Available online at www.sciencedirect.com
ScienceDirect
Talanta 75 (2008) 1151–1157
Talanta
www.elsevier.com/locate/talanta

Encapsulation of glucose oxidase within poly(ethylene glycol) methyl ether methacrylate microparticles for developing an amperometric glucose biosensor

J.P. Hervás Pérez [a], E. López-Cabarcos [b], B. López-Ruiz [a,*]

[a] *Sección Departamental de Química Analítica, Facultad de Farmacia, Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain*
[b] *Departamento de Físico-Química Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*

Received 18 September 2007; received in revised form 21 December 2007; accepted 7 January 2008
Available online 21 January 2008

Abstract

Poly(ethylene glycol) methyl ether methacrylate (PEGMEM) microparticles were synthesized and glucose oxidase (GOx) was immobilized within the microparticles. An amperometric biosensor was fabricated using the microparticles with GOx as biological component. The enzyme immobilization method was optimized by investigating the influence of monomer concentration and cross-linker content used in the preparation of the microparticles in the response of the biosensor. The best analytical results were obtained with the microparticles prepared with 0.21 M PEGMEM and 0.74% cross-linking. Furthermore, we have investigated the influence on the biosensor behaviour of parameters such as working potential, pH, temperature and enzymatic load. In addition, analytical properties such as sensitivity, linear range, response time and detection limit were determined. The biosensor was used to determine glucose in human serum samples and to avoid common interferents present in human serum such as uric and ascorbic acids. A Nafion layer was deposited on the electrode surface with satisfactory results. The useful lifetime of the biosensor was at least 520 days.

Keywords: Amperometric biosensor; PEGMEM; Glucose oxidase; Microparticles

1. Introduction

Glucose biosensors have attracted great interest because of the increasing incidence of diabetes in the population of developed countries [1,2]. The performance of a biosensor depends on the materials and fabrication techniques. The use of biocompatible materials will allow the monitorization *in vivo* of the different compounds such as glucose or cholesterol. Future progress and development in biosensor design will inevitably focus upon the technology of new materials that promise to solve the biocompatibility problems. Materials with hydrogel-like properties are generally biocompatibility due to their high water content [3] and can be used to immobilize enzymes such as GOx. The immobilization of enzymes with complete retention of its biological activity in matrices with good diffusion properties for substrates is a decisive factor for the development of biosensors. Various methods for enzyme immobilization have been reported, such as covalent binding [4,5], entrapment in a suitable matrix [6,7], adsorption onto insoluble materials [8], conjugation [9], ionic–covalent hybridization [10,11], etc.

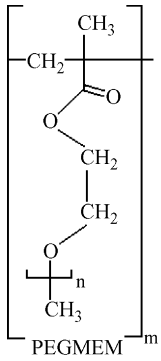
Besides, GOx (EC 1.1.3.4) is a very robust enzyme that is often used as model for testing and developing new immobilization systems such as polymer films, polymer gels, conducting polymers, and methacrylate-based polymers that have been assayed for developing glucose biosensors [12–17].

After the pioneer work in the 1960s [18], the use of methacrylate hydrogels in biomedical applications has made remarkable progress in the field of biosensors, drug delivery systems, contact lenses and synthetic membranes [7,11,19–23].

PEGMEM (see Scheme 1) has been copolymerized with different cross-linkers to form comb-like copolymers with hydrogel

* Corresponding author. Tel.: +34 91 394 1756; fax: +34 91 394 1754.
E-mail address: bealopru@farm.ucm.es (B. López-Ruiz).

0039-9140/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2008.01.030



Scheme 1. Structure of PEGMEM.

properties because it is a non-cytotoxic, monofunctional and biocompatible polymer [24–26].

In this work we have prepared biocompatible microparticles of *p*-PEGMEM and we have used them as a new system of immobilization of GOx for application as biological component of an amperometric glucose sensor. The biosensor was optimized by studying the influence on the biosensor response of parameters used in microgel synthesis such as monomer concentration and cross-linking content as well as working conditions such as working potential, pH of the medium, temperature, and enzymatic load.

2. Experimental

2.1. Chemicals

PEGMEM, GOx (425 IU/mL) from *Aspergillus niger*, D+glucose, ascorbic acid, uric acid and Nafion 5 wt.% were purchased from Sigma (St. Louis, MO, USA). *N*,*N*′-Methylenebisacrylamide (BIS) from Aldrich (St. Louis, MO, USA), ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED) and the surfactant Span 80 from Fluka (Buchs, Switzerland). Acetate/phosphate buffer solutions were prepared from stock solutions of sodium dihydrogen phosphate and sodium acetate (Panreac). The dialysis membrane (12,000–14,000 MWCO) was purchased from Spectrum Medical Industries. All reagents were used as received and the water was Milli Q quality (Millipore, Milford, MA, USA).

2.2. Apparatus and measurements

The microgel particles were examined using scanning electron microscopy (SEM) in a JEOL JSM-6400 microscope operating at an acceleration voltage of 20 kV and 5000 magnification. The grid with the microparticles was dried, and replicas were produced by shadowing with gold deposited with a Balzers Sputter Coater (SCD-004). Particle size measurements in the range 2–150 μm were performed with a Galai-Cis-1 particle analyzer system. The instrument consists of a laser-based analyzer which evaluates the particle diameter from the time it takes to cross a laser beam and a video-based analyser shows the shape of the microparticle. Amperometric measurements at constant potential were carried out at a Metrohm Polarecord potentiostat, Model E-506. The pH of the buffer solution was adjusted using a Metler Toledo MP-230 pH-meter. Electrochemical measurements were performed in 0.05 M acetate/0.05 M phosphate buffer, in a three-electrode cell with a platinum electrode as working electrode, a SCE reference electrode and a platinum counter electrode.

2.3. Emulsion preparation

p-PEGMEM microgels with varying amounts of BIS were prepared using the concentrated emulsion polymerization method [27]. The immobilization of GOx was carried out by adding the enzyme (425 IU/mL) in the aqueous phase of the concentrated emulsion. The amount of cross-linking $\eta\,(\%) = n_{BIS}/(n_{BIS} + n_{monomer})$ where n_{BIS} and $n_{monomer}$ are the number of moles of BIS and monomer, respectively was varied between 0.34% and 5.82%. Since the time of synthesis is about 1.5 h, the temperature was controlled and kept below 25 °C to preserve the enzyme properties.

2.4. Overall reaction of the glucose biosensor

The electrode surface (diameter 3 mm) was polished with 0.05 μm alumina slurry paste. After polishing, any residual abrasive particles were removed ultrasonically in ethanol and subsequently in distilled water. An exactly weighed amount of microgel particles was placed on the electrode surface and fixed with a dialysis membrane. The resulting electrode was washed with phosphate buffer and overoxidized at +0.6 V versus SCE until the background current decreased to a constant level.

The biosensor response is based on an indirect measurement that correlates the amount of glucose with the concentration of hydrogen peroxide. Because the redox centres in GOx are insulated within the enzyme molecule, direct electron transport to the surface of the electrode does not occur to any measurable degree. GOx is first reduced by the substrate glucose and then reoxidized by oxygen. Oxygen behaves as electron acceptor for reduced GOx leading to the formation of hydrogen peroxide that is oxidized at the electrode and results in the current that is detected by amperometry.

3. Results and discussion

3.1. Characterization of the microparticles

Fig. 1 shows a micrograph of *p*-PEGMEM microparticles with entrapped GOx prepared with $\eta = 0.74\%$. The microgels are spherical and polydisperse.

As Fig. 2 shows, the concentrated emulsion polymerization method produces microparticles with entrapped GOx whose diameters lie between 2 and 12 μm. The average size is 5.2 μm very close to the average size 5 μm of the microparticles without GOx and cross-linking $\eta = 0.74\%$. As Fig. 2 illustrates, 50% of the microparticles present a size comprised between 2 and 4 μm.

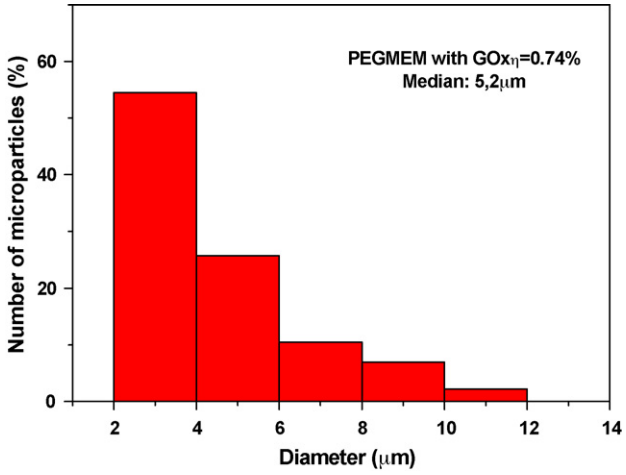


Fig. 1. SEM micrograph of freeze-dried *p*-PEGMEM microparticles with entrapped GOx.

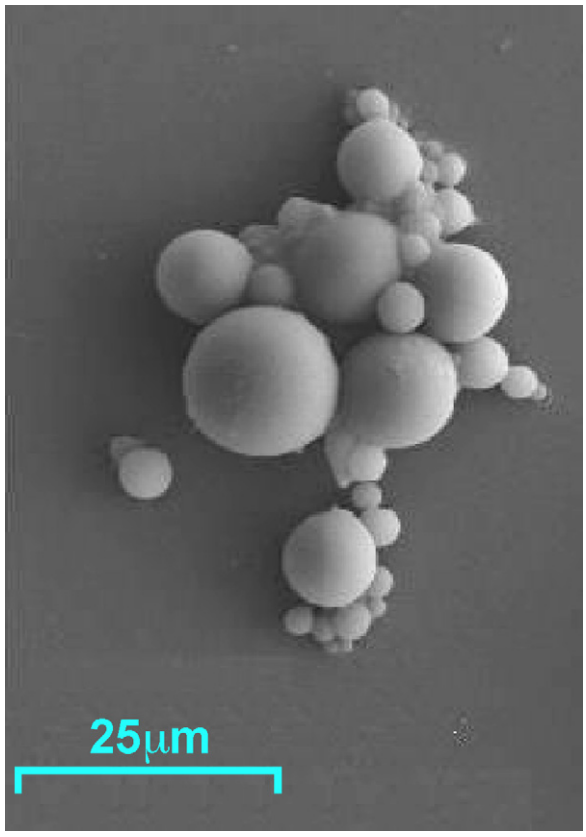


Fig. 2. Size distribution of the *p*-PEGMEM microparticles with entrapped GOx, in phosphate buffer pH 6.5 and 25 °C.

3.2. Optimization of the synthesis method

3.2.1. Monomer concentration

The steady-state response curves, obtained at the platinum electrode with *p*-PEGMEM microparticles synthesized at pH 6.5, $\eta = 0.74\%$, containing 425 IU/mL of GOx and different proportions of monomer in the aqueous phase (0.10, 0.21, 0.83 and 1.17 M) are illustrated in Fig. 3a. The maximum current response was found when the monomer concentration was 0.21 M. A further increase in the monomer concentration decreases the current response and we attribute this behaviour to a higher diffusional barrier that hinders the motion of the substrate towards the enzyme catalytic site as well as the diffusion of the product of the enzymatic reaction towards the electrode. Decreasing the monomer concentration at 0.10 M also results in a decrease of the response, due to the loss of immobilized enzyme when small amount of monomer is used. The enzymatic activity observed in the supernatant liquid obtained after the synthesis of the microparticles when the monomer concentration is 0.10 M seems to support this interpretation.

3.2.2. Effect of the cross-linking contents

In order to find the optimum pore size of the polymer matrix, biosensors based on microparticles with different amount of cross-linking (η) were prepared. Fig. 3b shows the calibration curves of the biosensor response versus glucose concentration for microparticles with cross-linking between 0.34% and 5.82%. The monomer concentration selected to obtain these curves was 0.21 M, and the amount of GOx 425 IU/mL.

The optimum response occurs in microgels with $\eta = 0.74\%$. Microparticles prepared with lower fractions of cross-linking present a pore size too large to efficiently retain the enzyme as it was confirmed by the enzymatic activity measured in the supernatant. The decrease of the response when the cross-linking fraction is higher than 0.74% is attributed to a higher substrate diffusional barrier imposed by the cross-linking. Accordingly

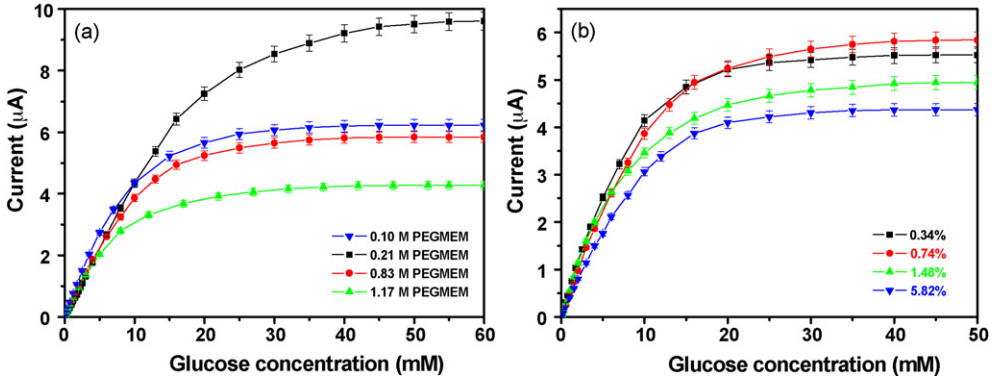


Fig. 3. Response of the glucose biosensor to different monomer concentrations (a) and to different cross-linking (b), by calibration plot for glucose in stirred 0.1 M phosphate buffer, pH 6.5, potential of +0.6 V vs. SCE and 25 °C.

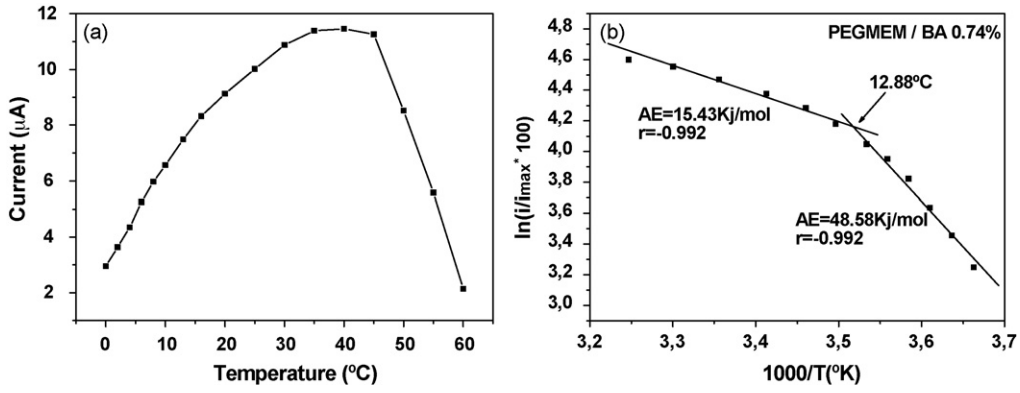


Fig. 4. Effect of working temperature (a) and Arrhenius plot (b) for GOx immobilized in *p*-PEGMEM $\eta = 0.74\%$, on the biosensor response to 30 mM of glucose. Experimental conditions: 0.1 M phosphate buffer, pH 6.5 and +0.6 V vs. SCE.

microparticles with cross-linking 0.74% and monomer concentration 0.21 M were selected for subsequent measurements performed in this work.

3.3. Optimization of the biosensor response

3.3.1. Working potential

The dependency of the biosensor response (steady-state current at the electrode) on the applied potential was investigated in 0.1 M phosphate buffer solution, pH 6.5 containing 0.25 mM glucose. The response of the enzyme electrode increased with the applied potential up to a value of +0.60 V versus SCE and levels off after this value. The operating potential of +0.60 V versus SCE was used in all subsequent measurements.

3.3.2. Temperature

The effect of temperature on the biosensor response at 30 mM glucose was studied in the interval 0–60 °C performing all measurements under oxygen saturation condition and keeping the pH constant at 6.5.

As it can be seen in Fig. 4a, the optimum temperature for enzymatic activity is between 35 and 45 °C. The temperature of maximum activity of the free enzyme is 30 °C [28]. It seems that the immobilizing support slightly protects the enzyme. Although we have checked that the highest enzyme activity takes place between 35 and 45 °C, we wanted to verify that to these temperatures the enzyme was staying stable. To probe this, the kinetics of degradation to different temperatures that suffers the biosensors with 30 mM of glucose was studied, keeping constant the temperature for 1 h, verifying the intensity on time 0 and on 60 min (Table 1). When the temperature was overcoming 30 °C, a decrease of the activity with time was observed, due to the enzyme denaturation.

The temperature of 30 °C was chosen as working temperature to all further experiments.

As Fig. 4b also illustrates, the Arrhenius plot for GOx immobilized in *p*-PEGMEM microparticles with $\eta = 0.74\%$ presents two regions. The activation energies were calculated from the slopes and were found to be 15.43 and 48.58 kJ/mol, respectively. The activation energy observed in the low temperature region is significantly higher than the one reported for the free enzyme, which was found to be 14.6 kJ/mol [29]. The activation energies values reported by Rubio Retama et al. [30] when GOx was immobilized in polyacrylamide were found to be 26.60 and 50.46 kJ/mol, quite similar than we observed.

As we reported in previous articles, the temperature at which the slope changes is related with the volume phase transition of the microgel around the enzyme [30–32]. At a temperature higher than the temperature of the volume phase transition, the microgel is swollen, the enzyme is surrounded by water and its activation energy comes closer to that of the enzyme in solution. However, in the shrunk phase the amount of water retained inside the hydrogel decreases, hindering access of the substrate to the enzyme and the enzyme–substrate union so the activation energy is increased. These results, as the ones obtained in previous works [30–32], seem to indicate that the changes in the Arrhenius plots are not due to changes in enzyme, but to the medium around it.

3.3.3. pH

The influence of pH on the glucose biosensor response to 30 mM glucose was checked in 0.05 M acetate/0.05 M phosphate

Table 1
Kinetics of degradation of enzyme entrapped in microparticles with 0.74% cross-linking

Temperature (°C)	Current, $t = 0$ s (nA)	Current, $t = 60$ s (nA)	Percentage of degradation
25	120	120	0.00
30	145	145	0.00
35	175	150	14.29
40	205	165	19.51

Experimental conditions: 0.1 M phosphate buffer, pH 6.5 and +0.6 V vs. SCE.

buffer solutions from pH 4.0 to 8.0 at 25 °C. The optimal pH was found to be 6.5 coincident with the optimum pH reported to the free enzyme, which indicates that the catalytic function of the GOx does not seem to be affected by the immobilization process proposed in this work.

3.3.4. Loading

The glucose levels of diabetic patients are higher than 5 mM, therefore, we have extended the calibration range of the biosensor studying the effect of enzymatic load on the biosensor response. Based on previous works [31] we have chosen an enzymatic load of 425 IU/mL and we tested two methods of modifying the amount of enzyme in the surface of the electrode: varying the amount of microparticles placed on electrode surface, and changing the amount of enzyme immobilized within the microgel.

The linear range reaches to 8.0×10^{-3} M when the amount of GOx in aqueous phase goes from 255 to 765 IU/mL and is slightly increased when the microparticles placed on the electrode surface from 1 up to 3 mg. In both cases the sensitivity is increased when the amount of GOx in aqueous phase goes from 255 to 765 IU/mL and when the microparticles placed on the electrode surface from 1 up to 3 mg. In base of the above results we have selected for later experiments 3 mg of microparticles loaded with 765 IU/mL GOx. Table 2 shows the analytical properties of the biosensor prepared with *p*-PEGMEM microgels as a function of the amount of microparticles placed on electrode surface and the enzyme immobilized into the microgel.

Under all the optimal experimental conditions (0.74% cross-linking, potential +0.6 V, pH 6.5 and 30 °C), we realized a calibration curve in which the glucose biosensor presented a sensitivity of 17.78 mA M^{-1} cm^{-2}, a maximum current density of 148.57 μA cm^{-2}, a linear range between 2.0×10^{-6} and 7.6×10^{-3} M and a detection limit of 1 μM. When comparing the results obtained with the proposed biosensors and that previously reported using a similar immobilization system (polymeric microparticles), an improvement is observed in the analytical properties as sensitivity, that was found to be 17.78 mA M^{-1} cm^{-2}, and the maximum current density which increases from 114.3 to 148.57 μA cm^{-2} [30,31]. Also when this device was compared with others using different immobilization system as layer-by-layer self-assembly films or deposition on the electrode surface [33–36], it appears that the stability, the detection limit and the linear range improved significantly. The detection limit decreased up to 10 times versus the reported devices, the linear range increased at least one order of magnitude and the stability changed from 30 and 150 days in the best of the case (microparticles) to 520 days observed with this biosensor.

3.4. Interference study

It is required that the enzymatic electrode operates at a potential of +0.6 V versus SCE for producing the oxidation of hydrogen peroxide. At this high potential, the H_2O_2 detection may be disturbed by the presence of interfering species such as uric acid (UA) and ascorbic acid (AA) when physiological samples are analyzed because these acids present a negative charge at the working pH (6.0–7.0). The interferences caused by electroactive molecules were reduced by the use of Nafion layer over the electrode surface. Nafion is a negatively charged polyelectrolyte whose effect on the negatively charged substrates is noticeable.

A layer of Nafion (50 μL) was applied on the electrode surface. The layer was dried in air for 15 min and, the electrode with Nafion film was kept at 80 °C for 45 min. Then microparticles of *p*-PEGMEM were placed over the Nafion film and subsequently covered with the dialysis membrane. For biosensor prepared without Nafion (GOx-biosensor), the interfering signals of 0.25 mM uric acid and ascorbic acid are significant (Fig. 5). On the contrary, when the GOx-Nafion-biosensor was used, the signals arising from interferents disappeared. However, current due to 0.25 mM of glucose was near constant, varying from 150 nm in the biosensor without Nafion to 145 nm in biosensor with Nafion.

3.5. Human serum sample analysis

The glucose concentration of five serum pools were analyzed by the GOx-biosensor and GOx-Nafion-biosensor. The glucose of these serums is analyzed also by the spectrophotometric method of hexokinase described by Young [37], which were used as reference method.

The result showed a good correlation with the reference methods, especially in the case of the biosensor prepared with Nafion. Measurements of glucose in serum samples

Table 2
Analytical properties of the *p*-PEGMEM biosensors as a function of the quantity of microparticles placed on electrode surface and the enzyme immobilized into the microparticles

Enzyme load		J_{max} (μA cm^{-2})	Sensitivity (mA M^{-1} cm^{-2})	Linear range (M)	R^2 (n)[a]
Quantity of microparticles in the biosensors (mg)	Quantity of enzyme in the microparticles (IU/mL)				
1	425	62.58	4.09	1.0×10^{-5} to 1.0×10^{-2}	0.9949 (15)
2	425	77.71	6.63	1.1×10^{-5} to 4.0×10^{-3}	0.9944 (12)
3	425	83.49	6.78	1.0×10^{-5} to 4.0×10^{-3}	0.9963 (12)
3	255	54.36	6.13	1.0×10^{-5} to 3.0×10^{-3}	0.9962 (11)
3	765	148.57	17.78	2.0×10^{-6} to 7.6×10^{-3}	0.9985 (15)

[a] Points of linear range in calibration curve.

Table 3
Quantitative results obtained from the analysis of the serum by the spectrophotometric method (reference), GOx-biosensor and GOx-Nafion-biosensor

Sample	Glucose (mg/dL) reference method	Glucose[a] (mg/dL) GOx-biosensor	R.S.D. (%)	GOx-biosensor deviations[b] (%)	Glucose[a] (mg/dL) GOx-Nafion-biosensor	R.S.D. (%)	GOx-Nafion-biosensor deviations[b] (%)
1	81	83 ± 1	1.7	+2.0	81 ± 1	1.3	−0.1
2	89	93 ± 2	2.3	+4.3	89 ± 2	2.2	−0.2
3	176	179 ± 1	1.2	+1.5	174 ± 1	1.9	−1.1
4	116	117 ± 1	1.2	+1.0	115 ± 1	1.8	−0.8
5	89	93 ± 1	2.0	+4.5	88 ± 1	1.3	−1.3

[a] Average of the three measurements.
[b] Deviation between the reference method and the biosensor results.

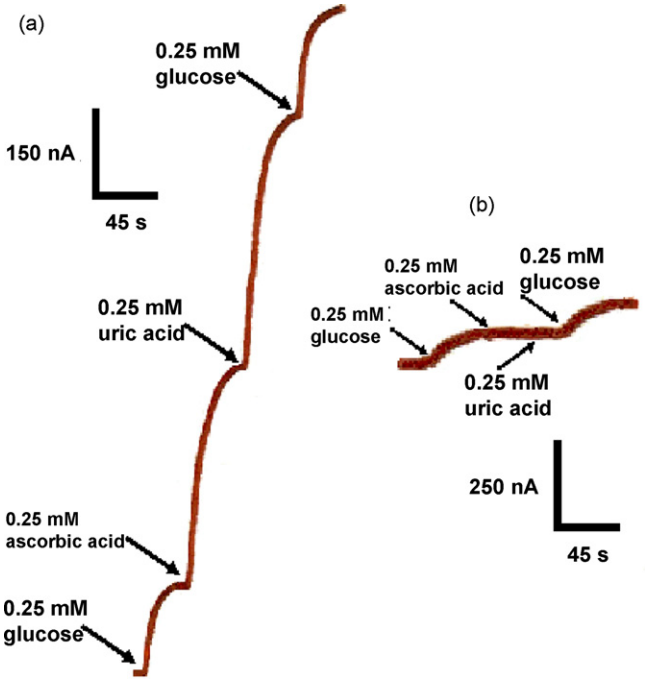


Fig. 5. GOx-biosensor response to 0.25 mM glucose, ascorbic and uric acids (a). GOx-Nafion-biosensor response to 0.25 mM glucose, ascorbic and uric acids (b).

obtained with GOx-Nafion-biosensor were similar with to those of the reference method, and differences between them never overcame 1.3% and with good precision (R.S.D. < 2.3%) (Table 3). The GOx-biosensor gave values 1.0–4.5% higher than those obtained by the reference methods that confirm that the serum samples have interferences. The GOx-Nafion-biosensor gave values −0.1/−1.3% smaller than those obtained by the reference methods, possibly because the layer of Nafion prevents the step of the whole glucose. The recoveries obtained

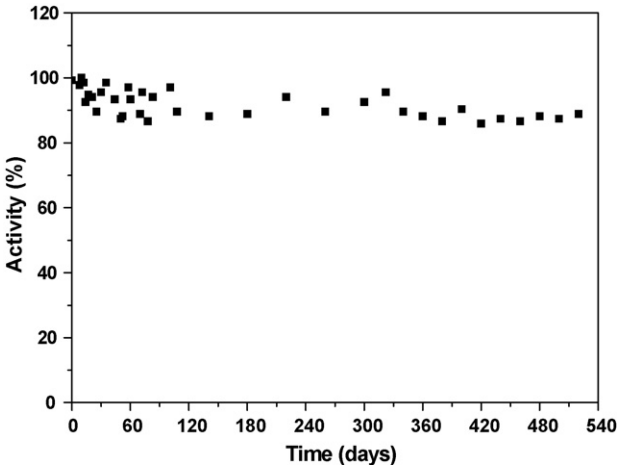


Fig. 6. Storage stability profiles for *p*-PEGMEM biosensor. The biosensor was stored frozen in phosphate buffer at −4 °C when not in use.

with both biosensors are between about 94.8 and 101.5% (Table 4).

3.6. Stability

To characterize the electrode stability, they were stored in a frozen phosphate buffer solution pH 6.0 and, periodically, the response of the biosensor to 0.25 mM glucose solution, at potential +0.6 V versus SCE, 30 °C was measured. We have found that biosensor exhibits an 85% of the initial signal after 520 days. This study is still on course (Fig. 6).

3.7. Analytical properties

The repeatability and reproducibility of glucose biosensors based on *p*-PEGMEM microparticles were evaluated in buffer

Table 4
Recovery studies using both glucose oxidase biosensors[a]

Sample	Glucose added (mg/dL)	GOx-biosensor		GOx-Nafion-biosensor	
		Glucose found[a] (mg/dL)	Recovery (%)	Glucose found[a] (mg/dL)	Recovery (%)
1	70	70 ± 1	100.2	71 ± 2	101.2
2	70	70 ± 1	100.1	71 ± 2	101.5
3	70	70 ± 1	97.5	70 ± 2	100.0
4	70	70 ± 1	94.8	70 ± 2	99.6
5	70	70 ± 1	99.3	70 ± 1	100.0

[a] Average of the three measurements.

solution. For the repeatability, 10 successive measurements of 0.25 mM glucose solution were performed. The relative standard deviation (R.S.D.) was 5.7%. The reproducibility of the analytical response obtained for three different electrodes when 5 mM of glucose was added (three measurements with each biosensor), obtained a R.S.D. of 0.88%.

4. Conclusions

Microparticles based on *p*-PEGMEM are presented as interesting matrix for GOx immobilization. We have demonstrated its application as enzyme immobilization support for an amperometric glucose biosensor. The optimal conditions of the synthesis medium were initiator's concentration of 10.96 mM, monomer concentration of 208.25 mM and 0.738% cross-linking content. Furthermore, the response of the biosensor was optimized for glucose detection. The optimal conditions of the biosensor response were: pH 6.5, 30 °C, 3 mg and 765 IU/mL. For eliminating the interferences caused by species with negative net charge, we have utilized a Nafion layer covering the electrode surface. The biosensor has been successfully applied to the determination of glucose in serum samples. The biosensor maintains 85% of the initial response 15 months later of the first use.

Acknowledgements

The authors acknowledge financial support from the Spanish Science and Education Ministry (MAT2006-13646-C03-01) from the CAM-UCM Program for "Consolidation of Research Groups" no. 950247 and no. 911033. We also thank M.E. Gil-Alegre (Department of Pharmacy and Pharmaceutical Technology, UCM) and A. Rodriguez (Electron Microscopy Centre, UCM) for valuable technical and professional assistance.

References

[1] B.D. Malhotra, A. Chaubey, Sens. Actuators B 91 (2003) 117.
[2] S. Chinnayelka, M.J. McShane, Biomacromolecules 5 (2004) 1657.
[3] V. Kudela, Encyclopaedia of Polymer Science and Engineering, vol. 7, Wiley, New York, 1987, p.783.
[4] L. Doretti, D. Ferrara, S. Lora, G. Palma, Biotechnol. Appl. Biochem. 29 (1999) 67.
[5] S.H. Kim, S. Kim, V.K. Yadavalli, M.V. Pishko, Anal. Chem. 77 (2005) 6828.
[6] S. Brahim, D. Narinesingh, A. Guiseppi-Elie, Anal. Chim. Acta 448 (2001) 27.
[7] S. Brahim, D. Narinesingh, A. Guiseppi-Elie, Biosens. Bioelectron. 17 (2002) 53.
[8] G. Bayramoğlu, E. Yalçin, M.Y. Arica, Process Biochem. 40 (2005) 3505.
[9] T. Konno, J. Watanabe, K. Ishihara, Biomacromolecules 5 (2004) 342.
[10] F.N. Kok, F. Bozuglu, V. Hasirci, Biosens. Bioelectron. 17 (2002) 531.
[11] F.N. Kok, V. Hasirci, Biosens. Bioelectron. 19 (2004) 661.
[12] M. Portaccio, M. El-Masry, N.R. Diano, A. De Maio, V. Grano, M. Lepore, P. Travascio, U. Bencivenga, N. Pagliuca, D.G. Mita, J. Mol. Catal. B: Enzyme 18 (2002) 49.
[13] L. Cen, K.G. Neoh, E.T. Kang, Biosens. Bioelectron. 18 (2003) 363.
[14] X. Liu, K.G. Neoh, L. Cen, E.T. Kang, Biosens. Bioelectron. 19 (2004) 823.
[15] H. Suzuki, A. Kumagai, K. Ogawa, E. Kokufuta, Biomacromolecules 5 (2004) 486.
[16] L.S. Bean, L.Y. Heng, B.M. Yamin, M. Ahmad, Thin Solid Films 477 (2005) 104.
[17] L.S. Bean, L.Y. Heng, B.M. Yamin, M. Ahmad, Bioelectrochemistry 65 (2005) 157.
[18] O. Wichterle, D. Lim, Hydrophilic gels for biologic use, in: Nature, St. Martin's Press Inc., NY, 1960, p. 117.
[19] Y. Yang, S. Mu, H. Chen, Synth. Metals 92 (1998) 173.
[20] P. Van der Wetering, E.E. Moret, N.M.E. Schuuemans-Nieuwenbrock, M.J. Van Steenbergen, W.E. Hennik, Bioconjug. Chem. 10 (1999) 589.
[21] A. Blandino, M. Macias, D. Cantero, Process Biochem. 36 (2001) 601.
[22] E.J. Calvo, R. Etchenique, L. Pietrasanta, A. Wolosiuk, C. Danilowicz, Anal. Chem. 73 (2001) 1161.
[23] C.A. Marquette, L.J. Blum, Sens. Actuators B 90 (2003) 112.
[24] M.A. Janney, O.O. Omatete, S.D. Walls, R.J. Nunn, R.J. Ogle, G. Westmoreland, J. Am. Ceram. Soc. 17 (1998) 581.
[25] M. Potoczek, E. Zawadzak, Ceram. Int. 30 (2004) 793.
[26] S.K. Mallapragada, B.C. Anderson, P.D. Bloom, V.V. Sheares-Ashby, US Patent 6,998,456 (2006) US 2002-357499P.
[27] B.J. Rubio Retama, B. López-Ruiz, E.J. López-Cabarcos, Biomaterials 24 (2003) 2965.
[28] L. Ying, E.T. Kang, K.G. Neoh, J. Membr. Sci. 208 (2002) 361.
[29] S. Cosnier, S. Szunerits, R.S. Marks, A. Novoa, L. Puech, E. Perez, I. Rico-Lattes, Talanta 55 (2001) 889–897.
[30] B.J. Rubio Retama, E.J. López-Cabarcos, B. López-Ruiz, Talanta 68 (2005) 99.
[31] M. Sánchez-Paniagua López, D. Mecerreyes, E. López-Cabarcos, B. López-Ruiz, Biosens. Bioelectron. 21 (2006) 2320.
[32] J.P. Hervás Pérez, M. Sánchez-Paniagua López, E. López-Cabarcos, B. López-Ruiz, Biosens. Bioelectron. 22 (2006) 429.
[33] S.A. Miscordia, J. Desbrieres, G.D. Barrera, P. Labbé, G.A. Rivas, Anal. Chim. Acta 578 (2006) 137.
[34] X. Chu, D. Duan, G. Shen, R. Yu, Talanta 71 (2007) 2040.
[35] B.Y. Wu, S.H. Hou, F. Yin, Zi.X. Zhao, Y.Y. Wang, X.S. Wang, Q. Chen, Biosens. Bioelectron. 22 (2007) 2854.
[36] L. Wu, X. Zhang, H. Ju, Biosens. Bioelectron. 23 (2007) 479.
[37] D.S. Young, Effects of Drug on Clinical Laboratory Test, third edition, AACC Press, Washington, 1990.