PVC as a sensor membrane material: influence of solvent casting variables

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Unplasticized polyvinyl chloride (PVC) has proved an especially difficult membrane material to form reproducibly. In its fabrication for sensor use, variable temperature solvent casting has been assessed and related to function as external membranes of a classical dual membrane amperometric oxidase electrode. The thermal history of the casting solution was shown to have an effect on both thickness and the permeability. With increasing temperature of the cast solution (4–37 °C) membrane thicknesses increased (5–30 μm). Amperometric responses to catechol and hydrogen peroxide decreased with increasing casting solution temperature whereas responses to ascorbate remained unchanged. These properties are tentatively attributed to the formation of discrete layers through differential changes in the rate of solvent evaporation. Scanning electron microscopy revealed distinct microstructure zones supporting this proposition and attesting to the need to factor in environmental temperature during casting.

1. Introduction

Polyvinyl chloride (PVC)-based membranes have been widely exploited for the improved functioning of potentiometric ion-selective electrodes [1]. The membranes here comprise an appropriate solvent plasticizer, neutral carrier species and/or ion exchanger, the latter being a major but not sole determinant of ion binding mediated interfacial potential generation in the presence of analyte. The PVC compositions used effectively separate the test aqueous solution, containing the target ion, from the internal reference solution. The activity-related interfacial potential translates into a measurable emf which can then be related to the concentration of the target ion in solution [1]. Plasticized PVC membranes have also been recently utilized by us in amperometric biosensors, where the incorporation of appropriate plasticizer has led to ''tunable'' permeability PVC membranes for the selective detection of lipophilic solutes either directly (e.g. glucose [2]) or as part of an amplified enzyme electrode transduction sequence [3]. These are lipophilic membranes in the main and their molecular selectivity has been rationalized on this basis [2–4]. However, little previous work has been conducted with unplasticized PVC (uPVC); it has been considered to be highly impermeable, unselective and irreproducible with regard to initial fabrication outcome and subsequent stability [5–7].

uPVC permeability can be shown to vary with the degree of crystallinity in the membrane [5, 8]. In the crystalline domain, the solute is essentially insoluble in the polymer matrix, and a subsequent low permeability can be regarded as being due to non-partitioning (molecular exclusion) in this lattice structure. PVC crystallinity is linearly proportional to the polymerization temperature and can only be altered post-polymerization [9] if temperatures exceed the glass transition temperature of the polymer ($T_g = 78 - 80\,^{\circ}C$). The T_g defines the temperature above which uPVC behaves rubber-like due to an increased freedom of chain movement [10]. Okuno *et al.* [11] demonstrated that the permeability of water vapor and ethanol through uPVC decreased with increased crystallinity of the membrane.

Another important parameter in determining permeability (other than polymer crystallinity) is the dielectric constant (ε) of the material, a measure of intermolecular forces and likely polarizability in an electric field. The closer ε is to 1, the more the material approximates to a situation where there is a vacuum between the polarizing plates (i.e. perfect insulation). The greater the deviation from 1, the less insulating the material. PVC has a dielectric constant of 3.3 comparable to that of teflon ($\varepsilon = 2.1$) and relative to polar water ($\varepsilon = 78.5$). Entry and diffusion of polar solutes through PVC from an aqueous solution might therefore be limited due to the contrasting dielectric properties of the two interfacing solvating media.

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Apparent reasons for variability in membrane permeability are investigated here with reference to casting solution temperature, and relation to the microstructure of the final membrane. The context of this work is the novel incorporation of uPVC into amperometric and not potentiometric sensors.

2. Materials and methods

2.1. Materials

High molecular weight (MW = 200 000 Da) poly(vinyl chloride) was obtained from BDH Chemicals (Merck Ltd, UK) and cellulose acetate powder (39.8% acetyl content) from Aldrich Chemicals. Succinic acid (free acid), bovine serum albumin (BSA; Fraction V), oxalic acid (disodium salt) and glutaraldehyde (50% v/v) were purchased from Sigma Chemical Co. (Poole, UK). Oxalate oxidase (OOD; from barley seedlings), specific activity: 0.25 units/mg lyophilizate, (ca. 5 units/mg protein) was purchased from Boehringer Mannheim (Lewes, UK). Ethanol (HPLC grade; $< 0.1\%$ water) and Tween-80 were obtained from Sigma Chemicals. Chemicals for electrointerferent studies were purchased from Aldrich Chemicals.

An electrode assembly designed for O_2 detection, (Rank brothers, Bottisham, Cambridge) was adapted for H_2O_2 detection, the working electrode (anode) being polarized at $+650$ mV versus Ag/AgCl. The cell consisted of a central 2 mm diameter platinum disc as the working electrode with an outer 12 mm diameter, 1 mm wide silver ring (Ag/AgCl) as a combined reference and counter electrode. An *x-t* chart recorder (Lloyd Instruments, plc, Fareham, UK) was used to record the amperometric responses of the electrode fed in from an in-house potentiostat current follower.

Scanning electron micrographs (SEM) were obtained using a Cambridge 360 scanning electron microscope. Dynamic contact angle and interfacial tension data were obtained using a Cahn 2000 Contact Angle Analyzer (Cahn Instruments, California, USA) based on the Wilhelmy plate method.

2.2. Polymer membrane casting

Cellulose acetate (0.2 g) was dissolved in acetone (10 ml) and the resulting solution poured into a Petri dish (7 cm i.d.) on a flat, horizontal surface. The dish was then covered with a glass plate, but not sealed and maintained under laminar air flow conditions to effect slow, more controlled, evaporation of solvent (2 days) to achieve weight constancy.

A stock solution of PVC (1.2 g) in tetrahydrofuran (THF) (100 ml) was prepared at room temperature ($22 \pm 2\,^{\circ}$C). Five ml aliquots of the resulting solution were then pipetted into 20 ml Pyrex glass bottles. The solutions were then temperature equilibrated (4–55 °C) for 30 min and then immediately poured into individual Petri dishes (9 cm i.d.) which were thermally pre-equilibrated at room temperature ($22 \pm 2\,^{\circ}$C). The dishes were again covered to allow membrane formation (2 days).

2.3. Selectivity studies

Before use, the electrode surface of the two-electrode cell was throughly polished with aluminum oxide, and then completely rinsed with distilled water. The electrode was polarized at a bias voltage of $+650$ mV versus the Ag/AgCl pseudo-reference electrode. The electrode was then treated with a saline solution for approximately 10 min. The surface was then rinsed with buffer and left moist. A Cuprophan dialysis membrane, wet with buffer, was positioned over the electrode; this ensured electrolyte contact between the working and reference electrodes and also served as a spacer. The Cuprophan layer was then covered with a 1 cm^2 portion of the membrane cut from the center of the cast materials. The electrode (in succinate buffer) was then exposed to individual solutions of interferents, and the sample chamber was washed thoroughly between each assay. The steady-state electrode response to each interferent was recorded in all cases.

2.4. Enzyme electrode construction

Oxalate oxidase (350 μg) was dissolved in a 50 mg/ml solution of bovine serum albumin (BSA) (250 μl), prepared in doubly distilled water, giving an enzyme solution of 12 U/ml activity. The enzyme solution (6 μl) was then rapidly mixed with glutaraldehyde (2.5% v/v; 3 μl) on a clean glass microscope slide. The resulting crosslinked mix was deposited in the center of a 1 cm^2 portion of a designated underlying membrane. A cellulose acetate inner selective membrane was used in all experiments. Upon this enzyme layer was immediately placed a 1 cm^2 cutting of a designated outer uPVC membrane. The resulting enzyme laminate was then compressed between glass slides with mild finger pressure (ca. 5 min) in order to complete membrane apposition with enzyme lamination. The glass slides, when prised apart, left a uniform enzyme laminate. The laminate was then rinsed with a jet of buffer to remove excess unreacted glutaraldehyde.

The laminate was then mounted on a Cuprophan membrane covered electrode with the underlying side in direct contact. Positioning of the rubber ''O''-ring sealant and fixing of the sample chamber screw cap afforded the completion of enzyme electrode construction.

2.5. Treatment of PVC with wetting agents

Two methods of pre-wetting were studied. In the first case, a square portion of the membrane was dipped in high pressure liquid chromatography (HPLC) grade ethanol for 15 s. The membrane was then removed and dipped in a bath of distilled water (15 s), to remove any excess ethanol from the surfaces of the membrane. The membrane was then layered on the electrode as previously described and then its selectivity tested with standard hydrogen peroxide, catechol and ascorbate solutions.

Alternatively, a square portion of the untreated membrane was directly layered onto the electrode, and then the sample chamber of the two-electrode cell treated with the wetting agent (either ethanol or surfactant

solution) for 30 min with stirring. The solution was then removed and the sample chamber completely washed with buffer. The membrane surface prepared in this way was again tested with standard interferents. Responses were monitored against time, with buffer washing between measurements over a period of 3 h.

3. Results and discussion

Increases in solution temperature, before casting, were envisaged as giving more ready mixing of solvent and polymer; temperature-dependent solubilization was consistent with this. However, the temperature of dissolution had no influence on subsequent outcomes. A correlation was observed between the cast membrane thickness (measured using an electronic micrometer) and the temperature of the casting solution (Fig. 1) despite the application of isovolumetric casting solutions over an equal area. A more open (less dense) polymer structure had thus resulted from increasing the solution temperature. SEM analysis of membrane edges (Fig. 2) confirmed the increase in membrane thickness with temperature. It also revealed a distinct change in the microstructure of the membranes; with a casting solution temperature of above the casting surface temperature (22 °C), stratification was seen, and multiple sheet-like layers were evident at a casting solution temperature of 37 °C (Fig. 2b). With slow cooling of the pre-heated polymer solutions to 22 °C (on a glass substrate), precipitation at more than one temperature would be inevitable and, here has resulted in stratified, parallel fault lines in membranes derived from higher casting solution temperatures. The extent of this, however, may vary with surfaces of different thermal conductivities and use of rapid cooling techniques.

In the present study, for purely operational reasons,

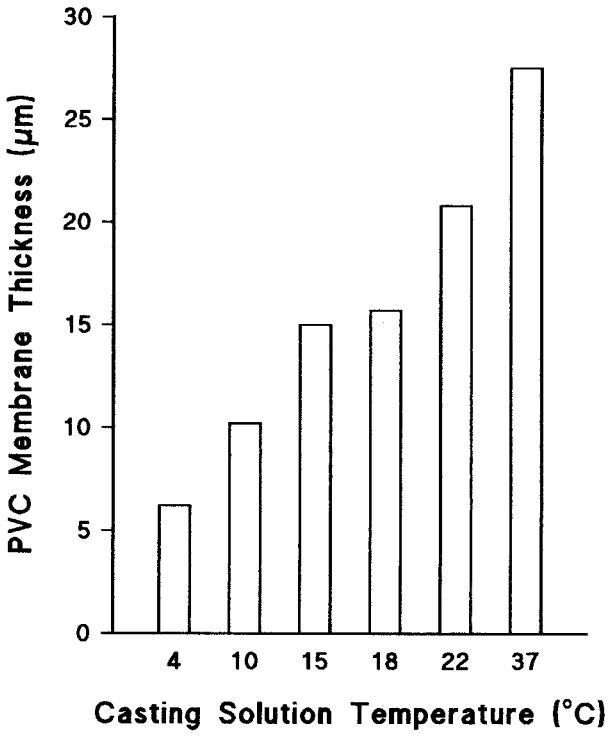
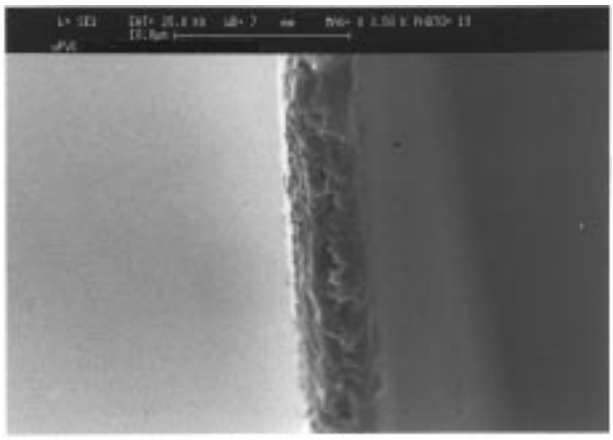
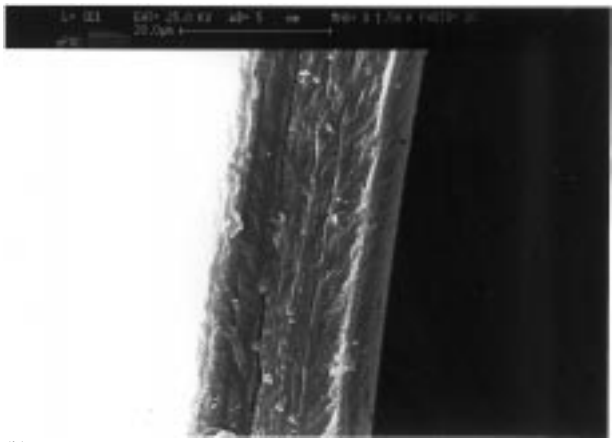


Figure 1 Plot of original casting solution temperature versus resultant cast membrane thickness.



(a)

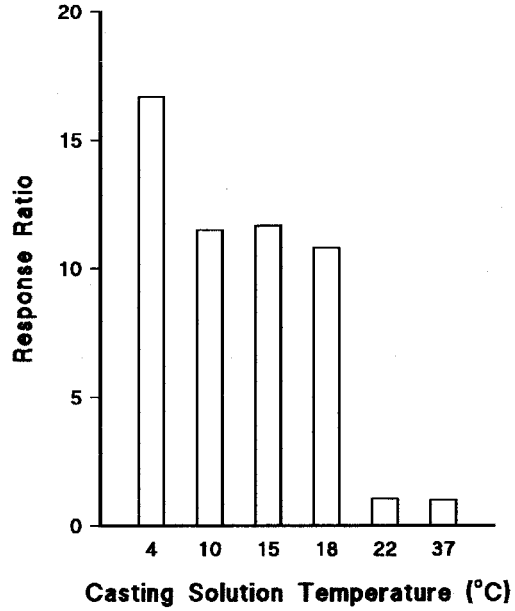


(b)

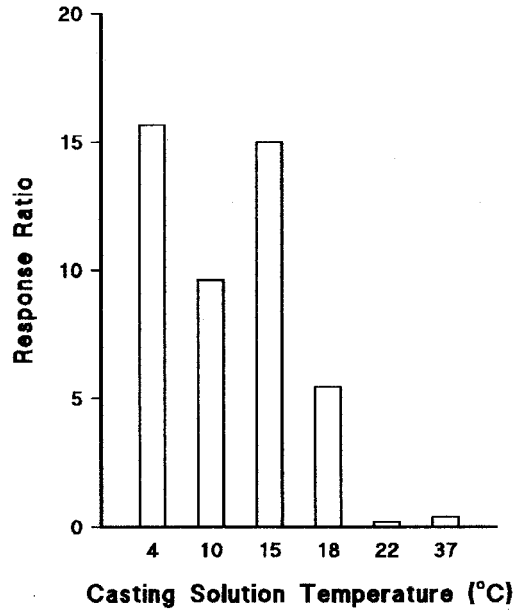
Figure 2 Scanning electron micrographs of: (a) 4 °C casting solution; (b) 37 °C casting solution; cast in glass Petri dish kept at 22 °C.

aqueous catechol and hydrogen peroxide permeability respectively were determined as a ratio of ascorbate permeability, as the latter is a major interferent ion in biological monitoring. Response ratios for each showed a similar trend (Fig. 3a,b). At casting solution temperatures between 4 and 18 °C, membranes exhibited comparable high selectivities; this would have significant practical value in amperometric sensor design. However, at 22 °C and above, membranes showed a marked reduction in peroxide and catechol permeability while ascorbate permeability remained at a low constant value (0.10 ± 0.02 nA for 100 μM ascorbate). Since hydrogen peroxide (MW = 34 Da) became less permeable than ascorbate (MW = 185 Da), such a change in permeability with increasing casting solution temperature clearly could not be attributed to a variability in the porous nature of the membrane.

Responses to 500 μM oxalate, when using the uPVC temperature-controlled membranes as outer membranes of an oxalate enzyme electrode, showed the reverse trend to the previous selectivity studies (Fig. 4). The low and constant permeability seen with ascorbate was also likely with the oxalate ion. This inverse effect was therefore possibly due to some other process. One possibility could be that the hydrogen peroxide product (from the enzyme reaction) was less able to escape through the external PVC membrane with increase in casting solution temperature (Fig. 3b). Consequently, more H_2O_2 was



(a)



(b)

Figure 3 Histogram of (a) catechol (100 μM): ascorbate (200 μM) and (b) hydrogen peroxide (100 μM): ascorbate (200 μM) response ratios for casting on a 22 °C surface.

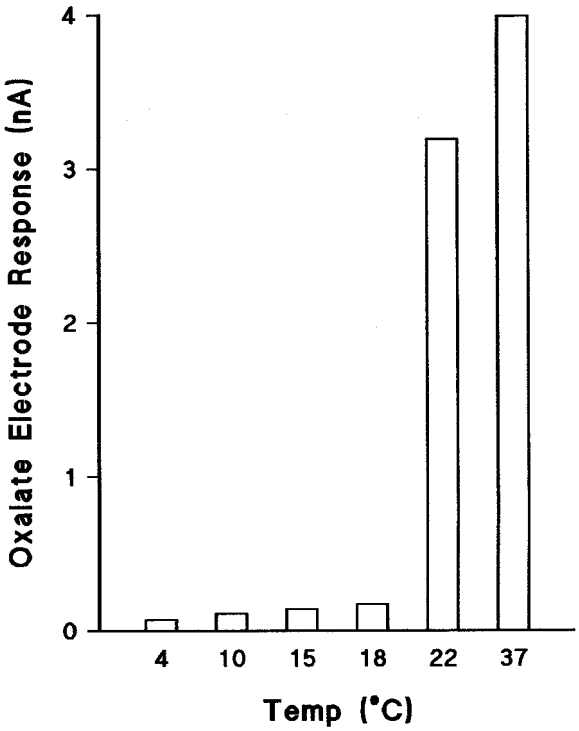


Figure 4 Effect of casting solution temperature on an oxalate enzyme electrode response (500 μM) response. Outer membrane was the designated temperature controlled membrane; an inner cellulose acetate membrane was used in all cases.

available to diffuse through the internal cellulose acetate membrane [3] to be degraded at the working electrode ''sink''.

It was apparent, therefore, that while putative membrane thickness influenced catechol and H_2O_2 permeability, ascorbate permeability was not affected. This was possibly caused by a different mode of transport of a charged species compared with neutral catechol or hydrogen peroxide. uPVC has inherent lipophilic properties [4] and neutral and uncharged species would certainly interact with the membrane to a greater extent than any charged species. One indicator of the solubility of various species in the membrane phase may be obtained by contact angle analysis and the determination of a PVC/water interfacial tension [12]. These are related according to the general equation [11, 12]

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cos \theta_{rec} \quad (1)$$

where the subscripts S, L and V refer to solid, liquid and vapor, respectively, and γ is the corresponding interfacial tension (N cm^{-1}), and θ_{rec} (°) is the receding contact angle (in the Wilhelmy plate method [12, 13]) between the solid–liquid and liquid–vapor interfaces. This wetting tension is a fundamental parameter used to identify phenomena such as penetration of liquids into porous media, solid–liquid adhesion and the stability of modified surfaces.

An increased interfacial tension with increasing PVC solution temperature would suggest an increase in hydrophobicity of the membrane, a probable consequence of a net decrease in the polar C–Cl groups oriented in the direction of the polar probe liquid [12].

TABLE I Contact angle and interfacial tension data for temperature controlled uPVC membranes (all in succinate buffer, pH 2.9)

Membrane (solution temp.→ surface temp.)	θ_{rec} (°)	γ_{rec} (N cm^{-1})
4 → 22 °C	43.9	0.00043
10 → 22 °C	41.8	0.00045
15 → 22 °C	37.0	0.00048
18 → 22 °C	35.0	0.00049
22 → 22 °C	13.5	0.00059
37 → 22 °C	51.0	0.00038
45 → 22 °C	47.1	0.00041
55 → 22 °C	46.3	0.00060
65 → 22 °C	31.2	0.00051

Contact angle analysis of the same membrane face as used in the electrochemical studies (using aqueous succinate buffer as the probe liquid), however, revealed no consistent correlation between casting solution temperature and the dynamic receding contact angle (Table I). This suggested that surface wettability had no apparent effect on membrane permeability and that bulk properties were (a) more reproducible (Fig. 3) and (b) a more dominant influence.

Differences in permeability to catechol and hydrogen peroxide with increasing temperature of the PVC cast may have been the result of a membrane bulk phenomenon, involving an increase in the packing density of the membrane.

The possibility that polymer solutions had the potential to retain varying degrees of order depending on solution temperature as a so called ''memory effect'' was tested by casting membranes with identical solution and membrane drying temperatures. The 4 °C solution cast at 4 °C exhibited the same solute permeability (not shown) as a 4 °C solution cast at 22 °C. Also, a 37 °C solution cast at 37 °C gave the same properties as a 37 °C solution cast at 22 °C. This showed the paramount importance of the casting solution temperature over the casting surface temperature.

PVC membranes, when treated with water, over a long period, develop a defined water rich layer extending into the membrane from the membrane/water interface [14, 15]. Fig. 5 reveals a progressive rise in PVC permeability to solutes over 6 h; this may well be due to slow water entry into the membrane. Pretreating a PVC membrane with a 0.2% (v/v) buffer solution of the surfactant, Tween-80 (Fig. 6a), and a 0.2% (v/v) solution of ethanol (Fig. 6b), respectively, reversed this situation, and produced an initial high in response to the species

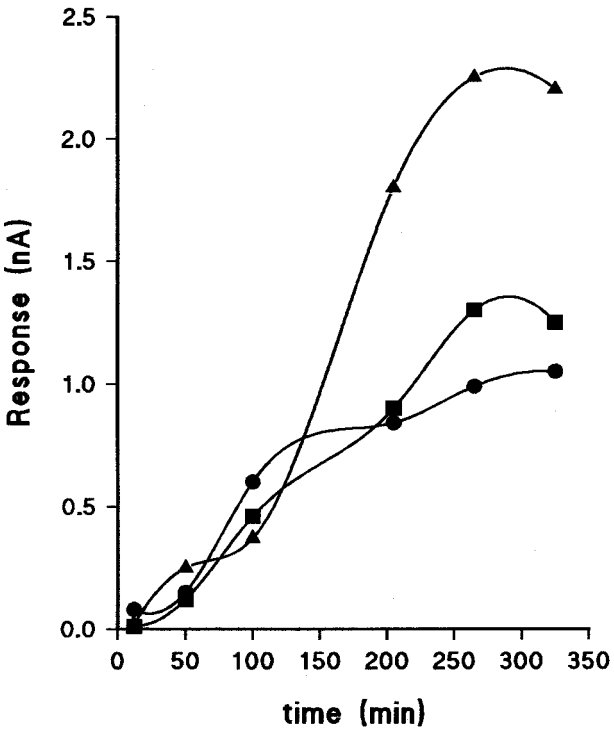


Figure 5 Effect on PVC (22 °C solution cast at 22 °C) covered electrode response to 0.5 mM ascorbate (●), 0.2 mM catechol (▲) and 0.2 mM hydrogen peroxide (■) with the membrane exposed to water over a 6 h period.

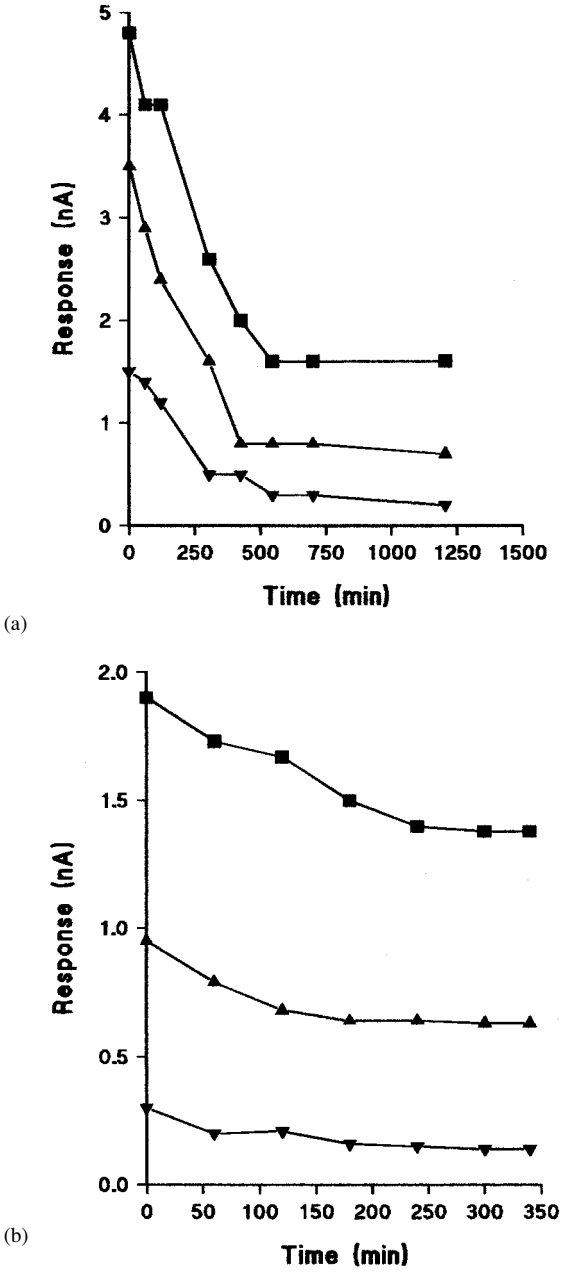
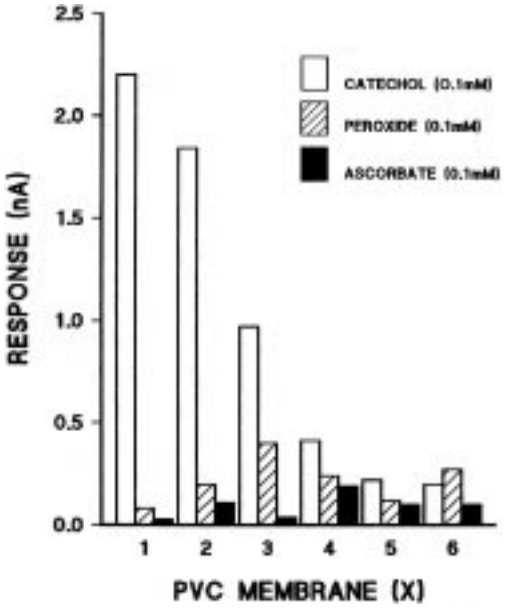


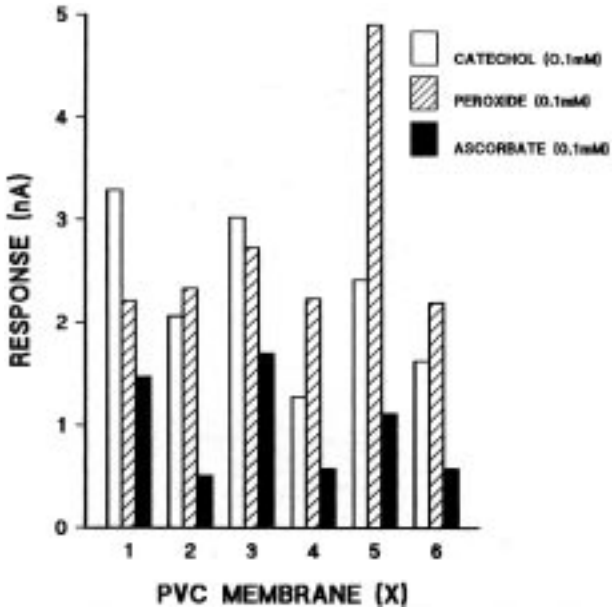
Figure 6 Effect on electrode response to catechol (▲), hydrogen peroxide (■) and ascorbate (▼) upon treating a uPVC (22 °C solution cast on
22 °C glass Petri dish) covered electrode with (a) Tween-80 (0.2% v/v) and (b) ethanol (0.2% v/v).

studied, which decayed with repeated exposure to pure buffer. The ethanol and surfactant treatment may have had some interfacial effect but undoubtedly a bulk intra-membrane effect would have been important; possible swelling by these agents undoubtedly was associated with an increase in the hydrophilic nature of the membrane, leading to greater permeability. Continued washing probably served to leach out surfactant or ethanol with less effective replacement of the void volume with water.

On the above assumption, membranes with casting temperatures of 4, 22 and 37 °C respectively were treated with ethanol (excess ethanol rinsed away with buffer) and then their permeability tested on the electrode. Fig. 7(a) shows the selectivity of each membrane (cast in duplicate) before ethanol treatment, and Fig. 7(b) after treatment with ethanol. It was evident that such treatment diminished solute discrimination, as might be expected if



(a)



(b)

Figure 7 Response of uPVC (4 °C solution cast at 22 °C) ($X = 1, 2$); uPVC (22 °C solution cast at 22 °C) ($X = 3, 4$); uPVC (37 °C solution cast at 22 °C) ($X = 5, 6$) to catechol, hydrogen peroxide and ascorbate: (a) before and (b) after 15 s ethanol treatment.

membrane hydrophilicity was to be temporarily increased.

4. Conclusions

PVC without plasticizer has considerable potential for solute discrimination and for extending the linear range of enzyme electrodes as an external membrane. Such membranes have been studied here, with permeability and dimensions controlled by the rate of polymer precipitation which affects the tendency to form intra-membrane domains. Use of polar organic solvent or a surfactant treatment of the cast membranes leads to standardized membrane permeability which is then independent of casting solution temperatures and which, after a series of washing stages, can be stabilized. It could be envisaged that the polar wetting agents had an effect which was present only at the interfacing PVC membrane surface, thereby offering a more compatible interface for the partitioning of polar solutes from bulk aqueous solution to an increasingly polar and hydrophilic membrane phase; the dielectric constant of ethanol ($\varepsilon = 24.3$) is intermediate between that of water and PVC, undoubtedly aiding this process, and solvent therefore serving as a semicompatible and metastable interface between the two media of contrasting polarities. Likewise, Tween-80, possessing a hydrophilic/lipophilic balance (HLB) of 15.0 is again intermediate to being completely soluble in either the aqueous phase or the hydrophobic PVC phase.

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