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Gold nanoparticles-induced enhancement of the analytical response of an electrochemical biosensor based on an organic–inorganic hybrid composite material

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ABSTRACT

The design and characterization of a new organic–inorganic hybrid composite material for glucose electrochemical sensing are described. This material is based on the entrapment of both gold nanoparticles (AuNPs) and glucose oxidase, which was chosen as a model, into a sol–gel matrix. The addition of spectroscopic grade graphite to this system, which confers conductivity, leads to the development of a material particularly attractive for electrochemical biosensor fabrication. The characterization of the hybrid composite material was performed using atomic force microscopy and scanning electron microscopy techniques. This composite material was applied to the determination of glucose in presence of hydroxymethylferrocene as a redox mediator. The system exhibits a clear electrocatalytic activity towards glucose, allowing its determination at 250 mV vs Ag/AgCl. The performance of the resulting enzyme biosensor was evaluated in terms of sensitivity, detection limit, linear response range, stability and accuracy. Finally, the enhancement of the analytical response of the resulting biosensor induced by the presence of gold nanoparticles was evaluated by comparison with a similar organic–inorganic hybrid composite material without AuNPs.

1. Introduction

Development of sensors based on enzyme immobilization onto transducer surfaces has become one of the most promising research areas in the field of bionanotechnology, mainly due to its potential applications in the development of analytical devices. In general, the retention of the enzymatic activity after the immobilization process is one of the most important issues in this field. The interaction between the enzyme and the solid surface can induce conformational changes in the adsorbed enzymes. The alteration of the three-dimensional conformation leads to a diminution of the catalytic activity and hinders the enzyme functionality.

During the last years, numerous works have been published concerning the use of the silica sol–gel technology as a strategy to preserve the catalytic activity of enzymes after the immobilization step [1–4]. In this sense, the polymeric network generated by sol–gel technology can be used as an adequate matrix [5,6] in which several compounds, including biological material, can be encapsulated through physical entrapment rather than by covalent bonding leading to a non aggressive approach. Particularly, the sol–gel network provides a biocompatible environment for enzyme protection exhibiting additional advantages such as simplicity of preparation, chemical inertness, high stability, physical rigidity, renewable surface and tuneable properties. The final properties of the sol–gel matrix play a key role in the biosensor performance and can be easily controlled by varying some parameters such as the precursor or the preparation conditions (pH, ratio of compounds, etc). Various precursors have been reported in the literature for the preparation of the sol–gel network, but among them the most used for enzyme encapsulation are oxysilanes such as methyltrimethoxysilane (MTMOS), tetramethoxysilane (TMOS), 3-aminopropyltriethoxysilane (APTOS) and tetraethoxysilane (TEOS) [7–12].

Recently, the integration of nanomaterials into (bio)sensors has become an attractive method for improving the properties of the resulting analytical device. From a practical point of view, gold nanoparticles (AuNPs) are widely used due to their interesting physicochemical properties, which are essentially different to those of bulk gold [13]. In particular, several works have demonstrated that AuNPs exhibit highly attractive catalytic properties toward several compounds in solution indicating an enhancement of the electrical communication with the electrode surface [14,15]. Other key properties of AuNPs are their large surface area in

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conjunction with an excellent biocompatibility. Both characteristics make this nanomaterial suitable to immobilize biomolecules, particularly enzymes, allowing the retention of the biological activity for a long period of time. Since the recognition of the use of nanoparticles is a versatile strategy for biomolecules immobilization [16], a great interest has been addressed to the development of biosensors based on AuNPs as a strategy of improving the biosensor performance [17].

In the present work we describe the preparation and characterization of a new organic–inorganic hybrid composite material from a three-dimensional silica polymer network, obtained by means of the sol–gel technology using tetraethoxysilane as precursor. This matrix provides an excellent network allowing the encapsulation of gold nanoparticles (AuNPs), a conductive material (graphite powder, C) and a biosensing molecule such as glucose oxidase (GOx), chosen as a model since it is a stable, inexpensive and well-studied enzyme. Moreover, GOx presents a wide range of applications in many fields such as biofuell cells [18] or clinical glucose determination [7,9,19,20]. The resulting hybrid materials were characterized by means of scanning electron microscopy (SEM) and atomic force microscopy (AFM) techniques. Both techniques allow obtaining information about not only the morphological properties of the surface but also about its compactness degree, homogeneity, rigidity and roughness. Finally, the analytical parameters of the resulting integrated glucose biosensor were studied.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*), lyophilized powder containing 15,200 U/g solid, was obtained from the Sigma Chemical Co. (St. Louis, MO). D-(+)-Glucose (99.5%) and Colloidal Gold (AuNPs, 20 nm particle size) were also purchased from Sigma Chemical Co. Tetraethoxysilane (TEOS), hydroxymethylferrocene (HMF, 97%) and polyethylene glycol (PEG, MW 400) were provided from Aldrich. Ultra pure spectroscopic graphite powder (C, particle size 50–50.3 μm) was from United Carbon Products Co. Inc. (Bay City, MI, USA). Other chemicals used in this work, such as sodium phosphate, acetaminophen, uric acid and ascorbic acid were reagent grade quality and used as received without additional purification steps. Sodium phosphate was employed for the preparation of buffer solutions (0.1 M, pH 7.0). Water was purified with a Millipore Milli-Q-System. All solutions were prepared just prior to use.

2.2. Experimental techniques

2.2.1. Scanning electron microscopy measurements

The scanning electron microscope was an ISI (DS-130C) operating at 40 kV. Measurements were carried out under high vacuum conditions.

2.2.2. Atomic force microscopy measurements

The atomic force microscope was a Nanoscope IIIa from Digital Instruments (DI, Santa Barbara, CA) used with D and J scanners (maximal scan range of ~14 μm and ~125 μm, respectively). Measurements were carried out in tapping-mode using Si cantilevers from Veeco. The samples were imaged with different cantilevers in order to ensure that the imaged structures were not due to tip artefacts. All measurements were carried out under ambient conditions.

2.2.3. Electrochemical measurements

All electrochemical measurements were performed on an Ecochemie Autolab PGSTAT12 system (Utrecht, The Netherlands). Experiments were carried out in an electrochemical cell with standard Teflon cover. All potentials are reported against a Ag/AgCl (Metrohm, 3.0 M KCl) reference electrode. The auxiliary electrode was a platinum wire and TEOS/GOx/C or TEOS/GOx/AuNPs/C composite electrodes were used as the working electrode. Cyclic voltammetry (CV) was the electrochemical technique applied to study the response of the composite electrodes in the presence of HMF (which acts as soluble mediator) and in the presence or absence of glucose. Solutions were deaerated with nitrogen gas before use, and the gas flow was kept over the solutions during experiments. Instrumental parameters were as follows: potential range from −0.1 V to 0.5 V and scan rate 10 mV s^{-1}.

2.3. Procedures

2.3.1. Sol–gel solution preparation

The sol–gel solution was prepared mixing 1.6 mL of TEOS with 1.0 mL of water and 200 μL of 0.1 M HCl. This solution was sonicated using an Ultrasons bath (P-Selecta) for 15 min at room temperature and stored overnight at 4 °C.

2.3.2. Activation of the graphite powder

The graphite powder used in the electrode preparation was previously activated by washing it with acetone, then with a mixture of HCl and HNO_3 (1:3) and finally with distilled water. The resulting paste was subsequently dried during 4 h at 400 °C [21].

2.3.3. Preparation of the biosensing organic–inorganic hybrid composite material

Two different kinds of biosensing organic–inorganic hybrid composite materials were prepared depending on the compounds encapsulated into the sol–gel three-dimensional network. The biosensing composite material denoted as TEOS/GOx/C was obtained by mixing 0.6 mL of the sol–gel solution with 0.4 g of graphite powder previously modified with enzyme. To obtain the glucose oxidase modified graphite, an amount of the conditioned graphite powder was mixed with GOx in phosphate buffer solution (PBS, pH 7) to adsorb the enzyme on the powder's surface after solvent evaporation (80 mg of GOx/0.5 g of graphite). This enzyme-modified graphite powder was allowed to dry for a week into a desiccator before being mixed with the sol–gel solution. The biosensing composite material including gold nanoparticles, denoted as TEOS/GOx/AuNPs/C, was prepared in a similar way, but using the gold colloidal solution (AuNPs, 20 nm average particle size) instead of water in the sol–gel solution preparation.

2.3.4. Electrode preparation procedure

The preparation of the electrode can be summarized as follows: 0.5 mL of the sol–gel solution was mixed with 0.4 g of graphite powder previously conditioned. The resulting inorganic composite material was packaged firmly into a glass body (15 mm large × 2 mm i.d.), leaving a little empty cavity (3 mm large × 2 mm i.d.) in one of the extremes. This was allowed to dry for five days at room temperature to achieve an adequate consistency. Subsequently, the little empty cavity was filled up tightly with the corresponding biosensing organic–inorganic hybrid composite material, leaving a little extra mixture sticking out of the tip to make easy the ulterior polishing step. Finally, a 0.5 mm diameter copper wire was inserted to the opposite extreme using silver tincture to establish the electrical contact and ensure the adhesion. The final electrode was allowed to dry for one day at room temperature and the surface was polished to remove any extra composite material, rinsed thoroughly with ultra pure water.

Before electrochemical measurements were made, electrode surfaces were conditioned by immersion during 2 h into 1 mM HMF

solution in PBS. This treatment avoids a posterior increment of the signal due to a mediator's adsorption.

3. Results and discussion

3.1. Optimization of parameters

As mentioned above, the first step involved in the preparation of the biosensing organic–inorganic hybrid composite material consists in a polymeric network generation by sol–gel technology, in which all the components integrating the biosensor will be subsequently encapsulated. The final properties of the resulting three-dimensional matrix depend strongly on both the chemical structure of the oxysilane precursor and the composition of the sol–gel solution. In this sense, two different silicon alcoxides (MTMOS and TEOS) were evaluated in order to study their influence on the final sol–gel properties. Among them, TEOS was selected as the most adequate because its use as precursor allows obtaining homogeneous sol–gel solutions without phase separation, relative short gelation times and adequate viscosity. Likewise, several volume ratios TEOS:H_2O were tested concluding that the optimal volume ratio TEOS:H_2O to reach the above requirements was 1.6:1.0. Under these conditions, the sol–gel process results in a homogeneous and transparent solution only under acid conditions using 200 μL of 0.1 M HCl as catalyser and ultrasonic agitation during 15 min. The obtained sol–gel solution is kept overnight at 4 °C and subsequently used to prepare the TEOS/GOx/C and TEOS/GOx/AuNPs/C composite electrodes. This preparation consists in thoroughly hand-mixing of the sol–gel solution with spectrographic graphite powder that is then allowed to gelify and to dry [21]. Different relations between graphite and sol–gel solution, unmodified and modified with GOx, were tested in order to obtain a uniform and suitable paste for its posterior packaging into the adequate electrode body. The optimal relation found were 0.4 g of graphite and 0.5 mL of sol–gel solution (0.8%, w/v), since the use of different ratios, 0.7% (w/v) and 0.5% (w/v), gives rise to a resulting paste whose consistency is not adequate for packaging into the glass tube. In the case of graphite modified with the enzyme, it was necessary to increase the volume of sol–gel solution up to 0.6 mL to ensure a good compactness, which is provided by the silicate continuum, without the necessity of any organic binder. The resulting composite material was packaged firmly into an electrode body. Among the diverse materials evaluated as candidates for electrode body, -plastic, Teflon and glass-, the last one was selected as the best material since, when the electrode body is filled up tightly with the sol–gel–graphite paste, the sol–gel solution bonds to the glass body, which results in an hermetic structure that only has contact with solutions through the electrode surface.

As mentioned above, gold nanoparticles are of extreme current interest since they have proved to be highly conductive (fast electron transfer) nanomaterials. In the preparation of TEOS/GOx/AuNPs/C composite electrodes, two different methods were used to achieve the objective of AuNPs incorporation in the composite: adsorption on graphite microparticles after solvent evaporation or direct incorporation in sol–gel precursors. In this case, although both methods were effective, the last one was selected because of its simplicity.

On the other hand, according to several authors, polyethylene glycol (PEG) enhances the hydrophilic character and permeability of the composite while reducing the sol–gel shrinkage over time [3,22]. After soaking in aqueous solution it forms microchannels within the composite because PEG leaches from the electrode. Thanks to the formation of these microchannels, PEG could be considered as "diffusion enhancer" of the analyte giving a higher current response [23,24]. In this sense, a volume of 250 μL of 10% (v/v) PEG was added to the sol–gel precursors but in this case, the AFM assays (discussed below) did not show significant structural differences between the two types of composites (in absence and in presence of PEG). As a consequence, PEG was not added to the sol–gel precursors.

3.2. Morphological characterization

As mentioned above, AFM and SEM techniques are very useful tools to obtain information about the morphological properties of the resulting surface obtained after each step involved in the ceramic composite electrodes preparation. The first step, the sol–gel formation, involves hydrolysis and polycondensation reactions leading to a SiO_2 lattice. Both the composition of the sol–gel mixture and the chemical structure of the precursor have a great influence on the morphological characteristics of the resulting surface, in particular roughness and size of pores. Fig. 1A shows a SEM image of a 40 μm × 40 μm sol–gel area, prepared as described above, which results to be rather flat and almost featureless. Fig. 1B and C shows AFM images taken on one of these flat areas. From these data a surface roughness in the 0.3–0.7 nm range is obtained, confirming the flatness of the surface. The higher resolution image displayed in Fig. 1C reveals a nanoporous surface with a typical external pore diameter of ~10 nm. The size and distribution of pores in the resulting sol–gel matrix is a relevant factor for enzyme encapsulation [25]. The pore diameter value obtained from the higher resolution AFM image confirms that the sol–gel preparation methodology leads to size pore diameters that will be not wide enough to allow the enzyme leakage, but adequate to facilitate the substrate permeation. As mentioned above, the influence of PEG addition to the sol–gel precursor on the final surface morphology was also evaluated by AFM (data not shown). In this case, the AFM measurements show that both preparation procedures (in absence and in presence of PEG) of the sol–gel lattice lead to a similar morphology of the resulting surface.

The addition of graphite powder to the sol–gel solution allows us to obtain a material (TEOS/C) that consists of a robust silica backbone entrapping interconnected carbon microparticles, which impart electronic conductivity to the material. As can be observed in Fig. 2A, it can be concluded that the addition of the graphite powder to the sol–gel solution causes a dramatic change on the micro-morphology of the surface. Large grains (between 25 μm and 50 μm of size) are observed on the surface, which correspond to those also observed by SEM on the raw graphite powder (data not shown). This rough morphology turns difficult the AFM measurements. It should be noted that the samples analyzed in this work were not specifically prepared for AFM characterization but they were the real surfaces generated by the sol–gel process. Notwithstanding, it is possible to carry out AFM analysis on restricted areas provided that special care is addressed to preserve the cantilever condition, particularly the tip size and shape during the imaging process. Under these conditions, images as that shown in Fig. 2B can be obtained. This figure shows micro and nanocrystalline flat graphite regions on top of one of the large grains observed in the SEM image. On one of these regions, we were able to obtain atomic resolution AFM images (Fig. 2C). The characteristic 0.25 nm graphite hexagonal lattice periodicity is clearly observed [26]. As mentioned above, we have essayed different ratios between graphite powder and sol–gel solution (0.8%, 0.7% and 0.5% (w/v)) to obtain the TEOS/C material. Subsequently, we have performed AFM measurements of the resulting composites. AFM images corresponding to TEOS/C material obtained by using graphite powder/sol–gel solution ratios of 0.7% and 0.5% (w/v) (data not shown) are similar than that presented in Fig. 2C for a 0.8% (w/v) ratio. Therefore, it can be concluded that a moderate change in the graphite powder/sol–gel solution ratio did not affect the final morphological characteristics of the resulting composite material.

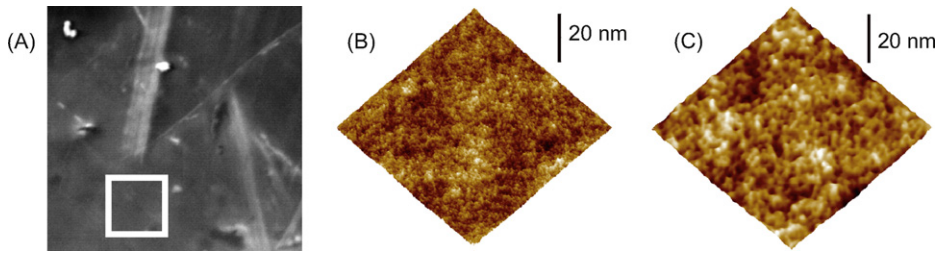

Fig. 1. (A) 40 μm × 40 μm SEM image of the sol–gel surface. (B) 7.5 μm × 7.5 μm and (C) 420 nm × 420 nm AFM images taken on a flat region as that marked in (A).

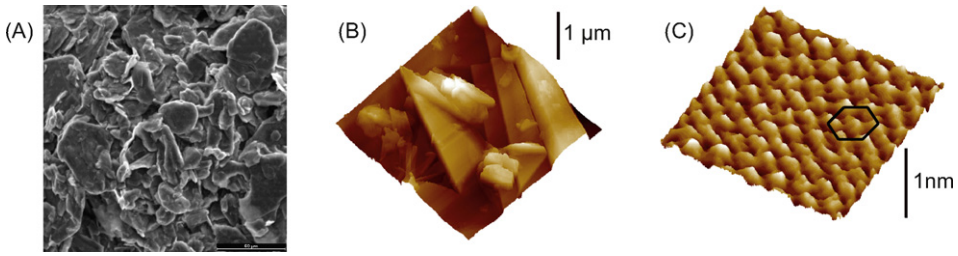

Fig. 2. (A) 150 μm × 150 μm SEM image of the TEOS/C surface. (B) 7.5 μm × 7.5 μm AFM image taken on a large grain. (C) 1.7 nm × 1.7 nm atomic resolution AFM image taken on a flat region of figure (B). The hexagonal graphite lattice, with a lateral side of 0.25 nm, is marked.

The presence of the enzyme (TEOS/GOx/C) implies a further change in the surface morphology of the sample at the micrometer scale (Fig. 3A) since it is formed by flat platelets delimited by deep crevices. Fig. 3B is an AFM image on two neighboring platelets separated by a deep groove in the middle of the image. At this scale, the platelet surface is rough and it is formed by rounded structures (Fig. 3C) that correspond to the globular GOx enzymes and aggregates covering the electrode surface. The measured lateral dimension of the smallest globular structures (14–16 nm) is in good agreement with the typical dimensions of GOx (6.0 nm × 5.2 nm × 7.7 nm) reported in the literature [27] taking into account tip widening effects due to the tip geometry and applied force. It is worth noting that AFM studies of the inner part of such sample, which was previously fractured, did show also globular structures. This fact indicates that the enzyme was successfully incorporated into the hybrid sol–gel matrix proving that the resulting sol–gel matrix is a good material for enzyme loading.

Finally, in order to assess the influence of the nanoparticles addition on the electrode nano-morphology we have compared the images obtained for the TEOS/GOx/AuNPs/C system (Fig. 4) with those obtained for the TEOS/GOx/C system (Fig. 3). From this comparison it is clear that both systems are analogous at the micron (Figs. 3A and 4A) and nano (Figs. 3B–C and 4B–C) scales.

3.3. Electrochemical performance of the glucose biosensor

Glucose biosensor is based on glucose oxidase, which in presence of oxygen catalyzes the oxidation of glucose into gluconolactone and hydrogen peroxide. The activity of the immobilized GOx can be electrochemically detected by monitoring the H_2O_2 produced in the enzymatic reaction. Accordingly, we have obtained the cyclic voltammetric response from −0.1 V to +0.8 V at 10 mV/s for the TEOS/GOx/AuNPs/C electrode in a 0.1 M pH 7.0 phosphate buffer solution containing 10 mM of hydrogen peroxide in order to test whether this strategy is adequate to glucose determination. The resulting cyclic voltammetric response (data not shown) exhibits an irreversible anodic wave with a peak potential at about +0.7 V. Therefore, the determination of glucose in real samples, such as blood, by direct oxidation of the hydrogen peroxide generated in the enzymatic reaction do not represent an adequate strategy because it can be affected by potential interfering species present in the sample, which could also be oxidized at the high potential required for H_2O_2 amperometric detection. In order to minimize the contribution of interfering substances to the glucose biosensor response, one of the most important approaches reported in the literature is based on the replacement of the natural electron acceptor (O_2) by an artificial mediator such as polyneutral red [11], celestin blue [20], or prussian blue [28,29]. Based on our previous experience with other oxidases [30], we have studied the ability of the hydroxymethylferrocene (HMF) to act as a redox mediator in solution. Fig. 5 (curve a) shows the cyclic voltammetric response from −0.1 V to +0.5 V at 10 mV/s for the TEOS/GOx/AuNPs/C electrode in a 0.1 M pH 7.0 phosphate buffer solution containing 1.0 mM of HMF in the absence of glucose. The redox response of the ferrocene/ferrocinium process in aqueous media is observed. Addition of glucose to a final concentration of 2.0 mM (curve b) leads to an enhancement of the anodic peak current concomitant with a decrease of the cathodic peak current consistent with a clear elec-

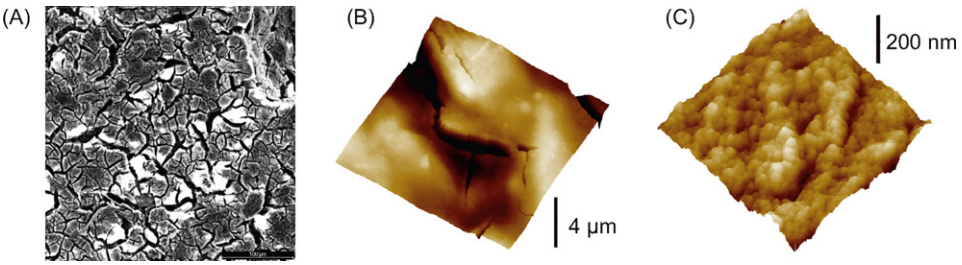

Fig. 3. (A) 350 μm × 350 μm SEM image of the TEOS/GOx/C surface. (B) 7.5 μm × 7.5 μm and (C) 1.0 μm × 1.0 μm AFM images taken on a platelet region.

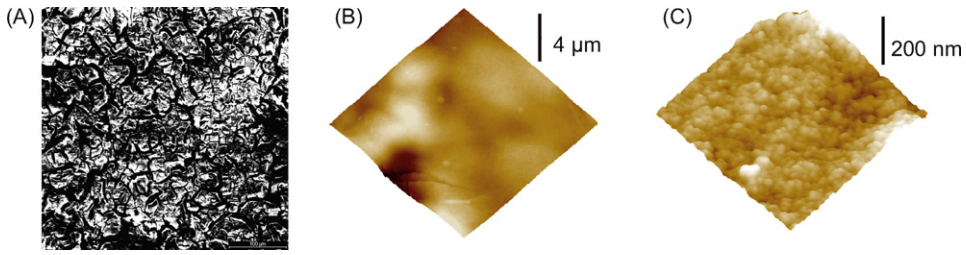

Fig. 4. (A) 350 μm × 350 μm SEM image of the TEOS/GOx/AuNPs/C surface. (B) 7.5 μm × 7.5 μm and (C) 1.0 μm × 1.0 μm AFM images taken on a platelet region.

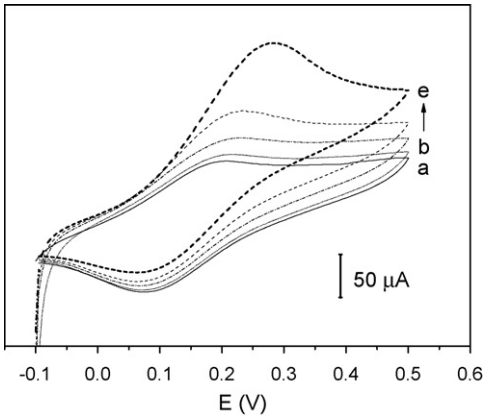

Fig. 5. (A) Cyclic voltammetric response at 10 mV/s for a TEOS/GOx/AuNPs/C composite electrode in a deareated solution of 1.0 mM HMF in 0.1 M phosphate buffer (pH 7.0) as a function of glucose concentration: (a) 0.0 mM, (b) 2.0 mM, (c) 7.0 mM, (d) 25 mM, (e) 55 mM.

trocatalytic effect. Fig. 5 (curves c–e) shows the increment of the magnitude of the catalytic current as a consequence of increasing the amount of glucose (final concentrations of 7 mM, 25 mM and 55 mM, respectively).

In order to ascertain the influence of the AuNPs on the enhancement of the analytical response of the designed organic–inorganic hybrid composite material, we have also obtained the voltammetric response (data not shown) of a similar composite material containing GOx but free of gold nanoparticles (TEOS/GOx/C). As one would expect, in this case the catalytic wave obtained after addition of 2.0 mM of glucose is smaller and less defined than that obtained when gold nanoparticles are present. Fig. 6 depicts typical calibration curves, in which catalytic current obtained from CV measurements was plotted vs glucose concentration for (A) TEOS/C, (B) TEOS/GOx/C and (C) TEOS/GOx/AuNPs/C ceramic carbon electrodes, respectively. Catalytic current was obtained by subtracting the current response in the absence of glucose to the response in the presence of glucose at a potential of +0.25 V. As one would expect, the response obtained (in the same conditions) for a hybrid composite material without enzyme (TEOS/C) is completely different. In this case, upon addition of glucose no catalytic waves are observed, confirming both the role of the enzyme in the catalytic response to substrate and the biocompatibility of the developed hybrid sol–gel material for immobilization of enzymes that retain their activity in a large extent. The analytical properties – linear response, sensitivity and detection limit – of both TEOS/GOx/C and TEOS/GOx/AuNPs/C biosensors were obtained from the linear part of the current vs glucose concentration plot. As shown in Fig. 6, the calibration plots were linear over a wide concentration range, 1–20 mM and 0.5–55 mM for TEOS/GOx/C and TEOS/GOx/AuNPs/C biosensors, respectively. The sensitivity values, calculated from the slope of the plot, were of 1.73 μA mM^{-1} and 2.43 μA mM^{-1}, respectively. The detection limit, calculated as the ratio between three times the standard deviation of background current and the sensitivity, was 1.9 mM for TEOS/GOx/C and 1.3 mM for TEOS/GOx/AuNPs/C. Since the normal glucose concentration in blood is in the 4.4–6.6 mM range [31], the detection limit obtained for the TEOS/GOx/AuNPs/C biosensor is medically useful for glucose level monitoring in blood. The accuracy of both TEOS/GOx/C and TEOS/GOx/AuNPs/C biosensors was evaluated from the relative standard deviation (R.S.D.) of eight different measurements of a 5 mM glucose concentration with the same biosensor. The R.S.D. values were 1.1% and 0.5%, respectively. As can be seen in Table 1, most of the analytical properties corresponding to both TEOS/GOx/C and TEOS/AuNPs/GOx/C are comparable or even better than results reported in the literature for similar sol–gel-based glucose biosensors [9,11,28,29,32–36]. The storage stability was also investigated by measuring the magnitude of the current response for a 10 mM glucose concentration every 5 days during a period of 30 days, keeping the biosensor stored at 4 °C between measurements. When the biosensor is reused, a gradual decay in the signal is observed, leading to a loss of 50% of the initial response after 30 days. Moreover, the developed biosensor exhibited a high degree of robustness since changes in the experimental conditions such as temperature, pH and electrolyte concentration do not critically affect its response.

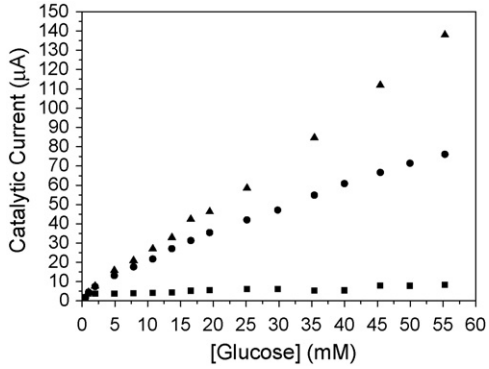

Fig. 6. Catalytic current vs glucose concentration for the following composite electrodes: (■) TEOS/C, (●) TEOS/GOx/C and (▲) TEOS/GOx/AuNPs/C. Data were obtained from cyclic voltammetric measurements at 10 mV/s in a deareated solution of 1.0 mM HMF in 0.1 M phosphate buffer (pH 7.0) for increasing glucose concentration.

In order to test the utility of the developed biosensors in the determination of glucose present in a real sample, we have carried out an exhaustive study of the influence of the more usual potential interferences. In this sense, we have obtained the biosensor response to 5 mM of glucose in absence and presence of several substances, which can be usually present in blood samples, such as acetaminophen (0.1 mM), ascorbic acid (0.1 mM) and uric acid (0.2 mM). While the presence of acetaminophen has a negligible effect to the biosensor response, addition of ascorbic acid or uric acid (both of them easily oxidized at electrodes) results in an increase of the catalytic current of 6% and 7%, respectively. These results, comparable or even better than other reported in the literature for similar biosensors [7,28,32,37], suggest that the developed

Table 1

Biosensor	Sensitivity ($\mu A\,mM^{-1}$)	Linear range (mM)	Applied potential (V)	Accuracy (R.S.D.)
TEOS/GOx/C (present work)	1.73	1–20	+0.25	1.1% ($n=8$)
TEOS/AuNPs/GOx/C (present work)	2.43	0.5–55	+0.25	0.5% ($n=8$)
Sol–gel/chitosan/GOx [32]	0.27	Up to 14	+0.35	2% ($n=7$)
Fc/Ormosil/GOx/GP [33]	1.76	Up to 35	+0.35	–
Sol–gel/CNT/GOx/Bppg [9]	0.20	0.2–20	+0.3	1.8% ($n=10$)
Sol–gel/GOx/chitosan/PB/GC [29]	0.42	0.05–26	−0.05	3.8% ($n=8$)
Sol–gel/PNR/GOx [11]	0.06	0.05–0.6	−0.25	–
Sol–gel/GOx/PtNPs-CNT [34]	0.28	1–25	+0.1	5.1% ($n=10$)
Sol–gel/PVA/GOx/SPE [35]	0.44	0–4.1	−0.5	–
Sol–gel/GOx/PB/GC [28]	0.84	0.01–5.8	0	1.8% ($n=8$)
Sol–gel/GOx/CNT/chitosan/PtNP/GC [36]	2.08	0.001–6.0	0.1	–

device can be successfully used to measure glucose concentrations in presence of possible interfering substances.

In the light of the results obtained concerning the performance of both TEOS/GOx/C and TEOS/AuNPs/GOx/C biosensors (summarized in Table 1), it is clear that addition of gold nanoparticles to the hybrid composite material improves the analytical properties of the resulting biosensor, particularly sensitivity, linear range concentration and accuracy. This improvement is a consequence of the advantages obtained through the combined use of gold nanoparticles and a biocompatible organic–inorganic hybrid material with a porous and open framework.

4. Conclusions

In this paper, we develop a novel organic–inorganic hybrid composite material for glucose electrochemical sensing based on integration of a biosensig element (GOx), nanomaterials (AuNPs) and graphite powder (C) into a sol–gel matrix. This composite material combines the advantages induced from both the silica matrix, which enables the incorporation of the other elements while keeping the enzymatic activity of the assembly, and the presence of nanostructures, which enhances the electroactive area. As a consequence, the resulting biosensor TEOS/AuNPs/GOx/C exhibits a wider linear range concentration, higher sensitivity and higher accuracy, when compared with a similar composite containing GOx but free of AuNPs (TEOS/GOx/C). Taking into account the good performance of the resulting biosensor, this approach is a promising route for designing a wide range of biosensors.

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