John, W.G., 1997. Ann. Clin. Biochem. 34, 17–31.
John, W.G., 2003. Clin. Chem. Lab. Med. CCLM/FESCC 41, 1199–1212.
Keil, P., Mortensen, H.B., Christophersen, C., 1985. Acta Chem. Scand. Ser. B: Org. Chem. Biochem. 39, 191.
Khin, Y.W., Si-Shen, F., 2005. Biomaterials 26, 2713–2722.
King, H., Rewers, M., 1993. Diabetes Care 16, 157–177.
Krishnamurti, U., Steffes, M.W., 2001. Clin. Chem. 47, 1157–1165.
Kuang, H.M., Deng, Z.X., Li, C.H., Sun, X.M., Zhuang, J., Li, Y.D., 2002. Acta Phys.-Chim. Sin. 18, 477–480.
Marko-varga, G., Emneus, J., Gorton, L., Ruzgas, T., 1995. Trends Anal. Chem. 14, 319–328.
Ogawa, K., Stollner, D., Scheller, F., Warsinke, A., Ishimura, F., Tsugawa, W., Ferri, S., Sode, K., 2002. Anal. Bioanal. Chem. 373, 211–214.
Rajkumar, R., Warsinke, A., Mohwald, H., Scheller, F.W., Katterle, M., 2007. Biosens. Bioelectron. 22, 318–3325.
Sode, K., Takahashi, Y., Ohta, S., Tsugawa, W., Yamazaki, T., 2001. Anal. Chim. Acta 435, 151–156.
Stöllner, D., St¨ocklein, W., Scheller, F., Warsinke, A., 2002. Anal. Chim. Acta 470, 111–119.
Sun, Q.Y., Vrieling, E.G., Van Santen, R.A., Sommerdijk, N.A.J.M., 2004. Curr. Opin. Solid State Mater. Sci. 8, 111–120.
Tian, F., Liu, Y., Hu, K., Zhao, B., 2003. J. Mater. Sci. 38, 4709–4712.
Tsang, S.C., Qiu, J.S., Harris, P.J.F., Fu, Q.J., Zhang, N., 2000. Chem. Phys. Lett. 322, 553–560.
Turner, A.P.F., Chen, B., Piletsky, S.A., 1999. Clin. Chem. 45, 1596–1601.
Wang, J., 1999. Anal. J. Pharm. Biomed. 19, 47–53.
Wyrna, D., Beyer, N., 2002. Nano Lett. 2, 419–421.
Zhang, Y., Zeng, G.M., Tang, L., Huang, D.L., Jiang, X.Y., Chen, Y.N., 2007. Biosens. Bioelectron. 22, 2121–2126.