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Collagen-based bioartificial materials – evaluation as membranes for extracorporeal blood purification

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The preparation of films from blends of collagen and poly(vinyl alcohol) is described and the transport properties of these films are evaluated. Different crosslinking procedures, dehydrothermal crosslinking and crosslinking by glutaraldehyde, have been used. The transport properties of these films have been studied using model solutes such as sodium chloride, vitamin B12 and bovine albumin. The results are compared with those obtained using commercial products such as Cuprophan® and poly(acrylonitrile) dialysis membranes. The aim of our study was to analyse the effects of different crosslinking methods and the effects of blending with poly(vinyl alcohol) upon the permeation characteristics of collagen-based membranes.

1. Introduction

Collagen is the primary structural component of mammalian tissues. Tendons, hide and bones of bovines and porcines are its most important industrial sources. Collagen-based materials have been extensively investigated for a variety of biomedical applications including dialysis membranes, wound dressing and artificial skin [1–3]. Haemodialysis membranes are prepared from collagen [4–9], extracted from calf skin by enzymatic degradation. The collagen membranes are produced by casting from acetic acid solutions and the membranes are crosslinked by heating at high temperatures or by UV irradiation. Poly(vinyl alcohol)-based membranes have been also proposed for extracorporeal blood purification. Various methods, including chemical crosslinking [10–12], have been investigated for overcoming the instability of poly(vinyl alcohol) in aqueous solutions. Thus, we have decided to prepare films of collagen and poly(vinyl alcohol) and to evaluate their transport properties in liquid systems for a possible use in dialysis. Two different crosslinking methods have been employed to prepare these films: dehydrothermal treatment and crosslinking by glutaraldehyde. A physico-chemical and biological characterization of these films is reported elsewhere [13, 14].

2. Experimental procedures

2.1. Materials

The materials used were: acid-soluble collagen (more precisely tropocollagen, TC), type I extracted from calf skin (Sigma Chemical Co., St. Louis, MO, USA); poly(vinyl alcohol) (PVA) (powdered, Aldrich Chemie, Steinheim, Germany) with an average molecular weight of 114 000 (determined by the viscosimetric method) and a hydrolysis degree of 100%; and 8% w/v glutaraldehyde (GTA) in water (Aldrich Chemical Co.).

2.2. Membrane preparation

A 1% (w/v) PVA solution in water and a 1% (w/v) TC solution in 0.5 M acetic acid were mixed at room temperature in different ratios to obtain a series of TC/PVA blends: 20/80, 50/50, 80/20 (w/w). These blends were used to prepare membranes by casting solutions at room temperature. Membranes of pure TC and pure PVA were also obtained by the same method. Afterwards the membranes were crosslinked according to the procedures described below.

2.3. Crosslinking methods

Two series of TC/PVA films were prepared: one series was crosslinked by GTA, the other was crosslinked by a dehydrothermal treatment DHT. In the former case, TC/PVA films obtained by casting were exposed to GTA vapours at 37 °C for 18h [14]. GTA is capable of reacting with the ε-amino groups of TC lysyl residues forming intermolecular crosslinks at room temperature [15], while GTA reacts with hydroxyl groups of PVA molecules only at high temperatures, at an acid pH and in the presence of a catalyst [16]. In the case of

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dehydrothermal crosslinking, TC/PVA films were placed in vacuum oven at 50 °C for 3 h, then at 90 °C for 30 min; subsequently the temperature was raised to 120 °C. The treated films were removed from the oven after 3 days [17]. The oven temperature was lowered to below 50 °C before the vacuum was released and then the TC/PVA films were removed. Removal of water from collagen by the dehydrothermal treatment results in the formation of interchain crosslinks which are the result of condensation reactions either by esterification or amide formation [18]. The amino acid residues that may be involved in such a reaction are aspartic acid, glutamic acid, serine, threonine, arginine and lysine.

2.4. PVA and TC release measurements

Both series of crosslinked TC/PVA films were placed in distilled water at 37 °C to measure the PVA and TC release after 24, 72 and 96 h. The PVA released was measured by a spectrophotometric method [19], whereas the amount of TC released was measured by the standard hydroxyproline method [20].

2.5. Swelling measurements

The same crosslinked samples, placed in distilled water, were removed, quickly blotted with tissue to remove excess surface water, and immediately weighed in a microbalance (0.1 mg accuracy). The membranes were then dried in an air oven at 40 °C for 24h and placed in a desiccator. The swelling ratio, Q, was calculated as

$$Q = \frac{\text{(weight of swollen samples)}}{\text{(weight of dry samples)}} \quad (1)$$

This procedure was repeated several times on the same samples to obtain an average value of Q.

2.6. Permeability measurements

Permeability measurements were performed at 37 °C using sodium chloride NaCl (MW 58 D), vitamin B12 (MW 1355 D) and bovine albumin (MW 69000 D). Solute permeability was measured in the experimental apparatus illustrated in Fig. 1. It consists of a diffusion cell made up of two chambers separated by the membrane to be tested (Fig. 2). The donor chamber (D) contains the permeate solution and the receptor chamber (R) contains deionized water. Each chamber has a volume of 1.5 cm^3 and the effective diffusion area of the cell is 0.64 cm^2. Both chambers are continuously fed (flow rates were kept at about 1.5 cm^3/min) and stirred. Two identical hydraulic heads, H1 and H2, permit to obtain across the membrane a pressure difference equal to zero. Diffused solutes were monitored on exiting the receptor chamber by conductometric methods in the case of NaCl, and by spectrophotometric methods in the case of vitamin B12 (280 nm) and bovine albumin (196 nm). The concentration of the solute on the permeate side of the membrane gradually increased until it became constant (i.e. until steady-state conditions were obtained). The time dependence

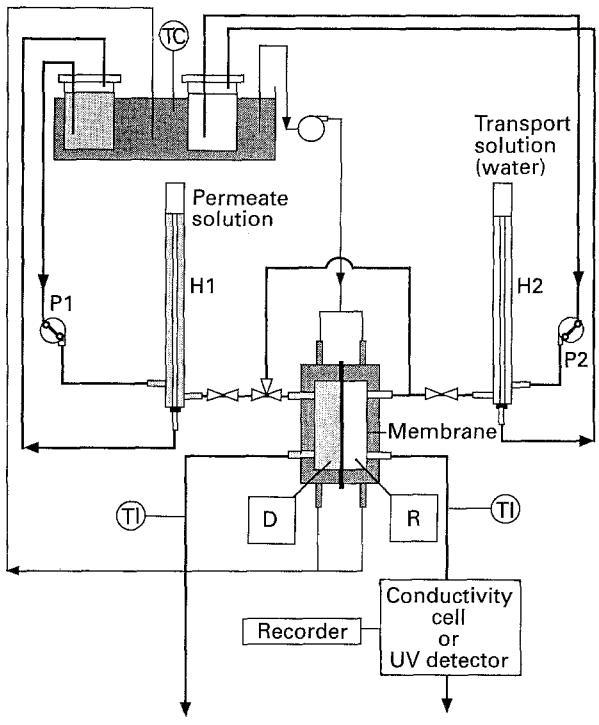

Figure 1 Schematic diagram of the experimental apparatus used for permeation studies: P1, P2 pumps; H1, H2 hydraulic heads; D donor and R receptor chamber of diffusion cell.

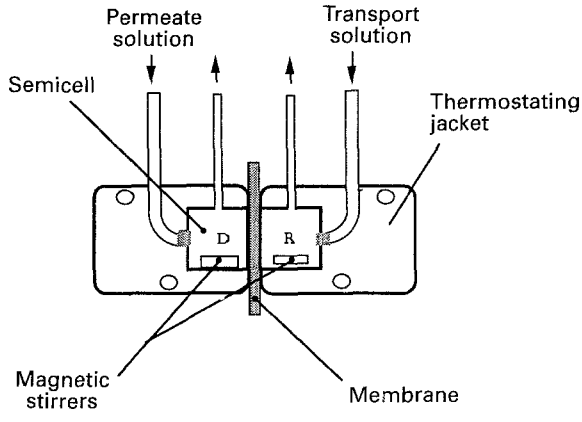

Figure 2 Diffusion cell.

of the solute concentration within the membrane is well established for a Fickian system [21]. When the steady state has been reached, the flux of solute across the membrane, J_s, may be written in terms of concentrations of the solute in the two chambers of the diffusion cell as follows:

$$J_s = (P/h)A(C_d - C_r)$$

where P is the solute permeability, h is the swollen membrane thickness, A is the membrane area, and C_d and C_r are the concentrations of the solute in the donor and the receptor chambers of the diffusion cell, respectively. The above equation has been used to calculate the solute permeability, P, provided that the concentration gradient, the flux under steady-state conditions and the swollen membrane thickness are known. The above equation also requires that the steady- state flux, J_s, has to be inversely proportional to the membrane thickness. This relationship will be

modified under experimental conditions where a boundary layer effect is obtained. In this case the relevant relationship assumes the form:

$$\frac{1}{J_s} = \frac{1}{P\Delta C}(h + P R_b)$$

where R_b is the boundary layer resistance and $\Delta C = C_d - C_r$. Thus, in systems where a boundary layer develops on both surfaces of the membrane, the flux will be modified, and the plot of $1/J_s$ as a function of h would show a positive intercept on the vertical axis. The data obtained show that $1/J_s$ is linearly dependent on h, with the curve passing through the origin. This indicates a negligible boundary layer effect.

The thicknesses of the water-swollen membranes were measured using a micrometer with an accuracy of ± 1 μm. The average thickness of wet films was 20–30 μm.

3. Results and discussion

The permeability values of TC/PVA membranes crosslinked by either GTA or DHT are reported in Fig. 3a–c., as a function of PVA percentage weight in the blend, for NaCl, Vit. B12 and bovine albumin. The results are compared with those obtained with commercial products such as Cuprophane and polyacrylonitrile (AN69) membranes. The permeability values are normalized to a thickness of 20 μm.

The results obtained from permeation tests showed that the permeability of TC/PVA membranes crosslinked either by GTA or by DHT, to all tested solutes, increases with increasing PVA content. This is due to the hydrophilic properties of PVA: the blending of PVA with collagen increases the water uptake and this favours the solute transport across the membrane.

The permeability of DHT-crosslinked samples (Fig. 3a–c) is linearly related to the weight fraction of the PVA component, unlike that of GTA-crosslinked samples. In addition, the permeability values of DHT-crosslinked films are lower than those of GTA-crosslinked films. GTA is able of binding stably with TC lysyl residues at 37 °C while GTA does not react with hydroxyl groups of PVA molecules in the operating conditions used. The PVA released from TC/PVA films is reported in Table I, as percentage of initial PVA content, for both GTA- and DHT-crosslinked samples. The data are relative to a release time of 4 days. The TC released from these films was negligible. This fact demonstrates that both DHT and GTA treatments proved to be effective crosslinking procedures for collagen. The pure PVA film, treated by GTA, is completely water-soluble; in contrast the PVA released from the pure PVA film, treated by DHT, is small compared to the initial quantity present in the films (Table I). The data of the GTA-samples suggest that the PVA is not crosslinked but remains physically trapped in a collagen network. Heat treatment of PVA, as reported in the literature [22], increases the crystallinity degree of PVA (as supported by thermal analysis data [13]) and thus decreases its amorphous content. Because of the resulting increase of the degree of crystallinity, the water-solubility of PVA decreases: the

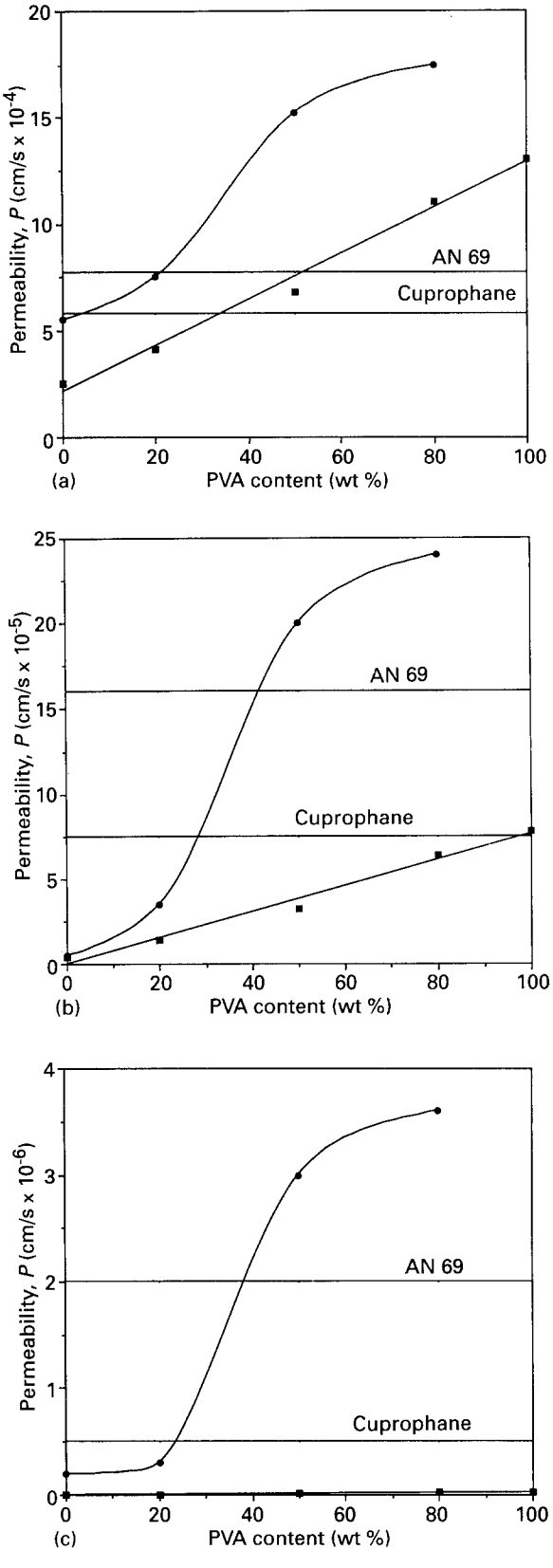

Figure 3 Permeability of TC/PVA crosslinked films for (a) NaCl, (b) Vit. B12, and (c) bovine albumin (● GTA; ■ DHT).

PVA pure film, treated by DHT, remains essentially insoluble in aqueous solution at 37 °C for 4 days (Table I).

The crystalline regions are impermeable even to small-molecule penetrants such as water, and thus the increase of PVA crystallinity in DHT-samples reduces the permeability to the tested solutes. The permeability values of DHT-samples, where the PVA crystallinity is

TABLE I PVA released by TC/PVA films, measured as percentage of the initial PVA content, for both GTA- and DHT-crosslinked samples

TC/PVA (w/w)	GTA-crosslinked samples (%)	DHT-crosslinked samples (%)
100/0	0	0
80/20	7.5	0
50/50	1.41	0.15
20/80	2.30	1.70
0/100	≈ 100	1.04

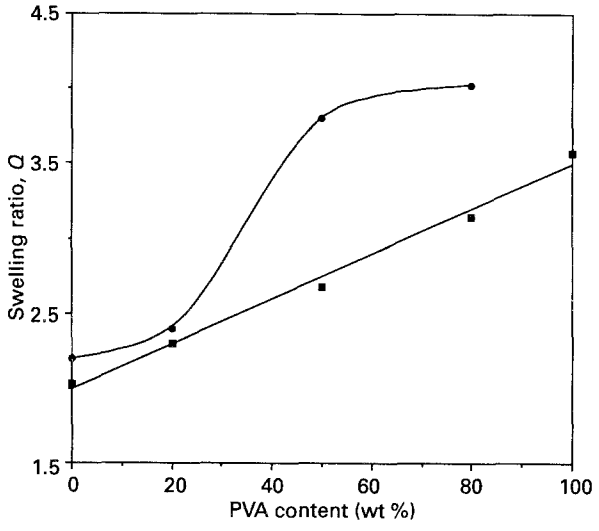

Figure 4 Swelling ratio Q of the TC/PVA crosslinked films versus percentage weight of PVA in the blend (● GTA; ■ DHT).

higher, are lower than those of GTA-crosslinked samples.

The permeability of both DHT- and GTA-samples decreases with increase in the molecular weight of tested solutes. The decrease of permeability is more evident in DHT-samples (Fig. 3a–c). The permeation characteristics of DHT-samples are more selective to solute transport, and thus these films are more suitable for possible use in haemodialysis. In fact a good dialysis membrane has to be permeable to low–medium molecular weight solutes such as urea, creatinine, uric acid, and sodium chloride, but at same time, it has to be impermeable to plasma proteins such as albumin (MW 69000 D) and other macromolecules.

The hydration of TC/PVA crosslinked films is measured by swelling ratio Q; Q, calculated from Equation 1, is reported in Fig. 4 as a function of the PVA weight fraction, for both GTA- and DHT-crosslinked samples. We note that Q increases with increasing PVA content in the same manner as permeability (Figs. 3 and 4). For high weight fractions of TC, the presence of PVA appears to have a negligible effect on Q. For low TC content, PVA molecules in GTA-samples maintain their capability to absorb large amounts of water and at the same time PVA reduces the crosslink density of TC present in the blend; whereas in DHT-samples the increase in crystalline content of PVA reduces the water uptake.

Finally, the results obtained from permeation tests showed that the permeability of these membranes to all tested substances increases with increasing PVA content for both DHT- and GTA-samples. This is due to the hydrophilic properties of PVA that favour solute transport across the membrane. The DHT-crosslinked samples showed permeability values lower than those of GTA-crosslinked samples.

The good solute permeability of TC/PVA films, in particular DHT-crosslinked films, compared with that of commercial Cuprophan® and AN 69 membranes, suggests that these TC/PVA crosslinked membranes could find applications in dialysis and/or hemodialysis processes.

Acknowledgements

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