GLUCOSA SENSORS BASED ON ENZYME ENTRAPMENT IN POLYACRYLMIDE MICROGELS.

Enrique López-Cabarcos, Jorge Rubio Retama, D.Mecerreyes[#], Beatriz López-Ruiz*
Departamento de Química-Física Farmaceútica. Facultad de Farmacia. Universidad Complutense de Madrid, 28040 Madrid, Spain
[#] CIDETEC, Parque Tecnológico Miramon, Paseo Mikeletegui, 61-1°, 20009 San Sebastián, Spain
*Departamento de Química Analítica. Facultad de Farmacia. Universidad Complutense de Madrid, 28040 Madrid, Spain.

Abstract.

Glucose oxidase has been entrapped in polyacrylamide microgels synthesized using the emulsion concentrated polymerisation pathway. This study shows that microgels provide an excellent matrix for glucose oxidase immobilization and they can be used as biological material in amperometric biosensors. To improve the biosensor response and to get rid off interferences, acrylic acid was added to the polymer matrix with the aim to charge negatively its surface. On other hand, the inclusion of polypirrole inside the polymer matrix let to a significative improvement in the reading potential and response time of the microgels.

Introduction.

The development of glucose sensors is an active research area due to the importance of glucose control in the treatment of diabetes. The most commonly used amperometric glucose sensors are based on the recognition of glucose by the enzyme glucose oxidase (GOx). Immobilization of the enzyme on the electrode surface is a key factor in fabricating a biosensor and various immobilization methods have been employed in the preparation of the amperometric enzyme electrodes. One method consists in encapsulation within polymers. In particular, GOx has been successfully immobilized on electropolymers (1-4), hydrogel polymers (5-7) and polymer latex (8). Microgel particles based on crosslinked polymers form an interesting group of hydrogels since they share properties with macrogels showing at the same time typical features of colloidal systems. Furthermore, reducing the gel size to mesoscopic dimensions decreases the time response by orders of magnitude which is very important when dealing with biosensors. In the present work, GOx is encapsulated in polyacrylamide microgels with the aim to preserve the enzymatic activity and to fabricate a biosensor (9-10) with these microparticles. The biosensor presents great stability, but when it is used to analysed clinical samples, interferences due to ascorbic and uric acid were observed. To remove this interferences, microgels with 50% of acrylic acid (which provides negative charge) were synthesized. The addition of small amount of the conducting polymer polypyrrole to the microgels reduces the reading potential and the response time of the biosensor. In this communication we characterized the microgels and we study the performance of the microgels as glucose sensors.

Enzyme immobilization.

The microgels were prepared using a concentrated emulsion polymerisation method. Two polymers were used, the first one contains acrylamide as monomer and bisacrylamide as cross-linker agent, in the second one, part of acrylamide has been changed by acrylic acid. The encapsulation of GOx was carried out solubilizing the enzyme in phosphate buffer pH 7.2 which constitutes the aqueous dispersed phase. W/O concentrated emulsions were produced by dropwise addition of the dispersed phase to the continuous oil phase (Dodecane plus Span 80). As is illustrated in Fig.1, redox polymerisation of the gel like emulsion produced entrapped enzyme microgels. Polyacrylamide microgels with GOx (see Fig1a) are named PMP1, poly(acrylamide-acrylic acid) microgels with GOx are named PMP2 (see Fig.1b) and polyacrylamide-polypyrrole microgels with GOx are named PMP3 (Fig.1c).

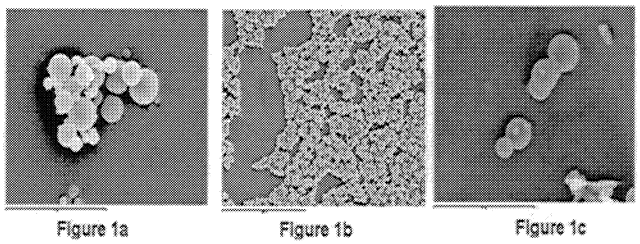

Figure 1. Scanning electron micrograph of microgels: a) PMP1, cross-linking (=3,2%), after one week of their synthesis, (b) PMP2, after 1 months of their synthesis, c) PMP3 microgels after two months of their synthesis.

Microstructure of the microgels.

We have recorded the X-ray diffraction patterns of microgels at room temperature. The diffractograms of samples with and without entrapped enzyme show slight differences. Fig.2 depicts the diffractograms of swelled microgels as a function of the number of cross-links (η = 0.021; 0.026; 0.035 and 0.039 mol/g). The diffraction patterns show two broad halos; one centred at around 2θ = 14degrees which appears only in crosslinked PAA samples and a more diffused one at 2θ = 21 degrees characteristic of polyacrylamide. The spacing of the peaks calculated using Bragg´s equation (1). is d_1=6.3± 0.1Å which roughly correspond to the length of the cross-linker bis(acrylamide) molecule and d_2=4.23± 0.05Å

$$2d \sin\theta = n\lambda \qquad (1)$$

Furthermore, one sees in Fig.2 that the position of the peaks is approximately constant as the cross-linking increases.

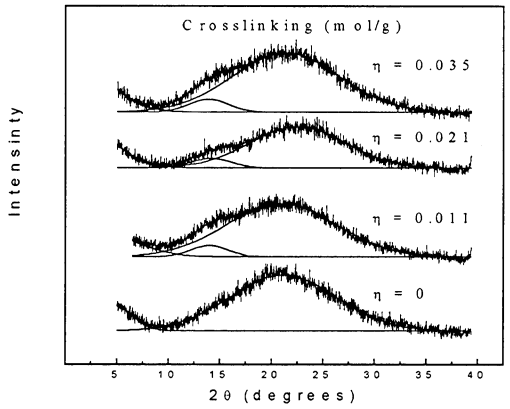

Figure 2 X-ray diffraction patterns of dried microgels with GOx as a function of the cross-linking.

GOx from *Aspergillus niger* crystallized in an hexagonal unit cell with lattice dimensions a = b = 67.54 Å and c = 215.38 Å. The absence of any crystalline peak in the diffraction patterns confirms that the packing of the enzyme is amorphous inside the microgels. A schematic representation of the gel particles with immobilized GOx, obtained from SEM, DSC and X-ray diffraction measurements, is presented in Fig.3. The outer layer of the microgel particle should be enriched in bis(acrylamide) aggregates because the polymerization is initiated in the droplets of the concentrated emulsion, employing ammonium persulfate radicals which diffuse from the continuous oil phase. The appearance of two maxima in the X-ray patterns of cross-linked microgels supports a two layer model (core and crust) for the microgel particle.

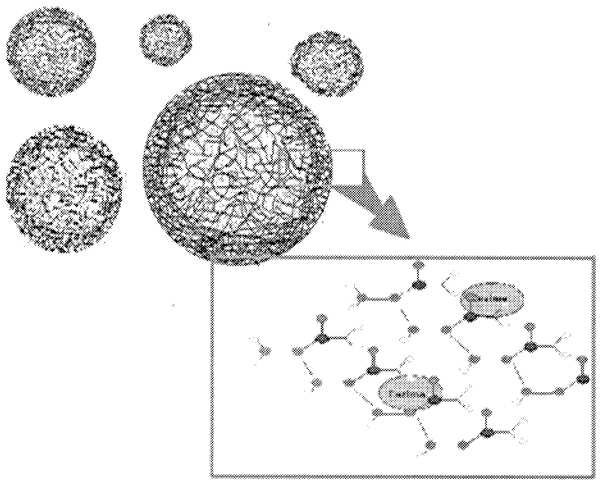

Figure 3. Schematic representation of microgels with immobilized GOx.

Electrode preparation.

An amount of microgel (3.0 mg) was placed on the surface of the platinum electrode. The microparticle layer was covered and flattened around the electrode surface using a dialysis membrane. The resulting electrode, see Fig. 4 was washed with phosphate buffer and overoxidized at + 0.6 V until the background current decreased to a constant level.

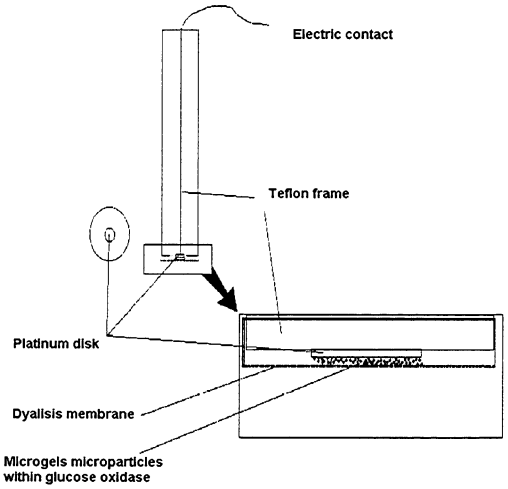

Figure 4.- Schematic view of the biosensor designed using the microgel with GOx as biological component.

GOx was entrapped inside microgels with different crosslinking content with the aim to select the microparticles with better performance as biosensor component. Fig.5 shows the biosensor response vs. glucose concentration at 35°C, for microgels with crosslinking ranging from η=0.7 to η=5.3.

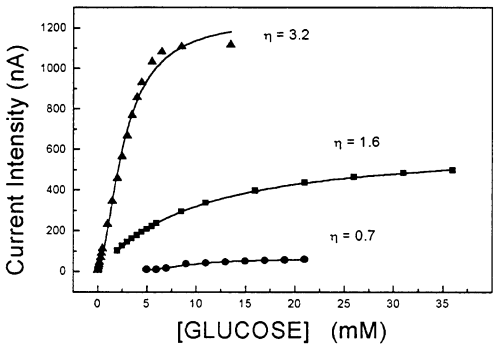

Figure 5.- Influence of crosslinking ratio on the response of the biosensor studied by calibration plot for glucose in stirred 0.1 M phosphate buffer. Potential +0.6 V vs. Ag/AgCl.

GOx is a homodimer glycoprotein with two identical subunits, each of which has a binding site for glucose, and cooperative interactions in this type of proteins are frequent. For cooperative interactions, the reaction velocity as a function of the substrate concentration can be described by the Hill equation.

$$V = \frac{V_{\max}[S]^n}{K_{app} + [S]^n} \qquad (2)$$

where [S] is the substrate concentration and the exponent, n, is referred to as the Hill coefficient. For the limiting case n=1 this is simple the Michaelis-Menten equation and K_{app} becomes K_M. The result of fitting the experimental data with equation 2 is showed in Fig. 5 (continuous lines) and the agreement is excellent.

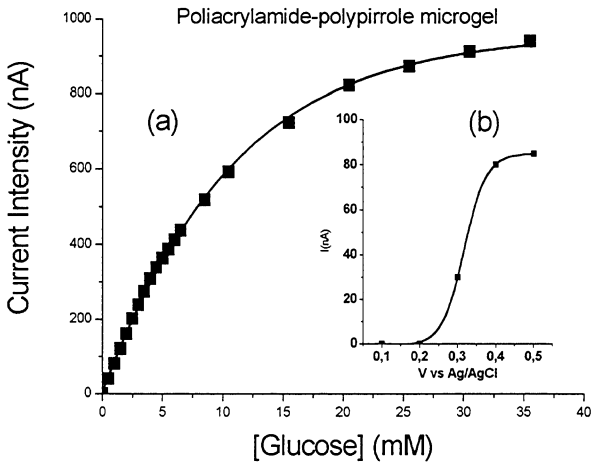

Figure 6. The Response of polyacrylamide-polypyrrole microgels is shown in fig. 6a and fig. 6b shows the response of polyacrylamide-polypirrole microgels at different reading potential.

At 35° C the response of the PMP1 and PMP2 biosensors is linear up to 5 mM glucose concentration. The incorporation of polypirrole inside the microgels allows to reduce the reading potential from 0.6V to 0.4V. Figure 6 shows the response of polyacrylamide-polypyrrole microgels by calibration plot for glucose. The response to glucose as function of different reading potential is also shown in the inset of figure 6. Because the added polypyrrole, the electrons released in the enzymatic reaction are transported directly from the microgel to the platinum electrode surface. This fact increases the velocity of the electrodic kinetics and concurrently decreases the response time from 40s to 14s.

The figure 7 shows a comparative response time between PMP1 and PMP3 microgels. The response time decreases from 40s in PMP1 to 14s in PMP3 microgels.

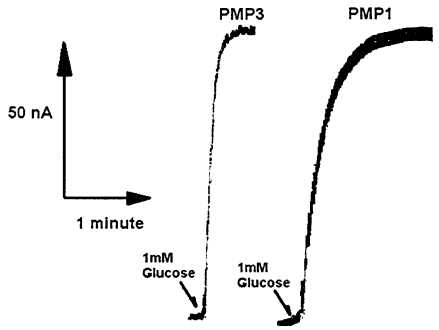

Figure 7. Response time of PMP3 and PMP1 microgels by addition of 1mM glucose stirred 0.1M phosphate buffer pH 6. The reading potential is +0.4V vs. Ag/AgCl when PMP3 microgels are used and +0.6 vs. Ag/AgCl when PMP1 microgels are used

Conclusions.

Microgel particles of polyacrylamide provide an excellent matrix for GOX immobilization when this biomaterial is part of a biosensor. With this immobilization method the enzymatic activity remains constant for at least one year. The biosensor prepared with PMP1, PMP2 and PM3 microgels can be used to measure glucose. The biosensor prepared with PMP2 microgel is very efficient to get rid off the interferences produced by ascorbic and uric acid, and can determine, with precision, glucose in complex samples like blood and serum. The stability of the biosensors is remarkable showing the same response the day of its preparation than four months later. Moreover, once the microgel with entrapped enzyme have been freeze-dried the enzymatic activity of the microgels remains unaltered for at least one year. The incorporation of some polypirrole inside de polyacrylamide microgels reduce the

reading potential from 0.6V to 0.4V and decrease the response time from 40s to 14s.

Acknowledgments.

The authors acknowledge financial support from DGI (BMF2000-0620 and PGC2000-2246-E) of the Spanish Science and Technology Ministry. We also thank A. Rodriguez (Electron Microscopy Center, UCM) and F. Conde (X-Ray Diffraction CAI, UCM) for valuable technical and professional assistance.

References

1. Cosnier, S., Biosens. Bioelectron. (1999) 14; 443-456
2. Adeloju, S. B. and Moline, A. N. Biosens. Bioelectron. (2001); 16, (3): 133-139.
3. Rhemrev-Boom, M. M.; Jonker, M. A.; Venema, K.; Jobst, G.; Tiessens, R., and Korf, J. Analyst (2001) 126, 1073-1079.
4. Kros A, Stephan W.F.M.van Hövell, Adv.Mater. (2001) 13 (20) 1555
5. Pishlo, M. V.; Michael, A. C., and Heller, A.. Anal. Chem. (1991) 63, 2268-2272.
6. Ohara, T. J.; Rajagopalan, R., and Heller, A.. Anal. Chem., (1994) 66, 2451-2457.
7. Calvo, E. J.; Etchenique, R.; Pietrasante, L.; Wolosiuk, A. , and Danilowicz, C. e. Anal. Chem. (2001) 73, 1161-1168.
8. Cosnier, Serge, Szunerits, Sabine, Marks, Robert S., Novoa, Andres, Puech, Laurence, Perez, Emile, and Rico-Lattes, I. Talanta 2001, 55, 889-897.
9. Zhang, G. Wright, Y. Yang (2000) Biosens. Bioelectron (2000), 15 , 273
10. Birch M E, Coury L A, Heineman W R , Anal. Chem.(1990) 62 1123