Different membranes were tested on screen printed graphite electrodes to improve signal quality when sensing oxygen at a –700 mV potential (vs Ag/AgCl). The use of a cellulose acetate membrane, drugged with quaternary ammonium salts to provide the necessary electrolyte, allows for a sensible reduction of noise for measurements at negative potentials.

1. Introduction

Biosensors which use thick or thin film electrodes carrying the biological mediator directly immobilized in various ways on their surface are very common in literature. This approach takes little care of the great number of interfering species which are often present in real samples. All the same the incidence of interfering species can be of great relevance on the measured signal. Some species can increase or decrease the signal if respectively discharge on the electrode or they're able to deplete oxygen concentration on the electrode surface. Some other interfering species do not affect directly the signal by a reduction reaction but may poison the electrode surface - phenols for example reduce themselves on the graphite electrode with a radicalic mechanism covering the electrode surface with a thin film which reduces oxygen diffusion, other species such as proteins can be adsorbed on the electrode with similar effect. The quality of signals improves even in use with standard solutions and in absence of interfering species with a membrane covering the electrode, as in absence of membranes oxygen can saturate the electrodes surface.

The possibility of covering μ-electrodes and capillary electrodes with a suitable membrane would be a great advantage for improving biosensors signal even with standard solutions and in absence of interfering species.

Different membranes were tested on screen printed graphite electrodes to improve signal quality when sensing oxygen depletion due to enzyme reactions in presence of suitable substrates. Both planar electrodes and front to back geometry μ-electrodes were tested using GOD as a model biomolecule, having in this way the possibility of studying the electrode behavior at both positive
and negative potentials, ranging from hydrogen peroxide oxidation potential (+700 mV vs. Ag/AgCl) to dissolved oxygen reduction potential (-700 mV vs. Ag/AgCl). Teflon, cellulose acetate and Araldite® membranes were tested even drugged with quaternary ammonium salts obtaining stabler signals, improved signal to noise ratio and, in some cases, extended linearity range for glucose.

2. Experimental

Electrodes - μ−electrodes were printed, assembled and pre-treated for stabilization, as stated in former work [1], while planar electrodes were printed using a classical circular layout. Cellulose Acetate Membrane: 3,96 g Cellulose acetate (Fluka) and 40 mg Polyvinyl acetate (AW 51000 droplets - BDH) were put in a solution of 20 ml tetra-hydrofurane (THF) and 30 ml acetone, and stirred continuously to complete dissolution. The solution so obtained was perfectly sealed and stored at 4°C until use. Quaternary ammonium salts in a percentage of 0,1%w/w were added to the solution before casting the membrane on the electrodes. Membranes were cast by dipping on μ-electrodes while in the case of planar electrodes, a drop of membrane solution was simply put on the electrode surface and used as a convenient “glue” to fix a Pall membrane to be used for enzyme immobilization in a second time. To make both membranes tightly adhere to the surface electrodes were pressed at 30Kg/cm².

Araldite® Membrane: (CIBA- resin :bisphenolA- epichlorohydrine, hardener: N(3-di-methyl-amino-propyl)-1,3-propylen-diamine) and solvent (either water or acetone). The paste obtained was casted on the electrode and eventually pressed.

Enzyme immobilization: Glucose oxidase (EC1.1.3.4 from Aspergillus Niger, activity180U/mg Fluka) was immobilized in different ways:

a) with epoxy resin (resin and hardener 1:2) mixing equal weights of Araldite, and solvent (either water or acetone were used),
b) with PAP (polyazetidine Hercules Polycup 172, 12% solids in water, from M. Delaney-New York 10010) dissolving directly the enzyme with the least quantity of PAP needed,
c) with BSA (FLUKA)/Glutaraldehyde (25% aqueous solution Fluka) after treating the electrode’s surface with APTES (3-amino-propyl-etoxyesane 98% Sigma),
d) with the amphiphilic Pall membrane previously fixed on the electrode (20µl GOD solution – 20mg/ml).

Free GOD was also used in solution in batch measurements.
3. Results and discussion

very high currents and a time lasting drift were obtained when bare electrodes coupled with GOD were based on oxygen depletion measurements (figure 1a).

The very high partial pressure of the gas which reaches the bare electrode surface, negatively affected the sensor responses to glucose giving as a result noisy and unstable signals.

A barrier to limit the diffusion of the gas and to lower the current on the electrode surface [14] with similar approach of commercial Clark electrodes, was adopted, putting a membrane onto the screen printed working electrode. As in this case a membrane could not be strictly fixed onto the tip of the electrode with an o-ring, it was cast close to the working electrode surface. Different membrane solutions were deposited with different techniques ranging from Teflon dispersed in aqueous solution, to silicon and cellulose acetate by dip and dry or screen printing or spin coating or casting (unpublished data). The best result, in terms of film adhesion, integrity and mechanical resistance, was obtained by casting a solution of CA (see materials and methods above) as shown in figure 1b where a stable baseline, a linear correlation of the current with glucose concentration, stable steady states for each glucose addition/concentration and higher signal to noise ratio, were obtained.

Figure 1. Glucose biosensor by oxygen detection (-700mV vs Ag/AgCl) with (b) and without (a) CA membrane casted onto the surface of screen printed graphite electrodes. Arrows report time and final concentration of the glucose addition. In (a) the recordings from two glucose biosensors without CA membrane are reported.

To assure the electrolyte needed for amperometric measurements and to keep the membrane closely adherent to the electrode surface without using a polyelectrolyte gel,
quaternary ammonium salts were added directly to the CA solution (0.1% w/v). Different salts were tested (Tetra-ethyl-ammonium perchlorate (TEAP), Tetra-ethyl-ammonium Bromide, Tetra-methyl-ammonium Bromide, Tetra-n-butyl-ammonium Bromide (TBAB)) and the ones with the best solubility in the CA solution (TEAP and TBAB) were chosen. Results obtained with different salts added to CA and different immobilization (simple physical adsorption on a Pall membrane or chemical immobilization using PAP) can be compared in Table 1.

Table 1. Sensitivity of glucose biosensors by chronoamperometry at –700 mV vs Ag/AgCl with different ammonium quaternary salts added to CA membrane. GOD immobilized on Pall membrane by adsorption or PAP procedures.

<table>
<thead>
<tr>
<th>Mem Electrolyte</th>
<th>GOD imm.</th>
<th>Detected Species</th>
<th>Flow/ Batch Electrode lay-out</th>
<th>Sensitivity (µA/mM cm²)</th>
<th>C.V.</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No No No</td>
<td>No</td>
<td>H₂O₂ batch</td>
<td>Front/back</td>
<td>1.42±0.03</td>
<td>2.1%</td>
<td>0.9981</td>
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<tr>
<td>No No No</td>
<td>No</td>
<td>H₂O₂ bath</td>
<td>Front/back</td>
<td>1.76±0.10</td>
<td>5.6%</td>
<td>0.9998</td>
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<tr>
<td>CA TBAB No</td>
<td>No</td>
<td>H₂O₂ bath</td>
<td>Front/back</td>
<td>1.76±0.14</td>
<td>8.0%</td>
<td>0.9995</td>
</tr>
<tr>
<td>CA TEAP No</td>
<td>No</td>
<td>H₂O₂ bath</td>
<td>Front/back</td>
<td>12.45±0.71</td>
<td>5.7%</td>
<td>0.9740</td>
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<tr>
<td>CA TEAP No</td>
<td>No</td>
<td>H₂O₂ bath</td>
<td>Front/back</td>
<td>7.80±0.60</td>
<td>7.3%</td>
<td>0.9739</td>
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<tr>
<td>CA TEAP No</td>
<td>No</td>
<td>H₂O₂ bath</td>
<td>Front/back</td>
<td>13.90±0.30</td>
<td>2.0%</td>
<td>0.9974</td>
</tr>
<tr>
<td>CA No Free O₂</td>
<td>bath</td>
<td>Front/back</td>
<td>11.34±1.42</td>
<td>12.5%</td>
<td>0.9941</td>
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<tr>
<td>CA TBAB Free O₂</td>
<td>bath</td>
<td>Front/back</td>
<td>7.38±0.42</td>
<td>5.8%</td>
<td>0.9723</td>
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</tr>
<tr>
<td>CA TEAP Free O₂</td>
<td>bath</td>
<td>Front/back</td>
<td>4.5±0.28</td>
<td>7.4%</td>
<td>0.9677</td>
<td></td>
</tr>
<tr>
<td>CA TEAP Free O₂</td>
<td>bath</td>
<td>Front/back</td>
<td>4.82±0.28</td>
<td>5.9%</td>
<td>0.9606</td>
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</tr>
<tr>
<td>CA TEAP PAP O₂</td>
<td>flow</td>
<td>Front/back</td>
<td>5.25±0.60</td>
<td>11.4%</td>
<td>0.9633</td>
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</tr>
<tr>
<td>CA TEAP PAP O₂</td>
<td>flow</td>
<td>Front/back</td>
<td>4.23±0.14</td>
<td>3.3%</td>
<td>0.9905</td>
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</tr>
<tr>
<td>CA TEAP PAP O₂</td>
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<td>1.2%</td>
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<td>Front/back</td>
<td>4.70±0.60</td>
<td>11.4%</td>
<td>0.9611</td>
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</tbody>
</table>

In fig 2 chronoaamperometry at -700 mV vs Ag/AgCl are reported for different quaternary salts (1% w/v) added to CA membrane with GOD immobilized on the Pall membrane (20 µl solution GOD 20 mg/ml).

Epoxy used as a membrane with entrapped glucose oxidase, showed a large limiting effect on glucose diffusion. With epoxy resins we were able to obtain an extended linearity to glucose till 50-100 mM. In figure 3 examples of extended linearity obtained with epoxy membranes of different thickness are reported.

4. Conclusions

Membranes (CA with ammonium quaternary salts as TBAB and TEAP or or epoxy resins) were cast on screen printed graphite electrodes obtaining better
performances of the graphite screen printed electrodes in terms of stability, signal to noise ratio, selectivity and extended linearity when oxygen detection at -700mV vs Ag/AgCl is used with oxidases.

![Figure 2: Chonoamperometry at -700mV vs Ag/AgCl comparing different quaternary salts (1%w/v) added to CA membrane. GOD was immobilized on the Pall membrane (20µl solution GOD 20mg/ml)](image)

![Figure 3: Calibration curves for glucose obtained with several thickness of the epoxy membrane](image)

**Acknowledgments**

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References


