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Amperometric carbon paste enzyme electrodes with Fe_3O_4 nanoparticles and 1,4-Benzoquinone for glucose determination

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Abstract

Two new amperometric carbon paste enzyme electrodes including Fe_3O_4 nanoparticles with and without 1,4-benzoquinone were developed for glucose determination. Electron transfer properties of unmodified and Fe_3O_4 nanoparticles and/or 1,4-benzoquinone modified carbon paste electrodes were investigated in 0.1 M KCl support electrolyte containing $Fe(CN)_6^{3-/4-}$ as redox probe by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) methods. Fe_3O_4 nanoparticles increased electron transfer at solution/electrode interface. The parameters affecting the analytical performance of the enzyme electrode have been investigated in detail and optimized for Fe_3O_4 nanoparticle modified enzyme electrode (Fe_3O_4–CPEE). Fe_3O_4 nanoparticles and 1,4-benzoquinone modified enzyme electrode (BQ-Fe_3O_4-CPEE) exhibited linear response from 1.9×10^{-7} M to 3.7×10^{-6} M, from 7.2×10^{-6} M to 1.5×10^{-4} M and from 1.3×10^{-3} M to 1.2×10^{-2} M with an excellent detection limit of 1.9×10^{-8} M. BQ-Fe_3O_4-CPEE was used for determination of glucose in serum samples and results were in good agreement with those obtained by spectrophotometric method.

Keywords: amperometry, biosensor, cyclic voltammetry, electrochemical impedance spectroscopy, glucose oxidase, mediator, nanoparticle

Introduction

Accurate, rapid, sensitive and simple determination of glucose is very important in biochemistry, clinical diagnostics, food industry and many different areas. Especially in clinical diagnostics, the concentration of glucose is used as an indicator for diabetes. Diabetes represents a serious worldwide public health problem and it leads to severe damage mainly in kidneys, heart and eyes (Lin, He, Zhao, & Zhang, 2009; Comba et al., 2010). Therefore, many techniques including colorimetry (Kabasakalian, Kalliney, & Westcott, 1974), gas chromatography-mass spectrometry (Di Gioia et al., 2004), chemiluminescence (Lv, Zhang, & Chen, 2003), voltammetry (Zhu, Lu, Li, Shao, & Zhu, 2009), liquid chromatography (Coquet, Veuthey, Haerdi, & Agosti, 1991) and spectrophotometry (Zaitoun, 2006) were reported for glucose quantification. However, these methods are usually laborious, expensive, time-consuming and/or complex to perform. An alternative method for glucose determination is the use of electrochemical enzyme electrodes, which allow direct, rapid, simple and low-cost measurement of glucose. Various types of enzyme electrodes have been reported for glucose determination since the work of Clark and Lyons in 1962 (Clark & Lyons, 1962; Saito & Watanabe, 1998; Tang et al., 2004; Cui et al., 2001).

The enzyme glucose oxidase (GOD) has been the most widely used biorecognition element in glucose enzyme electrodes. GOD catalyzes the oxidation of glucose to gluconolactone and reduces the natural mediator, oxygen, to hydrogen peroxide. H_2O_2 formed during this reaction can be determined with amperometric sensors (Yang, Ren, Tang, & Zhang, 2009; Ali, Nur, Willander, & Danielsson, 2010).

Unfortunately, the amperometric determination of H_2O_2 requires high anodic potential. In this case, other electroactive species present in the sample such as ascorbic acid acetaminophen and uric acid can also be oxidized and interfere with the analysis. This drawback can be eliminated by designing electrochemical glucose biosensors working at lower potentials. In glucose biosensors, redox-active species (mediators) can be used as artificial electron acceptors for $FADH_2$ in place of O_2. With the use of the right mediator, it is not necessary to apply a high potential to the electrode since mediators can be oxidized at potentials slightly higher than their redox potentials (Saito & Watanabe, 1998).

Carbon paste electrodes have been widely used for electroanalytical applications since their introduction by Adams in 1958. These electrodes offer the advantages of low background current, low cost of fabrication and ease of modification (Švancara, Vytas, Barek, & Zima, 2001). A number of glucose biosensors based on carbon paste matrix have been reported (Wang & Walcarius, 1996; Liu, Lu, & Wang, 1999;

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Wang, Mo, Li, & Porter, 2001). The use of redox mediators in carbon paste electrodes is a promising approach (Yang et al., 2004; Koyuncu, Erden, Pekyardımcı, & Kılıç, 2007). Especially, quinones are one of the most commonly used mediators in mediated biosensors (Yang et al., 2004; Casero, Darder, Pariente, & Lorenzo, 2000).

Recently, applications of nanomaterials in biosensors have aroused much interest. These interesting materials exhibit desirable properties such as large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency and strong adsorption ability that are useful in biosensing applications. Nanomaterials have the unique ability of promoting fast electron transfer between electrode and the active site of the enzyme. Among the various nanomaterials, magnetite nanoparticles have recently gained more interest due to biocompatibility, strong superparamagnetic property and low toxicity (Kaushik et al., 2009). Glucose biosensors based on magnetite nanoparticles have been reported (Kaushik et al., 2008; Baby & Ramaprabhu, 2010). However, preparation of a glucose biosensor based on magnetite nanoparticles and 1,4-benzoquionone, to our best knowledge have not been reported.

In this work, we developed simple and low-cost enzyme electrodes working at low potentials and performed reliable glucose analysis in real samples. We constructed two different modified carbon paste enzyme electrodes by incorporating Fe_3O_4 nanoparticles or Fe_3O_4 nanoparticles and 1, 4-benzoquinone with glucose oxidase within a carbon paste matrix. We investigated; the parameters that influence the electrode performance and the analytical characteristics for Fe_3O_4 nanoparticles modified enzyme electrode. Analytical characteristics, operational stability, interference effect and application of Fe_3O_4 nanoparticles and 1,4-benzoquinone modified enzyme electrode to real samples were also investigated.

Experimental

Equipment and reagents

The electrochemical studies were carried out with IVIUM electrochemical analyzer (www.ivium.nl) and a three-electrode cell stand (www.basinc.com). The working electrode was a modified carbon paste electrode. The counter and the reference electrodes were a Pt wire (BAS MW 1034) and Ag/AgCl (BAS MF 2052) electrode, respectively (www.basinc.com). The pH values of the buffer solutions were measured with ORION Model 720A pH/ion meter and ORION combined pH electrode (www.thermoscientific.com).

Glucose oxidase (E.C. 1.1.3.4 from *Aspergillus niger* sp. with a specific activity of 39 Units/mg solid), Fe_3O_4 nanoparticles, uric acid, ascorbic acid and glutaraldehyde were purchased from Sigma (www.sigmaaldrich.com). Sodium monohydrogenphosphate and sodium dihydrogenphosphate were supplied from Riedel-de Haën (www.riedeldehaen.com). 1,4-Benzoquinone, bovine serum albumin (BSA), graphite powder, paraffin oil and glucose were from Fluka (www.sigmaaldrich.com). All other chemicals were obtained from Merck (www.merckgroup.com). Standard solutions of glucose were prepared by dissolving glucose in phosphate buffer solution and allowed to mutarotate at room temperature for 24 hours before use. The standard glucose solutions were prepared freshly every day. All the measurements were carried out at room temperature.

Preparation of carbon paste and modified carbon paste electrodes

Carbon paste was prepared in the following proportions for unmodified electrode (UCPE): 77.64% graphite powder and 22.36% paraffin oil. Fe_3O_4 nanoparticles modified carbon paste electrode (Fe_3O_4–CPE) was composed of 59.52% graphite powder, 18.12% Fe_3O_4 nanoparticles and 22.36% paraffin oil. 1,4-Benzoquinone modified electrode was composed of 64.48% graphite powder, 13.16% 1,4 benzoquinone and 22.36% paraffin oil (BQ–CPE). Fe_3O_4 nanoparticles and 1,4-benzoquinone modified carbon paste (BQ–Fe_3O_4–CPE) was composed of 46.58% graphite powder, 18.12% Fe_3O_4 nanoparticles, 12.94% 1,4-benzoquinone and 22.36% paraffin oil. The modified electrodes were prepared by hand-mixing graphite powder with the nanoparticles and then adding paraffin oil and thoroughly mixing for approximately 20 minutes to form homogeneous modified carbon paste electrodes.

Graphite powder and Fe_3O_4 nanoparticles were mixed and enzyme solution (25 μL glucose oxidase (10 Unit), 1.5 mg BSA and 25 μL 1.25% glutaraldehyde) was added for Fe_3O_4 nanoparticles modified enzyme electrode (Fe_3O_4–CPEE). Paraffin oil was added after the evaporation of water and mixed for approximately 20 minutes until a uniform paste was obtained. Fe_3O_4 nanoparticles and 1,4-benzoquinone modified enzyme electrode (BQ–Fe_3O_4–CPEE) was also prepared in a similar way. In all cases, the paste was placed into the bottom of the working electrode body and the electrode surface was polished with a smooth paper layer to have a smooth surface. The electrodes were washed with distillated water and working buffer between measurements. Electrodes were stored in refrigerator at +4°C when not in use.

Amperometric measurements

Electron transfer properties of unmodified and modified electrodes were determined in 0.1 M KCl solution containing 1 mM $K_3[(Fe(CN)_6]$ + 1 mM $K_4[(Fe(CN)_6]$ by Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). The cyclic voltammograms of unmodified carbon paste electrode (UCPE) and Fe_3O_4-CPE were recorded at (−0.2)V-(+ 0.6)V, BQ-CPE at (−0.25)V-(+ 0.6)V and BQ-Fe_3O_4-CPE at (−0.3)V-(+ 0.6)V. EIS measurements were performed at the frequency range of 10^5- 10^{-2} Hz with 5 mV amplitude under open circuit potential (E_{OCP}) conditions.

All other amperometric measurements were performed in phosphate buffer solution (0.05 M pH 7.5). The electrochemical response of the UCPE to H_2O_2 was investigated first. 5.0 mL buffer solution was added to the cell. After the application of + 0.30 V potential, the background current was allowed to decay to constant value. Then an aliquot of 10^{-2} M H_2O_2 stock solution was added to the cell and the solution was purged with nitrogen and stirred prior to each measurement. The current values against H_2O_2 concentrations were plotted in order to determine the sensitivity of the

electrode to H_2O_2. The same experiment was performed with Fe_3O_4-CPE and BQ-Fe_3O_4-CPE.

Determination of glucose in human serum samples

Standard addition method was used to determine the glucose concentration in human serum. Serum samples were mixed with 10% trichloroacetic acid (TCA) for deproteinization. After a 10-minute centrifugation at 3000 rpm, supernatants were filtered and diluted. BQ-Fe_3O_4-CPEE was placed in the cell containing 5.0 mL buffer and after the stabilization of background current, 10 µL sample was added. The solution was purged and stirred. The response of the electrode against glucose was measured in oxygen-saturated medium at +0.30 V. After the current reached a steady-state value, increasing amounts of standard glucose solution were added to this solution and standard addition curve was plotted. The glucose concentration of the serum sample was calculated from this curve.

Results and discussion

Electrochemical Characterization of Unmodified and Modified CPEs

CV of the ferricyanide system is a convenient and valuable tool to monitor the characteristics of the surface modified electrodes. The electron transfer properties of UCPE, Fe_3O_4-CPE, BQ–CPE and BQ–Fe_3O_4–CPE were investigated in 0.1 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$ at 10 mVs^{-1}. The cyclic voltammograms are shown in Figure 1A. While the well-defined redox peaks of $Fe(CN)_6^{3-/4-}$ were observed for UCPE and Fe_3O_4-CPE, redox peaks of BQ/H_2BQ (about −0.08 V and −0.17 V) were present for BQ–CPE and BQ–Fe_3O_4–CPE in addition to redox peaks of $Fe(CN)_6^{3-/4-}$. Fe_3O_4 nanoparticles increased current intensity and decreased peak-to-peak separation ($E_{p,a} - E_{p,c} = \Delta E_p$) for $Fe(CN)_6^{3-/4-}$ waves. After the modification of electrode with BQ, no significant change was observed in current intensity and peak-to-peak separation compared to UCPE. The ΔE_p for UCPE, Fe_3O_4–CPE, BQ–CPE and BQ–Fe_3O_4–CPE were 280 mV, 170 mV, 290 mV and 250 mV, respectively. The lower ΔE_p value for Fe_3O_4–CPE indicates that electron transfer of this electrode is faster than the other electrodes (Yang, Ren, Tang, & Zhang, 2009; Yao & Shiu, 2007; Lu & Chen, 2006; Lin et al., 2008). Lu and Chen revealed that nano-Fe_3O_4 promoted electron transfer between the electroactive Fe^{3+}/Fe^{2+} species and electrode surface (Lu & Chen, 2006). Yang et al. reported that Fe_3O_4 nanoparticles increased the current response of Fe^{3+}/Fe^{2+} redox couples, indicating the enhancing effect of the Fe_3O_4 nanoparticles on the electric conductivity of the electrode (Yang, Ren, Tang, & Zhang, 2009). These results confirm our findings. The electrochemical surface area of Fe_3O_4–CPE was calculated from the voltammetric peak current by using Randles – Sevcik equation to support the experimental results and to investigate the effect of Fe_3O_4 on the electrochemical surface area of the electrode (Yao & Shiu, 2007; Wang, 2006; Ding, Chang, Wu, Lai, & Chang, 2005). According to the Randles - Sevcik equation, the peak current is directly proportional to the concentration in bulk solution and increases with the square root of the scan rate. Figure 1B shows the peak current versus the square root of the scan rate and cyclic voltammograms for $Fe(CN)_6^{3-/4-}$ redox probe at scan rates ranging from 10 to 150 mVs^{-1} (inset in Figure 1B) for Fe_3O_4-CPE. The peak potential shifts slightly while the peak current increases with increasing the scan rate. The effective electrochemical surface area of Fe_3O_4-CPE is calculated by using the slope of the curve. The effective electrochemical surface area of Fe_3O_4-CPE (0.125 cm^2) is much higher than the surface area of the UCPE (0.071 cm^2). The curve is linear, which suggests that the mass transfer phenomenon in the double layer region of the electrode is mainly controlled by diffusion (Song et al., 2010; Du, Li, Mei, & Liu, 2009; Khan et al., 2008).

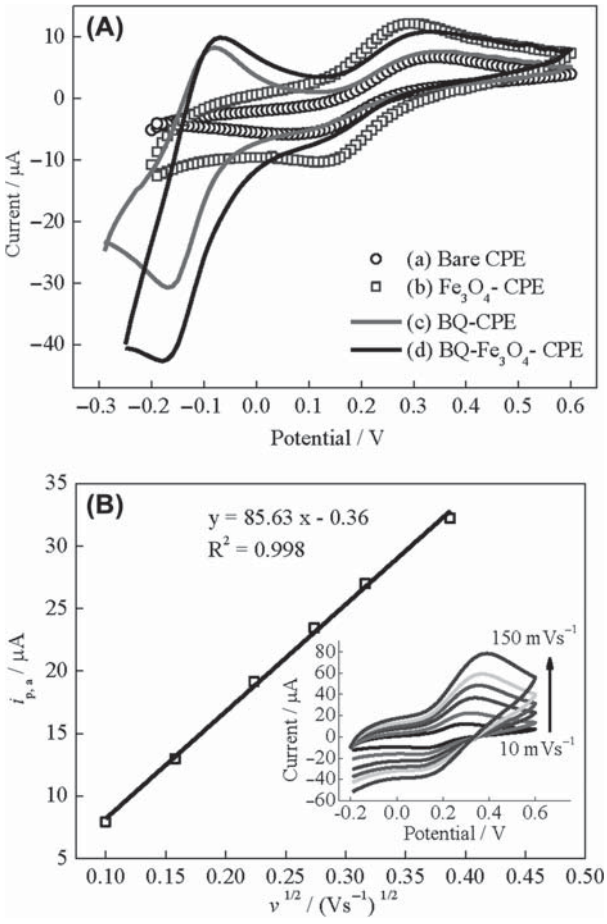

Figure 1. (A) Cyclic voltammograms of (a) UCPE, (b) Fe_3O_4-CPE, (c) BQ-CPE and (d) BQ-Fe_3O_4-CPE at 10 mVs^{-1}, (B) curve of the anodic peak current versus the square root of the scan rate for Fe_3O_4-CPE (inset: cyclic voltammograms at scan rates of 10, 25, 50, 75, 100 and 150 mVs^{-1}) in 0.1 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$.

EIS is an effective method to investigate the electrical properties of modified electrodes and to determine electrochemical reaction rates (Wang, 2006). Figure 2 shows electrochemical impedance spectra, Nyquist curves, of UCPE, Fe_3O_4-CPE, BQ-CPE and BQ-Fe_3O_4-CPE. The Nyquist curves include a depressed semicircle portion and a linear portion. The semicircle portion at high frequencies corresponds to the electron transfer limited process, and the linear portion at low frequencies corresponds to the diffusion process. The diameter of the semicircles is equal to the electron transfer resistance (R_{ct}) value (Wang, 2006; Liu et al., 2006). The R_{ct}

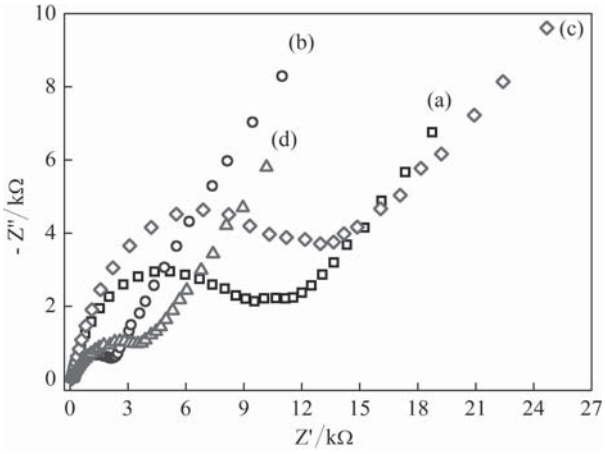

Figure 2. The Nyquist curves of (a) UCPE and (b) Fe_3O_4-CPE (c) BQ-CPE and (d) BQ-Fe_3O_4 CPE in 0.1 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$.

values for UCPE, Fe_3O_4-CPE, BQ-CPE and BQ-Fe_3O_4-CPE are approximately 10.8, 2.0, 11.7 and 3.1 kΩ, respectively. The results indicate that while Fe_3O_4 nanoparticles dramatically increase electron transfer at solution/electrode interface, BQ slightly decreases the electron transfer (Yang, Ren, Tang, & Zhang, 2009; Lu & Chen, 2006). However, R_{ct} values of Fe_3O_4-CPE and BQ-Fe_3O_4-CPE are lower than that of UCPE, so these two modified electrodes promise better results in the preparation of enzyme electrodes.

Response characteristics of CPEs and CPEEs

The quantification of glucose is based on the electrochemical detection of enzymatically generated H_2O_2 in oxygen-saturated medium, thus the sensor sensitivity is dependent on the electrochemical response of the electrode to H_2O_2. Electrodes with high catalytic efficiency to H_2O_2 would have high sensitivity to glucose. Figure 3A shows the current difference values versus H_2O_2 concentration obtained with UCPE, Fe_3O_4–CPE, BQ–CPE and BQ–Fe_3O_4–CPE in nitrogen-saturated solution. The sensitivities of the Fe_3O_4–CPE, BQ–CPE and BQ–Fe_3O_4–CPE are higher than that of unmodified carbon paste electrode to H_2O_2. The highest sensitivity was obtained with BQ–Fe_3O_4–CPE. This can be explained by the synergistic action of the electrocatalytic activity of 1,4-benzoquinone and Fe_3O_4.

The response of UCPEE, Fe_3O_4–CPEE, BQ-CPEE and BQ–Fe_3O_4–CPEE to glucose was also determined at + 0.30 V in oxygen-saturated solution (Figure 3B). The sensitivities of the Fe_3O_4–CPEE and BQ–Fe_3O_4–CPEE are higher than that of unmodified carbon paste electrode similar to H_2O_2 responses. We investigated the glucose response of different enzyme electrodes in nitrogen-saturated buffer solution and compared these results with those obtained in oxygen-saturated solution to clarify the detection mechanism of glucose. UCPEE and Fe_3O_4–CPEE showed very small catalytic activity toward glucose in nitrogen-saturated solution in contrast to the response in oxygen-saturated solution. This indicates that Fe_3O_4 nanoparticles cannot catalyze the electron transfer between the reduced enzyme and electrode surface efficiently. BQ–CPEE and BQ–Fe_3O_4–CPEE

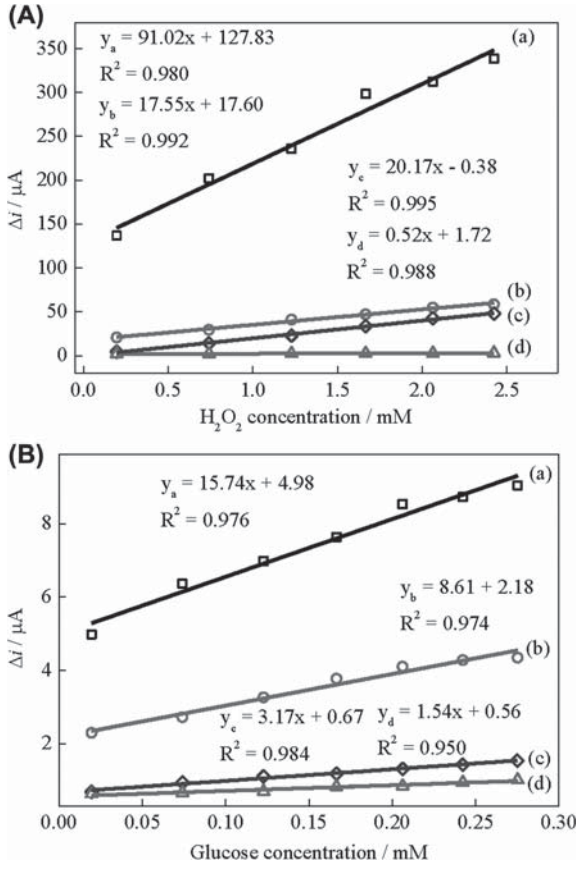

Figure 3. (A) Calibration curves for H_2O_2: (a) BQ-Fe_3O_4-CPE, (b) Fe_3O_4-CPE (c) BQ-CPE and (d)UCPE (0.05 M pH 7.5 phosphate buffer, + 0.30 V, nitrogen-saturated solution). (B) Calibration curves for glucose: (a) BQ-Fe_3O_4-CPEE, (b) Fe_3O_4-CPEE (c) BQ-CPEE and (d) UCPEE (0.05 M pH 7.5 phosphate buffer, + 0.30 V, oxygen-saturated solution).

exhibited good response to glucose both in nitrogen and in oxygen-saturated solutions. However, the sensitivities of the electrodes were higher in oxygen-saturated solution, indicating the synergistic action of the electrocatalytic activity of 1,4-benzoquinone and oxygen. This is a clear indication of 1,4-benzoquinone acting as a mediator for glucose determination. 1,4-Benzoquinone reduces to hydroquinone (H_2BQ) and hydroquinone is also reoxidized to 1,4-benzoquinone on carbon paste electrode surface at + 0.30 V. Our results are in good agreement with the data reported (Yang et al., 2004). Consequently, possible electron transfer behavior of the BQ-Fe_3O_4-CPEE may occur as following:

$$\text{Glucose} + \text{GOD(FAD)} \rightarrow \text{Gluconolactone} + \text{GOD(FADH}_2\text{)} \quad (1)$$
$$\text{GOD(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOD(FAD)} + \text{H}_2\text{O}_2 \text{ and/or} \quad (2)$$
$$\text{GOD(FADH}_2\text{)} + \text{BQ} \rightarrow \text{GOD(FAD)} + \text{H}_2\text{BQ} \quad (3)$$
$$\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}^- \text{ and/or} \quad (4)$$
$$\text{H}_2\text{BQ} \rightarrow 2\text{H}^+ + \text{BQ} + 2\text{e}^- \quad (5)$$

The detection mechanism is based on equations (1), (3) and (5) in nitrogen-saturated solution and (1), (2), (3), (4) and (5) in oxygen-saturated solution. It can be suggested that the mechanism of glucose determination is based on the electrochemical oxidation of H_2O_2 and H_2BQ in oxygen-saturated solution. However, in nitrogen-saturated solution only electrochemical oxidation of H_2BQ occurs. Furthermore, the responses of the UCPEE and Fe_3O_4-CPEE in

nitrogen-saturated solution shows that the detection mechanism is not based on the direct electrochemistry of glucose oxidase. In this study, the highest sensitivity was obtained with BQ-Fe_3O_4-CPEE (Figure 3B) which may be attributed to the fact that Fe_3O_4 nanoparticles increases the surface area and electric conductivity of the electrodes thus enhancing the sensitivity of the electrode.

Optimum working conditions and electrode composition of Fe_3O_4-CPEE

The amount of enzyme loading can affect the amperometric response of an enzyme electrode. Therefore, it is important to determine the enzyme amount in carbon paste matrix. The responses of Fe_3O_4-CPEE were measured at five different enzyme amounts by keeping the other conditions constant and varying the glucose oxidase amount between 4 and 12 Units. Gradual increase in the current differences was observed as the amount of glucose oxidase increased. However, over 10 Unit of enzyme, the response current tends to be saturated. The optimum enzyme loading was specified as 10 Unit because further increase of enzyme loading would be a waste of this expensive reagent.

Investigation of the effect of pH on the response of the enzyme electrode is of great importance, since the activity of the immobilized GOD is pH dependent (Wang et al., 2009). The pH dependence of the Fe_3O_4-CPEE response was evaluated at 0.09 mM glucose concentration over the pH range from 6 to 9 (Figure 4). The response current increases from pH 6 to 7.5 and then decreases with increasing pH. The proton is critical in the redox behavior of GOD-FAD. The decrease of the response at high pH values may be due to the decrease of proton concentration and activity of the immobilized GOD (Yang, Ren, Tang, & Zhang, 2009). The highest response was obtained at pH 7.5 as shown in Figure 4. Therefore, pH 7.5 was selected as the optimum pH and all the following measurements were performed at this pH. Good responses for free enzyme have been reported in the pH range of 4–7 (Tang et al., 2004). The results obtained from pH study indicate that immobilization procedure has a little influence on the properties of glucose oxidase. Different pH values such as 6.0 (Yu, Yu, Zhao, & Zeng, 2008; Che et al., 2010), pH 6.5 (Wang, Wang, Di, & Tu, 2008), pH 7.0 (Wang et al., 2009; Xue, Xu, Zhou, & Zhu, 2006), pH 7.2 (Yang, Ren, Tang, & Zhang, 2009), pH 7.4 (Tang et al., 2004), pH 8.0 (Chuang, Wang, & Lan, 1997), pH 9.0 (Luo et al., 2010) were also reported in the literature as optimum pH for glucose enzyme electrodes. The difference of the optimum reported pH values may be attributed to different mediators, enzyme supplies, immobilization methods or electrode preparation procedures. The amperometric response of Fe_3O_4-CPEE was determined at different phosphate concentrations of 0.05 M, 0.10 M, 0.15 M and 0.20 M and the best response for Fe_3O_4-CPEE was obtained at 0.05 M. Above this concentration, the current difference was found to show a significant decrease. The optimized parameters were also used for the BQ-Fe_3O_4-CPEE to compare the performance of the electrodes. However, we checked the optimum working pH also for BQ-Fe_3O_4-CPEE and the maximum response was obtained at pH 7.5 similar to the Fe_3O_4-CPEE.

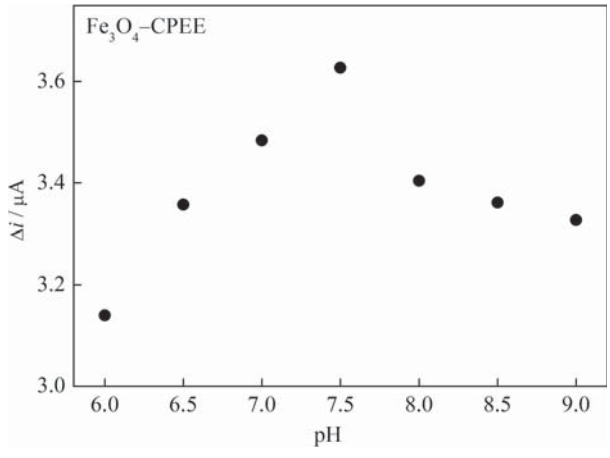

Figure 4. The effect of buffer pH on the response of Fe_3O_4-CPEE (0.05 M phosphate buffer, + 0.30 V).

The response of Fe_3O_4-CPEE to constant glucose concentration (0.09 mM) was determined at different working potentials between (0) - (+ 0.70) V. Potentials higher than + 0.70 V were not investigated due to the increasing risk of interference effects. Amperometric current was increased gradually for Fe_3O_4-CPEE in the range from (+ 0.20) to (+ 0.70) V. Although the maximum current difference was obtained at + 0.70 V, + 0.30 V was selected for the amperometric determination of glucose since we obtained sufficient response and calibration curves with good linearity. This working potential also could minimize the risk for interfering reactions of other oxidizable species in real samples.

Performance parameters of Fe_3O_4-CPEE and BQ-Fe_3O_4-CPEE

The response time of the enzyme electrode depends on the glucose concentration. The amperometric response times of Fe_3O_4-CPEE and BQ-Fe_3O_4-CPEE for glucose were determined at two different glucose concentrations. The current differences for 1.0×10^{-5} M and 1.0×10^{-4} M glucose versus time were plotted. The response time was shorter at lower concentrations than at higher concentrations. The current reached 95% of the steady-state current within about 120 seconds (t_{95}) for Fe_3O_4-CPEE and 100 seconds (t_{95}) for BQ-Fe_3O_4-CPEE. The shorter response time of BQ-Fe_3O_4-CPEE may be attributed to the faster electron transfer feature of nanoparticle-mediator composite.

The repeatability of Fe_3O_4-CPEE and BQ-Fe_3O_4-CPEE was also investigated. Five calibration curves were plotted by the use of the same electrodes sequentially. The relative standard deviation of the sensitivities was 2.8% for Fe_3O_4-CPEE and 4.1% for BQ-Fe_3O_4-CPEE.

We checked the long-term stability of BQ-Fe_3O_4-CPEE prepared under optimum conditions. The electrode was stored in dry atmosphere at + 4°C when not in use. The BQ-Fe_3O_4-CPEE lost only 4.5% of its initial sensitivity after 21 days. This shows that Fe_3O_4 nanoparticles and 1,4-benzoquinone ensure the stability of the enzyme electrode. The stability of the BQ-Fe_3O_4-CPEE electrode is much better compared to the stability of another carbon material electrode with a 26.5% loss of response after 22 days. (Tang et al., 2004).

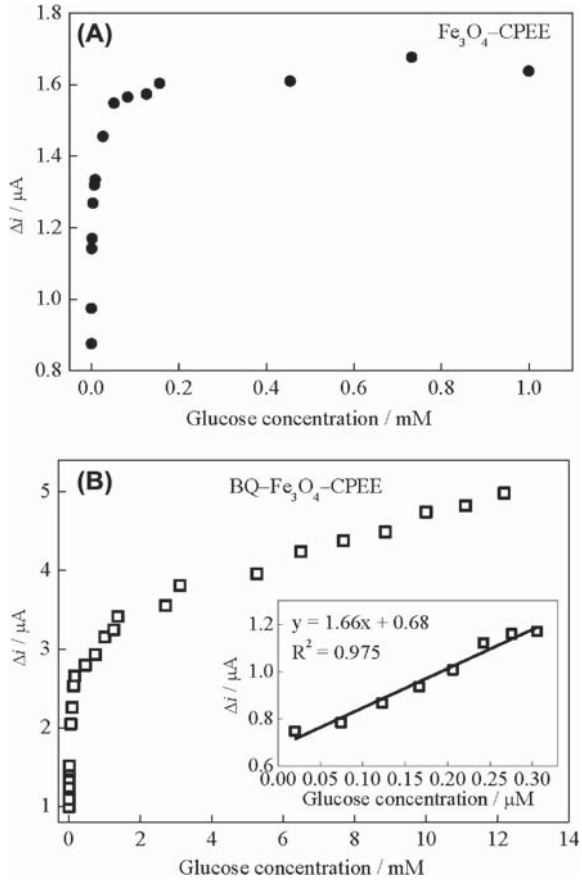

Figure 5. (A) Effect of glucose concentration on the response of Fe_3O_4-CPEE (B) Effect of glucose concentration on the response of BQ-Fe_3O_4-CPEE (inset: glucose response of the BQ-Fe_3O_4-CPEE at low concentrations) (0.05 M, pH 7.5 phosphate buffer, + 0.30 V, oxygen-saturated solution).

Figure 5A shows the amperometric response curve of the Fe_3O_4-CPEE recorded as a function of glucose concentration under optimized experimental conditions. The response current increases with increasing concentration of glucose up to 7.3×10^{-4} M. The limit of detection is 1.9×10^{-7} M. The curve is composed of three linear parts ranging from 1.9×10^{-7} M to 1.9×10^{-6} M, from 3.8×10^{-6} M to 5.2×10^{-5} M and from 8.3×10^{-5} M to 7.3×10^{-4} M.

The relationship between glucose concentration and response current of the BQ-Fe_3O_4-CPEE is shown in Figure 5B. The BQ-Fe_3O_4-CPEE exhibited a linear response in the range from 1.9×10^{-7} M to 3.7×10^{-6} M, from 7.2×10^{-6} M to 1.5×10^{-4} M and from 1.3×10^{-3} M to 1.2×10^{-2} M. The limit of detection is 1.9×10^{-7} M. The linear working range of BQ-Fe_3O_4-CPEE is wider than that of Fe_3O_4-CPEE. This may be attributed to the synergetic effect of Fe_3O_4 nanoparticles and 1,4-benzoquinone. We also investigated the response of BQ-Fe_3O_4-CPEE at lower glucose concentrations. The enzyme electrode showed linear response between 1.9×10^{-8} M- 3.1×10^{-6} M (Figure 5B inset). This response at lower concentrations is important when working with dilute samples to eliminate the effects of interferences. It can be concluded that GOD ($FADH_2$) can react with O_2 and BQ at different concentrations of glucose as clearly indicated from the three ranges of linearity of the calibration curves. This study show that the use of BQ and Fe_3O_4 together as a modifier in CPEE's improves the detection limit and linear working range remarkably compared to other magnetite-nanoparticle-modified glucose biosensors recently reported (Yang, Ren, Tang, & Zhang, 2009; Kaushik et al., 2008; Li, Wei, & Yuan, 2009).

The oxidizable compounds such as uric acid, urea, glucose, creatinine and ascorbic acid can interfere with the amperometric measurement of enzyme electrodes. In our study, the effect of interferences was evaluated for two common interfering substances, namely ascorbic acid and uric acid, normally present in human serum and urine. Interference effect was only determined for BQ-Fe_3O_4-CPEE. Addition of 0.3 mM ascorbic acid and 0.3 mM uric acid to 0.3 mM glucose increased the response current by about 65% and 38%, respectively. However, when the concentration of ascorbic acid and uric acid decreased to 2 μM the interference effect also decreased to 2% and 7%, respectively. Therefore, it can be concluded that the response to glucose at + 0.30 V was not affected by addition of 2 μM ascorbic acid significantly and dilution reduces the effect of interferences as it was reported in the literature (Martinez-Peréz, Ferrer, & Mateo, 2003). In this study, we performed the real sample measurements in the range of 5.4×10^{-7} M- 9.8×10^{-7} M and in this range, we did not observe a significant effect of interferences.

Determination of glucose in serum

In Table 1, results obtained from four serum samples by using BQ-Fe_3O_4-CPEE are presented together with those obtained from spectrophotometric technique. Results are in good agreement and show that BQ-Fe_3O_4-CPEE can be successfully used for the detection of glucose concentration in serum samples. We checked the accuracy of the method by *t*-test. The *t* value is 0.33 for BQ-Fe_3O_4-CPEE at 95% confidence level, for which t_{critic} is 3.18 (Skoog, West, Holler, & Crouch, 2004). It is clear from the *t*-test that there is no difference between the results of two methods at a confidence level of 95%.

Conclusion

In this study, we have presented the use of magnetite nanoparticles and 1,4-benzoquinone together in electrochemical enzyme electrodes for the first time. The BQ-Fe_3O_4-CPEE demonstrated an excellent catalytic activity toward glucose determination through the synergetic action of magnetite nanoparticles and 1,4-benzoquinone. It was shown that the mechanism of glucose determination is based on the electrochemical oxidation of H_2O_2 and H_2BQ in oxygen-saturated medium and only electrochemical oxidation of H_2BQ in nitrogen-saturated solution. 1,4-benzoquinone acted as a

Table 1. Comparison of glucose concentration in serum samples using the modified enzyme electrode and the spectrophotometric method.

	Glucose (mg/dL)	
	BQ-Fe_3O_4-CPEE[a]	Spectrophotometric
Sample 1	70.05 ± 1.1	69
Sample 2	108.93 ± 2.6	109
Sample 3	123.15 ± 1.5	124
Sample 4	114.28 ± 1.7	115

[a]Each value is the mean of three measurements.

mediator and magnetite nanoparticles increased electric conductivity and surface area of the electrode. BQ-Fe_3O_4-CPEE provided the determination of glucose at a low potential (+0.30 V) and low concentration range hence reducing the risk of interference. It can be concluded that BQ-Fe_3O_4-CPEE shows promise for glucose determination in real samples. The incorporation of magnetite nanoparticles with different mediators can be extended to other enzyme-based biosensors.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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