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Evaluation of the permeability of modified cellulose acetate propionate membranes for use in biosensors based on hydrogen peroxide detection

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Abstract

Phase inversion cellulose acetate propionate membranes showed low permeability to hydrogen peroxide aqueous solutions. Their permeability was increased by alkaline hydrolysis of the ester linking units. However, the permeability remained lower than that of an unsubstituted cellulose membrane. The inclusion of hydroxypropyl cellulose in the membrane formulation, followed by an alkaline hydrolysis step, increased permeability to hydrogen peroxide aqueous solutions to 29% of that of an unsubstituted cellulose membrane.

Abbreviations: BA – *n*-butyl acetate, CA – cellulose acetate, CAP – cellulose acetate propionate, HPC – hydroxypropyl cellulose, THF – tetrahydrofuran.

Introduction

Polymeric membranes have been used in conjunction with enzyme-based biosensors since the biosensor concept was created (Clark and Lyons 1962; Updike and Hicks 1967). These membranes have played the main roles of (i) matrices for immobilising enzymes and mediators and (ii) permselective barriers (Wang 1992; Alvarez-Icaza and Bilitewski 1993).

Cellulose acetate propionate (CAP) is a mixed cellulose ester developed for coating applications. It is soluble in both organic solvents and in alcohol/water mixtures, has a fast solvent release rate and is the basis of an excellent film-forming polymer (Eastman Chemical Co. 1994). CAP membranes have been used in separations by reverse osmosis (Cadotte et al. 1970; Kutowy et al. 1976; Sand 1990), and by pervaporation (Luo et al. 1977).

The porosity and the degree of hydrophilicity of membranes prepared from cellulose esters can easily be altered by simple alkaline hydrolysis of the ester functions. When these membranes are used in electrochemical biosensors, this approach should allow control over the selectivity of the biosensor by rejecting undesired interferent species, either by size or polarity exclusion effects. When compared to cellulose acetate (CA), CAP allows the preparation of permselective barriers of higher hydrophobicity and has better film-forming properties.

In order to evaluate the suitability of CAP membranes to be used in biosensors based on hydrogen peroxide detection, their permeability to hydrogen peroxide aqueous solutions was evaluated and the membranes were modified with the aim of improving permeability.

Methods

Preparation of cellulose acetate propionate membranes

The casting of a CAP solution was followed by controlled evaporation of the solvent and coagulation in

Table 1. Formulations of the CAP membranes prepared (BA = *n*-butyl acetate; t_{evap} = evaporation time before coagulation).

Formulation	CAP (wt. %)	Solvents (wt. %)		Additives (wt. %)		t_{evap} (min)
		Acetone	Water	BA	Na_2CO_3	
Doctor blade gap: 200 μm						
A	18.0	82.0	–	–	–	5
B	19.9	75.57	0.5	4.0	0.03	3
C	19.9	75.57	0.5	4.0	0.03	0.5
D	19.9	75.59	0.5	4.0	0.01	3
E	19.9	75.59	0.5	4.0	0.01	0.5
Doctor blade gap: 500 μm						
F	20.0	72.0		8.0		3

a non-solvent bath. These procedures were based on conditions published for the preparation of membranes of CAP and CA by phase inversion (Kutowy et al. 1976; Kuhn et al. 1989).

CAP (CAP 504.02) was supplied by Eastman Chemical Co., Kingsport, TN, USA, with $\bar{M}_n = 15000$ g/mol, hydroxyl content: 5.0% (by weight), acetyl content: 0.6% (by weight) and propionyl content: 42.5% (by weight). It was used to prepare solutions in acetone (Aldrich, Poole, UK), with or without additives such as sodium carbonate (BDH, Poole, Dorset, UK) or *n*-butyl acetate (Aldrich) (Table 1). These solutions were manually cast on levelled, clean glass plates by slowly drawing an aluminium doctor blade. After controlled evaporation of the cast solution, coagulation was subsequently performed by slowly immersing the plate in a large, shallow enamel trough containing distilled water at room temperature. The coagulation was allowed to proceed beyond the moment the membrane was spontaneously released from the plate, for a total immersion time between 30 and 60 min. Formulations containing sodium carbonate were treated with a 0.1 M acetic acid solution following the coagulation step, in order to increase the removal rate of sodium carbonate. The membrane was finally removed from the bath and let to dry between paper towels.

Additionally, a mixed solution of CAP and hydroxy-propyl cellulose (HPC, Klucel EF, Aqualon, Wilmington, DE, USA) in tetrahydrofuran (THF, Aldrich) was assembled. This was composed of 9.9% (by weight) of CAP, 1.1% (by weight) of HPC and 89% (by weight) of THF. The formulation was cast with a doctor blade gap of 500 μm. An evaporation time of 5 min was allowed before coagulation was commenced. The coagulation conditions were similar to those described above.

Alkaline hydrolysis

Membranes were hydrolysed in a NaOH solution (0.1–1.0 M), under reflux conditions or at other temperatures, for different time intervals (see the Results section). After hydrolysis, the membranes were immersed in distilled water at room temperature, transferred to a 0.1 M HCl solution, rinsed with distilled water and, finally, transferred to a 0.1 M sodium phosphate buffer solution (pH 7.0) until tested.

Evaluation of permeability to aqueous hydrogen peroxide

The permeability of the membranes to hydrogen peroxide aqueous solutions was evaluated by chronoamperometry, using a hydrogen peroxide electrode (Yellow Springs Instruments Co., Yellow Springs, OH, USA) poised at + 700 mV vs. Ag/AgCl with the aid of a potentiostat (Ursar Scientific Instruments, Oxford, UK). When a CAP membrane was assayed, the original cellophane membrane of the electrode was replaced by a sample of the CAP membrane that had been left overnight in a 0.1 M sodium phosphate buffer at pH 7.0. A baseline (current intensity vs. time) was obtained with 50 μL of phosphate buffer in the recess of the hydrogen peroxide electrode, held upside down, a volume large enough to cover the membrane surface. A portion (20 μL) of a solution of hydrogen peroxide in the same buffer (concentration range used: 0.1–10 mM) was then added. The value of the current intensity at the plateau was used as the response of the electrode to hydrogen peroxide. The permeability of the CAP membranes was expressed as a percentage of the permeability of a cellophane membrane, based on the relative response.

Table 2. Response of the hydrogen peroxide electrode covered with the CAP membranes hydrolysed under reflux (membrane formulations are given in Table 1).

Membrane formulation	[NaOH] (M)	Hydrolysis time (h)	Response[a] (%)
B	0.1	1	8
B	0.1	6	8
C	0.1	1	8
C	0.1	6	8
D	1.0	0.25	8
D	1.0	0.5	8
E	1.0	0.7	17
F	0.5	2	0
F[b]	0.5	4	23

[a]Percentage of the response obtained with a cellophane-covered electrode.
[b]A hydrolysis time of 5 h did not increase the permeability. Hydrolysis times above 5 h resulted in membrane disintegration.

Results and discussion

Permeability to hydrogen peroxide aqueous solutions

The membranes were tested in a situation that was similar to that found in an enzyme-based amperometric biosensor. Thus, the CAP-covered electrodes were first used in an activity assay of an enzyme-catalysed reaction producing hydrogen peroxide (oxidation of β-D-glucose to gluconic acid and hydrogen peroxide, catalysed by the enzyme glucose oxidase). The enzyme activity obtained with a CAP membrane-covered electrode (formulation A, Table 1) was approximately one-half of that obtained with the standard colorimetric glucose oxidase assay.* This behaviour did not depend on which side of the membrane was facing the electrode. As expected, the results of the assay performed with the cellophane-covered electrode reproduced those of the standard activity assay, indicating that an unsubstituted cellulose matrix was not responsible for the lack of sensitivity. This reduced response of the CAP membrane-covered electrode is thought to be due to a lack of permeability of the CAP membrane to hydrogen peroxide. Since CA membranes are permeable to hydrogen peroxide, and as the flux of water through CA membranes decreases when acetate groups are substituted by propionate groups (Cadotte et al. 1970), the lack of permeability to water could also play a role. This inferior permeability seen with the CAP membranes could be related to (i) the presence of the more hydrophobic propionate groups in the CAP, (ii) a high degree of substitution of CAP, and (iii) a more dense structure. Three approaches to increasing the permeability of the CAP membranes were then evaluated. These were: (i) alkaline hydrolysis of the ester linkages in CAP membranes, (ii) addition of a water-soluble cellulose derivative to the formulation, and (iii) alkaline hydrolysis of the ester linkages of the membrane obtained by route (ii).

Alteration of membrane permeability by alkaline hydrolysis of ester linkages

An alkaline hydrolysis study was undertaken, assessing the effect of different NaOH concentrations, temperatures and reaction times on different membrane formulations. *n*-Butyl acetate was included in the formulations to improve the flexibility of the resultant membranes. Without such a plasticiser, the membranes became rather fragile. For an alkali concentration of 0.1 M, membranes thought to be the most porous (formulations B and C) gave poor responses (Table 2). Increasing the hydrolysis time under reflux from 1 to 6 h and increases in the alkali concentration made little difference to this situation. However, hydrolysis of a membrane prepared with a lower evaporation time produced a higher response (formulation E, Table 2).

As the membranes became thin and fragile after the alkaline treatment under reflux, thicker membranes were prepared by increasing the doctor blade's gap. After hydrolysis, the best membranes (formula-

* A version of Trinder's colorimetric assay, based on hydrogen peroxide detection with 3,5-dichloro-2-hydroxy-benzenesulphonic acid, 4-aminoantipyrine and horseradish peroxidase, was used (Foulds et al. 1990).

tion F, Table 2) showed a response that was 23% of that of a cellophane-covered electrode.

Alteration of membrane permeability through inclusion of hydroxypropyl cellulose in the formulation

A cellulose derivative soluble in both organic solvents and water (HPC) was employed with the role of a polymeric pore former. The membranes obtained were almost not permeable to hydrogen peroxide aqueous solutions. The permeability did not increase when immersion in distilled water was extended from 2 to 48 h. This result should be related to difficulties in the solubilisation of HPC in the coagulation bath, probably due to interchain enmeshment between CAP and HPC.

The effect of alkaline hydrolysis on the permeability of the CAP/HPC composite membranes was studied. A hydrolysis temperature of 50 °C was used, since reaction under reflux resulted in membranes that often were too fragile to be inserted in the electrode's recess. The best result, a response that was 29% of that of the cellophane-covered electrode (Table 3), was obtained for membranes that had experienced hydrolysis in 0.5 M NAOH for 9 h. Hydrolysis times above 9 h resulted in a membrane that was too fragile to be handled. Shorter hydrolysis times using more concentrated alkali solutions (1.0 M) did not improve the permeability. As HPC is water-soluble only for temperatures below 40–45 °C, it remains in the membrane, maintaining the structural integrity of the membrane after hydrolysis and increasing membrane hydrophilicity.

This approach resulted in membranes having the highest permeability to hydrogen peroxide aqueous solutions of this study. However, this low permeability limits the use of these membranes in enzyme-based, electrochemical biosensors based on hydrogen peroxide detection.

Conclusions

Phase inversion membranes of CAP showed poor permeability to aqueous hydrogen peroxide solutions. The permeability of the membranes was increased to a limited extent by alkaline hydrolysis of the ester bonds. However, the permeability remained much lower than that of an unsubstituted cellulose membrane and increased membrane fragility. The alkaline hydrolysis of a CAP/HPC composite membrane provided membranes with the highest permeability (29% of that of an unsubstituted cellulose membrane).

Table 3. Response of the hydrogen peroxide electrode covered with the CAP/HPC composite membranes after alkaline hydrolysis at 50 °C.

[NaOH] (M)	Hydrolysis time[a] (h)	Response[b] (%)
0.1	5	1
0.1	22	6
0.1	29	6
0.5	2	6
0.5	6	18
0.5	9	29
1.0	2	12

[a]Hydrolysis times above the higher time given for each set resulted in a membrane that was too fragile to be inserted in the electrode's recess.

[b]Percentage of the response obtained with a cellophane-covered electrode.

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