Studies on Modified Chitosan Membranes. II. Dialysis of Low Molecular Weight Metabolites

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SYNOPSIS

The permeabilities of low molecular weight metabolites were determined through chitosan and a series of chitosan–poly(vinyl pyrrolidone) (PVP) membranes. The dialysis studies were carried out *in vitro* using a diaphragm-type test cell. The basic metabolites (urea, creatinine, and glucose) show higher permeation rates than do the acidic metabolites (uric acid and phosphate) through all the modified membranes. The hydrophilicity of the membranes, molecular weight, and chemical nature of the metabolites were important parameters in determining transport properties of the membranes. It was observed that higher permeation rates can be obtained by manipulating the amount of PVP in the blended membranes. The PVP weight loss in the aqueous medium was negligible.

INTRODUCTION

Chitosan is a biopolymer and consists of $\beta(1 \rightarrow 4)$-2-amino-2-deoxy-D-glucose) repeat units. It is normally obtained by the alkaline deacetylation of chitin, which is the second most abundant, naturally occurring structural material found in the shells of crustacea and cuticles of insects and also in the cell walls of some fungi and microorganisms. The crystalline structure of chitin is analogous to cellulose. The physical and chemical properties of chitin and chitosan have been studied by Muzarrelli.[1,2]

The film-forming properties of chitosan were recognized by several workers and different end uses of chitosan membranes have been proposed, e.g., in reverse osmosis, ion exchange, metal ions uptake, diffusion of dyes, and separation of water–alcohol mixtures.[3-8] Chitosan has also been modified by graft copolymerization and blending with water-soluble polymers.[9]

Many workers agree that any membrane that meets three basic criteria—biocompatibility, higher dialysis rates, and selectivity and strength—should be adequate from the standpoint of hemodialysis.[10] Chitosan membranes already possess some of the above-mentioned properties.[11]

In pursuit of fulfilling the requirements of an ideal hemodialysis membrane, an investigation of a preliminary nature is conducted. A dialysis test cell has been designed to study the permeabilities of low molecular weight metabolites (urea, creatinine, uric acid, phosphate, and glucose) through chitosan–PVP and chitosan membranes. The preparation and characterization of such membranes have already been described.[11] It is hoped that prospective modified membranes will be biocompatible and will exhibit higher dialysis rates.

EXPERIMENTAL

Materials

The degree of *N*-deacetylation and molecular weight ($\bar{M}_v$) of chitosan were 89.7% and 1.2×10^6 g/mol, respectively. Chemicals and reagents used in the spectrophotometric studies were AnalR or ACS reagent grade and were obtained from Aldrich and BDH Chemical Cos. All the metabolites were used as supplied. The dialysis test cell and other glassware were washed first with diluted HNO_3 and then with distilled water. All the membranes were stored in a

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Table I Molecular Weights of the Metabolites and Their Concentrations Used in the Membrane Permeability Evaluation

Sample No.	Metabolite	Molecular Weight (g/mol)	Concentration (mg %)
1	Urea	60.06	150
2	Creatinine	113.12	20
3	Phosphate[a]	136.09	10
4	Uric acid[b]	168.11	15
5	α-D-Glucose	180.16	150

[a] Prepared from KH_2PO_4.
[b] Solubilized with 14.4 mg % Li_2CO_3 to remove turbidity.

desccicator over P_2O_5 at room temperature. The wet thicknesses of the membranes were kept between 22 and 25 μm and were measured with a digital micrometer (RS Stock No. 601-906).

Preparation of Metabolite Stock Solutions

A set of five different metabolites were chosen for the evaluation of permeabilities of the membranes under investigation. The concentrations and their molecular weights are presented in Table I. The metabolite stock solutions were stored in a refrigerator at 3–4°C.

Dialysis Apparatus and Procedure

The dialysis permeability measurements were conducted using a continuous flow dialysis cell made up of two detachable glass compartments (Figs. 1 and 2). The membrane under investigation was placed in between the two compartments and the two sides were clamped together with plastic-coated clips. Silicone grease was used to avoid any leakage. In the left-hand side (lhs) of the cell metabolite, solution was placed and in the right-hand side (rhs), distilled water was circulated using a peristaltic pump (Watson-Marlow Ltd. MHRE 200) at a rate of 10 cm^3/min. The water in the rhs was pumped in an once-through pass and then allowed to drain. Both sides of the cell were stirred at 550 rpm using glass stirrers. The dialysis experiment was carried out at 37.0 ± 1.0°C in a waterbath (Grants Instruments Ltd., Cambridge, Type SS40). Before the start of each run, metabolite solution and the membrane was preconditioned at the required temperature and the test side (lhs) of the cell was washed twice with distilled water before loading a new test solution. Samples (0.5–1.0 cm^3) from the lhs were taken out every hour for 4 h and analyzed on a spectrophotometer. After each run, dialyzed solution was carefully siphoned out and its volume was measured for any osmosis or dilution effects. Care was taken not to stretch the membrane during mounting. Triplicate runs were made and the permeabilities were

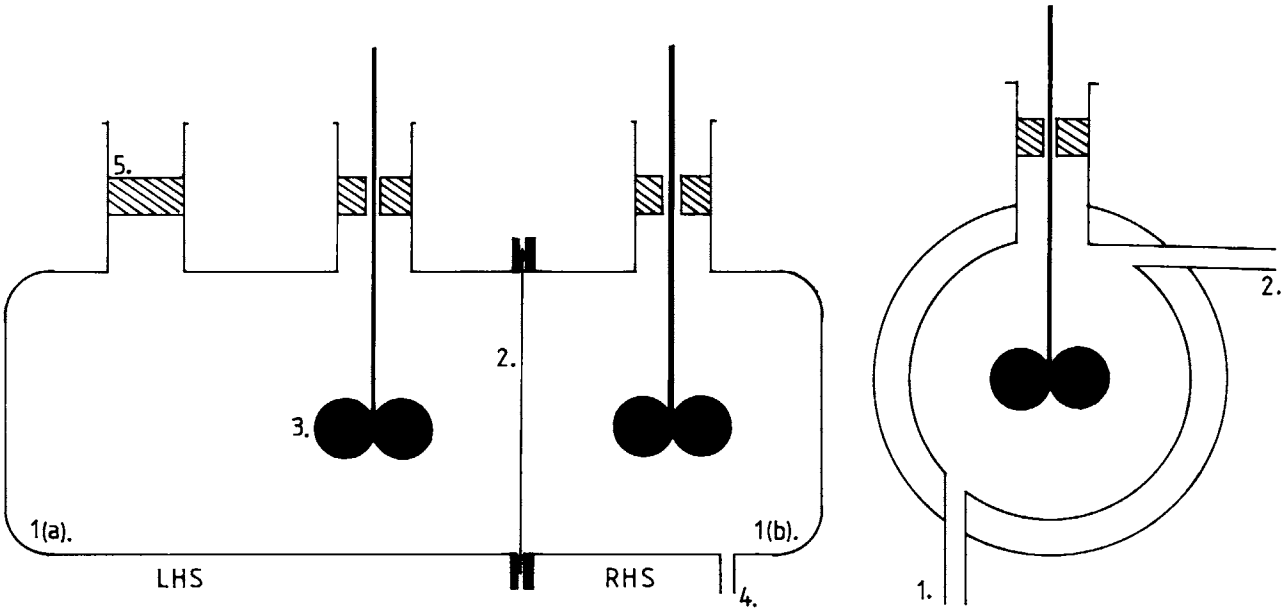

Figure 1 (a) Dialysis test cell: (1a) metabolite; (1b) distilled water; (2) membrane; (3) glass stirrer; (4) water inlet port; (5) glass stopper. (b) Side view of right-hand side of the test cell: (1) water inlet port; (2) water outlet port.

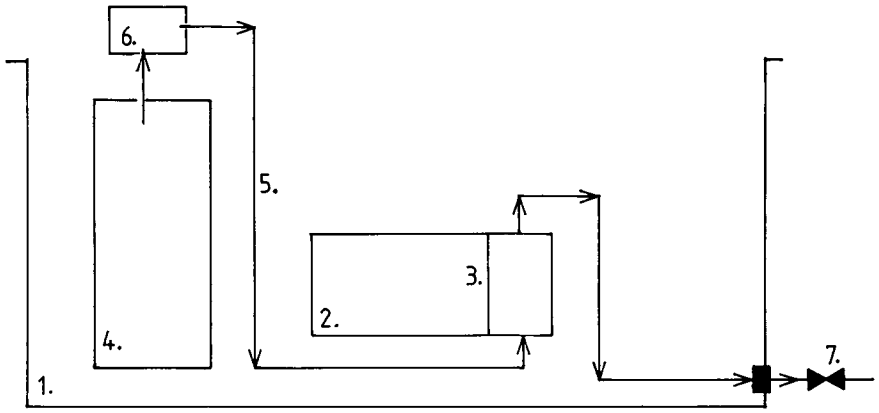

Figure 2 Dialysis apparatus: (1) water bath; (2) test cell; (3) membrane; (4) water reservoir; (5) silicone rubber tubing; (6) peristaltic pump; (7) drain control valve.

reported, as an average of two best runs, from the relationship[12]

$$\ln C_0/C_t = P \cdot A \cdot t/l \cdot V \qquad (1)$$

where C_0 = initial concentration of the metabolite (mg %); C_t = concentration at time "t"; P = permeability (cm^2/min); A = area of the membrane available for dialysis (9.62 cm^2); V = volume of the metabolite used (120 cm^3); t = dialysis time (min); l = wet thickness of the membrane (cm). The plots of ln C_0/C_t against time/membrane thickness were constructed and slope of the line was used to calculate permeability.

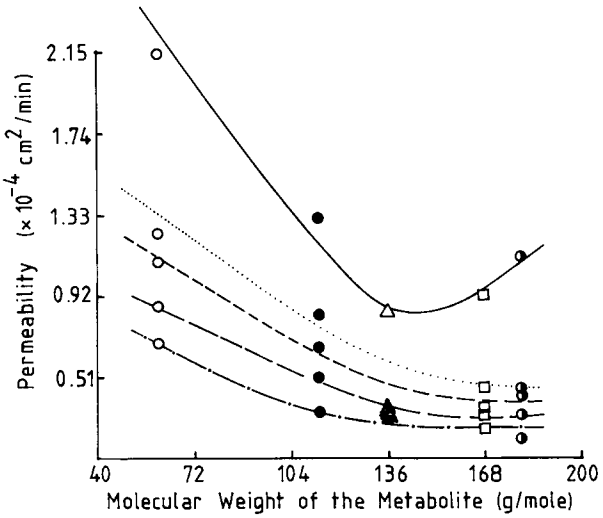

Figure 3 Permeability and the permeant–molecular weight relationships of (○) urea, (●) creatinine, (△) phosphate, (□) uric acid, and (◑) glucose for chitosan–PVP membranes (wt/wt): (-·-) 100/0; (– –) 80/20; (----) 60/40; (· · · · · · · · ·) 40/60; (——) 20/80.

The dialysis half-life, $t_{1/2}$ (min), was calculated from

$$t_{1/2} = k(l/P) \qquad (2)$$

where constant k = 8.65 cm.

Spectrophotometric Determination of the Metabolites

For the estimation of the metabolites, a Philips PU 8820 UV-VIS spectrophotometer was used. The absorbances of uric acid and creatinine were measured against distilled water as blank at 290 nm and 235 nm, respectively, whereas urea, phosphate, and glucose were estimated according to the methods given in the literature.[13]

RESULTS AND DISCUSSION

The permeability and permeant–molecular weight relationship for chitosan and different blended membranes is shown in Figure 3. Generally, permeabilities appear to decrease as the molecular weights of the metabolites are increased. Urea, due to its lowest molecular weight, has faster dialysis rates than does any other metabolite through all the five membranes. Uric acid diffuses less rapidly. The permeabilities of phosphate more or less remain unchanged except for the 20/80 wt/wt† blend. It is difficult to establish a general trend in permeation characteristics except for basic metabolites: urea,

† In this paper, the percentage of chitosan in the blends is always given first.

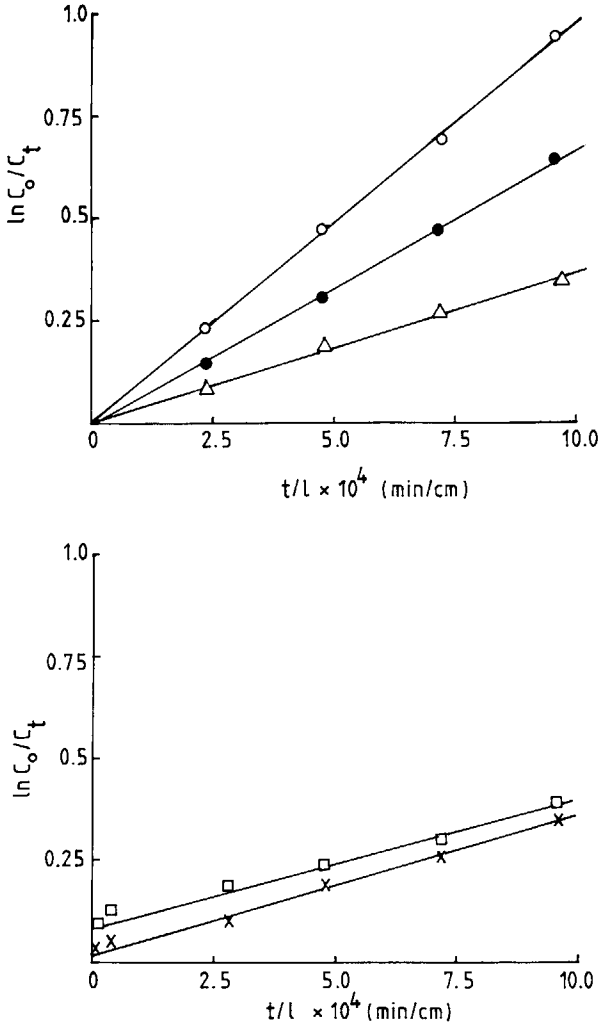

Figure 4 (a) and (b) Typical dialysis rates of (○) urea, (●) creatinine, (△) glucose, (**X**) uric acid, and (□) phosphate through a chitosan–PVP membrane.

creatinine, and glucose. All these show a steady decline in dialysis rates as the molecular weight increases [Fig. 4(a)]. On the other hand, acidic metabolites (uric acid and phosphate) show unsatisfactory behavior. Dialysis rates in Figure 4(b) clearly indicate that during first hour, after the addition of the test solution, large quantities of the acidic metabolites are adsorbed on the surface of the membrane (indicated by the presence of an intercept and deviation from linearity), followed by a slow diffusion. This could be discussed in terms of their chemical nature and physiochemical interactions with the membrane. It is known that the solubility of a compound plays an important role in determining its permeability. Uric acid is sparingly soluble in water, and its solubility is improved by adding Li_2CO_3 salt, whereas phosphate solution, prepared from KH_2PO_4, is inorganic in nature and possesses anionic character. It may be that both the metabolites interact with the chitosan membrane, which is basic in nature, and increase "residence time" during diffusion through the membrane. The improvement in the permeabilities of these acidic metabolites are observed only when PVP above 60% is present in the blends. This is because PVP itself has a basic character and further enhances the basicity of the blends. In contrast, the 20/80 wt/wt blend shows a large improvement in phosphate and uric acid permeation rates due to a considerable increase in the hydrophilic character.

The dialysis half-lives and permeant–molecular weight relationships, calculated from eq. (2), are shown in Figure 5. The $t_{1/2}$ values appear linearly proportional to the molecular weights of the metabolites except for acidic metabolites. The general trend in dialysis half-lives is glucose > creatinine > urea. Phosphate and uric acid show higher $t_{1/2}$ values due to their strong anionic nature and poor solubility, respectively.

Effects of the Blending and Water Contents

Figure 6 shows that the dialysis rates of urea, creatinine, and glucose almost linearly increase as the amount of PVP in the blends is raised, except for acidic metabolites. Addition of 25 wt % PVP into the casting solution improves the permeabilities of uric acid and glucose and levels them with the permeation rate of phosphate. Further addition of PVP significantly increases the dialysis rates of glucose, but phosphate and uric acid show marginal improvements. An abrupt increase in the permeabili-

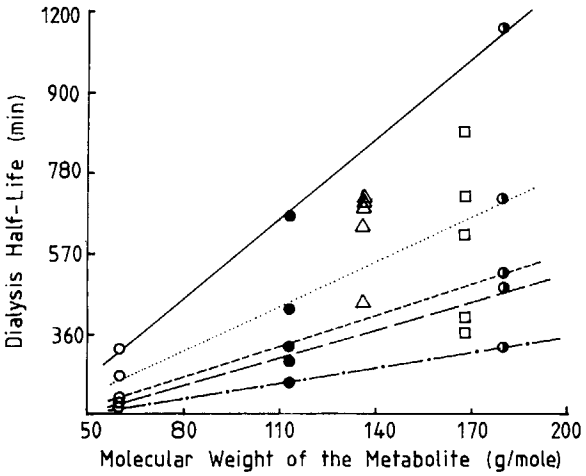

Figure 5 Dialysis half-life and the permeant–molecular weight relationships of (○) urea, (●) creatinine, (△) phosphate, (□) uric acid, and (◑) glucose for chitosan–PVP membranes (wt/wt): (-·-) 100/0; (– –) 80/20; (----) 60/40; (·····) 40/60; (——) 20/80.

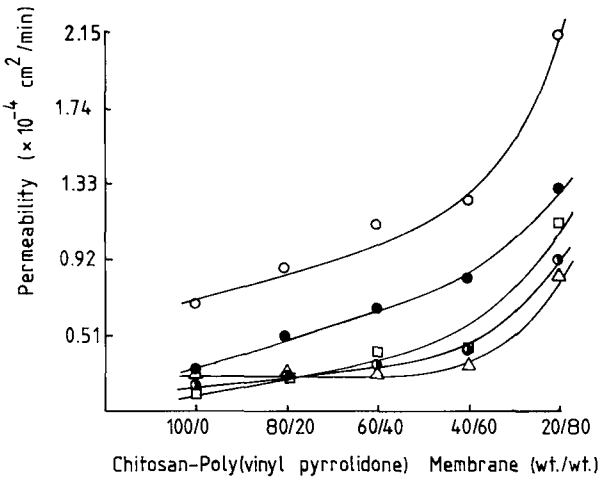

Figure 6 Effect of membrane composition on the permeabilities of metabolites: (○) urea; (●) creatinine; (△) phosphate; (◑) uric acid; (□) glucose.

ties of all the metabolites is observed when 20/80 wt/wt membrane is employed. The increased permeation properties of the blends can be seen in terms of improved hydrophilic character and formation of more porous structure.

The water-absorption property of a membrane is described in terms of its water contents.[11] According to Figure 7, when water contents are improved by 64% (on moving from chitosan to 40/60 wt/wt membrane), the permeabilities of urea, creatinine, and glucose increase by 82, 148, and 137%, respectively. There is also an 80% increase in the permeation of uric acid. However, there is only 11.4% improvement in the permeability of phosphate. The water contents of 20/80 wt/wt membrane show a 76% improvement, with respect to chitosan, which causes above a 200% increase in the permeabilities of all the metabolites apart from phosphate, which shows only a 167% increase in its permeability.

To envisage the effect of blending on dialysis rates of the metabolites, percent improvement is calculated according to the following relationship:

$$\% \text{ Improvement in the permeability of the metabolite} = (P_c - P_m/P_c) \times 100 \quad (3)$$

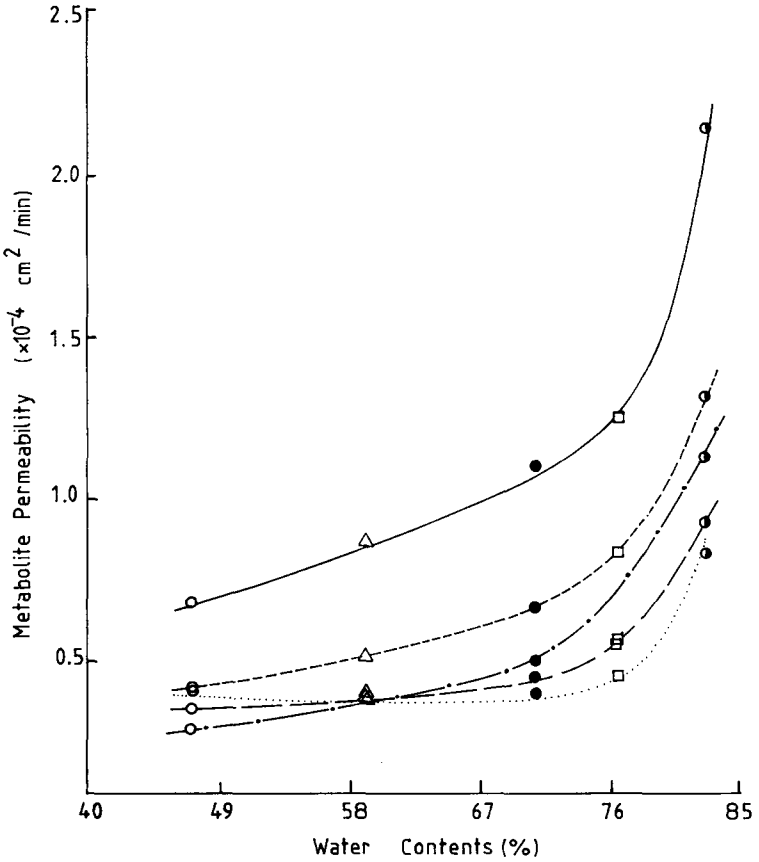

Figure 7 Dependence of the permeabilities of (——) urea, (- - - - -) creatinine, (· · · · · · · · ·) phosphate, (– –) uric acid, and (– · –) glucose upon the water contents of chitosan–PVP membranes (wt/wt): (○) 100/0; (△) 80/20; (●) 60/40; (□) 40/60; (◑) 20/80.

where P_c and P_m are the permeabilities of a given metabolite through chitosan and modified chitosan membranes, respectively. These percent improvements are shown in Figure 8, which indicate that superior dialysis rates are achieved by blended membranes when compared with chitosan.

The dialysis half-life is another important parameter on which the performance of a given membrane is compared with others. Figure 9 shows half-lives of the metabolites decrease as blends of higher PVP contents are employed. Urea, being the fastest, shows the lowest $t_{1/2}$ values, due to its lower molecular weight. On the other hand, glucose, due to its high molecular weight, takes the longest time to reduce its concentration by half; hence, it shows the highest $t_{1/2}$ values. The general trend varies for different metabolites, but for basic metabolites, the trend, for dialysis half-lives, in increasing order is urea < creatinine < glucose. Phosphate takes a relatively longer time in diffusing through all the blends except for the 80/20 wt/wt membrane. The time required for glucose, uric acid, and phosphate for permeating through the 80/20 wt/wt blend is the same. A similar explanation can be given here to that which was described earlier for permeability and permeant–molecular weight relationships.

CONCLUSIONS

Chitosan membrane exhibited superior dialysis rates toward uric acid and phosphate, but it was comparatively less permeable toward urea, creatinine, and glucose. The relationship between permeability and permeant–molecular weight appeared inversely proportional, indicating selectivity and semipermeability of the membranes toward each metabolite.

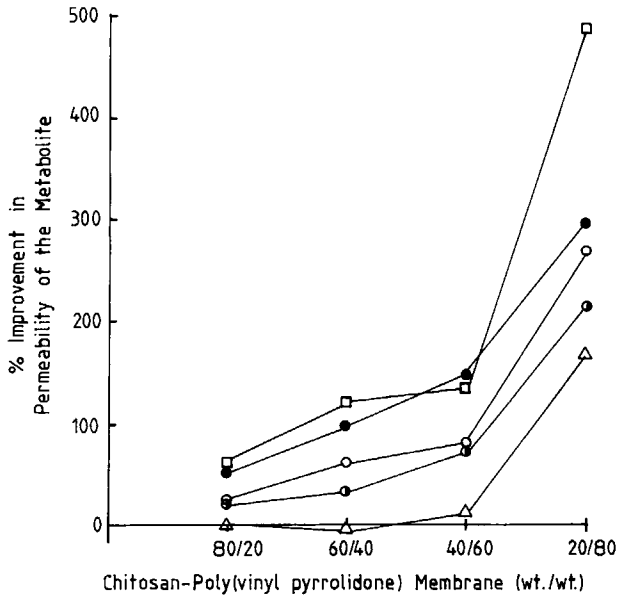

Figure 8 Percent improvements in the permeabilities of (○) urea, (●) creatinine, (△) phosphate, (◑) uric acid, and (□) glucose through modified membranes compared with chitosan.

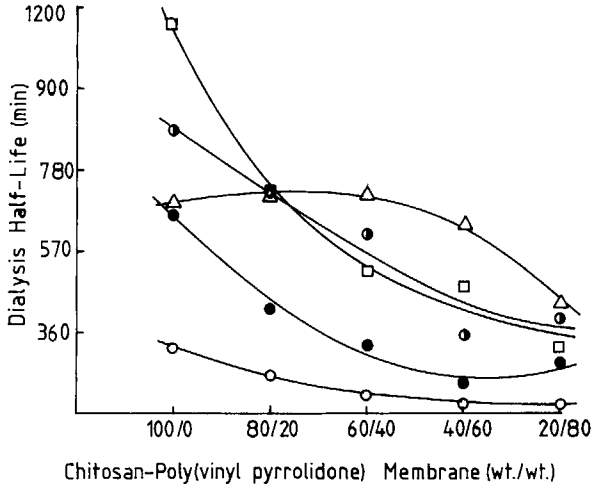

Figure 9 Effect of membrane composition on the dialysis half-lives of (○) urea, (●) creatinine, (△) phosphate, (◑) uric acid, and (□) glucose.

Higher permeabilities of basic metabolites and uric acid were observed through all the blended membranes other than chitosan except for the 20/80 wt/wt blend. Phosphate dialyzed at a slower rate due to its acidic nature and a possible interaction with the membranes. Superior permeation rates of phosphate and uric acid were achieved through blending (above 40% improvement observed for both the metabolites). Dialysis rates of all the metabolites increase as the amount of PVP in the blends was raised due to a corresponding increase in hydrophilicity and loss of the crystalline structure to some extent.

The study indicates a possible use of modified chitosan membranes in hemodialysis. To further investigate the potential of application of these membranes, more work is in progress.

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