Permeability of Vitamin B-12 in Chitosan Membranes. Effect of Crosslinking and Blending with Poly(vinyl alcohol) on Permeability

SHUJI NAKATSUKA and ANTHONY L. ANDRADY*

Department of Polymer Science, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709

SYNOPSIS

The permeability and diffusion of vitamin B-12 in chitosan, crosslinked chitosans, and chitosan/poly(vinyl alcohol) (PVA) blends were studied using "lag time" technique. Apparently the diffusion coefficient, D, for both crosslinked and blended chitosan membranes depends solely upon the equilibrium swelling ratio, Q, of the material in water. The functional dependence of D on Q was obtained from the data. The partition coefficients calculated for vitamin B-12 in all membranes studied were nearly constant ($K \approx 0.4$). The results are shown to be consistent with the "pore type" transport mechanism for vitamin B-12 in these chitosan membranes.

INTRODUCTION

Chitin and chitosan represent the second largest class of naturally occurring polymers. Chitosan (β-(1-4)-2-amino-2-deoxy-D-glucose), derived from chitin by deacetylation, is a unique basic polysaccharide. While the structure of chitosan is generally represented as a homopolymer, the deacetylation process is rarely complete and most commercial and laboratory products tend to be a copolymer of chitosan repeat units and repeat units typical of chitin, that is (β-(1-4)-2-acetamido-2-deoxy-D-glucose). The copolymer ratio depends on the source and preparation of chitosan but the glucosamine units predominate. The hydrophilicity of the polymer, due to amine functionalities in most repeat units, makes the polymer soluble in dilute acid solutions and yields a rubbery hydrogel in water.

Hydrogels in general have high biocompatibility and are good candidates for use in medical implant devices, wound dressings, and as matrices for the controlled delivery of drugs such as antibiotics,[1] steroids,[2-4] narcotic antagonists,[5] and others.[6,7] Chitosan and modified chitosan hydrogels[8-10] have some advantages in this regard, because of their nontoxicity and, being biopolymers, their biocompatibility and biodegradability. The ability of chitosan to swell in water into a soft rubbery consistency, resembling body tissue, may enhance its biocompatibility[10-12] and make it a good matrix for hydrophilic pharmaceuticals, which permeate only poorly in silicone matrices popularly used for drug delivery.[13] Films of chitosan have good mechanical properties[14,15] and their swelling and equilibrium stress-strain properties have been reported.[16] Hirano et al.[17] discussed the permeability of chitosan gels; the permeability of the membrane to pharmaceuticals has been reported. Sawayanagi et al.[18] studied the permeation of a series of drugs through chitosan hydrogel membranes and found the permeation rates to decrease with increasing molecular volume of the drug. The decrease was linear in the range of molecular volumes of 270 to 350 mL/mol.

In general, permeation in hydrogels is described in terms of two mechanisms: the pore mechanism[3,4,19] and the partition mechanism. These mechanisms may not operate exclusively, but one may be expected to dominate for a given drug/membrane pair. In those hydrogels where the "partition" mechanism predominates, the solute dissolves in the polymer itself and progresses across the membrane via diffusion in the polymer fraction.

* To whom correspondence should be addressed.

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Solubility parameters for the system determine the permeability for the most part in such a model based on solution–diffusion.[3,4,19]

In the "pore" mechanism, the solute is presumed to diffuse through microchannels within the membrane structure. The permeability then would be determined by the average pore size in relation to the molecular volume of the solute and the water solubility of the solute.[3,4,19] Not being a glassy system, these water-filled "pores" or channels are fluctuating in volume and are not fixed in definite locations. In spite of its macroscopic homogeneity, biopolymer gels such as chitosans are likely to have bound as well as free water. Water in biological polymers is proposed to exist as bound, free, and interfacial water.[20] A similar model has been forwarded for synthetic hydrogels by Andrade et al.[21] Diffusion of solute in the pore model might be expected to occur primarily via the bulk-like water within the hydrogel.

The object of the present work is to study the mechanism of small-molecule transport across chitosan matrices and to determine the effect of crosslinking, and blending with compatible synthetic polymers upon the permeation characteristics. Vitamin B-12 will be used as the model compound in the study. While some work has been reported on the permeation of model compounds (including vitamin B-12)[22] through chitin and chitosan, no information on the effect of modifying the matrix with crosslinking is available in the literature. Variation of the diffusion coefficient, the partition coefficient, and the swelling ratio with crosslinking and blending of chitosan with poly(vinyl alcohol) was studied in the present effort.

EXPERIMENTAL

Membrane Preparation

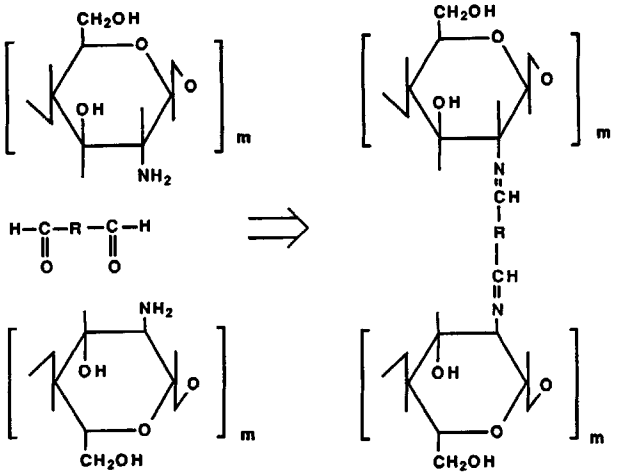
Chitosan solutions were prepared by dissolving about 3–4 g of chitosan flakes (Protan Inc., Commack, NY) in about 100 mL of 0.75% aqueous acetic acid solution at ambient temperature with stirring overnight. The solution was filtered through a cheese cloth and then through a 70 μm mesh filter in order to remove undissolved chitosan and debris. The mass fraction of chitosan in the resulting solution was 0.031. The solution was degassed under vacuum and cast on a clean glass plate. Drying of the cast film was carried out in an air oven at 60°C for 20 h. The dry films of chitosan easily separated from the glass surface and were immersed in a mixture of 2 N aqueous sodium hydroxide and methanol (1 : 4 v/v), to remove residual acid. The chitosan membrane was repeatedly washed with several portions of distilled water to remove all traces of alkali and was stored at 5°C in deionized water until use.

Crosslinking

Discs (~ 22 mm in diameter) of water-swollen chitosan membranes were placed in a solution (10 mL) of either gluteraldehyde (Aldrich Chemical Co.) or glyoxal (Aldrich Chemical Co.) in distilled water, and were allowed to react for a period of 20 h. In the case of gluteraldehyde solutions, the initial and the final concentrations were measured by UV spectrophotometry (Gilson Model HM Holochrome) using the absorption band at 280 nm. This allowed the calculation of the amount of crosslinking agent incorporated per mole of repeat unit of chitosan, assuming that all such units were glucosamine. In the case of glyoxal which cannot be accurately monitored spectrophotometrically, the actual amounts of crosslinking agent which reacted with the chitosan were not known.

The concentration of the dialdehyde solution was varied to obtain membranes with different crosslink densities. With gluteraldehyde, the concentration range used was 40 ppm to 150 ppm, and for glyoxal it was 15 ppm to 150 ppm.

The reaction leading to crosslink formation was as follows:



$R = (CH_2)_n$: $n = 3$ for Gluteraldehyde; $n = 0$ for Glyoxal

Blend Preparation

Poly(vinyl alcohol) PVA (Sigma Chemical Co.), with an average of M_n of 10,000 was used to prepare chitosan/PVA blends. Aqueous PVA solutions

(5.0% w/w) were mixed with chitosan solutions (3.1% w/w in 0.75% acetic acid) in appropriate proportions to obtain a series of blends. The two solutions were readily miscible in all proportions at ambient and they yielded clear films. The resulting solution was used for membrane preparation as described previously in the "Membrane Preparation" section. The thicknesses of the water-swollen membranes were measured using a micrometer with an accuracy of ± 1 μm.

Swelling Measurements

Discs ($\sim$ 22 mm in diameter) of water swollen chitosan membrane were equilibrated overnight in phosphate buffered saline (PBS) solution of pH 7.4 at 23 $\pm$ 1°C. The membrane samples were then removed, quickly blotted with tissue to remove excess surface water, and weighed immediately in a microbalance (0.1 mg accuracy). The membrane was then placed in a large volume of deionized water to remove the buffer salts and dried in an air oven at 80°C for 24 h. The swelling ratio, Q, was calculated as

$$Q = (\text{weight of swollen sample})/(\text{weight of dry sample}) \quad (1)$$

This procedure was repeated several times to obtain an average value of Q for each sample type.

Determination of Permeability and Diffusivity

Vitamin B-12 (5,6 dimethylbenzimidalyl cyanocobamide) (Sigma Chemical Co.), (molecular weight 1355) was dissolved in PBS at a concentration of 1.0% by weight. This solution was used in all membrane transport experiments.

The experimental set up is illustrated in Figure 1. It essentially consists of a diffusion cell fabricated from a block of Teflon and made up of two chambers separated by the hydrogel membrane. The upper (donor) chamber contains the vitamin B-12 solution and is unstirred. The lower (receptor) chamber contains a rotating magnetic stirrer and has a volume of 0.90 mL. A buffer solution sweeps the lower chamber continuously and is monitored using a continuous recording UV spectrophotometer (measuring absorbance at 361 nm) for vitamin B-12 permeating through the membrane. The effective diffusional area of this cell was 2.10 cm^2.

In a typical run, a disc of swollen chitosan membrane, approximately 22 mm in diameter, was mounted between the donor and receptor halves of the cell. A steady flow of buffer solution was maintained through the lower receptor half of the cell and its flow rate was adjusted to 0.02 mL/s using the variable pump settings. Particular care was taken to ensure that no air bubbles existed in any part of the receptor half of cell, in the teflon tubing, or within the spectrophotometer. A known volume of vitamin B-12 solution (1 mL) was then introduced into the upper half of the cell and the increase in absorbance due to permeation of solute was continuously recorded. The determination of diffusion coefficients using the so-called "lag-time" method was employed.[23-24]

Determination of Distribution Coefficient

A disc ($\sim$ 22 mm in diameter) of uncrosslinked chitosan membrane was placed in a 1.0% (w/w) solution of vitamin B-12 for a period of 48 h at ambient. The membrane was removed, quickly blotted on the outer surface to remove excess vitamin B-12 solution, and placed in a known volume of PBS (100 mL) at ambient. After 48 h of equilibration, the

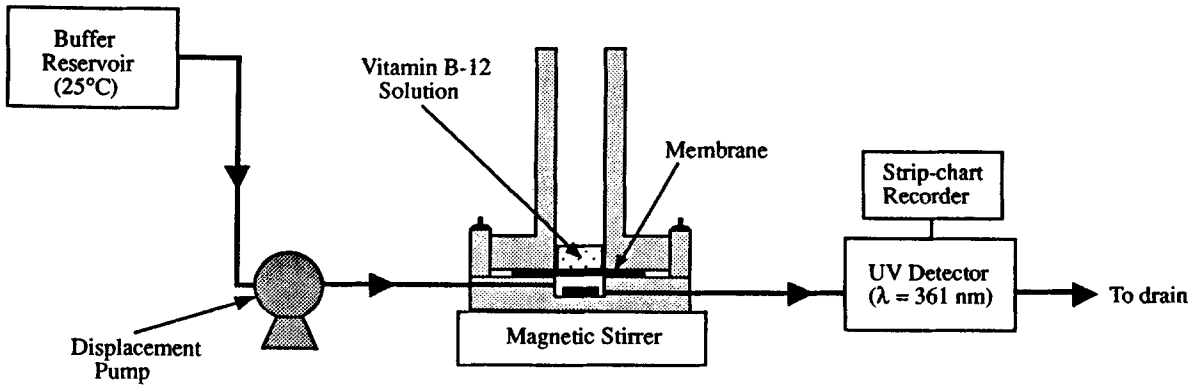


Figure 1 Schematic diagram of the experimental apparatus used for permeation studies.

concentration of vitamin B-12 in the buffer (C_2) was determined spectrophotometrically. Membrane was then placed in vitamin solution again. From known concentrations of vitamin B-12 after equilibration with chitosan membrane (C_1), it was possible to estimate the amount of solute sorbed into the swollen membrane. The distribution coefficient for the system was calculated as follows:

$$K_d = \frac{C_2 V}{V_p(C_1 - C_2)} \tag{2}$$

where V and V_p refer to the volume of the buffer and of swollen chitosan membrane, respectively. The K_d is called the experimentally determined distribution coefficient to distinguish it from the partition coefficient, K, calculated from D and P values obtained in lag-time experiments.

RESULTS AND DISCUSSION

Figure 2 shows the variation of the concentration of vitamin B-12 permeating through chitosan hydrogel membranes with time, for three different membrane thicknesses, h. Concentration of the solute on the permeate side of the membrane gradually increased until that in the membrane became constant (i.e., until steady state conditions were obtained). The time-dependence of the solute concentration within the membrane is well established for a Fickian system. When the steady state has been reached, increase in the amount of diffusant, M, with time, t, is given by the expression[24]:

$$M = \frac{DC_0A}{h}\left(t - \frac{h^2}{6D}\right) \tag{3}$$

where C_0 is the concentration of the solute on the high-concentration surface of the membrane, h is the membrane thickness, A is the membrane area and D is the diffusion coefficient. A plot of cumulative amount of solute permeated, which is essentially obtained by integrating the time-dependent concentration curve in Figure 2, is linear in time under steady state conditions. The intercept of this part of the curve, or "lag time" $\frac{h^2}{6D}$, allows the calculation of the diffusion coefficient, D. Typical plots used in the lag-time calculation for various thicknesses of chitosan membranes are shown in Figure 3. The steady state portion of the curve used to determine lag-time showed a high degree of linearity for all samples (correlation coefficient > 0.99).

At steady state, the concentration of the solute changes linearly across the membrane ($C_{m1} > C_{m2}$), where C_{m1} and C_{m2} are the concentrations of solute at the donor and receptor surfaces of the membrane.

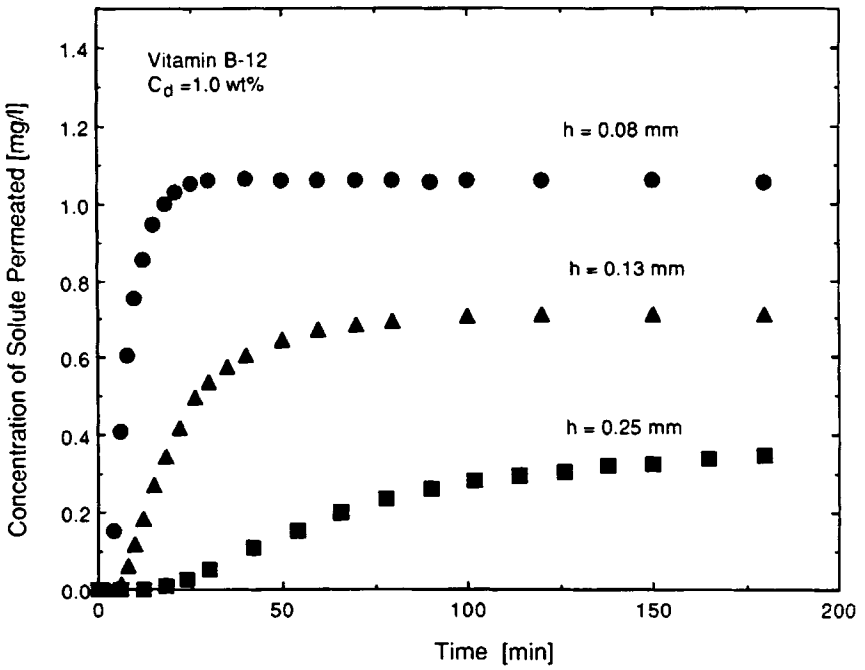


Figure 2 Variation of concentration of vitamin B-12 in permeate with time, for various thicknesses of chitosan membranes.

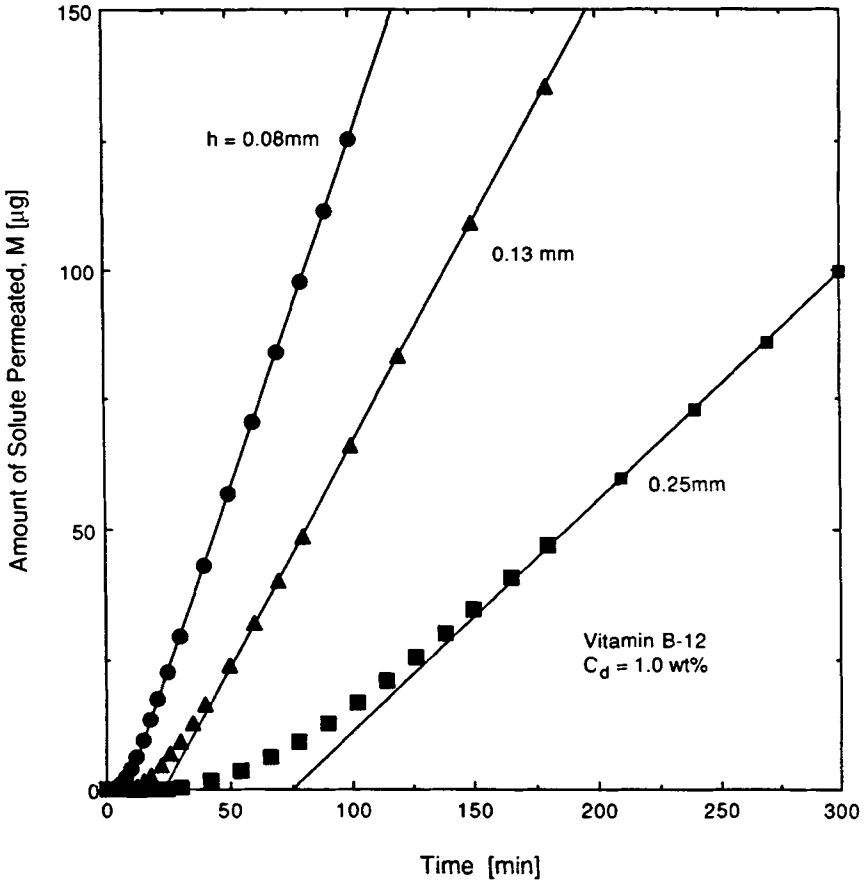


Figure 3 Variation of cumulative amount of vitamin B-12 in permeate with time, for various thicknesses of chitosan membranes.

Provided the partition coefficient, K is independent of the concentration, the flux of solute across the film, J, might be written in terms of concentrations of the solute in the two compartments of the cell as follows.

$$J = \frac{1}{A} \cdot \frac{dM}{dt} = \frac{DK}{h} (C_d - C_r) \tag{4}$$

$$P = D \cdot K \tag{5}$$

The concentrations C_d and C_r of the solute are for the solutions in donor and receptor compartments of the cell, respectively. This equation yields the permeability, P, provided the concentration gradient, flux under steady state conditions, and the swollen membrane thickness are known. The eqs. (3), (4), and (5) were used to calculate D, K, and P from the data.

Equation (4) also requires the steady state flux J_s to be inversely proportional to the thickness of the membrane. This relationship will be modified under experimental conditions where a boundary layer effect is obtained. In this case the relevant relationship is of the form,

$$\frac{1}{J_s} = \frac{1}{P\Delta C} (h + PR_b) \tag{6}$$

where R_b is the boundary layer resistance and $\Delta C = C_d - C_r$.

In systems where a boundary layer develops on either surface of the membrane, the flux will be modified, and such a plot will yield a positive intercept on the vertical axis. As shown in Figure 4, the present data showed $1/J_s$ to be linearly dependent on h, with curve passing through the point of origin. This indicates a negligible effect of a boundary layer.

Crosslinked Chitosan Membranes

Crosslinking a polymer matrix generally leads to a reduction in permeability to solutes. This decrease

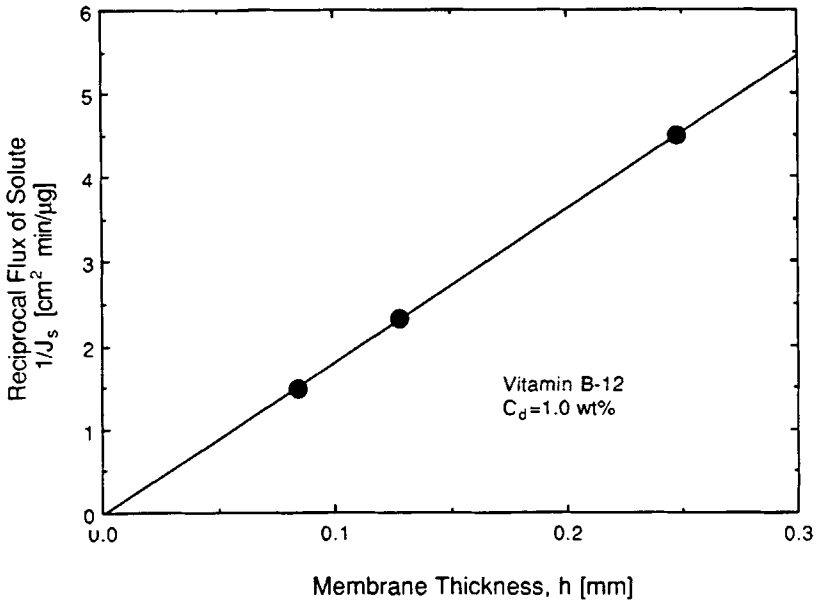


Figure 4 Relationship between the reciprocal of steady-state flux and membrane thickness.

may be a result of a number of causes, including reduced diffusion coefficient, decreased crystallinity due to the introduction of junction points, or even the chemical modification of the original polymer matrix by the altered chemistry at crosslink points. In the case of hydrogels where the permeation occurs via a "pore" mechanism, the reduction in water uptake by the membrane accompanying increased crosslink density is likely to be an important factor.

The crosslinking of the chitosan membranes was carried out using gluteraldehyde at room temperature while the membrane was in the swollen state. Some of the very highly crosslinked samples were too brittle for testing in the swollen state and only those networks containing 1.3 to 7.1 mole percent of the dialdehyde were investigated. Figure 5 shows typical lag-time curves obtained for crosslinked chitosan networks. As expected, the amount of solute permeated at any given time decreased with increasing crosslink density. The values of D, K, and P for these networks, obtained from experimentally determined lag-times, are listed in Table I.

As with cellulose substrates[3,25] that are structurally similar to chitosan, the mechanism of permeation in chitosan is likely to be predominantly of the "pore" type. This is supported by measurements of distribution coefficient of swollen chitosan membrane using an equilibration technique as described in the "Experimental" section. The average value of the coefficient obtained was 0.36, indicating there was little, if any, preferential dissolution of the solute in the polymer phase. This value is very close to K values given in Table I. This is not surprising as K should be the same as K_d in the absence of a boundary layer. Values less than unity have been interpreted as indicating transport via a "pore" diffusion mechanism.[3,4] Furthermore, as seen from Table I, the partition coefficient, K, is not significantly affected by crosslinking, suggesting low interaction between vitamin B-12 and the polymer fraction in the hydrogel.

The main effect of crosslinking is therefore to reduce the degree of equilibrium swelling achieved by the network under the experimental conditions. Flory[26] has discussed the effect of crosslink density on the equilibrium swelling of networks. Under equilibrium conditions, the degree of swelling in ideal networks is governed by the crosslink density, the temperature, molar volume of solvent and the polymer–solvent interaction parameter. The degree of swelling decreases nonlinearly with increasing crosslink density,[26,27] provided that the other variables remain constant. The extent of swelling can be conveniently quantified by the swelling ratio, Q, of the hydrogel membrane.

Figure 6 shows the decrease in diffusion coefficient and the swelling ratio with increase in mole percent of crosslinking agent added to the chitosan matrix. In this range of crosslink densities, the swelling ratio is linearly dependent upon the level of crosslinking agent incorporated. A plot of the diffusion coefficient, however, shows some curvature

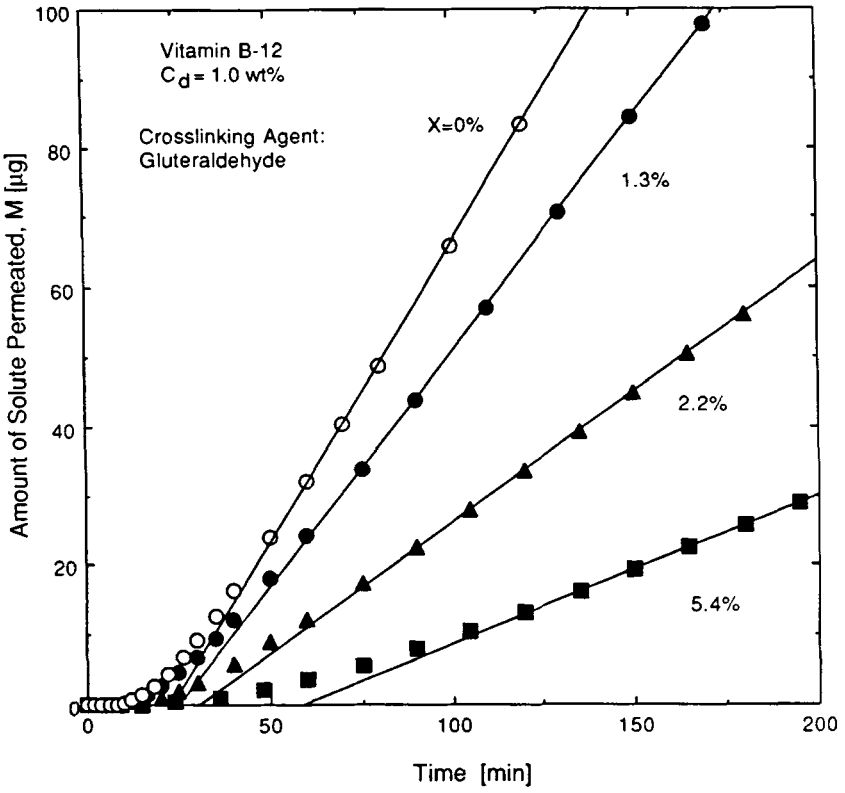


Figure 5 Variation of cumulative amount of vitamin B-12 in permeate with time for chitosan membranes with various degrees of crosslinking (chitosan crosslinked with gluteraldehyde).

indicating that the coefficient is particularly sensitive at low levels of crosslinking. In some hydrophilic polymers, the diffusion coefficient for the solute shows a limiting value at high crosslink densities.[28] The existence of a such a limit at very high crosslink densities cannot be ruled out for chitosan hydrogels in view of the curvature in Figure 6. However, chitosan hydrogels were extremely brittle at higher crosslink densities and the measurement of transport characteristics was impractical. In any event, the existence of a limiting value of diffusion coefficient at high degrees of crosslinking is not necessarily inconsistent with the pore mechanism.

Chitosan–Poly(vinyl alcohol) Blends

As crosslinking and the consequent decrease in the swelling ratio decreases the diffusion coefficient of the hydrogel, it is of interest to study the effect of increasing the swellability of chitosan. This can be conveniently achieved by blending in a hydrophilic polymer with a higher diffusion coefficient for vitamin B-12, compared to uncrosslinked chitosan.

Poly(vinyl alcohol) is a water-soluble polymer, readily miscible with chitosan. Being more hydrophilic, the blending of PVA with chitosan tends to increase the water uptake by the latter. The blend itself remains essentially insoluble in nonacidic aqueous media due to hydrogen bonding that occur between the hydroxyl groups in PVA and the amine groups of chitosan. The increased swellability of the blends is illustrated in Figure 7, where the swelling ratio Q is shown to be linearly related to the weight fraction of the PVA component. The logarithmic values of the permeability P also increased linearly with the weight fraction of PVA (Figure 7). The partition coefficient K was again found to be insensitive to the composition of the blend, as can be seen in Table I. In these "looser," more hydrophilic blends, the mechanism of transport is likely to remain of the pore type, hence the negligible effect of blending on K. Unlike in heavily crosslinked networks, these blends take up large amounts of water ensuring a high level of bulk-like water in the matrix occupying the voids or pores.

Blair et al.[16] reported the gel swelling index, which is the same as $(Q-1)$, for chitosan/PVA blends, with 0–70 wt % PVA. Their values for swelling ratio agree

with the present data. They also measured the tensile properties of the blends as dry films, and observed a reduction in tensile strength in the presence of PVA. As pointed out by them, this is possibly due to reduced crystallinity of chitosan in the blends. Miya et al.[29] also studied similar blends, and found the strength of blends show a maximum value around 20 wt % chitosan content in blend. Based on FTIR spectroscopic data, they found that the presence of PVA molecules in a chitosan system tended to disrupt crystallinity of chitosan and PVA in the blend. The disruption of crystallinity would increase the amorphous content, and therefore both Q and P of the blends. The present data suggests such an increase (Table I) and that the hydrogen bonding interactions between chitosan-PVA does not lead to a "tighter" network structure.

Relationship between Diffusivity and Swelling Ratio

In free-volume theory[30,31] for diffusion of solutes in polymer membranes, the transport of solute is interpreted in terms of a solute molecule progressing across the membrane by moving from "hole" to "hole." For hydrogel/solute systems where transport is exclusively via a pore type mechanism (i.e., for the case where the effective free volume is essentially the free volume of the water), Yasuda et al.[30] developed an expression relating the diffusion coefficient D with the degree of hydration, H, of the membrane. The natural logarithm of the relative diffusivity of a given solute, compared to that for water, changes proportionally to the reciprocal of the hydration of the membrane.

$$\ln\left(\frac{D}{D_o}\right) = \ln \varphi\,(q) - \frac{Bq}{v_f}\left(\frac{1}{H} - 1\right) \qquad (7)$$

where D_o is the diffusion coefficient of solute in water, q is the effective cross-sectional area of solute, $\varphi(q)$ is the probability of occurrence of a void (or a pore) with a volume larger than that of the molecular dimensions of the solute in the polymer matrix, v_f is the free volume of water and B is a constant. The hydration H in eq. (7) is the volume fraction of water

Table I Permeability and Swelling Data for Chitosan and Chitosan/PVA Membranes

Material	Membrane Thickness (mm)	% Mole Crosslinking Agent	% Weight PVA in Blend	D ($\times 10^{-8}$ cm^2/s)	K_d	P ($\times 10^{-7}$ cm^2/s)	Q
Chitosan	0.084	—	—	2.39	0.418	9.98	2.227
	0.128	—	—	2.36	0.389	9.18	2.238
	0.248	—	—	2.29	0.398	9.11	2.245
Crosslinked Chitosan							
Gluteraldehyde	0.110	1.25	—	1.39	0.443	6.16	2.198
	0.123	1.55	—	1.37	0.384	5.26	2.149
	0.109	2.20	—	1.09	0.378	4.12	2.122
	0.117	3.45	—	0.85	0.463	3.94	2.097
	0.114	4.63	—	0.69	0.438	3.02	2.040
	0.112	5.37	—	0.59	0.395	2.33	2.020
	0.098	7.10	—	0.45	0.448	2.02	1.957
Glyoxal	0.138	1.2[a]	—	2.01	0.376	7.56	2.207
	0.112	3[a]	—	1.35	0.368	4.97	2.131
	0.101	6[a]	—	0.83	0.378	3.12	2.070
	0.102	10[a]	—	0.52	0.376	1.96	2.007
Chitosan/PVA Blend	0.181	—	16.7	4.23	0.374	15.8	2.373
	0.218	—	33.3	5.77	0.380	21.9	2.483
	0.232	—	50.0	9.97	0.416	41.5	2.647
	0.198	—	66.7	16.0	0.401	64.2	2.710
	0.242	—	83.3	20.2	0.462	93.3	2.806
	0.234	—	100	25.9	0.438	113.4	2.894

[a] These moles are of glyoxal added per mole chitosan.
D = Diffusion coefficient; K_d = Partition coefficient; P = Permeability; Q = Swelling ratio.

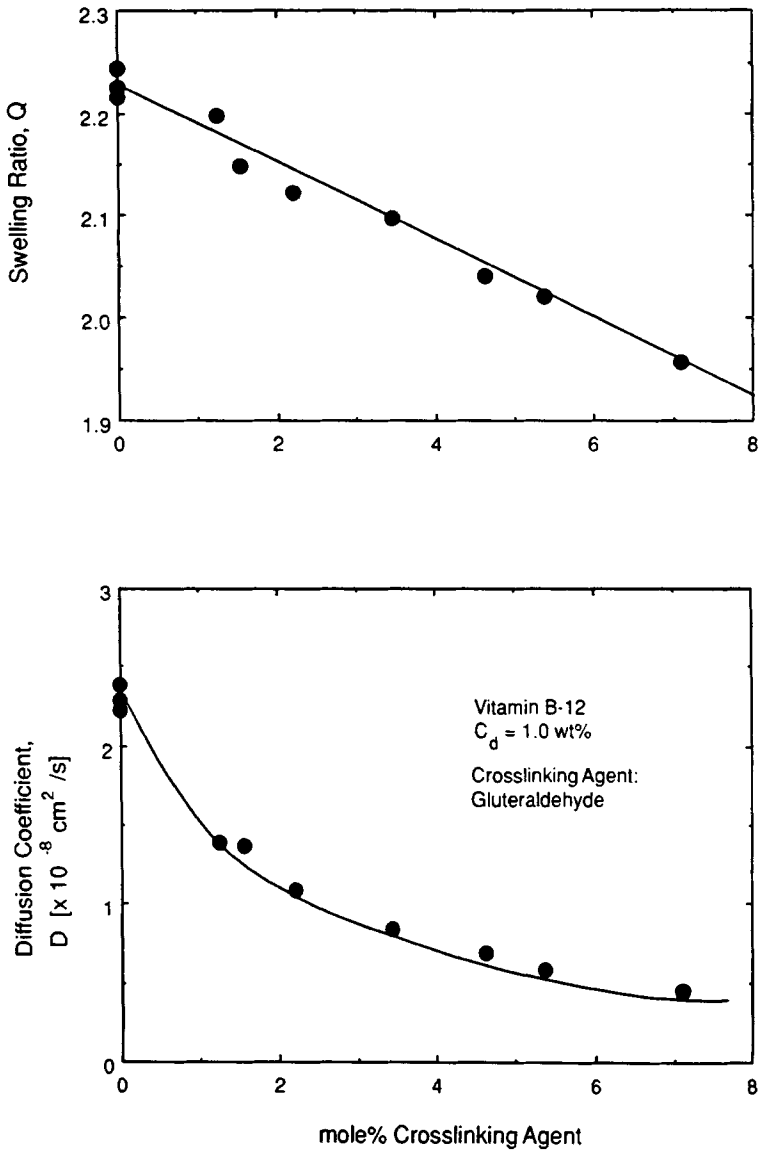


Figure 6 Diffusion coefficient of vitamin B-12, through crosslinked chitosan membranes and the swelling ratio of the membranes, vs. mole percent of crosslinking agent (gluteraldehyde).

in the swollen membrane and is related to the swelling ratio, $Q(=W_s/W_d)$, as follows:

$$H = 1 - \frac{W_d/\rho_p}{(W_s - W_d)/P + W_d/\rho_p}$$

$$= 1 \Big/ \left[1 + \frac{\rho}{\rho_p(Q-1)}\right] \qquad (8)$$

where W_d and W_s are the weights of dry and buffer swollen membranes respectively, and ρ and ρ_p are the densities of buffer and polymer, respectively.

Equation (7) can be rewritten in terms of the swelling ratio Q as follows:

$$\ln\left(\frac{D}{D_o}\right) = \ln \varphi(q) - \frac{k}{Q-1} \qquad (9)$$

where k is a proportionality constant. Assuming the density of the membrane to be invariant with crosslinking, and blending with PVA, the present data in Table I might be used to check the applicability of eq. (9) to chitosan networks and blends. Figure 8 shows a plot of data according to the equation.

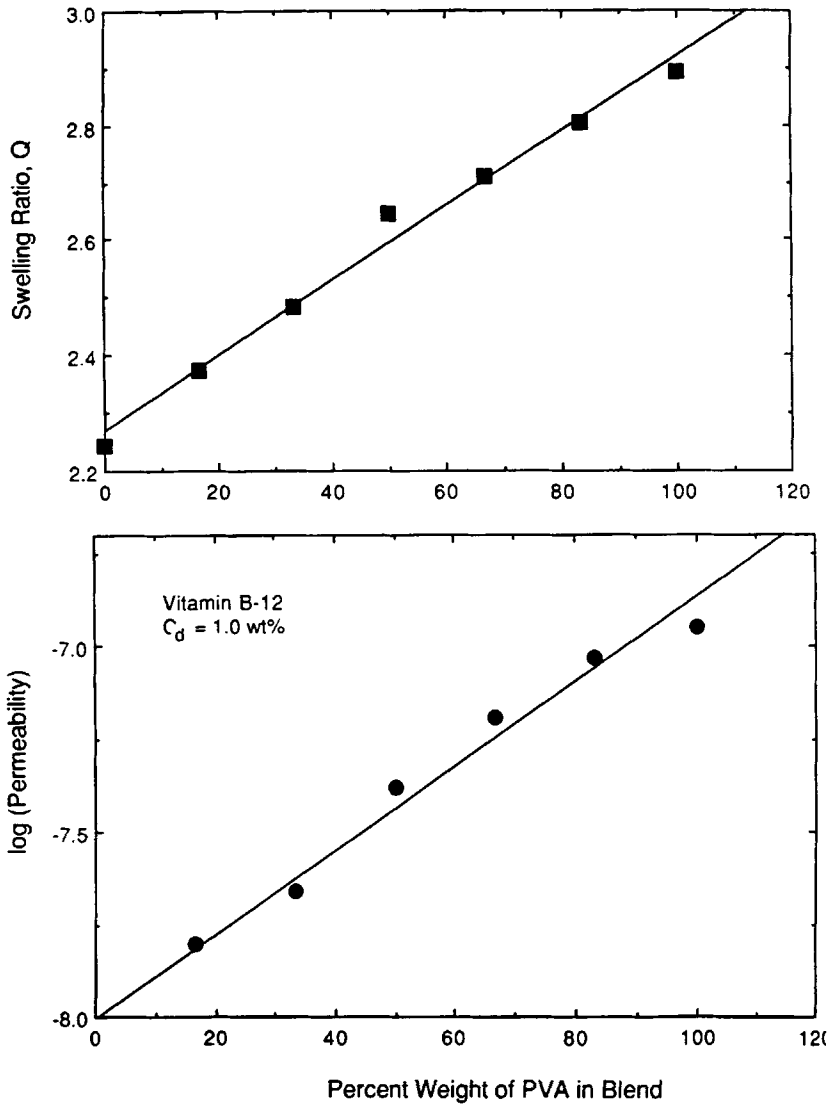


Figure 7 Logarithm of permeability of chitosan/PVA blend membranes and the swelling ratio of the membranes, vs. percent weight of PVA in the blend.

The present data yields a linear plot in agreement with the form equation and, interestingly, allows the data points for crosslinked chitosans as well as chitosan/PVA blends to be represented on the same curve. The intercept of the plot shows $\varphi(q)D_0$ to be the same for the range of crosslinked chitosan membranes and the blends examined here. Without an accurate value for the diffusion coefficient of vitamin B-12 in buffer, the probability factor $\varphi(q)$ cannot be extracted from the present data. The $\varphi(q)$ and B, both of which are characteristics of the swollen membrane, are little influenced by modifications (crosslinking and blending with PVA) of the polymer. Interestingly, the nature of crosslinking agent appears to have little effect as well. While gluteraldehyde is a larger, more flexible, crosslinker compared to glyoxal, this difference is not reflected in the transport properties of crosslinked membranes. Apparently, the probability of pore formation is not affected by the presence of low levels of crosslinks.

Linearity of the plot in Figure 8 therefore indicates all chitosan systems studied to transport vitamin B-12 via a predominantly pore type mechanism.

CONCLUSIONS

The hydration of chitosan hydrogels as measured by swelling ratio, can be altered over a wide range,

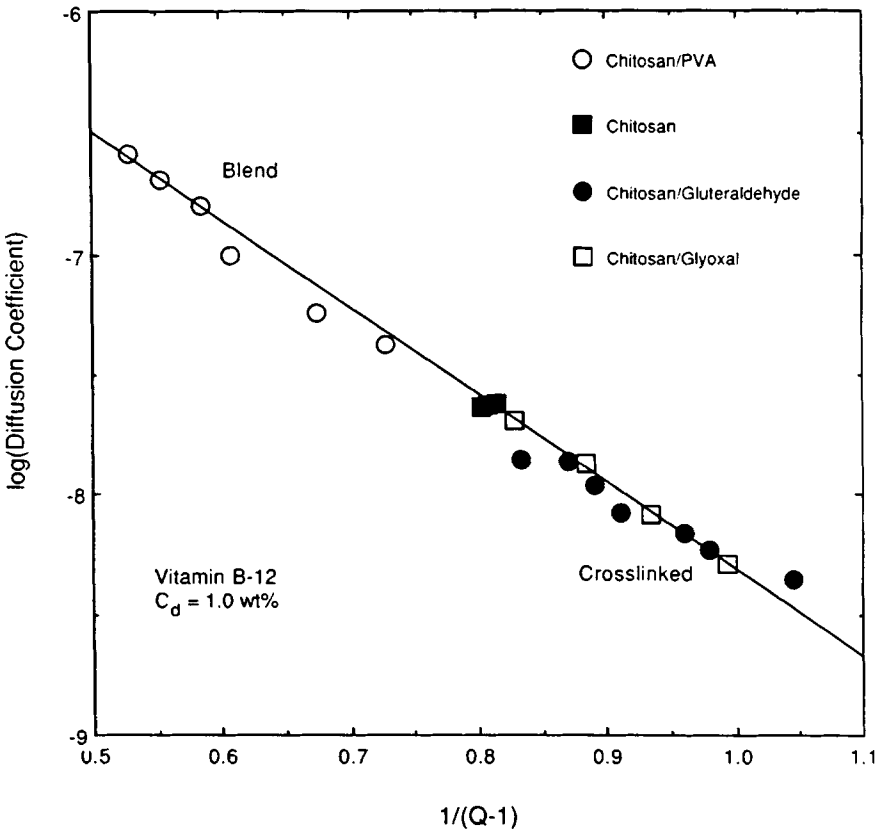


Figure 8 Logarithm of diffusion coefficient of vitamin B-12 through crosslinked chitosan membrane and chitosan/PVA blends, vs. $1/(Q-1)$ (Q: swelling ratio); ○ blend, ● crosslinked with gluteraldehyde, □ crosslinked with glyoxal, ■ uncrosslinked chitosan.

either by crosslinking or by blending with a poly(vinyl alcohol). Crosslinking decreases the equilibrium swelling of the hydrogel while blending increases it in proportion to the PVA content in the blend.

The diffusion coefficient of vitamin B-12 in the hydrogels was found to depend solely upon the degree of hydration of the membrane. The degree of crosslinking and the presence of PVA in blends affected the diffusion coefficient insofar as they altered the swelling of the hydrogel. The partition coefficient, on the other hand, was unaffected by the crosslinking and blending modifications, as might be expected for a "pore type" system.

All data generated during the study suggest the transport mechanism in chitosan (crosslinked and blended chitosan)/vitamin B-12 system to be predominantly of the pore mechanism.

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