Prostaglandin E_1-Immobilized Poly(Vinyl Alcohol)-Blended Chitosan Membranes: Blood Compatibility and Permeability Properties

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SYNOPSIS

A series of membranes are prepared by air drying thin films, which were composed of poly(vinyl alcohol) blended with chitosan [a (1 → 4) 2-amino-2-deoxy-β-D-glucan] (PVA–Chit) in different ratios. The PVA-blended chitosan membranes showed improved strength properties and permeability functions for low-molecular-weight compounds. Nonthrombogenic PVA–Chit (4 : 6) membranes were derived by immobilizing bioactive molecules like PGE_1 on heparin-modified membranes, via free radical mechanisms, by N_2 plasma. This novel membrane demonstrated good permeability properties for small molecules and showed a dramatic reduction in platelet attachment. The prostaglandin E_1-immobilized substrate also indicated an increase in albumin surface attachment and a reduction in fibrinogen binding. This may be one of the parameters for a reduced platelet-surface attachment, which may also improve the blood compatibility of the substrate. It is also postulated that the total water content of membranes need not be the prime factor governing the permeability of solutes through water-swollen membranes. However, many other parameters govern the solute permeability, like the amount of solutes dissolved in bound water and the status of water in the polymer matrix.

INTRODUCTION

Hemodialysis, an extracorporeal blood purification procedure, utilizes polymeric membranes to achieve desired levels of solutes and water from the blood. The active part of the artificial kidney is the semipermeable membrane itself, where the commercial regenerated cellulose and cuprophan are still being used due to their good solute permeability and mechanical strength. Since molecular transfer is due primarily to the passage of solute through "microholes" or "pores," the permeability is directly related to the molecular weight of the diffusing substance and there is little selectivity in the separation of two closely related molecules[1,2] through cellophane or cuprophan. Thus, new membranes need to be developed for better control of transport, ease of formability, good mechanical properties, and blood compatibility.

Hirano et al.[3,4] prepared a series of membranes from chitosan and its derivatives that showed improved dialysis properties. The most serious limitation of using these artificial membranes is the surface-induced thrombosis, which requires heparinization of blood to prevent clotting. Current research has shown that on exposure of synthetic surfaces to whole, flowing blood, most materials are rapidly coated with a layer of blood proteins,[5,6] in parallel with the adhesion of platelets[7] leading to thrombus formation. Lyman and Kim[8] and Packham et al.[9] suggested that albuminated surfaces adhere less platelets, but on the other hand γ-globulin and fibrinogen-adsorbing surfaces intensify the adhesion of platelets. Thus, determining the composition of the adsorbed protein layer that forms on a polymer surface is important in the development of membranes, which are inherently blood compatible.

Recent studies have indicated that protein-

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blended chitosan membranes have superior permeability properties.[10] Surface modification of albumin–chitosan-blended membranes have been reported via immobilization of bioactive complexes[11] or coupling biomolecules on liposome-modified[12] membranes through carbodiimide functional moieties. Such novel types of membranes demonstrated good permeability properties for small molecules and also showed good blood compatibility. However, the wet tear strength of chitosan-based membranes needs to be improved along with their nonthrombogenic functions.

Chemical crosslinking of poly(vinyl alcohol) with paraformaldehyde has been developed and standardized to make it suitable for fabricating quality membranes having excellent mechanical properties.[13] In this work, chitosan, one of the most abundant polysaccharides in nature, was blended with poly(vinyl alcohol) to improve its wet tear strength. The optimum blended membrane was further modified via immobilization of PGE_1 on heparinated substrates, using N_2 plasma, and evaluated for their antithrombotic and permeability properties. Interaction studies with plasma proteins and platelets to develop an understanding of this modified surface were also attempted. It appears that these biomolecule-immobilized membranes exhibit superior permeability, mechanical properties, and nonthrombogenic character as compared with standard cellulose membranes.

MATERIALS AND METHODS

Chitosan [a (1 → 4) 2-amino-2-deoxy-β-D glucan], one of the abundantly available polysaccharides in nature, was obtained as a gift from the central Institute of Fisheries Technology, Cochin, India. Chitosan, derived from prawn and crab shell chitin by greater than 85% deacetylation, with particle size of 1–3 mm and an inherent viscosity of 5.13 for 0.5% solution in 2% acetic acid at 30°C, was used as received. Poly(vinyl alcohol), PVA (88% mol hydrolyzed having weight average M_w 125,000) and paraformaldehyde were obtained from BDH Chemicals Ltd., Poole, England. Albumin (human, fraction V. 96–99% pure), γ-globulin (human, Cohn fraction II), fibrinogen (human fraction I, 95% protein clottable), and PGE_1 were from Sigma Chemical Co., USA. Radiolabeled proteins used were iodinated (^{125}I) human albumin and (^{125}I) human fibrinogen from Amersham, UK. Heparin (25,000 IU in 5 mL, Biological Evans Ltd. India), and all other chemicals used were of the highest quality available and were pure for such studies as reported elsewhere.[10–12]

Preparation of the Membranes

PVA–chitosan-blended membranes were prepared similar to our method reported previously[10,11] for chitosan–albumin blend. In summary, a PVA solution in distilled water was prepared and mixed well with paraformaldehyde, which was dissolved in 5 g % NaOH and kept for crosslinking. The excess paraformaldehyde was removed by dialyzing the solution against water overnight. Chitosan was dissolved in 2% acetic acid and well mixed in presence of 2% PVA solution (crosslinked PVA, where excess paraformaldehyde was removed) in various ratios at room temperature. This was spread over a clean glass plate and dried at 50°C to form a uniform thin film. The membranes were removed from the plate by soaking in 0.5*M* NaOH solution at room temperature (30°C), washed with water, then finally with ethanol, and kept at vacuum until use.[10–12]

Immobilization of PGE_1 on Heparinized Membranes

PVA–chitosan (4 : 6)-blended membranes were chosen for surface modification studies. Heparinized substrates were prepared by exposing the membranes to 50 mg/100 mL heparin as described elsewhere.[14] Briefly, the PVA–chitosan-blended membranes were dipped in 0.1*M* phosphate buffer, pH 7.4, and shaken for 10 min to avoid air/water interface. Heparin dissolved in phosphate buffer was added and shaken to make up 50 mg/100 mL solution as described earlier.[14] After 3-h exposure at room temperature, they were taken out and rinsed with buffer, and finally dried at vacuum for 5 h.

Glow Discharge Treatment

Edwards' vacuum coating unit E306 A was employed for glow discharge treatment. The plasma reactor of this unit consisted of a cylindrical dome of diameter 30 cm. The electrodes were in ring form positioned at a height of 12 cm from the base plate. Plasma glow was generated with nitrogen gas at a pressure of 10^{-1} m bar. The heparinated membranes were exposed to N_2 plasma for 15 min,[15] and the freshly generated free groups were formed on the surface.

100 μg PGE_1 was dissolved in phosphate buffer pH 7.4, added to the previously plasma activated heparin-modified PVA–chitosan membranes, and

kept overnight at 4°C for coupling. At the end of this process, the unbound PGE_1 was washed off the membranes with buffer and the samples were then kept at −20°C at vacuum until needed. These membranes were studied for their mechanical properties, permeability, protein adsorption kinetics, and platelet adhesion.

Water Content Determination

The membranes were immersed in distilled water for several days and the water on the membrane surfaces was blotted off prior to weighing. The weights of the dry membranes were determined by drying them to constant weight at about 100°C. The water content was expressed as the ratio of the weight of water in the water-swollen membrane to that of the dry membrane. The inherent viscosity of the PVA–chitosan blends were also recorded using a standard viscometer technique.

Platelet Adhesion Studies with Washed Platelets

Calf blood platelets were isolated by differential centrifugation, within 2 h after collection, from citrated blood as described elsewhere[15,16] and washed with tyrode solution (0.055*M* glucose, 0.138*M* NaCl, 0.012*M* Na_2CO_3, 0.0018*M* $CaCl_2$, 0.0049*M* $MgCl_2$, 0.0027*M* KCl, 0.00036*M* NaH_2PO_4, pH 7.4) for the adhesion studies.[16,17] Briefly, 10 mL blood was collected and added to 1.0 mL 3.8% sodium citrate and centrifuged at 700 × G for 10 min. The supernatant liquid, containing platelet-rich plasma (PRP), was centrifuged at 1,000 × G for 10 min and the white blood cell button was removed. The PRP was again centrifuged at 2,000 × G for 10 min to spin down the platelets, which were then washed three times with tyrode solution and suspended in the same solution. The concentration of platelets was determined by treating them with trypan blue and counting the unstained viable cells in a hemocytometer.[18]

The platelet suspensions (approx. 1.0×10^8 platelets/mL) were exposed to various PVA-blended chitosan membranes for 15 min at room temperature (~ 30°C). The platelets were then rinsed with 0.1*M* phosphate buffer, pH 7.4. Next, the platelets were fixed with 1% carbodiimide and stained with Coomassie Blue G. The number of platelets adhering to the substrates were counted using an optical microscope. A minimum of 30 fields were counted from three separate experiments and the data were expressed as the number of platelets observed per mm^2 on the surface, including the standard deviation.

Trace-Labeled Studies

The kinetics of protein binding to PGE_1-modified PVA–chitosan membranes with time was investigated using ^{125}I-labeled albumin and ^{125}I-labeled fibrinogen as described elsewhere.[19,20] In short, the clean films of size 2 × 1.5 cm were exposed to a protein mixture (avoiding the air/H_2O interface) containing 0.25 mg/mL albumin, 0.15 mg/mL γ-globulin, and 0.075 mg/mL fibrinogen, with a known amount of the single-labeled protein in phosphate buffer (pH 7.4) at 37°C (concentration of labeled albumin in the medium was 40%, 0.46 μcurie/mL, and fibrinogen 7%, 0.51 μcurie/mL).

The films were dipped in buffer and shaken. Furthermore, the protein mixture in buffer was added to obtain the respective concentrations of them inside the media to reduce the air/water interface.[19,21] The amounts of γ-globulin and fibrinogen used in the mixture are higher than that of plasma concentrations, as reported elsewhere.[21] Experiments were run over a period of about 3 h. The films were taken out and were shaken and rinsed in three separate beakers filled with approximately 30 mL distilled water and a final rinse in flowing water (under a flow rate of 150 mL/min for 60 sec). A uniform rinsing procedure was adopted for all samples, which was necessary to achieve an irreducible level of surface concentration.[20]

The films were counted in a γ-counter and are reported as surface concentration of protein (μg cm^{-2}) computed from the equation[20]:

$$\lambda = \frac{C_p R_b}{A R_s},$$

where C_p = bulk concentration (μg mL^{-1}), Rb = count rate of surface, A = area of surface (cm^2), and Rs = count rate per mL protein solution. Values represent the mean of three separate experiments and most of them are within 95% confidence limits.

Proteins that had been preadsorbed for 3 h underwent exchange for 3 h in a protein mixture containing 0.25 mg/mL albumin, 0.15 mg/mL γ-globulin, and 0.075 mg/mL fibrinogen. The films were taken out with time and the surface albumin concentration was quantitated as described earlier.

Permeability Test for the Membranes

A dialysis chamber was used for determining the permeability of membranes to various compounds, as a function of time, at room temperature. The

membrane was clamped between the two compartments using multiple supporting and sealing devices. One compartment was filled with distilled water and the other with a mixtures of solutes containing urea (100 mg%, M_w, 60), creatinine (10 mg%, M_w, 113), uric acid (10 mg%, M_w, 168), inulin (25 mg%, M_w, 5,000), albumin (100 mg%, M_w, 69,000), K^+ (5 meq/L), and Na^+ (140 meq/L) in 0.05M phosphate buffer, pH 7.4. The permeability of solutes through the membrane was analyzed spectrophotometrically at intervals of 1, 2, 4, 6, and 16 h, employing a diacetyl monoxime reagent for urea,[22] alkaline picric acid for creatinine,[23] the Folin–Wu modified method for uric acid,[23] phenol–sulphuric acid for inulin,[24] and Folin and Ciocalteu's method[25] for albumin. The permeability percentages were then calculated from triplicate experiments. The degree of permeability was expressed as:

$$\text{Permeability (\%)} = \frac{\text{Concentration of the compound in the filtrates}}{\text{Concentration of the compound in the initial solution}} \times 100.$$

The mechanical properties of the wet membranes were also determined by the ASTM standard method protocol using a Chatillon Universal test stand model UTSE-2.[26] The membranes were dipped in water for at least 48 h and cut in the form of standard dumbbell-shaped specimens, having as length between the grips of 2.5 cm, a width of 0.5 cm, and employing a crosshead speed of 1 in/min. The tensile stress and the tensile strength (percentage of elongation) were calculated.

RESULTS AND DISCUSSION

Table I gives the inherent viscosity, percent water of hydration, and platelet adhesion to various modified chitosan membranes. It seems the inherent viscosity of the blended polymer varies with changes in PVA ratio in the blend. The percent water of hydration also increased with increase in PVA content in the blend. Thus, it is apparent that the solution blending of PVA with chitosan generates membranes having a large range of physical characteristics.

The results of platelet adhesion to various modified chitosan membranes are also shown in Table I. It appears that the rate of adhesion of platelets onto the PVA-blended chitosan membranes are similar. However, the number of adhering platelets seen on PGE_1-immobilized PVA-blended chitosan membrane surfaces have been apparently reduced compared with their bare-blended membrane and the standard cellulose membrane.

It is believed that heparin has anticoagulant properties and that their action may be dependent on a factor (presumably AT-III) present in the blood plasma.[27] Thus, it is conceivable that these substrates may bind AT-III from the plasma and are

Table I Inherent Viscosity, Percent Water of Hydration, and Platelet Adhesion to Various PVA-Blended Chitosan Membranes

Membranes[a]	Inherent[b] Viscosity	Percent of[c] Water of Hydration	Mean Platelets[d] ± SD
I. Chitosan bare (CM)	5.13	102 ± 7.5	28.2 ± 2.6
II. PVA-blended CM (2 : 8)	4.63	170 ± 7.1	27.9 ± 3.5
III. PVA-blended CM (3 : 7)	4.02	157 ± 18.0	27.7 ± 2.9
IV. PVA-blended CM (4 : 6)	3.73	181 ± 14.0	27.7 ± 3.0
V. PVA-blended CM (5 : 5)	3.40	173.6 ± 6.7	27.4 ± 3.0
VI. PVA bare	0.88	165 ± 7.4	26.5 ± 2.7
VII. PVA-blended CM (4 : 6)–Hep–GDT–PGE_1	—	175 ± 11.6	13.4 ± 2.4
VIII. Std. cellulose membrane	—	—	23.5 ± 3.1

[a] Paraformaldehyde-crosslinked PVA was mixed well with chitosan solution in various ratios and PVA-blended chitosan membranes were developed (membranes II–V). PVA-blended CM (4 : 6) was exposed to heparin for 3 h at room temperature and glow discharge treated under N_2 plasma for 15 min. Finally, the generated free radicals were used for coupling PGE_1 at 4°C overnight (membrane VII).

[b] 0.5 g % solution of the polymers in 2% acetic acid at 30°C.

[c] Water content was expressed as the ratio of the weight of water in the water-swollen membrane to that of the dry membrane.

[d] Values expressed as the average of the number of platelets attached to the surface per mm^2 with standard deviation (at least 30 observations from triplicate experiments).

Table II Thickness and Mechanical Properties of Various PVA-blended Chitosan Membranes

Membranes[a]	Thickness[b] (μm ± SD)	Tensile Strength[b] (kg/cm^2 ± SD)	Tensile Strain (% elongation) ± SD
I. Chitosan bare (CM)	141 ± 21	76.3 ± 6.2	83.6 ± 11.9
II. PVA-blended CM (2 : 8)	96 ± 6	94.1 ± 8.2	97.6 ± 14.3
III. PVA-blended CM (3 : 7)	93 ± 20	95.6 ± 10.9	107.6 ± 9.7
IV. PVA-blended CM (4 : 6)	124 ± 14	106.8 ± 3.1	115.8 ± 4.8
V. PVA-blended CM (5 : 5)	137 ± 21	110.6 ± 4.8	153.5 ± 21.4
VI. PVA bare	120 ± 12	127.9 ± 5.5	286.8 ± 27.3
VII. PVA-blended CM (4 : 6)–Hep–GDT–PGE_1	137 ± 11	104.6 ± 5.5	114.7 ± 4.6
VIII. Std. cellulose membrane	163 ± 3	163.4 ± 20.9	49.6 ± 10.0

[a] For membrane details, refer to Table I.
[b] Thickness, tensile strength, and strain were noted after dipping the membranes in water for about 48 h (wet membranes).

thus able to neutralize the activity of clotting factors generated at the blood–material interface. Grode et al.[28] demonstrated that surface-bound prostaglandin E_1 is capable of inhibiting platelet adhesion in comparison with control surfaces. Prostaglandins such as PGI_2, PGE_1, PGD_2, etc.[29] are believed to stimulate membrane-bound adenyl cyclase and thereby raise intracellular levels of cyclic AMP within platelets, resulting in an inhibition to platelet-surface binding. Thus, it seems these biomolecules—heparin and PGE_1—have substantially improved the blood compatibility of the PVA–chitosan-blended membrane, which may be due to their combined action, i.e., anticoagulant and antiplatelet functions at the interface.

The studies related to the mechanical properties of membranes under stress are of particular interest after various modifications since they relate to the ultimate tensile strength of the material and are a measure of its ability to hold, without rupturing, under the pressure of hemodialysis. Thus, the mechanical properties of various PVA–chitosan-blends and modified membranes under wet conditions have also been evaluated, as indicated in Table II. It appears that PVA blending improves the stress and strain characteristics of the membrane significantly. Standard cellulose membranes demonstrated maximum wet strength, but the strain was higher with PVA membranes. Thus, it is conceivable that the blending of chitosan with PVA has improved the

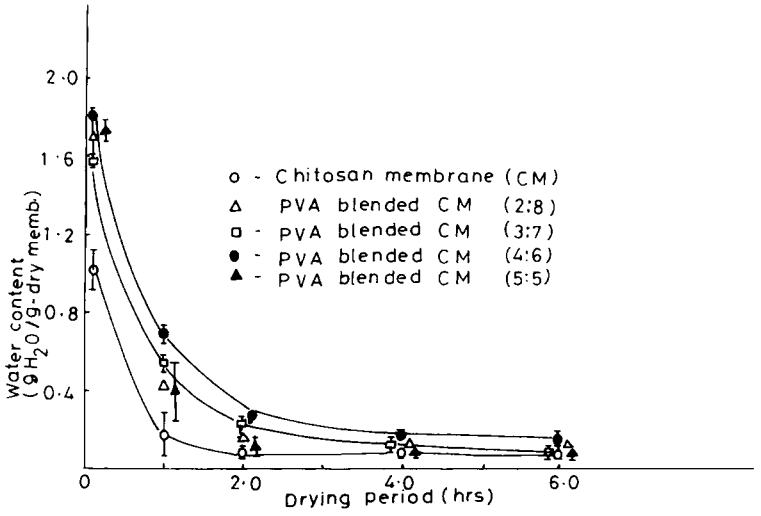

Figure 1 Effect of drying period and the dependence of water content for various PVA-blended chitosan membranes. I 95% confidence limits.

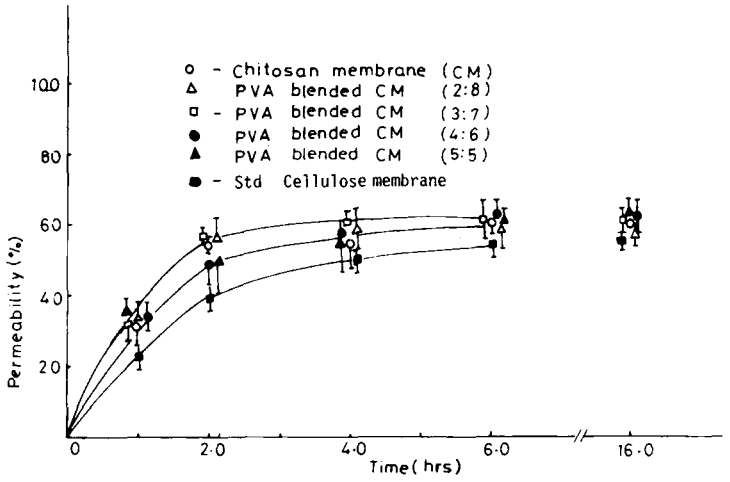

Figure 2 Permeability of urea through various PVA-blended chitosan membranes (from a mixture of urea creatinine, UA, inulin, alb, NaCl, and KCl pH 7.4). I 95% confidence limits.

mechanical properties, making the blend comparable to that of standard membranes. Table II also provides the thickness of various membranes investigated. It is evident that the thickness vary from 95 to 145 μm for chitosan membranes or PVA-blended membranes, and the standard membranes have 163 μm thickness.

The effect of drying period and the dependence of water content for various PVA-blended chitosan membranes are shown in Figure 1. All PVA-blended chitosan membranes demonstrated an increase in water content during the initial drying period and reached a constant value for all cases on drying them for 6 h. It appears the amount of water in the swollen membranes of PVA blends are higher compared to bare chitosan.

The permeability of various molecules—like urea, creatinine, uric acid, inulin, and albumin—through PVA-blended chitosan membranes, as a function of time, is shown in Figures 2–6, respectively. It appears that the permeability of all these molecules through the PVA-blended membranes is greater than for the unblended chitosan membranes. Moreover, the permeability of those molecules is higher in all the PVA-blended chitosan membranes compared to standard cellulose membranes, as is evident from Figures 2–6. PVA blended to chitosan in the ratio of 4 : 6 has been selected from the optimum mechanical thick-

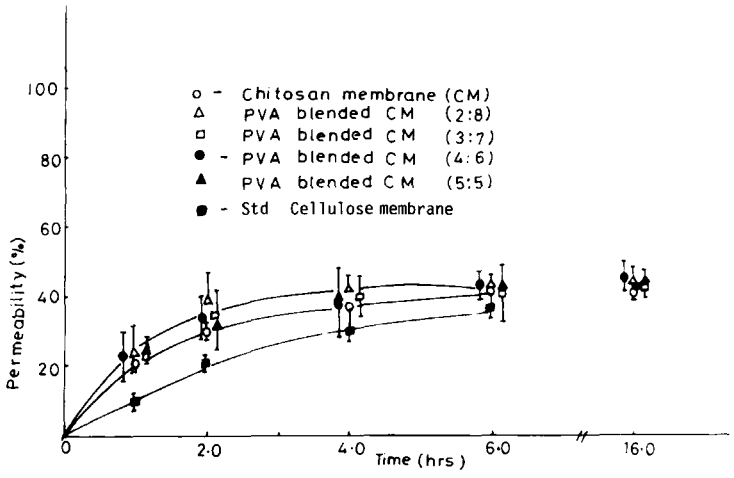

Figure 3 Permeability of creatinine through various PVA-blended chitosan membranes from a mixture of solutes. I 95% confidence limits.

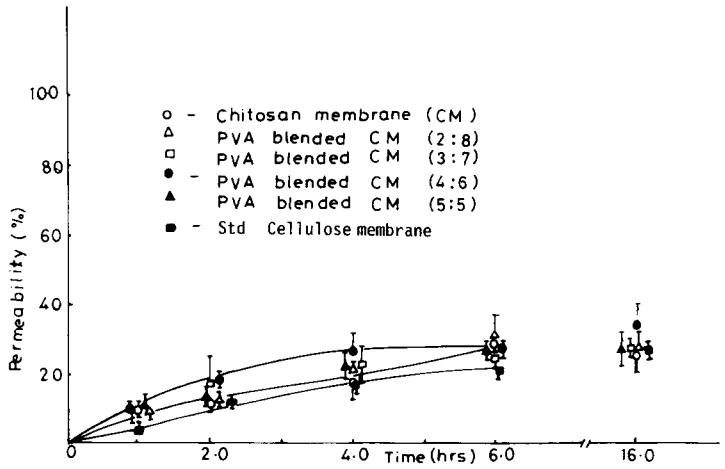

Figure 4 Permeability of uric acid through various PVA-blended chitosan membranes from a mixture of solutes. I 95% confidence limits.

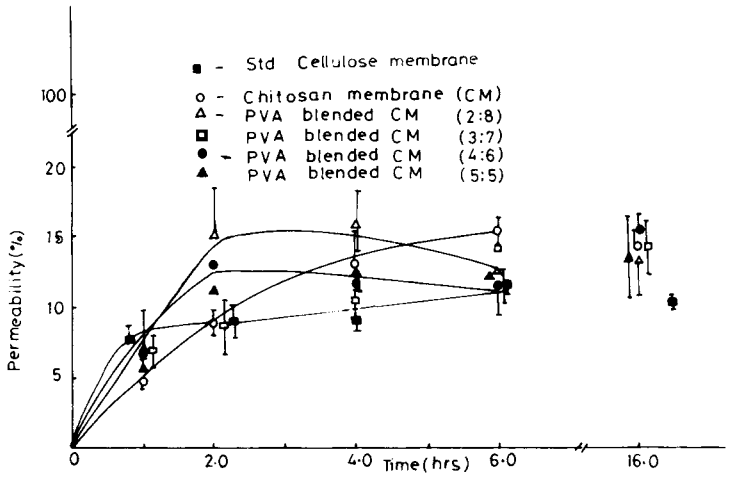

Figure 5 Permeability of inulin through various PVA-blended chitosan membranes from a mixture of solutes. I 95% confidence limits.

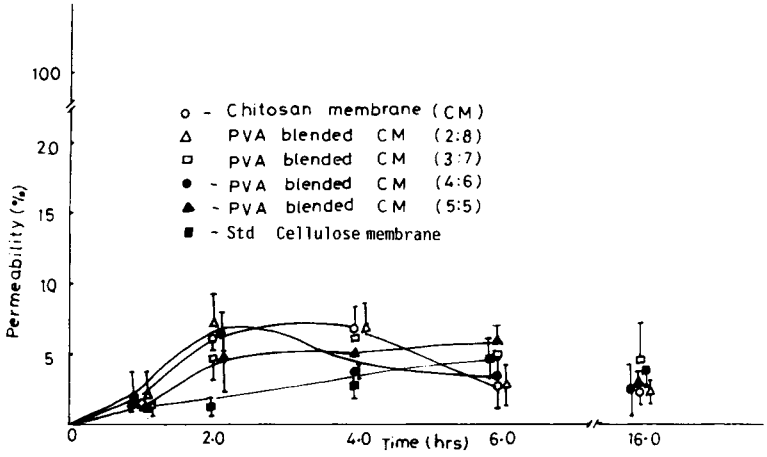

Figure 6 Permeability of albumin through various PVA-blended chitosan membranes from a mixture of solutes. I 95% confidence limits.

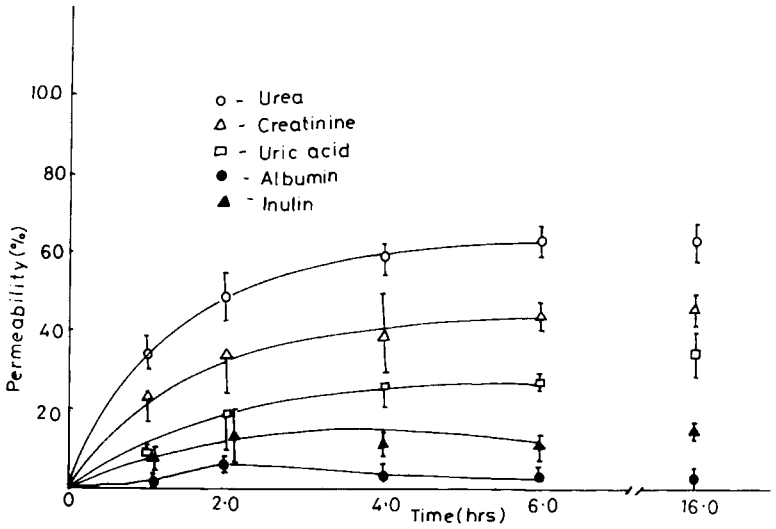

Figure 7 Permeability of various molecules through PVA-blended chitosan (4 : 6) membrane from a mixture of solutes. I 95% confidence limits.

ness and permeability properties of the membranes for further studies since these functionalities are very close to standard membranes.

The permeability of various molecules through PVA-blended (4 : 6) and PGE_1-immobilized to heparin-modified membranes, as a function of time, is summarized in Figures 7 and 8. It is evident from the studies that the biomolecule-modified membranes have similar permeability properties for small molecules compared to those of only PVA-blended membranes.

It appears the permeability properties for small molecules through all PVA-blended chitosan membranes do not vary significantly with varied ratio of PVA in the blend. However, the amount of initial water content of the swollen membranes and the percent of water of hydration have been different to these cases as is evident from Table I and Figure 1. It is assumed that water-soluble solutes diffuse only through water phases in water-swollen membranes.[30,31] Based on the free volume theory of diffusion, Yasuda et al.[30,31] indicated that the diffusive permeability of solutes through membranes is explained by water content. Many workers[30–32] have reported different states of water in membranes, referred to as "bound water," "free water," and "secondary bound water" or "intermediate water."

At the present, it is only clear that water-soluble

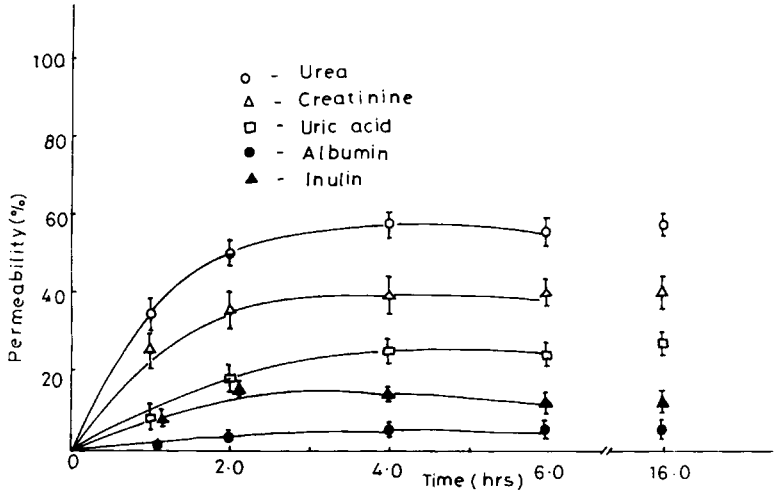

Figure 8 Permeability of various molecules through heparin–PGE_1-modified PVA-blended chitosan (4 : 6) membrane from a mixture of solutes. I 95% confidence limits.

solutes are difficult to diffuse through intermediate water regions and even more difficult to diffuse through bound water regions.[32] It seems the amount of free water allowing the diffusive permeation of solutes may be same in all the PVA–chitosan-blended systems, although the total water content varies, proposing a similar membrane permeability. However, further studies are needed to understand the relationship between the diffusive permeability of solutes through the membranes and the states of water in them.

The adsorption and desorption kinetics of albumin as a function of time to PVA-blended chitosan and PGE_1-modified substrates are indicated in Figures 9 and 10, respectively. Albumin is adsorbed rapidly and has reached a constant surface concentration within 1 h to the bare chitosan membranes and PVA-blended chitosan membranes; however, it has increased slowly up to 3 h with bare PVA. The adsorption of albumin to PGE_1-modified surface was very rapid and increased up to 24 h, as is evident from Figure 9. The desorption of albumin from 3-h preadsorbed substrates is shown in Figure 10. Preadsorbed albumin from the PGE_1-modified polymer substrate is not desorbed much compared to other surfaces. However, from the bare PVA and PVA–chitosan membrane systems the preadsorbed albumin is rapidly removed. It is possible that initially adsorbed albumin is removed by exchange with other proteins or with unlabeled albumin. Thus, it may be postulated that more stable layer of albumin exists on the PGE_1-modified substrate compared to other cases at all times.

The adsorption/desorption pattern of fibrinogen as a function of time is depicted in Figures 11 and 12, respectively. Fibrinogen is adsorbed rapidly up to 3 h and has slowly increased up to 24 h of study, as demonstrated in Figure 11. Fibrinogen–surface binding was highest with bare chitosan membranes; however, lower levels were observed with the other three cases. The desorption patterns of fibrinogen from various polymer substrates as a function of time are depicted in Figure 12. The initially adsorbed fibrinogen is subsequently removed from the surfaces or exchanged with the proteins in the media. Large amounts of fibrinogen is removed from chitosan membranes compared to other systems; however, lower fibrinogen levels always exist in bare PVA or PGE_1-modified cases. Thus, it is concluded that fibrinogen surface concentration on PGE_1-modulated substrate was lower compared to bare chitosan or PVA-blended chitosan membranes.

It has been extensively studied and indicated that the surface concentration is not affected by the tracer level and that the labeled protein acts as a tracer of the protein mixture.[19,20] Kinetics of protein adsorption studies indicated that PGE_1-immobilized substrate substantially reduced the surface–fibrinogen concentration from a mixture of proteins. This modified substrate also enhanced the albumin–surface concentration.

It is known that fibrinogen adsorption mediates

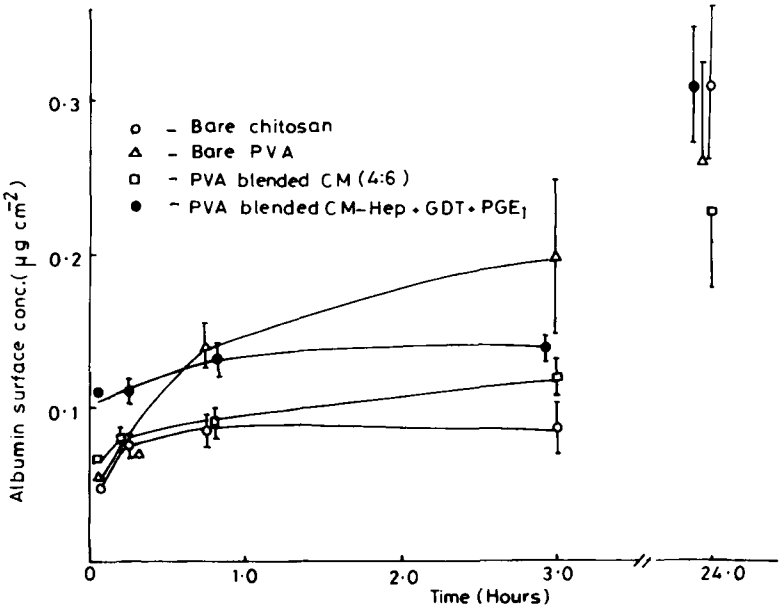

Figure 9 Adsorption of albumin on the various membranes containing blends of PVA : chitosan (4 : 6) from a mixture of proteins. I 95% confidence limits.

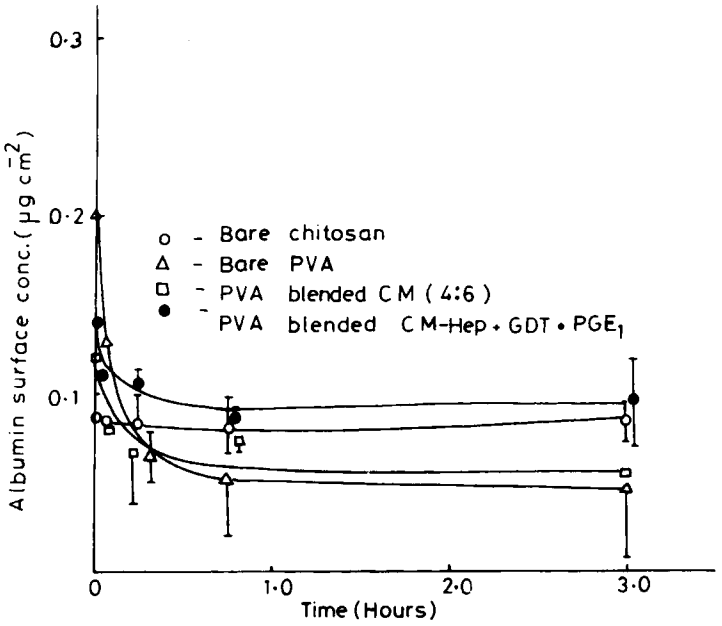

Figure 10 Desorption of albumin from the various membranes containing blends of PVA : chitosan (4 : 6) to a mixture of proteins. I 95% confidence limits.

platelet adhesion[5] and is a major factor in clot formation. On the other hand, albumin-coated surfaces have been shown to inhibit fibrinogen binding and reduce platelet adhesion. This increase in albumin–surface binding due to PGE_1-modified substrates appears to be good from the blood compatibility point of view.

In conclusion, the present studies suggest that

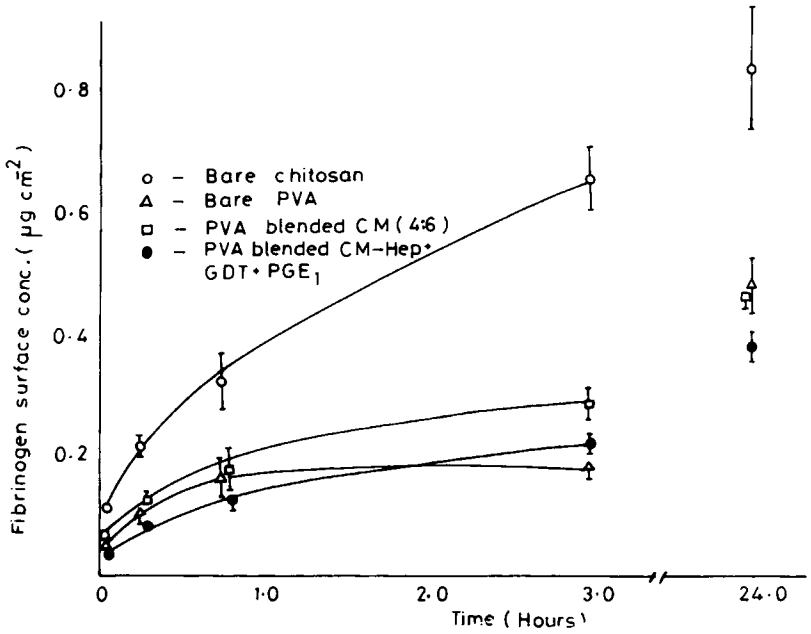

Figure 11 Adsorption of fibrinogen on the different membranes studied from a mixture of proteins. I 95% confidence limits.

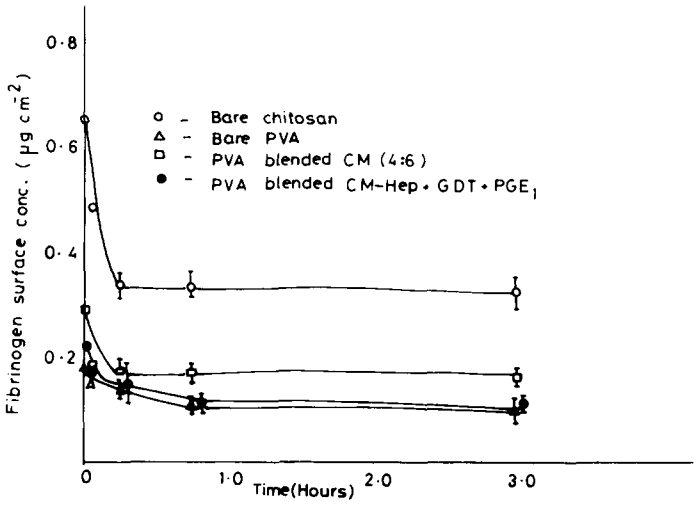

Figure 12 Desorption of fibrinogen from the different membranes studied to a mixture of proteins. I 95% confidence limits.

PVA-blended chitosan membranes have superior permeability for small molecules as compared to standard cellulose membranes with adequate mechanical properties. It also appears that membranes having biomolecules like heparin and PGE_1-immobilized on them via plasma treatments may have wider applications in the hemodialysis of patients by offering improved permeability and blood compatibility. This may also be useful for patients who are at risk of internal hemorrhaging on heparinization since a reduction or elimination of the use of soluble heparin during dialysis may be possible. Finally, it is suggested that the above modification using plasma treatments would be useful toward improving the blood compatibility of existing membranes.

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