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Alginate copper oxide nano-biocomposite as a novel material for amperometric glucose biosensing

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ABSTRACT

A novel amperometric glucose biosensor based on alginate-CuO nano-biocomposite and glucose oxidase (GOD) film was developed and characterized. The properties of the alginate-CuO-GOD film were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Amperometric measurements were employed to characterize the analytical performance of the biosensor. Several parameters including amount of alginate, concentration of GOD and cross-linkers, amount of CuO nanoparticles, and effect of pH were studied and optimized. Under optimal conditions, the developed alginate-CuO-GOD biosensor was shown to have two linear ranges; from 0.04 mM to 3 mM (with a correlation coefficient of 0.9996 and the sensitivity of 30.443 μA mM^{-1} cm^{-2}) and from 4 mM to 35 mM (with a correlation coefficient of 0.9994 and the sensitivity of 7.205 μA mM^{-1} cm^{-2}). The overall detection limit was estimated to be 1.6 μM (signal-to-noise ratio of 3) and the K_m value of 2.82 mM. The biosensor exhibited rather good performance with long-term stability (remainder of activity is 78% after 15 days) and significant specificity for glucose when compared to possible interfering molecules such as ascorbic acid, uric acid and acetaminophen.

1. Introduction

Diabetes mellitus is a common metabolic disorder characterized by hyperglicemia due defects in insulin secretion/action; this long lasting pathology can lead to major complications, such as diabetic neuropathy, retinopathy and cardiovascular diseases [1,2]. The main indicator for diabetes mellitus is the level of blood sugar which could be higher or lower than the normal range (80–120 mg/dL). The most effective way, up to now, to treat diabetes mellitus is to severely restrict the diet and to regularly control the blood sugar levels; therefore, there is a significant need of fast, reliable, and inexpensive detection methods for glucose [3,4]. In spite of the influential advances in glucose biosensor technology, there are still several challenges associated to the achievement of reliable glucose monitoring. Glucose detection is essential in not only clinical diagnostics but also biotechnology and food industry [5,6].

Electrochemical biosensors have many advantages such as limited complexity, robustness and high range of detection limit [7]. Enzyme-based electrochemical glucose biosensors is the most commonly studied fiels due to its simplicity, high selectivity and low cost making this an ideal model in new technology development. One of the most important issues, in electrochemical biosensor technology, to be dealt with is the stable immobilization of the biorecognition element (i.e. enzyme) onto the electrode surface in order to maintain its biological activity and, at the same time enhance the performance of the biosensor [8,9]. Covalent binding, cross-linking, physical/ionic adsorption, microencapsulation, gel entrapment, Langmuir-Blodgett or layer-by-layer (LBL) techniques have been all applied to enzyme immobilization [10–12]. In enzyme biosensors nanomaterials, with their large surface area and good electrical conductivity, have been shown to considerably improve the electron transfer, to provide mechanically and chemically durable systems [13,14] and to decrease the influence of interferences (e.g. ascorbic acid and uric acid) [15]. Among many diverse nanomaterials, oxide nanoparticles are quite attractive due to their mechanical hardness, excellent electrical properties, chemical passivity and thermal stability [16].

Copper (II) oxide (CuO), is an attractive and interesting transition metal oxide, and important p-type semiconductor with a narrow band gap of 1.2 eV. High stability and low cost of CuO, together with its electronic and catalytic properties [17], make this an attractive material for use in solar cells, lithium ion batteries for gas sensing and biosensor applications [18]. Several types of CuO nanostructures with different morphologies and sizes such as nanoparticles, nanoflowers, nanowires, nanocubes, hierarchical nanostructures and nano arrays, have been successfully prepared and demonstrated for sensing applications [19–24].

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Alginate is a linear unbranched copolymer consisting of 1,4-linked β-D-mannuronic (M) and α-L-guluronic acid (G) residues in different sequences and proportions. The physical properties change with the ratio of M and G acids [25]. Alginate is a pH shift polymer, which has two kinds of carboxylic acid groups of pKa values 3.38 and 3.65, respectively. So that its solubility and net charge depends on pH [26]. Due to features such as non-toxicity, biocompatibility, mild gelation conditions, hydrophility and low cost, alginate is an important biomaterial mostly used in areas of drug delivery, wound healing, in vitro cell culture and tissue engineering [27].

In this work we report on the development of a novel electrochemical glucosensor based on the combination of alginate, CuO nanoparticles and GOD. Alginate hydrogel was used as the immobilization matrix, via covalent crosslinking, for the GOD and CuO nanoparticles were used to provide stability to the hydrogel and, more importantly, to introduce significant catalytic properties to the hydrogel itself. The surface morphologies of the alginate, alginate-CuO, alginate-CuO-GOD were studied with scanning electron microscopy (SEM). Fourier transform infrared (FTIR) spectrophotometer was used for characterization of alginate, CuO, GOD, alginate-GOD, alginate-CuO nano-biocomposite and its interaction with GOD.

Several parameters affecting the biosensor performance have been studied in detail. In addition, the electrochemical behavior of the sensor has investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The developed biosensor has been finally applied to the detection of glucose in real serum samples.

2. Materials and methods

2.1. Chemicals and reagents

Glucose oxidase (E.C.1.1.3.4 GOD), Alginic acid from *Macrocystis pyrifera* (kelp) with %61 mannuronic acid and %39 guluronic acid (240 kDa), Copper (II) oxide (CuO), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbondiimide (EDC), sodium chloride (NaCl), acetic acid (CH_3COOH), potassium chloride (KCl), glucose, Potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), ascorbic acid, and uric acid were purchased from Sigma. Sodium dihydrogen orthophosphate (NaH_2PO_4), and disodium hydrogen orthophosphate (Na_2HPO_4) were purchase from Merck. The solutions were prepared using de-ionized water purified using a MilliPore Simplicity unit to a resistivity ≥18.2 MΩ cm. The glucose stock solutions were prepared with de-ionized water, left at +4 °C overnight to allow mutarotation before use.

2.2. Characterization and instrumentation

The surface morphologies of the alginate, alginate-CuO and alginate-CuO-GOD films were investigated using a Nova Nanosem 430 - FEI model scanning electron microscope (SEM) after being sputter-coated with gold. Fourier transform infrared (FTIR) measurements were carried out on a Bruker Tensor 27 (ATR - FTIR). A Gamry Instrument with Framework Version 5.50 Software was used to carry out all electrochemical measurements.

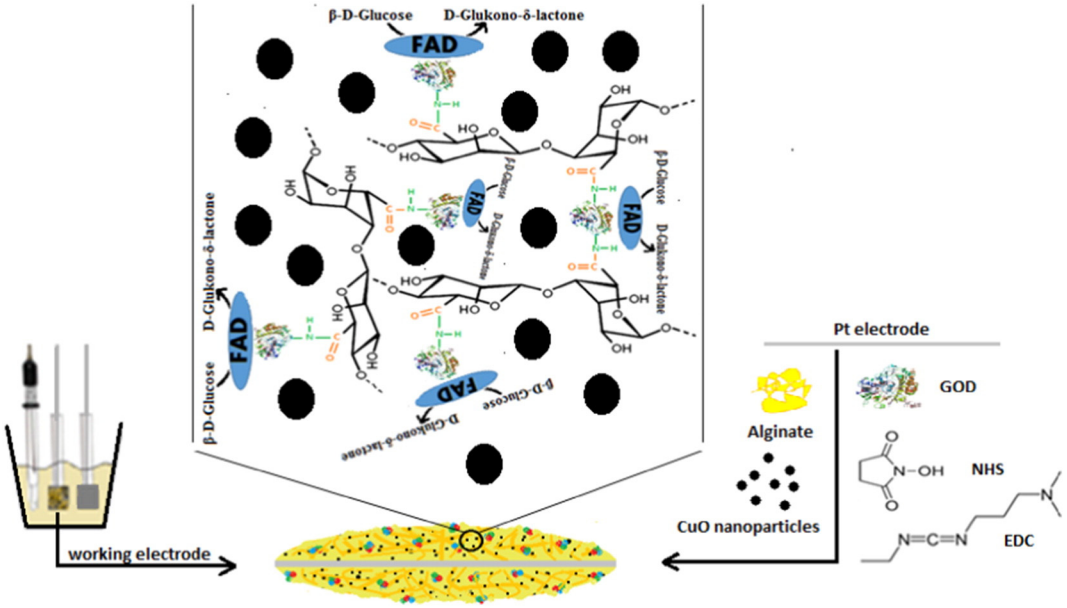
2.3. Electrochemical measurements

2.3.1. Fabrication of the alginate-CuO-GOD electrode

The surface of the bare platinum foil electrode (area 1 cm^2) was polished to mirror smoothness with 0.05 μm alumina slurry and rinsed thoroughly with ethanol first and with deionized water after; finally, the electrode was dried in air.

0.0002 g CuO and alginate $1.5 \times 10^{4-}$ g were added in an Eppendorf tube with acetate buffer (0.05 M, pH 5.5) and mixed vigorously for 5 min using a vortex to obtain a homogeneous black dispersion. Following 10 U of GOD were added to the Eppendorf containing the alginate CuO nano-biocomposite. The resulting alginate-CuO-GOD mixture was mixed for 3 s, in order not to denature of the enzyme; this mixing step was repeated three times. 0.28 M ratio of NHS/EDC mixture, in acetate buffer, was added to the alginate-CuO-GOD suspension to obtain a final volume of 50 μL. Following a short vertex mixing 25 μL of the obtained gel solution were drop-casted onto each side of the dry platinum electrode [28]. The resulting alginate-CuO-GOD electrode was left to dry overnight at +4 °C. Schematic illustration of the fabrication process can be seen in Scheme 1.

All electrochemical experiments were performed using a three-electrode electrochemical cell system comprised of a bare platinum foil electrode (area 1 cm^2) as a counter electrode, a bare or modified platinum foil electrode as a working electrode and an Ag/AgCl, introduced into the cell via a Luggin capillary as a reference electrode. Amperometric measurements of glucose with alginate-CuO-GOD and alginate-GOD electrodes was accomplished by adding known concentrations of



Scheme 1. Schematic illustration of the biosensor construction.

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glucose after the system reaches the steady state current into electrochemical cell contains 0.05 M PBS solution at pH 6.5 (0.1 M KCl and 0.1 mM $Fe(CN)_6^{4-/3-}$) under the applied potential of 0.65 V. EIS and CV were performed in PBS (0.05 M, pH 6.5) containing 0.1 M KCl and 5 mM $[Fe(CN)_6]^{3-/4-}$ redox couple. The Nyquist plots were obtained within the frequency range of 0.01 Hz − 100 kHz. In Cyclic voltammetry, the potential was cycled from −0.2 to 0.6 V with a scan rate of 100 mV s^{-1}.

3. Results and discussion

3.1. Surface characterization of modified electrode with SEM

Morphology of the surfaces of the biosensor was studied by SEM imaging; Fig. 1 compares the images of the alginate, alginate-CuO and alginate-CuO-GOD systems, respectively. Fig. 1A illustrates the alginate film which displays rough and homogenous morphology on the Pt electrode surface; the obtained rough morphology can be explained with the poor mechanical rigidity of the alginate hydrogel [29]. Changes in the surface morphology were observed after addition of CuO nanoparticles. As it can be seen from Fig. 1B, distribution of nanoparticles is homogenous and the surface is smoother. CuO nanoparticles behave like a filler in the alginate hydrogel matrix, contributing, in this way, to increase the stability of the polymeric film. Furthermore, the addition of CuO in the hydrogel is expected to improve its electrochemical properties and to facilitate the immobilization of the enzyme. The SEM image of the alginate-CuO-GOD nano-biocomposite is shown in Fig. 1C. When the GOD enzyme is assembled within the gel matrix, a major change is observed in comparison to highly smooth alginate-CuO matrix due to the globular shape of the enzyme.

3.2. Characterization of the nano-biocomposite material using FTIR

Interactions between alginate, CuO nanoparticles and GOD were investigated using FTIR spectroscopy. Fig. 2 shows the FTIR spectrum of alginate (a), CuO nanoparticles (b), alginate-GOD hydrogel (c), alginate-CuO nano-biocomposite (d) and alginate-CuO-GOD nano-biocomposite (e). In the spectrum of the pure alginate hydrogel the bands at 1587 cm^{-1} and 1409 cm^{-1} can be ascribed to the symmetric and asymmetric stretching vibration of the carboxylate group and the band at 1026 cm^{-1} was assigned to the stretching vibration of C—O—C— groups [30,31]. The same absorption bands were also observed in the spectrum of alginate-CuO nano-biocomposite; no further bands were recorded confirming the FTIR silence of the CuO and to the absence of significant chemical interaction between the metallic nanomaterial and the alginate [32]. The spectrum of alginate and alginate-CuO nanoparticles were changed significantly when GOD was covalently immobilized in the hydrogel. GOD immobilized alginate and alginate-CuO nano-biocomposite presented two new bands at 1624 cm^{-1} and 1537 cm^{-1} due to the formation of amide bonds during the EDC/NHS crosslinking of the alginate and enzyme, which corresponded to the amide I (the C—O stretching vibrations of the peptide bond groups) and amide II (the N—H in plane bending and C—N stretching modes of the polypeptide chains), respectively [33]. These results confirmed that GOD was covalently immobilized, successfully, in the hydrogel.

3.3. Optimization of alginate-CuO-GOD nano-biocomposite composition

Nature and composition of the immobilization matrix have a great influence on the biosensor response providing this not only a barrier to protect the enzymes but it also influences the conformation of the enzymes decreasing and subsequently their catalytic activity. Different parameters that could affect the performance of the biosensor, namely amount of the alginate, GOD, NHS/EDC concentration and the amount of CuO nanoparticles were studied using amperometry. As a first step the optimum amount of alginate was determined. For this purpose, different electrodes were prepared with different amounts of alginate while the amounts of the other components were kept constant; the same procedure was applied for optimizing all the other parameters. The optimum value of the alginate matrix was determined to be $1.5 \cdot 10^{-4}$ g/50 μL immobilization gel (Fig. S1). Low alginate concentrations increased the nanoparticle and/or enzyme leakage due to increased pore size, and smooth film formation cannot be occurred in low polymer concentrations. At higher levels the response increases due to an increase in the amount of nanoparticle/enzyme bondage.

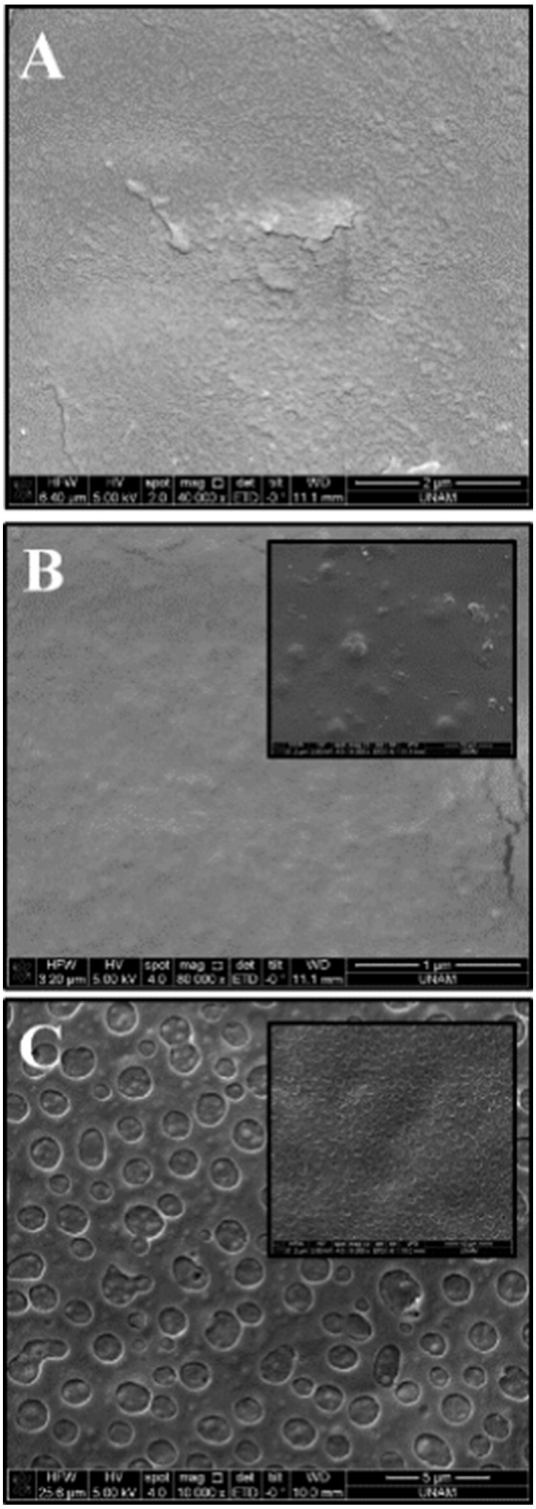

Fig. 1. SEM images of (A) alginate, (B) alginate-CuO, and (C) alginate-CuO-GOD films.

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Lower amounts of alginate lead to decreased amperometric responses since the insignificant amounts of GOD might be bond to the matrix. Higher amounts of alginate revealed decreased signal, again. It can be attributed to the decreased conductivity of the transducer as a result of accumulation of the excess amount of alginate. However, a thick matrix would cause a GOD activity due to enzymatic conformational limitation. Enzyme optimization was performed using different concentrations of GOD where the optimum concentration was found to be 10 U (Fig. S2). The amperometric response increased with increasing GOD concentration up to a limit followed by a decrease in the signal. Increased GOD concentrations caused a decrease in the amperometric response probably due to the formation of protein clusters and increase in enzyme-enzyme cross-linking that resulted in, on one side, oversaturation of the matrix and, on the other side, in limited conformational flexibility of the enzyme with subsequent reduction in their activity [34,35].

Responses of the biosensors prepared using different molar ratio of NHS/EDC are presented in Fig. S3. The optimum value was determined to be 0.28. Increased molar ratio of cross-linker decreased the response of the biosensor which can be explained on the basis of GOD deactivation, due to the stiff compact nature of the alginate based hydrogel. Further increase causes decrease of the biosensor response, probably due to excess nanoparticle/enzyme binding, leading to inactivation of the surface. Increasing the cross-linker concentration reduced the response of immobilized GOD, due to deactivation of the enzyme molecules and formation of a tight gel structure because of the excess cross-linker. Adversely, the low cross-linker concentration decreased the amperometric signal due to insufficient immobilization of GOD to alginate.

Amperometric responses of biosensors prepared using different amounts of CuO nanoparticles are presented in Fig. S4. The optimum CuO value was found to be 0.0002 g. As it can be seen increasing amounts of nanoparticles decreased the response of biosensor. The excess amounts of nanoparticles occluded the pores of alginate gel, and caused decreased electrical signal of the biosensor due to the deterioration of even distribution of the nanoparticles within the alginate matrix and reducing the conductivity. Finally low levels resulted in low amperometric signals due to insufficient electron transfer through electrode in similar with high levels which caused by aggregation and thus forming extra resistance.

To summarize the optimal mixture composition for the preparation of the alginate-CuO-GOD was found to be: 10 U GOD, 0.28 M ratio of NHS/EDC, 0.0002 g CuO nanoparticles and $1.5 \cdot 10^{-4}$ g alginate in a 50 μL immobilization gel.

3.4. Electrochemical properties of the modified electrodes

Electrochemical characterization of the biosensor and of the nano-biocomposite composition were performed by EIS, cyclic voltammetry and amperometry. The EIS measurements of the electrode coated with different hydrogel compositions are shown in Fig. 3A. As it can be seen, an increase in R_{CT} value is observed after coating Pt electrode with alginate; this can be considered as a good indication of the successful formation of the hydrogel. When CuO nanoparticles were present in the alginate hydrogel, the R_{CT} value decreased significantly (b). This result suggests that the CuO nanoparticles promote electron transfer within the permeable structure of the hydrogel. When the surface of Pt electrode was covered with alginate-GOD composite (e), the R_{CT} value dramatically increased. Significant decrease in R_{CT} was recorded, as expected, when CuO nanoparticles were present in the nanocomposite (d) confirming that proposed nano-biocomposite provides a permeable surface with enhanced electron transfer abilities [36].

Corresponding cyclic voltammetric measurements, recorded in PBS (0.05 M, pH 6.5) solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$, are shown in Fig. 3B. The coating of the Pt electrode with the alginate hydrogel resulted in a small reduction of the peak current intensity but not significant changes in the peak to peak separation. This was different when GOD was added to the alginate hydrogel; in this case a significant loss in peak current was also associated to a significant increase in the peak to peak separation (from ca. 154 mV to 234 mV); this increase in the ΔE_p, together with the significant loss in peack current, was a clear indication of the immobilization of the GOD [37]. On the other hand, when CuO nanoparticles were added within the alginate-GOD matrix a significant increase in the voltammetric peak current and a decrease of the peak to peak separation (ΔE_p; ca. 179 mV) were recorded. These results indicate the significant catalytic properties of the CuO nanoparticles.

Cyclic voltammograms of electrodes modified with the optimum alginate-CuO-GOD nano-biocomposite and with alginate-GOD hydrogel were measured at various scan rates (from 50 to 500 mV s^{-1}) in PBS (0.05 M, pH 6.5) solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ to further understand the importance of the CuO nanoparticles in the electrochemical properties of the nano-biocomposite As it can be seen from the Fig. 4A and C, both anodic and cathodic peak currents increased linearly with increased scan rate; furthermore the data shows a more significant current in the presence of the CuO nanoparticles anodic confirming this the catalytic properties of CuO nanoparticles.

The electron-transfer-rate constant (k_s) and electron-transfer coefficient (α_s) of the GOD were estimated using the Laviron's theory [38].

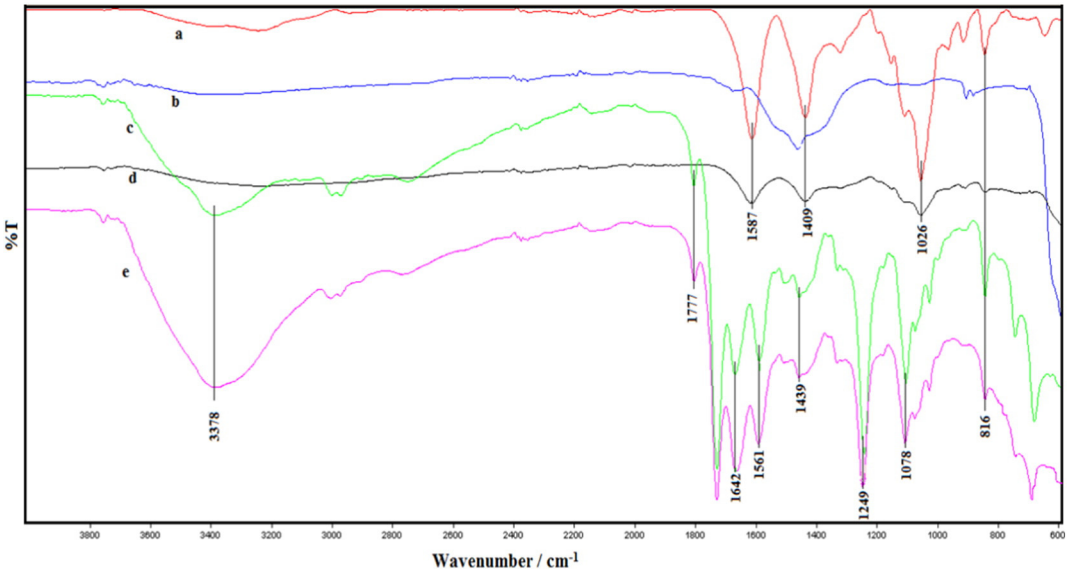

Fig. 2. FTIR spectras of alginate (a); CuO nanoparticles (b); GOD immobilized alginate film (c); alginate-CuO nano-biocomposite (d); and GOD immobilized alginate-CuO film (e).

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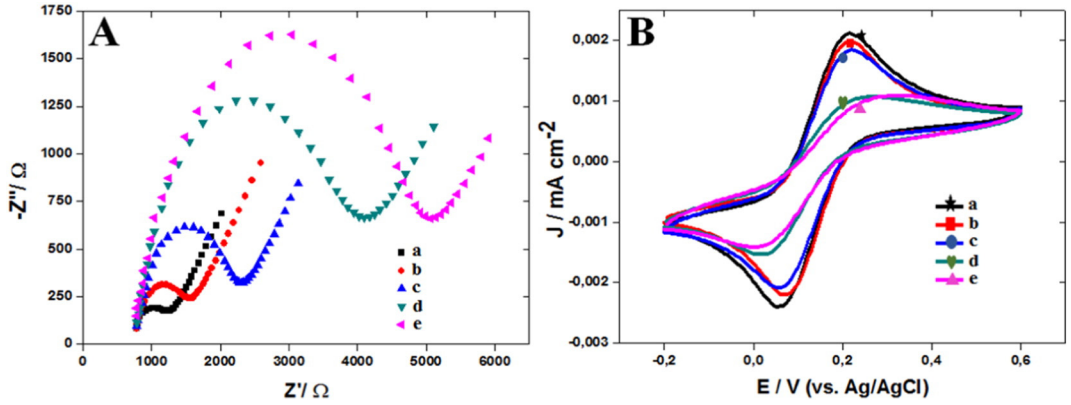

Fig. 3. (A) Nyquist diagrams of: bare Pt electrode (a); alginate-CuO nano-biocomposite electrode (b); alginate modified Pt electrode (c); alginate-CuO-GOD electrode (d); GOD immobilized alginate electrode (e) in the 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{4-/3-}$, pH 6.5, at the frequency range 0.01 Hz to100 kHz at 220 mV open circuit potential (E_{oc}) (B) Cyclic voltammograms of: bare Pt electrode (a); alginate-CuO electrode (b); alginate modified Pt electrode (c); alginate-CuO-GOD electrode (d); alginate-GOD electrode (e) in the 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{4-/3-}$, pH 6.5, at a scan rate 100 mVs^{-1}.

Plots of the Epa and Epc vs. the natural logarithm of the scan rates gave two straight lines with slopes of $RT/(1-\alpha)nF$ and $-RT/\alpha nF$ at high scan rates for the anodic and cathodic peaks, respectively, as it can be seen in Fig. 4B and D. Relevant kinetic parameters were obtained of the electrodes could be determined from the potential scan rate dependence of the anodic and cathodic peak potentials. If $n\Delta Ep > 200$ mV, the heterogeneous electron-transfer-rate constant (k_s) could be determined by the Laviron's equation; $k_s = \alpha nF\nu/RT$ [38]. Linear regression equations and Epa and Epc vs. the natural logarithm of scan rates equations are given as a table in the Fig. S5. Thus, the anodic (k_{sa}) and cathodic (k_{sc}) electron-transfer-rate constants and, the anodic (α_a) and cathodic (α_c) transfer coefficients were estimated for the alginate-CuO-GOD electrode and the alginate- GOD electrode. The k_{sa} value of the alginate-CuO-GOD electrode is 6. 675 s^{-1} which is larger than k_s value of the alginate-GOD electrode and other value reported in literature as for example: 2.83 s^{-1} of the GOD-graphene-chitosan modified electrode [39], and 2.85 s^{-1} of the GOD immobilized cage-like PbS nanostructure [40]. This result supports the advanced catalytic effect of CuO nanoparticles. In addition, both the cathodic and anodic peak currents of alginate-CuO-GOD and the alginate-GOD electrode were linear versus the square root of the scan rate, indicating the diffusion-controlled redox process (S6).

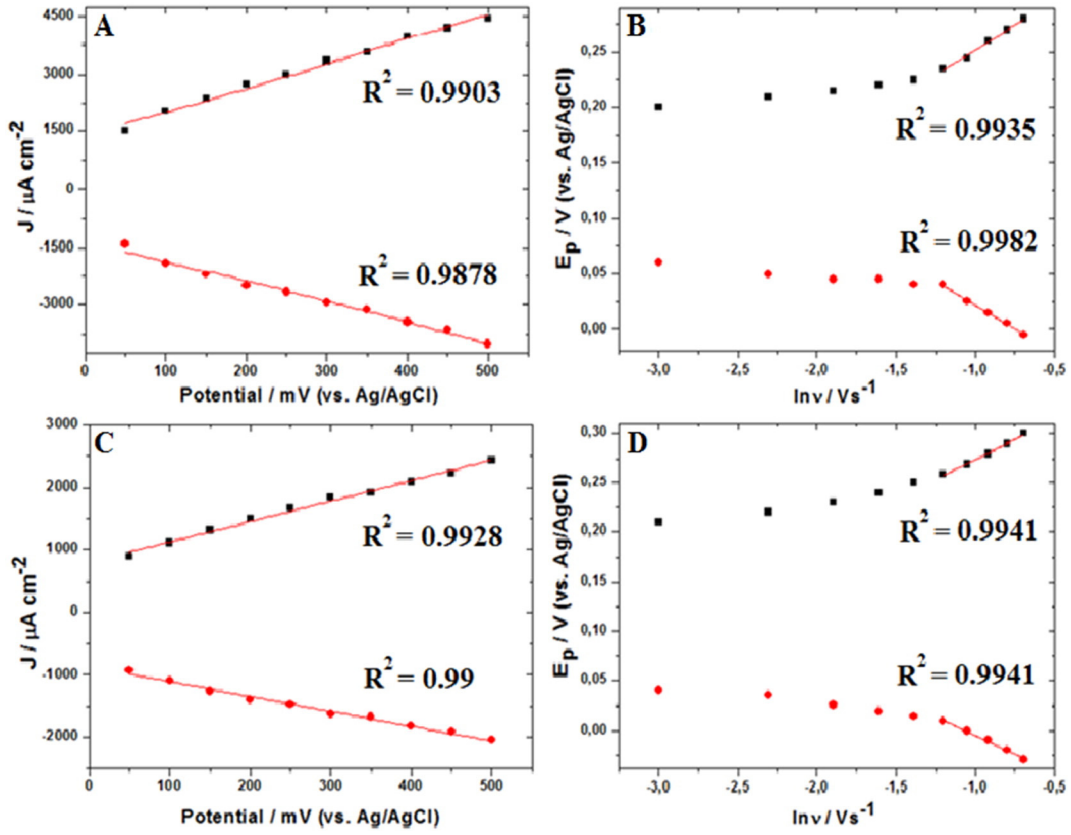

Fig. 4. Linear dependence of anodic and cathodic peak currents with scan rate (ν) for cyclic voltammograms obtained at (A) alginate-CuO-GOD electrode, (C) alginate-GOD electrode. Plots of the peak potentials (E_{pa}, E_{pc}) versus the natural logarithm of scan rate (ln ν) for (B) alginate-CuO-GOD electrode, (D) alginate-GOD electrode. Scan rate: 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mV s^{-1}. Solution composition: PBS (0.05 M pH 6.5) containing 0.1 M KCl 5 mM $Fe(CN)_6^{4-/3-}$.

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3.5. Effect of pH

The pH of the measurement media can significantly influence the response of the enzyme-biosensor influencing this significantly the enzymes' activity and stability [41]. The optimum pH for glucose oxidase has been reported to 5.5 [42]; the effect of pH on our biosensor was investigated and as it can be seen in Fig. S7 the optimum pH shifted to 6.5 probably due to the nature of the alginate hydrogel.

3.6. Amperometric determination of glucose and calibration curve

The obtained amperometric calibration curves are shown in Fig. 5. As it can be seen the amperometric response of the alginate-CuO-GOD electrode showed two linear relationships; the first with the glucose concentration ranging from 0.04 mM to 3 mM with a correlation coefficient of 0.9996 and sensitivity of about (30.443 ± 0.250) μA mM^{-1} cm^{-2} (Fig. 5B), and a second with the glucose concentration ranging from 4 mM to 35 mM with a correlation coefficient of 0.9994 and the sensitivity of (7.205 ± 0.063) μA mM^{-1} cm^{-2} (Fig. 5A inset). The detection limit of the alginate-CuO-GOD nano-biocomposite electrode was estimated to be 1.6 μM (signal-to-noise ratio of 3). The amperometric response of the alginate-GOD electrode showed a linear relationship with the glucose concentration within a range of 0.04 mM to 10 mM with a correlation coefficient of 0.9983 and sensitivity of (17.647 ± 0.243) μA mM^{-1} cm^{-2} (Fig. 5E–F). The detection limit of the biosensor was estimated to be 8 μM (signal-to-noise ratio of 3). These experimental results clearly indicated the advantages, in term of sensitivity and dynamic range, of the proposed nano-biocomposite. This may be attributed to the fact that the CuO nanoparticles substantially promote the electron transfer and enhance the electrocatalytic activity of the biosensor [43].

The amperometric results of the alginate-CuO-GOD and alginate-GOD biosensors showed the dependence on substrate concentration and it was well agreement with Michaelis-Menten kinetics. The apparent Michaelis-Menten constant (K_m^{app}) of GOD in the proposed nano-biocomposite was calculated using the Lineweaver-Burk equation [44]:

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

where i_{ss} is the steady-state current after addition of the substrate, i_{max} is the maximum current measured under saturated substrate conditions, and C is the bulk concentration of the substrate. The K_m values for the alginate-CuO-GOD nano-biocomposite and the alginate-GOD electrodes were calculated to be 2.82 mM and 0.15 M, respectively. The K_m value of the alginate-CuO-GOD nano-biocomposite is smaller (2.82 mM) than other value reported in literature as for example those obtained at the flower-shaped CuO nanostructures composed of thin nanosheets based sensor (8.7 mM) [45] and of those reported for multilayer films of chitosan-gold nanoparticles (10.5 mM) [46]. Alginate-CuO-GOD nano-biocomposite seems to provide better environment for GOD to perform its catalytic activity.

3.7. Selectivity, reproducibility and stability of the alginate-CuO-GOD electrode

The selectivity of the developed biosensor was tested using possible common blood interfering species such as ascorbic acid, uric acid, acetaminophen and phenylalanine. As it can be seen in Fig.6A no significant results were obtained from addition of ascorbic acid (0.01 mM), uric acid (0.01 mM), acetaminophen (0.01 mM), and phenylalanine (0.01 mM). No significant responses were recorded upon addition of

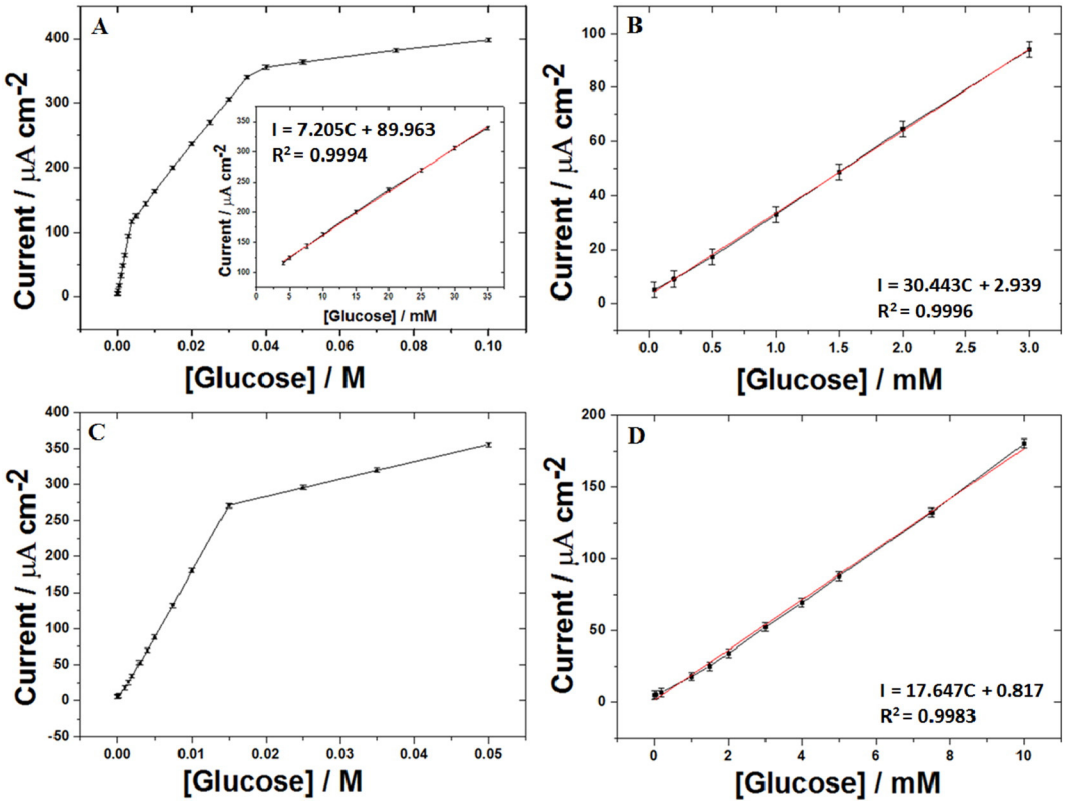

Fig. 5. Amperometric responses of the biosensor for the addition of different glucose concentrations. (A) shows the calibration curve of the alginate-CuO-GOD electrode (Inset of A shows the high linear range of the calibration curve with the sensitivity of (7.205 ± 0.063) μA mM^{-1} cm^{-2} and the correlation coefficient of 0.9994). (B) shows the small linear range of the alginate-CuO-GOD electrode with the sensitivity of (30.443 ± 0.250) μA mM^{-1} cm^{-2} and the correlation coefficient of 0.9996. (C) shows the calibration curve of the alginate-GOD electrode (F) shows the linear range of the alginate-GOD electrode with the sensitivity of (17.647 ± 0.243) μA mM^{-1} cm^{-2} and the correlation coefficient of 0.9983. Applied potential: 0.65 V and pH 6.5.

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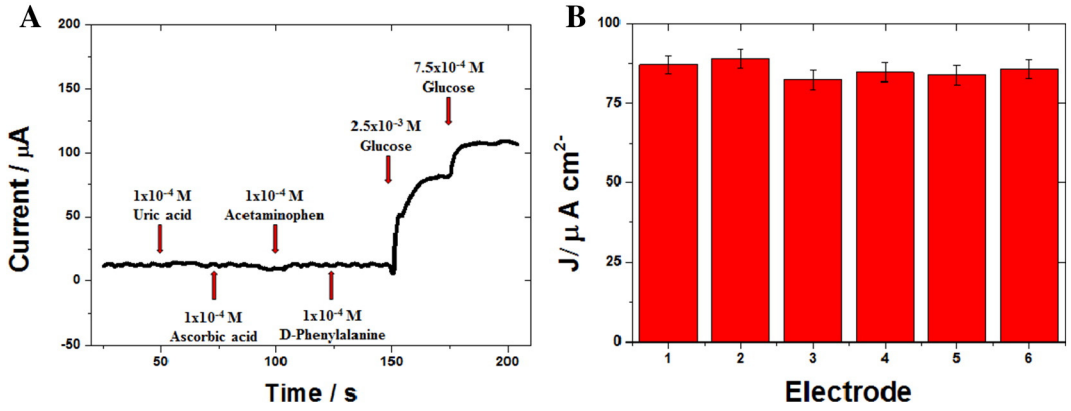

Fig. 6. (A) Amperometric i-t response of alginate-CuO-GOD modified Pt electrode for the addition of the interferencing species followed by various glucose concentrations into 0.1 M KCl solution containing 0.1 mM $Fe(CN)_6^{4-/3-}$, pH 6.5. (B) Reproducibility of the alginate-CuO-GOD electrode.

the interfering molecules; on the other end a significant increase in the amperometric response was obtained after addition of glucose confirming the specifically of the proposed biosensor for the detection of glucose [47,48]. Selectivity could be attributed to ability of alginate to create and efficient chemical/physical barrier for the interfering molecules.

The reproducibility of the alginate-CuO-GOD nano-biocomposite electrode was investigated by preparing six electrodes and using them for the detection of 40 mM glucose, in triplicate, under the same conditions (Fig. 6B). The relative standard deviation (RSD) of the six electrodes was found to be 0.94%, revealing good reproducibility in the fabrication process.

To evaluate the stability of the developed alginate-CuO-GOD nano-biocomposite biosensor, the prepared electrode was stored at +4 °C when not in use and its stability was evaluated by monitoring the response for 15 days (Fig. S7). The activity of the electrode remained at about 98%, 97%, 93%, 86% of its initial response at days 2, 4, 6 and 10, respectively and decreased to 78% after 15 days. The status of alginate in the composite can heighten the adhesive ability of the electrode and biocompatibility of the microenvironment for the immobilized GOD. However, CuO aggregated with alginate can control the release of immobilized biomolecules from the composite matrix during storage [34,49,50].

The satisfactory reproducibility and stability of the constructed biosensor may due to the effect of alginate hydrogel to create a friendly environment for the enzyme.

3.8. Real serum samples

In order to demonstrate the applicability of the developed alginate-CuO-GOD nano-biocomposite biosensor for real sample analysis, the biosensor was tested with human blood serum. Three different human serum samples from the local hospital were tested with the developed biosensor. Results are summarized in Table 1. The recovery rates were between 97.9% and 99.4%. The results demonstrate the potentiality of the developed biosensor for real sample analysis applications.

Table 1
Determination of glucose in human serum samples with alginate-CuO-GOD biosensor.

Samples	Determinded by hospital (mM)	Measured by alginate-CuO-GOD biosensor[a] (mM)	RSD (%)	Relative error (%)	Recovery (%)
1	3.77	3.76 ± 0.02	0.53	0.27	99.7
2	6.49	6.36 ± 0.14	2.18	2.00	97.9
3	20.00	19.87 ± 0.46	2.3	0.65	99.4

[a] The average value of three successive measurements.

4. Conclusion

In this study, a novel biosensor based on alginate-CuO-GOD nano-biocomposite has been developed and demonstrated for the detection of glucose. SEM characteristic showed that CuO nanoparticles were well seeded and dispersed in the alginate matrix. The developed biosensor exhibited good sensitivity with a detection limit of 1.6 μM glucose and low K_m value of 2.62 mM. The biosensor also showed good reproducibility (0.94%) and long-term-stability (retained of 78% of response after 15 days of storage at 4C). The responses to interference species such as uric acid, ascorbic acid, acetaminophen and phenylalanine were negligible compared with those to glucose. The recorded performances of the biosensor are attributed to synergic effect of the CuO nanoparticles (i.e. enhanced surface area, and electron transfer rate) and of the alginate (i.e. improved environment for GOD immobilization and physical/chemical barrier for interfering). Human serum samples were tested with the fabricated biosensor and the obtained results were in good agreement with those obtained using reference techniques. The developed alginate-CuO-GOD nano-biocomposite-based biosensor is expected to have great potential for biomedical applications thanks to its good properties, low cost, simplicity, high sensitivity and selectivity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2016.12.003.

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