Glucose Sensors Based on Glucose Oxidase Immobilized in Polypyrrole-Polyacrylamide Microparticles

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ABSTRACT

We have synthesized polyacrylamide-polypyrrole (PPy/PA) microparticles which present conductivity in the range of the semiconductor materials (around 10^{-5} S/cm). Glucose Oxidase was immobilized within microparticles with composition 50/50 (w/w) PPy/PA. These microparticles were used as biological component of an amperometric biosensor which shows similar glucose response in both aerobic and anaerobic conditions.

Keywords: polypyrrole-polyacrylamide microparticles, immobilization of glucose oxidase, glucose biosensor

1 INTRODUCTION

Intrinsically conducting polymers have become of great scientific and technological importance because of their electrical, electronic, magnetic and optical properties. Great effort has been dedicated to improve their processibility and nowadays the fabrication of films and fibbers of controlled thickness has been achieved [1-4]. However, to our knowledge, conducting polypyrrole microparticles have not yet been synthesized probably because colloidal polymer particles are produced by polymerization in the internal phase of emulsions and pyrrole is slightly soluble in water. Therefore, after polymerization the polymer would precipitate making difficult the formation of conducting microparticles. The approach that we present in this paper involves the immobilization of soluble polypyrrole into microscopic polyacrylamide particles using the concentrated emulsion polimerisation method, which has been recently employed in our laboratory to encapsulate enzymes [5-6]. The main novelty of this method in comparison with conventional emulsion polymerisation lies in the large volume fraction of the dispersed phase, which in the concentrated emulsion is larger than 74% . The aims of this contribution are twofold: First, to synthesise polyacrylamide-polypyrrole conducting microparticles following the W/O concentrated emulsion polymerisation method and to study their conductivity as a function of the polypyrrole content. The second objective is to encapsulate GOx in these microparticles for its use as the biological component of a glucose sensor.

2 POLYPYRROLE-POLYACRYLAMIDE MICROPARTICLES WITH ENTRAPPED ENZYME

Soluble, doped polypyrrole has been synthesized following the procedure outlined in the literature [7]. The soluble polypyrrole was incorporated in the aqueous phase of a W/O concentrated emulsion. The content of polypyrrole in the concentrated emulsion was varied and micropaticles with 10/90, 15/85, 25/75, 30/70, 40/60 and 50/50 polypyrrole/acrylamide (w/w in the aqueous phase) were produced. Adding TEMED started the polymerization of the aqueous dispersed phase, during which temperature was controlled and kept below 35° C. After 1 h of reaction, the polymerization was complete and the microparticles were isolated by centrifugation (5000 rpm for 10 min at 10°C).

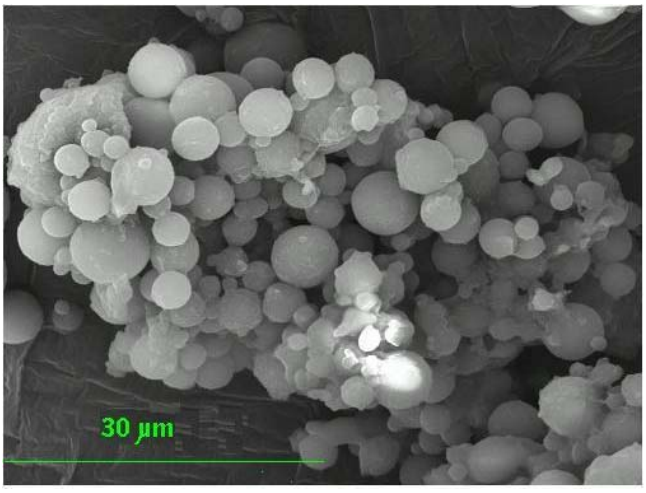


Figure 1. Electron micrograph of polypyrrole-polyacrylamide (50/50 w/w) microparticles with entrapped Glucose Oxidase (polyacrylamide crosslinking is 3.2%)

Glucose Oxidase was immobilized in the PPy/PA microparticles by incorporating the enzyme in the aqueous phase (a solution of polypyrrole, monomer and cross-linker in phosphate buffer at pH 6.0) of the concentrated emulsion before beginning the polymerisation. As it is shown in Fig.1 redox polymerization of the gel-like emulsion produced polyacrylamide microparticles that contain polypyrrole and GOx entrapped inside them.

The electrical conductivity of the microparticles was measured by the four-point probe method at room temperature using disks (2mm thickness) prepared in a press under the application of $2 \cdot 10^7$ Pa during 1 min. The conductivity increases with the amount of PPy entrapped, from 10^{-12} S/cm for pure polyacrylamide, up to 10^{-5} S/cm (semiconductivity range) for the 50/50 PPy/PA microparticles. The conductivity was also investigated by IR spectrometry looking for polypyrrole conducting bands in the 10/90 and 50/50 PPy/PA samples. As is illustrated in Fig. 2, the sample with 50% PPy shows typical bands of polypyrrole such as: 1) polaron at 646 cm^{-1}, 2) bipolaron at 978 cm^{-1}, 3) and 4) vibration modes of the ring at 1236 and 1478 cm^{-1}, 5) and 6) stretching vibration of the C=C at 1565 and 1619 cm^{-1}, while the peaks associated to PPy segments are scarcely seen in samples with 10% PPy content.

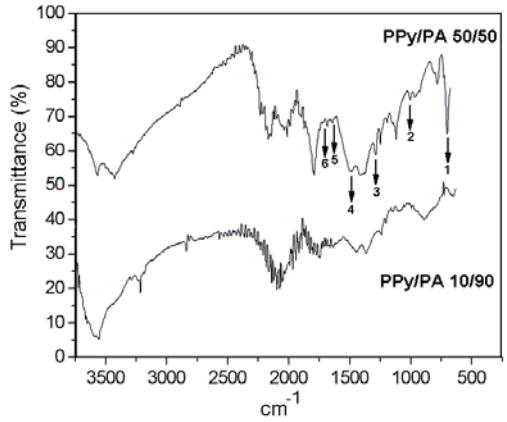


Figure 2. Infrared spectra of the 10/90 and 50/50 (% w/w) PPy/PA microparticles. The arrows indicate some IR typical bands of polypyrrole.

3 GLUCOSE BIOSENSOR

The microparticles with immobilized GOx were used as the biological component of an amperometric glucose sensor. To prepare the electrode, the 50/50 PPy/PA microparticles with GOx were selected because they showed the best conductivity properties. The enzyme electrode was prepared by depositing 3.0 mg of microparticles on the surface of a platinum electrode. The microparticles layer was covered and flattened around the platinum electrode surface using a dialysis membrane. The resulting electrode was subsequently placed in a three-electrode cell as working electrode. The electrode was then washed with phosphate buffer and a potential of +400mV *vs* (SCE) was applied to the sensor until the background current had decreased to a constant level. More details about the electrode preparation are given elsewhere [5-6].

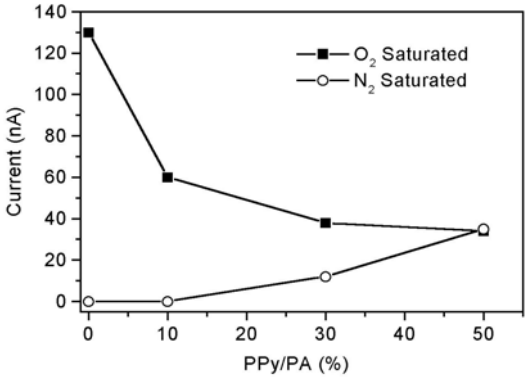
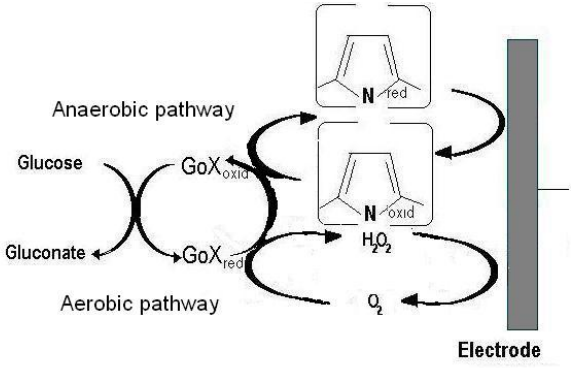


Figure 3. Biosensor current response to glucose as a function of the microparticle PPy content. The biosensor prepared with 50/50 PPy/PA microparticles gave the same response under both aerobic and anaerobic conditions.

The maximum current response to glucose of the 50/50 biosensor, measured in stirred solutions at 25ºC, under aerobic and anaerobic conditions is shown in Fig.3 as a function of the PPy content. To work in anaerobic conditions, oxygen was removed from the cell by purging with nitrogen during 20 min. The absence of oxygen was confirmed by using a biosensor prepared with PA microparticles with GOx but lacking pyrrole, which doesn't show response to glucose as is illustrated in Fig.3 (PPy/PA=0). In O_2 saturated solutions the biosensor current response decreased when the PPy content increased and the opposite bahaviour was observed in N_2 saturated solutions.



Scheme1. Proposed working mechanism of the 50/50 PPy/PA glucose biosensor when the electrode is polarized at 0.4V *vs* (SCE).

The 50/50 PPy/PA biosensor gave similar response in both cases (aerobic and anaerobic), which indicates that in anaerobic conditions electrons are transferred from the active site of the enzyme to the electrode *via* the polypyrrole, whereas in aerobic conditions the mediator is the O_2 molecule as in illustrated in the scheme 1.
The biosensor prepared with 50/50 PPy/PA microparticles shows a current intensity considerably smaller than that obtained when pure polyacrylamide with GOx microparticles were used in the biosensor. To improve the performance of the electrode, the cross-linking of polyacrylamide was reduced from 3.2% (selected as optimum in pure PA biosensors) to 2% with the result shown in Fig.4. As can be seen in Fig. 4 the reduction of cross-linking produced an increase of the current from 500nA up to 2000nA.

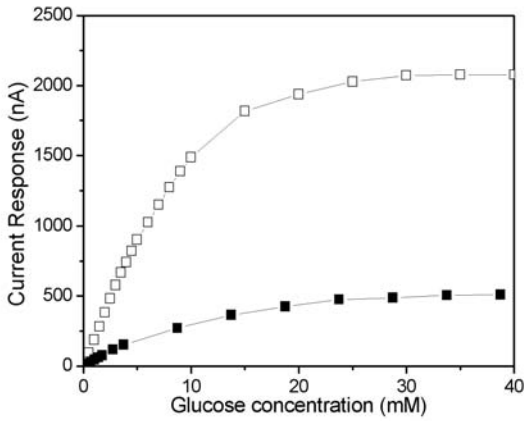


Figure 4 Response to glucose concentration of the 50/50 PPy/PA biosensor. Decreasing the polyacrylamide cross-linking from 3.2% to 2% increases the current intensity by a factor close to four.

The figures of merit of the 50/50 PPy/PA biosensor can be summarized as follows: I) The sensitivity to glucose is 189 nA/mM in aerobic and 92 nA/mM in anaerobic conditions. II) The limit of detection (signal/noise=3) is 0.03 mM in both cases (aerobic and anaerobic). III) The linear range of glucose concentration dependence (dynamic range) goes from 0.1mM to 10mM. IV) The average response time is 11s, which is smaller than the time of 32s obtained using glucose sensors based on GOx immobilized in PA microgels. Because the diffusion time of substrates is proportional to the square of the traveled length, the response time of this type of biosensors is similar to the response time obtained with biosensors prepared with enzymes entrapped in very thin membranes (10-15μm thick) which is around 30s [8] and is smaller than the response time commonly reported with polymeric membranes, which is around 2 min [9].

4 CONCLUSIONS

Using the concentrated emulsion polymerization method, we have synthesized PPy/PA microparticles which show conductivity in the range of the semiconductor materials. The conductivity increases seven orders of magnitude from 10^{-12} S/cm in pure PA microparticles up to 10^{-5} S/cm in 50/50 PPy/PA microparticles. Glucose Oxidase was immobilized in the 50/50 PPy/PA microparticles and an electrode was prepared having these microparticles as biological component. The 50/50 PPy/PA biosensor gave similar response in both, aerobic and anaerobic conditions which indicates that the electrons are transferred from the active site of the enzyme to the electrode *via* the polypyrrole.

5 ACKNOWLEDGEMENTS

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