

# Cellulose derivatives membranes as supports for immobilisation of enzymes

**D. MURTINHO**

Department of Chemistry, University of Coimbra, 3000 Coimbra, Portugal

**A. R. LAGOA, F. A. P. GARCIA AND M. H. GIL\***

Department of Chemical Engineering, University of Coimbra, 300 Coimbra, Portugal

---

Cellulosic derivatives (cellulose acetate, cellulose propionate and cellulose acetate-butyrate) as membranes, were prepared in different ways. These were then characterised by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and contact angle evaluation. Subsequently, catalase ( $\text{H}_2\text{O}_2$  :  $\text{H}_2\text{O}_2$  oxireductase; EC 1.11.1.6), alcohol oxidase (Alcohol : oxygen oxireductase; EC 1.1.3.13) and glucose oxidase ( $\beta$ -D-Glucose : oxygen 1-oxireductase; EC 1.1.3.4) were covalently linked to these membranes. The catalytic activity and stability of these enzymes, when immobilised, were examined. The results obtained showed that the immobilisation efficiency and the stability of the coupled enzymes could be correlated with the studied properties of the supports. The cellulose acetate membrane which was prepared by evaporation gave the more active conjugate support-enzyme. Membranes prepared by the immersion technique were more crystalline and therefore less suitable for enzyme immobilisation. The highly hydrophobic membranes, obtained from the propionate and the butyrate esters of cellulose reduced the activities but gave better storage stability.

KEYWORDS: glucose oxidase; alcohol oxidase; catalase; cellulose derivatives; enzyme immobilisation.

## INTRODUCTION

Enzymes, after being immobilised, offer several advantages over their 'free' form equivalent. Examples include better stability, possible reuse, greater sensitivity and greater reproducibility of effectiveness. Immobilisation brings about the possibility of their use in bioprocessing, in analysis systems and in enzyme dependent therapy. The choice of support for fixation of the biocatalysts is very important (Ozden and Hasirci, 1990; Garcia-Segura *et al.*, 1987; Piedade *et al.*, 1995).

Different enzymes and cells have been fixed in fibers, in membranes and in gels of cellulose or cellulose derivatives (Gemeiner, 1993). Graft copolymers of cellulose (Hebeish and Guthrie, 1981) have served as supports for the coupling of cells, acid

\*To whom correspondence should be addressed.

phosphatase, glucose oxidase and trypsin (Beddows *et al.*, 1986), alkaline phosphatase (Beddows *et al.*, 1984), ascorbate oxidase (Gil *et al.*, 1993) and other biomolecules (Alves da Silva and Gil, 1988).

Membrane systems have an important part to play in the behaviour of many types of biosensors (Gil *et al.*, 1993; Cardwell *et al.*, 1993; Cass, 1990; Gil *et al.*, 1992; Kostov *et al.*, 1993; Piedade *et al.*, 1995; Alves da Silva *et al.*, 1991). We are interested in such membrane support systems. Protein immobilisation onto filters and membranes has been shown to be important in bioaffinity purification processes such as affinity filtration (Kennedy and Paterson, 1993) and membrane bioreactor operation (Rucka *et al.*, 1990).

In this work we wish to report the immobilisation of selected enzymes, that are of importance to biosensor technology, on well characterised membranes derived from cellulose derivatives. A qualitative explanation of the enzyme behaviour, based on the physical and chemical characteristics of the membranes studied, is presented.

## EXPERIMENTAL

### *Materials*

Cellulose propionate and acetate-butyrate were purchased from Aldrich, Dorset (UK) and cellulose acetate from Sigma, Dorset (UK). Glucose oxidase ( $\beta$ -D-Glucose: oxygen 1-oxireductase; EC 1.1.3.4) from *Aspergillus niger*, catalase ( $H_2O_2$ :  $H_2O_2$  oxireductase; EC 1.11.1.6) from bovine liver and alcohol oxidase (Alcohol: oxygen oxireductase; EC 1.1.3.13) from *Candida boidinii* were supplied by Sigma. Sodium periodate, hexamethylene diamine and glutaraldehyde were obtained from Merck, Darmstadt (Germany). All other reagents were of analytical grade.

### *Preparation of the membranes*

Solutions of the cellulose derivatives in tetrahydrofuran (10% w/v) were cast on glass plate (20 × 20 cm), by using a casting knife (0.33 mm). The solvent was evaporated at room temperature. Other membranes were prepared from the same solution by precipitation in a water bath. The water was either at 5 °C or at 25 °C.

### *Determination of water vapour sorption*

The prepared membranes were conditioned over a saturated solution of copper sulphate (98% R.H.) at 25 °C until constant weight was achieved. Afterwards the polymer was, in each instance, weighed every minute for ten minutes. The initial sorption capacity was obtained graphically after extrapolation to zero time. Each sample was dried to constant mass, under reduced pressure, at 100 °C. The percentage of water uptake is given by,

$$\% \text{ sorption} = (M_i - M_f)/M_f \times 100$$

where  $M_i$  is the initial mass at zero time and  $M_f$  is the final dry mass.

### *Determination of static contact angles*

Static contact angles were determined with a Contact- $\theta$ -Meter (Leverreel, Consett, Durham, England). A sessile drop of water was placed on the surface of the membranes and the contact angle was determined at 20 °C.

### *Characterisation of the membranes by DSC and SEM*

Differential scanning calorimetric analysis was carried out on the membranes using a Polymer Laboratories, PI-DSC analyser. Sample pieces of the membrane, weighing 5-6 mg, were sealed in aluminium pans. The samples were heated at a rate of  $10\text{ }^{\circ}\text{C min}^{-1}$  in a  $\text{N}_2$  gas purged atmosphere, using a flow rate of  $10\text{ cm}^3\text{ min}^{-1}$ .

In order to analyse the surface morphology by SEM, a Joel 820 unit coupled to a Link EDX analysis unit was used, at 15 kV, with the samples previously coated with a thin film of gold by vacuum deposition to a depth of 20 nm. Specimens selected for SEM analysis were chosen to be representative of the total sample.

### *Diffusion coefficients*

A method for measuring the diffusion coefficient of water in different kinds of polymeric membranes was developed. A cell (Fig. 1) with two compartments was assembled. One compartment, A, was completely filled with distilled water. The other, B, was filled with poly(ethylene glycol) 400. The compartments were separated by a particular cellulose derivative membrane. A capillary, positioned in the upper side of the cell, measures the variation of water volume during the diffusion process. The poly(ethylene glycol) 400 in compartment B, continuously stirred, maintains the concentration of water on this side of the membrane as zero.

To ensure that equilibrium was really achieved, the membranes were immersed in water for 24 hours before the beginning of their use in a particular experiment.

The water diffusion coefficients were determined by following an adaptation of Fick's 1st law:

$$dh/dt = Ds_m/(a_c l)$$

where  $dh/dt$  is the variation in the length of the column of water in the capillary tube with time,  $s_m$  is the membrane surface area,  $a_c$  is the capillary area and  $l$  is the membrane thickness.

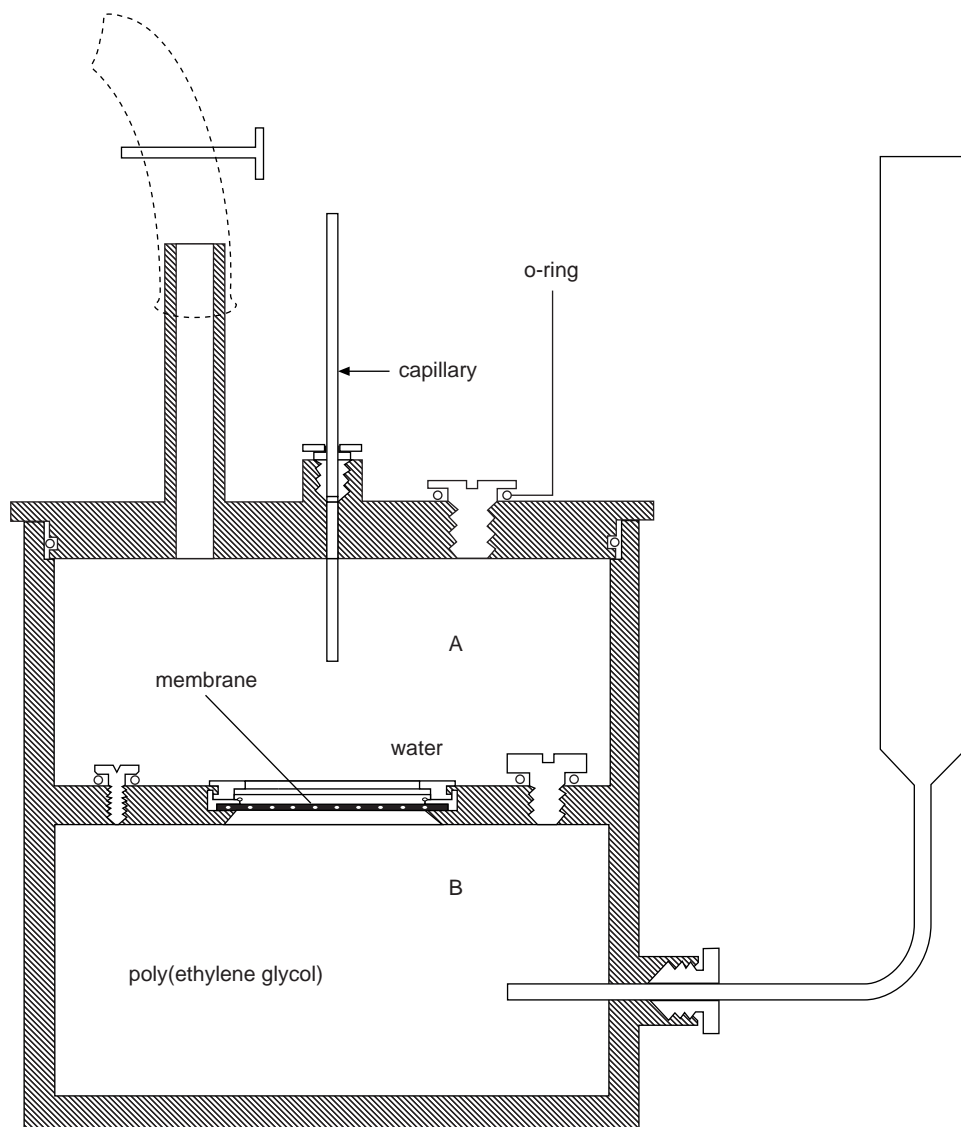
### *Enzyme immobilisation*

Membrane sections were allowed to react with  $10\text{ cm}^3$  of sodium periodate (0.5 M) for 2 h in the dark. They were then treated with  $10\text{ cm}^3$  hexamethylene diamine 1% (w/w) solution for 18 h. This step was followed by 2 h at  $4\text{ }^{\circ}\text{C}$  in  $10\text{ cm}^3$  of glutaraldehyde (5% (v/v) solution) in 0.05 M phosphate buffer at pH 7.5. The membranes were thoroughly washed with distilled water between each step.

The enzyme solutions in 0.05 M phosphate buffer, pH 7.5 (glucose oxidase  $9\text{ cm}^3$   $2\text{ mg/cm}^3$ ; alcohol oxidase  $15\text{ cm}^3$   $4\text{ mg/cm}^3$ , and catalase  $15\text{ cm}^3$   $2\text{ mg/cm}^3$ ), were allowed to couple to the activated membranes over a period of 20 h at  $4\text{ }^{\circ}\text{C}$ . The membranes were thoroughly washed with 0.05 M phosphyate buffer, pH 7.5, and stored in the same buffer at  $4\text{ }^{\circ}\text{C}$  until the activity assay process.

### *Enzyme activity assays*

The activity of the immobilised glucose oxidase (GOX) and of the free glucose oxidase was determined by the *o*-dianisidine procedure (Hugget and Nixon, 1957) (1 U.I. = 1 mmol D-glucose oxidised per minute).  $50\text{ cm}^3$  free enzyme solution were added to a buffered reaction mixture ( $50\text{ mM}$  acetate buffer, pH 5.1) that consisted of:  $2.4\text{ cm}^3$  of *o*-



**FIGURE I.** Diffusion cell.

dianisidine 0.21 mM; 0.5 cm<sup>3</sup> D-glucose 10%; and, 0.1 cm<sup>3</sup> peroxidase 60 U/cm<sup>3</sup>. In the case of the immobilised enzyme, because the chromogen adsorbs onto the membranes, the process was separated into two steps. A weighed membrane portion containing the immobilised glucose oxidase was immersed in 5 cm<sup>3</sup> D-glucose (10% solution in 50 mM acetate buffer, pH 5.1). Then 100 cm<sup>3</sup> aliquots were withdrawn from the reaction vessel and reacted with 2.4 cm<sup>3</sup> of *o*-dianisidine 0.21 mM and 0.1 cm<sup>3</sup> peroxidase 60 U/cm<sup>3</sup>.

The activity of the free alcohol oxidase (AOD) and of the immobilised alcohol oxidase was determined by the 4-aminoantipyrine procedure, at pH 7.5, 50 mM phosphate buffer,

using methanol as the substrate (1 U.I. = 1 mmol methanol oxidised per minute). 100 cm<sup>3</sup> of free alcohol oxidase were added to 1 cm<sup>3</sup> of peroxidase, 6 U/cm<sup>3</sup>, and a indicator solution that consisted of 0.4 mM 4-aminoantipirine, 11 mM phenol and 1.5 M methanol. In the case of immobilised enzyme, weighed portions of membranes were immersed in 3 cm<sup>3</sup> of peroxidase solution and 6 cm<sup>3</sup> of indicator solution.

The activity of the immobilised catalase was determined by following the decomposition of H<sub>2</sub>O<sub>2</sub> at 240 nm, (1 U.I. = 1 mmol of H<sub>2</sub>O<sub>2</sub> degraded per minute). A weighed portion of membrane was immersed in 15 cm<sup>3</sup> of H<sub>2</sub>O<sub>2</sub> 100 mM solution in 50 mM phosphate buffer, pH 7.0.

#### *Storage stability of free and immobilised glucose oxidase*

The storage stability of glucose oxidase was assessed. A 0.2 mg/cm<sup>3</sup> of free enzyme solution, in 50 mM acetate buffer, pH 5.1, as well as the membranes containing the immobilised enzyme in the same buffer, were stored at 4 °C for 3 days. The residual activity was then determined.

## RESULTS AND DISCUSSION

### *Membrane characterisation*

All the membranes prepared by the evaporation technique were characterised by scanning electron microscopy. Cellulose acetate (CA), cellulose propionate (CP) and cellulose acetate-butyrate (CAB) membranes are symmetric and almost nonporous.

The cellulose acetate membranes prepared by immersion in a water bath (CA-5 and CA-25) are non-symmetric with a dense top layer and an open structured sub-layer. As the temperature of the coagulation bath was increased from 5 to 25 °C (CA-5 and CA-25 respectively) the porosity of the top layer increased due to the rapid exchange of the solvent by the non-solvent.

### **Interaction of membranes with water**

The diffusion coefficient obtained for the water/cellulose acetate combination (Table 1) was of the same order of magnitude as that obtained by other authors (Long and Thompson, 1955) and was almost three times the value obtained for cellulose propionate. This fact, as well as the results obtained for the water sorption capacity and contact angle, can be explained by the increase in the number of hydrophobic alkyl groups in the cellulose propionate.

**TABLE I. Membrane water interaction**

Membrane	Water Sorption (%)	Contact Angle (°)	Diffusion Coefficient × 10 <sup>10</sup> (cm <sup>2</sup> /s)
CA	13.6 ± 0.5	73 ± 3	163.0 ± 5.4
CA-5	11.8 ± 0.9	63 ± 2	282.0 ± 1.0
CA-25	12.1 ± 0.2	55 ± 1	∞
CP	7.7 ± 0.1	78 ± 3	58.2 ± 2.8
CAB	4.6 ± 0.4	79 ± 1	104.0 ± 4.4

With the cellulose acetate butyrate, one must consider: a) the increasing number of alkyl groups and b) the presence of two different types of substituent groups. An increase in the number of alkyl groups should decrease the diffusion coefficient and the water sorption capacity. There should also be an increase in contact angle value. However, the diffusion coefficient of cellulose acetate butyrate is higher than that of cellulose propionate. This is probably because there are two different substituent groups to increase the free volume within the membrane. One theory (Rebenfeld *et al.*, 1976) of the diffusion process refers to the diffusion of small molecules and can be interpreted in terms of 'diffusional pops'. A 'diffusional pop' relates to a place in the polymer, near the permeate compound, with enough thermal energy to open momentarily a volume of a size that the migration of the permeate to a new position in the polymer is achieved. This free volume allow the molecules of the permeate to jump between the 'holes' of the polymer, promoting diffusion.

CA-5 and CA-25 membranes have the lower contact angle values (better wetting) and higher diffusion coefficients. This is due to the more 'open' structure of these membranes. In the CA-25 membrane, the water permeated so fast that the diffusion coefficient value could not be measured using the diffusion cell. Thus, a value of  $\infty$  appears in Table 1.

This combination of easier wetting and rapid transport is important to the design of effective membrane sensor systems (Guthrie and Lin, 1996).

### Thermal characteristics of the cellulosic membranes

Some of the thermal characteristics of the various cellulosic ester membranes are given in Table 2 and Fig. 2 (DSC results).

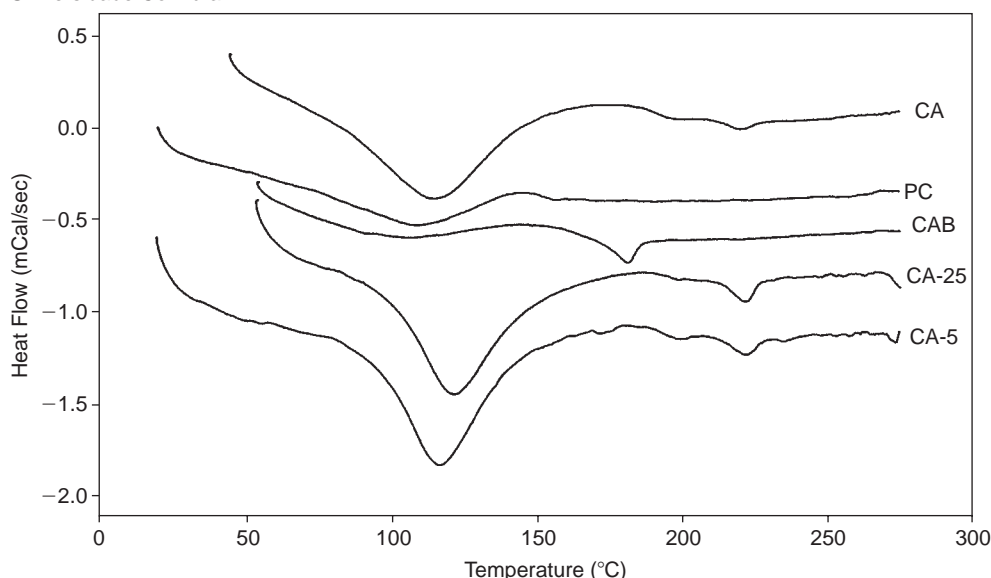
It is clear that:

- (1) All the membranes have a  $T_g$ . Cellulose acetate has the highest value probably because this polymer is more ordered than cellulose propionate or cellulose acetate-butyrate. The increase in the  $T_g$  value in the CA-25 membrane may be due to the higher temperature at which the membranes were conditioned. Higher temperatures allow a more efficient exchange of the solvent with the non-solvent. Consequently, a greater amount of water penetrates the membrane. This water can interact with any free hydroxyl groups in the cellulose derivative by hydrogen bonding, allowing the acetate groups to reorganise in a more ordered way (Buntjakov and Averyanova, 1972).
- (2) Cellulose acetate membranes are partially ordered as shown by the melting enthalpy results. The membranes prepared by immersion are more ordered than

**TABLE 2. Thermal properties of the membranes**

Membrane	$T_g$ (°C)	$T_m$ (°C)	$\Delta H$ (J/g)
CA	191.2	210.6	2.05
CP	152.2	–	–
CAB	173.8	–	–
CA-5	192.4	212.5	3.35
CA-25	196.3	212.9	4.65

Universidade Coimbra



**FIGURE 2.** DSC profiles of the cellulose ester membrane systems **CA-cellulose acetate**, **CP-cellulose propionate**, **CAB-cellulose acetate-butyrate**, **CA-25-cellulose acetate (25 °C - see text)**, **CA-5-cellulose acetate (5 °C - see text)**.

those prepared by solvent evaporation, due to water penetration during the preparation.

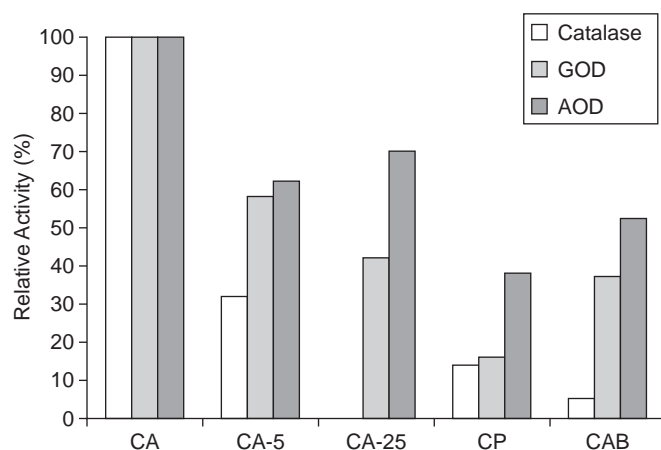
Cellulose propionate and cellulose acetate butyrate are amorphous membranes, as shown by their DSC profiles.

#### *Enzyme immobilisation*

Membranes taken from the same batch as those that were subjected to characterisation by DSC, contact angle and diffusion studies were used as supports in the covalent immobilisation of catalase, glucose oxidase (GOX) and alcohol oxidase (AOD). The anhydroglucose units of the membranes were oxidised with sodium periodate. The resulting aldehyde groups were then made part of an extender arm of hexamethylene diamine. The residual  $-NH_2$  groups were then activated with glutaraldehyde, before the coupling of the enzymes was undertaken.

The relative activities of the enzyme/membrane composites are shown in Figure 3. CA linked to glucose oxidase had an activity of 1.9 U.I./g polymer; alcohol oxidase had an activity of  $169 \times 10^{-3}$  U.I./g and catalase had an activity of 1120 U.I./g.

The membrane enzyme activity results indicate that the immobilisation survival efficiency is dependent on the support used. The enzyme itself has a minor influence. On the contrary, the activity of the coupled enzymes is dependent on the nature of the support and, therefore, on the physical and the chemical characteristics of the membranes.



**FIGURE 3. Relative activity of immobilised enzymes in the different membranes. Relative Activity =  $100 \times (\text{membrane activity}/\text{membrane with the highest activity (CA)})$ .**

The cellulose acetate membranes are better supports for enzyme coupling than acetate-butyrate. The worst is cellulose propionate, with the exception of catalase that has more activity on CP than on CAB. Of the acetate membranes, those prepared by evaporation gave better activities than did those prepared by coagulation in water.

The more hydrophilic membranes have better opportunities for activation and for coupling of the enzymes. This is because they allow better contact between the polymeric chains of the membranes, the activating reagents and the enzyme molecules, in the aqueous solutions. In the same way, larger lateral groups and carbon chains, retard the access to the sites on cellulosic ester polymer.

The CA membranes gave superior enzyme activities than those provided by CP and CAB. This is because of their more hydrophilic character (using the water sorption as a measure), their lesser substituent group content and their degree of substitution. Thus, a larger quantity of enzyme becomes linked.

The CA membranes prepared by immersion are more difficult to activate and link to the enzyme than those prepared by evaporation. This is because they are less hydrophilic and have a more organised structure. Interaction between the polymeric chains is reduced. The coupling yield of CA-5 and CA-25 was less than that of CA.

The diffusion coefficient provides another useful parameter in establishing the performance of immobilised enzyme systems. The CA-5 membranes and the CA-25 membranes are very permeable and would be expected to be more active catalysts than CA. However, the quantity of enzyme immobilised is very low, because of the polymer suprastructure. With CP and CAB membranes, diffusion is a determining factor on the effective activity of the system. The CAB membranes are very hydrophobic and have a longer lateral group (four carbons), suggesting a potentially lower immobilisation yield. Nevertheless, the AOD and GOX activities are higher than those provided by CP. This is because the CAB membranes are much more permeable than the CP, which offers resistance to the diffusion of both the substrates and products of the enzymatic reaction. With immobilised catalase, permeability of the support is not a limiting factor of the



reaction. This is because the substrate, hydrogen peroxide, has great mobility, allowing it to diffuse rapidly into the membranes, to reach the immobilised catalase.

The storage stability of GOX was investigated by monitoring the activity after 3 days of storage at 4 °C (Table 3). The results confirm that covalent immobilisation stabilises the enzymes. A close look at the stability results, and comparison with the initial activities, shows that the supports with the greater activity are those that give less stabilisation of the enzyme.

**TABLE 3 Storage stability of free (U.I./protein g) and immobilised (U.I./membrane g) glucose oxidase.**

Membrane	Day 0	Day 3	
	Activity (U.I./g)	Activity (U.I./g)	Activity Retention (%)
Free enzyme	0.144	$21.4 \times 10^{-3}$	14
CA	1.9	$266 \times 10^{-3}$	14
CA-5	1.1	$236 \times 10^{-3}$	21
CA-25	0.8	$239 \times 10^{-3}$	30
CP	0.3	$236 \times 10^{-3}$	79
CAB	0.7	$239 \times 10^{-3}$	34

The enzyme immobilised in acetate cellulose membranes is less stable than that stabilised on the CP and CAB membranes. This implies that hydrophobic microenvironment reduces the enzyme hydration and, also, contact with the aqueous solution, thus diminishing the conformational mobility leading to denaturation.

The stability of the CP-immobilised GOX is distinctly greater as a result of specific interaction between the enzyme and support. Alternatively, processes that could lead to denaturation may become diffusion limited. Immobilised biocatalytic systems, in which the activity is limited by the diffusion of the reactants, can have an apparent stability which is greater than the stability of the free enzyme molecules.

## CONCLUSIONS

Characteristics such as crystallinity, hydrophilicity and permeability can be correlated with the activity and stability of enzymes that are immobilised in membranes. The cellulose-derivative membranes used are a good model for the study of the factors that influence the behaviour of immobilised enzyme systems.

Fusion enthalpies, water sorption values and diffusion coefficients are useful in helping to establish practical parameters for the creation of effective supports for the immobilisation of enzymes. Contact angle phenomena are of limited importance in this context.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by JNICT (Portugal) for R. Lagoa.

## REFERENCES

- Alves da Silva, M. and Gil, M. H. (1988) Graft copolymers as supports for the immobilisation of biological compounds, *Analytical Uses of Immobilised Compounds for Detection, Medical and Industrial Uses* (Guilbault, G. G. and Mascini, M. eds), 177–185.
- Alves da Silva, M., Gil, M. H., Redinha, J., Brett, A. and Pereira J. (1991) Immobilisation of glucose oxidase on nylon membranes and its application in a flow-through glucose reactor, *J. Polym. Sci.: Part A: Polym. Chem.* **29**, 275.
- Beddows, C. G., Gil, M. H. and Guthrie, J. T. (1986) Immobilization of BSA, enzymes and cells of *Bacillus Stearothermophilus*, onto cellulose, poly(galacturonic acid) and starch based graft copolymers containing maleic anhydride, *Biotech. Bioeng.* **28**, 51.
- Beddows, C. G., Gil, M. H. and Guthrie, J. T. (1984) Investigation of the immobilisation of bovine serum albumin, trypsin, acid phosphatase and alkaline phosphatase to poly(hydroxyethyl acrylate)-co-cellulose and poly(hydroxyethyl acrylate)-co-pectin, *Polymer Bulletin*, **11**, 1.
- Buntjakov, A. S. and Averyanova, V. M. (1972) The structure of solutions and films of cellulose acetate, *J. Polym. Sci. (Part C)*, **38**, 109.
- Cardwell, T. J., Cathral, R. W., Deady, L. W., Dorkos, M. and O'Connell, G. R. (1993) A fast response membrane - based pH indicator optode, *Talanta* **40**, 765.
- Cass, A. E. G. (1990) *Biosensors, Practical Approach*, 1st Ed., Oxford University Press.
- Garcia-Segura, J. M., Cid, C., Liano, J. M. and Gavilanes, J. G. (1987) Sepiolete-supported urease, *British Polymer Journal*, **19**, 517.
- Gemeiner, P., Stefuca, V. and Bales, V. (1993) Biochemical engineering of biocatalysts immobilised on cellulosic materials, *Enzyme Microb. Technol.*, **15**, 551.
- Gil, M. H., Alegret, S., Alves da Silva, M., Alegria, A. C. and Piedade, A. P. (1993) The use of modified cellulose for biosensors, *Cellulosics: Materials for Selective Separations and Other Technologies*, (Kennedy, J. F., Philips, G. O. and William, P. A. Eds), p. 163–171, New York, Ellis Horwood Ltd.
- Gil, M. H., Piedade, A. P., Alegret, S., Alonso, J., Martinez-Fàbregas, E. and Orellana, A. (1992) Covalent binding of urease on ammonium-selective potentiometric membrane, *Bios. and Bioel.* **7**, 645.
- Guthrie, J. T. and Lin, L. (1996) *J. Membrane Sci.*, **113**, 89–92.
- Hebeish, A. and Guthrie, J. T. (1981) *The Chemistry and Technology of Cellulosic Copolymers*, New York, Springer-Verlag.
- Hugget, A. and Nixon, D. (1957) *Lancet*, **2**, 368.
- Kennedy, J. F. and Paterson, M. (1993) *Polymer International*, **32**, 71.
- Kostov, Y., Tzonkov, S., Yotova, L. and Krysteva, M. (1993) Membranes for optical pH sensors, *Anal. Chimica Acta*, **280**, 15.
- Long, F. A. and Thompson, L. J. (1955) Diffusion of water vapour in polymers, *J. Polym. Sci.*, **15**, 413.
- Ozden, M. Y. and Hasirci, V. N. (1990) Enzyme Immobilisation in Polymer Coated Liposomes, *British Polymer Journal*, **23**, 229.
- Piedade, A. P., Gil, M. H., Cavaco, M. C. and Andrade, M. E. (1995) Behaviour of catalase immobilised on poly (acrylonitrile)-g.co-hydroxyethyl methacrylate when used in a continuous system, *Polymer International*, **38**, 269.
- Piedade, A. P., Guthrie, J. T., Kazlanciunas, A. and Gil, M. H. (1995) Characterisation of cellulose derivatives - relevance to sensor development, *Cellulose*, **2**, 243.
- Rebenfield, L., Makarewicz, P. J., Weigmann, H. D. and Wilkes, G. L. (1976) Interactions between solvents and polymers in the solid state, *J. Macromol. Sci.-Macromol. Chem.* **C15(2)**, 279.
- Rucka, M., Turkiewicz, B. and ZuK, J. (1990) Polymeric Membranes for Lipase Immobilisation, *JAOCs*, **67** 887.