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Research paper

Drug diffusion and binding in ionizable interpenetrating networks from poly(vinyl alcohol) and poly(acrylic acid)

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Abstract

Hydrogels of poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), and their interpenetrating networks (IPNs) were prepared using glutaraldehyde and ethylene glycol dimethacrylate as crosslinking agents. The hydrogels were characterized by measuring their equilibrium polymer volume fraction, equilibrium swelling ratio, and mesh size. Drug and protein diffusion through these hydrogels were studied. Solutes studied included theophylline, vitamin B_{12} and myoglobin. The ratio of PVA and PAA in the IPNs was varied to study the effect of ionic polymer content on the polymer/drug interactions and on the drug diffusion rate. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to analyze the polymer/drug binding interactions. It was concluded that drug diffusion may be impeded by associated drug binding, especially in IPN hydrogels containing high amounts of PAA.

Keywords: Theophylline; Vitamin B_{12}; Myoglobin; Poly(vinyl alcohol); Poly(acrylic acid)

1. Introduction

1.1. Hydrogels

Hydrogels have become increasingly important materials for pharmaceutical and biomedical applications [1]. They are used in a variety of applications including diagnostic, therapeutic, and implantable devices (e.g. catheters, biosensors, artificial skin, controlled release drug delivery systems, and contact lenses) [2]. Hydrogels have been widely used in such applications because of their biocompatibility with the human body and also because they exhibit characteristics similar to natural tissue [3].

Selection of hydrogels used in such processes depends on the characteristics of the gel and the drug or protein. Hydrogels have several important characteristics that play an important role in drug diffusion including ionization of the gel, swelling ratio, and specific mesh or pore size. Functional groups along the polymer chain can react to the external environment (e.g. temperature, ionic strength, and pH of the swelling agent). The swelling ratio is also a very important parameter because it describes the amount of water that is contained within the hydrogel at equilibrium and is a function of the network structure, crosslinking ratio, hydrophilicity, and ionization of the functional groups [4]. It is calculated from swelling studies and can be used to determine the molecular weight between crosslinks and the mesh size of the hydrogels. The mesh or pore size is the space available for drug transport.

The drug characteristics are as important as those of the gel. The size, shape, and ionization of the drug affects its diffusion through the membrane. In the case of ionization, if the gel and the drug are ionized, interactions may occur that may hinder or assist in the diffusional process, depending on the charges on the gel and drug. If the charges are the same,

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the gel repels the charges of the drug and does not hinder, and in some cases assists, transport. If the charges are opposite each other, interactions between the gel and the drug may take place hindering transport. This behavior was observed by Khare and Peppas [5] in studying poly(2-hydroxyethyl methacrylate-*co*-methacrylic acid) and poly(2-hydroxyethyl methacrylate-*co*-acrylic acid) using dynamic and equilibrium swelling studies. Chou et al. [6] and Kuo et al. [7] also observed this behavior in poly(2-hydroxyethyl methacrylate-*co*-methacrylic acid), *N*,*N*-dimethylaminoethyl methacrylate, and poly(hydroxyethyl methacrylate). Through swelling studies Hariharan and Peppas [4] were able to develop a model showing the effects of pH, ionic strength of the solution, and concentration of ionizable groups on polymer.

Ogawa et al. [8] used dilatometry to study the total volume gel change. They concluded that values of the maximum total volume change could be related to the maximum heat of swelling, even though the volume changes of the hydrogel itself were not related to enthalpy changes. To account for this, they proposed using Flory's theory for enthalpy and polymer/solute interactions.

The purpose of this research was to prepare and characterize neutral and hydrophilic gels and study their diffusional properties. Factors that may affect drug diffusion through the gel include mesh size, equilibrium swelling ratio, solute size, and hydrophilicity of the gel and drug. To support the diffusion studies, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was used to qualitatively analyze these interactions.

1.2. Drug transport in hydrophilic networks

Drug transport through hydrogels has been extensively studied in recent years. Much of the work has led to theories used to describe drug transport. Yampol'skii and Platé [9] can predict drug transport properties of gels based upon the chemical structure of the polymer. am Ende et al. [10] studied the drug transport through ionic hydrogels as a function of mesh size and environmental conditions (i.e. pH and ionic strength), and determined that each factor plays a very important role in drug transport. They also concluded that hydrogels may be tailor-made for a release of a specific drug, protein, or peptide. Other studies showing the effect of pH on drug transport from ionized hydrogels were done by Brannon-Peppas and Peppas [11]. They found that pH-dependent hydrogels could be prepared to exhibit zero or near zero-order release, this behavior could be attributed to the effect of the pH on the relaxation, swelling and release mechanism of the hydrogel.

In biological systems, another important factor is polymer/drug interactions when both the polymer and drug are ionized. Collins and Ramirez [12] studied these interactions and determined that polymer/solute interactions decrease solute transport through a gel. Gudeman and Peppas [13] also studied these interactions using well characterized interpenetrating networks of poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA) by varying the content of PAA (the ionic component) in the gel. They also compared the permeation of solutes through interpenetrating polymer networks (IPNs) at pH 3 (below the pK_a of PAA) and pH 6 (above the pK_a of PAA) and determined that permeation is a function of size exclusion as well as polymer/solute interactions.

Some of the early work in solute transport through hydrogels was carried out by Renkin [14] who studied solute diffusion through porous cellulose membranes. He compared experimental results to predictions based on the theory proposed by Pappenheimer [15] and found that they were in close agreement. He concluded that solute diffusion was a function of pore and solute size.

Yasuda et al. [16,17] studied the relationship between drug rejection and water flux of non-ionic [16] and ionic [17] gels using reverse osmosis. Anderson and Quinn [18] developed hydrodynamic equations governing transport in microporous systems ($r \leq 1$ μm) accounting for Brownian motion and steric restrictions. They showed that a 1-dimension diffusion/convection analysis could be used for such systems and developed a series of equations to account for the effect of the pore wall on the solute/solvent drag.

Peppas and Reinhart [19] developed a theory based upon the free volume theory for a three component system (water, solute (drug) and polymer gel). It predicted the dependence of the drug diffusion coefficient on drug size, mesh size, swelling ratio and other structural characteristics of the hydrogels as shown in Eq. (1).

$$\frac{D_{SM}}{D_{SW}} = k_1\left(\bar{M}_c - \bar{M}_c^*/\bar{M}_n - \bar{M}_c\right)\exp\left(k_2 r_s^2/Q_{m-1}\right) \quad (1)$$

Here, D_{SM} and D_{SW} are the diffusion coefficients of the drug in the hydrogel and water, respectively, $\bar{M}_c$ is the molecular weight between crosslinks, k_1 and k_2 are structural parameters of the polymer/water system, $\bar{M}_c^*$ is the critical molecular weight between crosslinks at which diffusion cannot occur, $\bar{M}_n$ is the molecular weight of the polymer before crosslinking, r_s is the Stokes hydrodynamic radius of the drug, and Q_m is the swelling ratio of the hydrogel. Using well characterized, amorphous PVA membranes [20] they were able to validate their theory.

Sassi and collaborators [21] used Monte Carlo simulations to develop a modified size exclusion theory based on statistical distribution of chains in the network. However, the theory does not consider ionic interactions or the effects of side groups on the structure, but focuses on chains in a large region of space.

1.3. Poly(vinyl alcohol)-based systems

The polymers studied in this research are poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA), a neutral and an ionic polymer, respectively. The two polymers were selected because the combination of their individual char-

acteristics helps to create a hydrogel that is desirable for pharmaceutical and biomedical processes. Also, the two polymers are miscible with each other and can be prepared in aqueous solution.

PVA is well known for its processability, strength, and long-term temperature and pH stability. The characteristics which make it ideal for biomedical use are its biocompatibility, non-toxicity, and minimal cell and protein adhesion. PAA is known to be a model hydrophilic system. Its carboxyl groups are ionized and swell considerably above the pK_a of 4.7. Because the chains are far apart above the pK_a, PAA is very fragile and breaks easily. To add strength to this hydrophilic system, PAA is polymerized and crosslinked with other polymers, in particular PVA.

Extensive work has been done on both polymers. The structure, characteristics, and drug diffusion of PVA have been studied by Reinhart and Peppas [20]. am Ende [22] has studied the characteristics of PAA and showed that solute diffusion increased as the pH increased. Gudeman [23] studied solute diffusion through PVA/PAA membranes as a function of temperature, ionic strength, and pH of the swelling agent.

The homopolymers can be prepared via a chemical technique where glutaraldehyde and ethylene glycol dimethacrylate (EGDMA) are used as crosslinking agents for PVA and PAA, respectively. Combinations of the two polymers can be prepared in the form of blends, copolymers, and interpenetrating networks (IPNs). Blends have been prepared via a freeze-thaw process where the polymers are frozen and thawed in a cyclic process [24, 25].

2. Materials and methods

2.1. Synthesis of homopolymers

A 10% w/v aqueous solution of poly(vinyl alcohol) (Elvanol 85-82, $\bar{M}_n = 48\,237$ and $\bar{M}_w = 103\,699$, (Lark Enterprise, Webster, MA; GPC analysis of PVA) E.I. duPont deNemours,Wilmington, DE) was prepared by dissolving PVA in water at 90°C for 6 h. The solution was removed from the oven and allowed to cool to room temperature.

In order to crosslink the PVA solution, glutaraldehyde was added as a crosslinking agent according to the desired crosslinking ratio. The crosslinking ratio, X, is defined as the ratio of moles of crosslinking agent to moles of PVA repeating units. In addition to adding 25% solution of glutaraldehyde, other solutions used were a 10% solution of sulfuric acid (the catalyst), a 50% solution of methanol (the quencher), and a 10% solution of acetic acid (the pH controller). They were added to the PVA solution in a 2:1:2:3 ratio, respectively. For a crosslinking ratio of $X = 0.01$, 0.2 ml sulfuric acid, 0.4 ml methanol, 0.6 ml acetic acid, and 0.4 ml glutaraldehyde were added to an aqueous solution containing 5 g of PVA and 50 g of water. The solution was mixed very slowly to prevent the formation of air bubbles which would create inhomogeneous membranes. Glutaraldehyde was then added to initiate crosslinking.

Immediately after carefully mixing the solution, the mixture was injected between two 75 × 50 × 1 mm microscope slides using a Pasteur pipet. The slides were separated by two microscope slides of the same dimensions to obtain uniform thickness. These were placed in a 60°C oven for 3 h, then cooled to room temperature. The hydrogels were placed in distilled water and washed for 5 days to remove uncrosslinked polymer.

Distilled acrylic acid (AA, Aldrich, Milwaukee, WI) and water were combined in a 1:1 ratio. A quantity of 0.5% ethylene glycol dimethacrylate (Aldrich) was added to the aqueous acrylic acid solution and thoroughly mixed. A quantity of 1% AIBN (Aldrich, Milwaukee, WI) was then added as the crosslinking agent.

Immediately after mixing the solution it was injected between two 75 × 50 × 1 mm microscope slides using a Pasteur pipet. The slides were separated by two microscope slides of the same dimensions to obtain uniform thickness. These were placed in a 60°C oven for 3 h and cooled to room temperature. The hydrogels were placed in distilled water and washed for 5 days to remove unreacted monomer.

The crosslinking of the homopolymers indicating the molecular weight between crosslinks, $\bar{M}_c$, and the mesh size, ξ, is shown in Fig. 1.

2.2. Synthesis of interpenetrating networks

PVA and AA solutions were prepared separately. The two solutions were then mixed together slowly to obtain membranes with VA:AA molar ratios of 60:40, 50:50 and 25:75. Like the homopolymers, the hydrogels were cast between two microscope slides, crosslinked in a 60°C oven for 3 h, and washed for 5 days. Fig. 2 shows the crosslinking of the IPNs. PVA crosslinks in the presence of AA, then AA polymerizes and then crosslinks to form the IPN.

2.3. Equilibrium swelling

The hydrogels were characterized from swelling studies

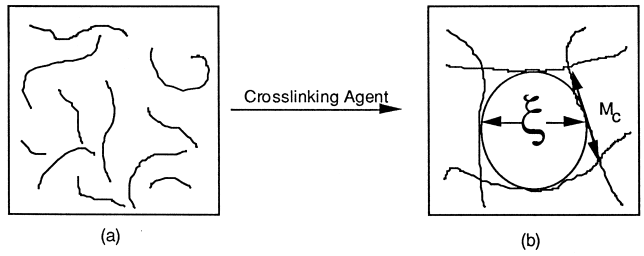

Fig. 1. Schematic of crosslinking of homopolymers. A crosslinking agent is added to the monomer or polymer in solution (a) to produce a crosslinked network (b) with a defined mesh size, x, and molecular weight between crosslinks, $\bar{M}_c$.

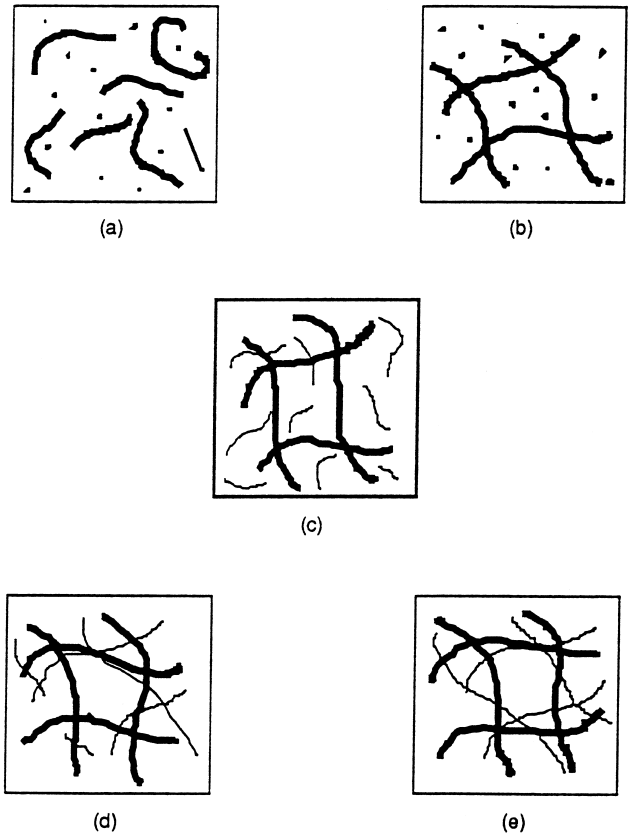

Fig. 2. Schematic of the crosslinking of interpenetrating networks. PVA (———) and PAA (■) plus their crosslinking agents were mixed together (a). PVA crosslinked in the presence of AA monomer (b), afterwhich AA polymerized in the presence of the PVA network (c). The crosslinking of PAA began (d) and continued until crosslinking of the IPN was completed (e).

immediately after crosslinking. Water was used as a swelling agent for PVA while pH 3 and pH 6 buffers were used as swelling agents for PAA and the IPNs.

To determine the equilibrium swelling ratio, Q, a sample of the membrane (1 cm^2 square) was cut immediately after crosslinking. This sample was weighed in air and heptane (a non-solvent). To obtain accurate weight measurements in heptane, the sample was placed in the stainless steel mesh basket which was suspended in heptane. The sample was then placed in deionized water at 37°C for 5 days to swell to equilibrium, and weighed in air and heptane. Before weighing, the sample was blotted to remove surface water. Finally, the sample was dried for 5 days at room temperature under a hood. Once again it was weighed in air and heptane. The equilibrium swelling ratio and the polymer volume fraction in the relaxed and swollen states were calculated using the weights measured.

Due to the ionization of PAA, buffer solutions of pH 3 and pH 6 were used in the equilibrium swelling studies for PAA and IPNs instead of water. The buffer solutions were prepared using a 0.1 M bb-dimethylglutaric acid (Sigma, St. Louis, MO). The pH was adjusted using 1.0 N NaOH. The equilibrium swelling was determined in the same manner as PVA.

2.4. Diffusion studies

Diffusion studies were performed at 37 ± 0.5°C using a Valia-Chen diffusion cell (Crown Glass, Somerville, NJ). Fig. 3 shows the setup of the diffusion cell. The apparatus consists of two half-cells with a volume of 3 ml and a side opening with a diameter of 9 mm. A magnetic stir bar was placed in each half-cell for continued agitation. Between the two half-cells a pre-equilibrated hydrogel membrane was securely placed and was protected from the atmosphere to prevent evaporation of the solvent from the membrane.

To the donor half-cell, 3 ml of the drug solution was added, and to the receptor half-cell 3 ml of the solvent was added. At regular intervals (i.e. 15, 30, 60 min) the contents of the receptor cell were moved and replaced with fresh solvent. To ensure constant temperature of 37°C, the half-cells were surrounded by a water jacket which was 37°C. An ultraviolet-visible light spectrophotometer (Spectronic 601, Milton Roy) was used to measure the absorbance of the samples taken from the receptor half-cell. Using a calibration curve derived from known concentrations of the drug solutions, the concentration of each sample taken from the receptor half-cell could be determined.

The solutes used in the diffusion studies varied in molecular weight, degree of ionization, and shape as shown in Table 1. Theophylline, vitamin B_{12}, and myoglobin were all purchased from Sigma, St. Louis, MO. Theophylline and vitamin B_{12} have a maximum wavelength around 274 and 359 nm, respectively. The concentration used in the donor cell was 1 mg/ml for both solutes. Myoglobin has a maximum wavelength around 390 nm and the concentration used was 10 mg/ml.

2.5. Determination of permeability coefficient

The drug or protein solute permeability coefficients were determined from the results of the permeation studies. After determining the concentration of the drug permeated using a calibration curve, these data were used to find the permeability coefficient.

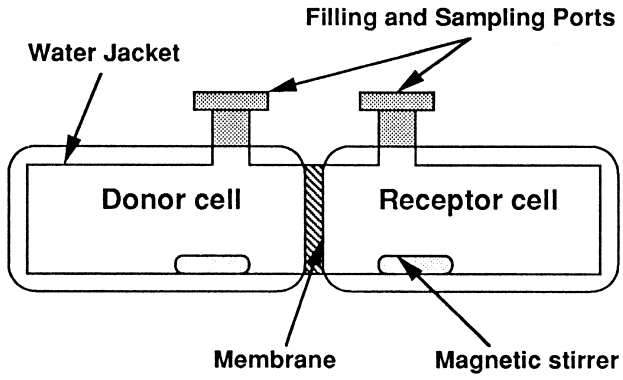

Fig. 3. Set-up of the diffusion cell.

Table 1
Description of the solutes used in the diffusion studies

Solute	Molecular weight	pK_a	Experimental diffusion (10^7 cm^2/s)	Shape and dimensions (Å)
Theophylline	180	8.6	–	–
Vitamin B_{12}	1355	Neutral	37.9 [27]	Spheroid [27] 9.5 × 9.2 × 7.5
Myoglobin	17200	7.0	12.8 [27]	Irregular [28] 12.5 × 17.5 × 22.5

The experimental diffusion coefficients were determined in water or saline at 37°C.

2.6. Determination of partition coefficient

The drug partition coefficients were determined by equilibrating the hydrogels (approximately 1 cm^2) in water at 37°C. They were then placed in 30 ml of solute of a known concentration at 37°C. After 3 days, the absorbances of the solutions were measured. Drug concentrations were determined for each solution using a calibration curve. The drug concentrations of the membranes were calculated using a mass balance for the solute.

2.7. ATR-FTIR spectroscopic studies

ATR-FTIR spectroscopy was used to qualitatively investigate the interactions between the ionized membranes and ionized solutes. This technique was employed to determine if drug diffusion was hindered due to the interaction between the ionized drugs and gel. The interaction would be noted by the shifts in the functional groups (i.e. carbonyl groups) of the membrane.

When using ATR-FTIR, the sample is placed in direct contact with the germanium crystal (Wilmad, Buena, NJ). Fig. 4 shows the setup of the sample on the crystal.

3. Results and discussion

3.1. Hydrogel synthesis

PVA was chemically crosslinked in a 60°C oven for 3 h. Glutaraldehyde was used as the crosslinking agent [29].

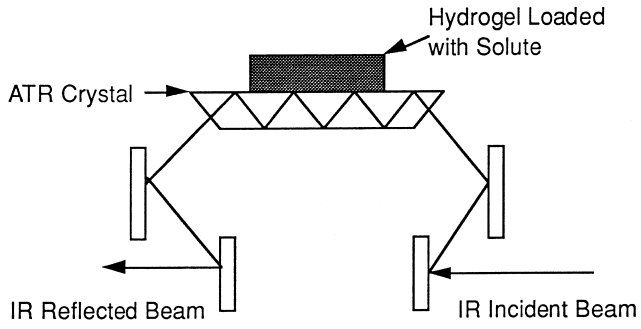

Fig. 4. Set-up of sample on the germanium crystal and infrared light path through ATR-FTIR.

Freeze-thawing [30–32] and γ irradiation [33] techniques have also been used to crosslink PVA. However, a technique was needed whereby IPNs containing PAA and PVA hydrogels could be made under acceptable conditions. Crosslinking using photopolymerization was tested but a crosslinked polymer (network) was not produced in a reasonable amount of time using glutaraldehyde as a crosslinking agent.

PAA was also chemically crosslinked for 3 h in a 60°C oven. EGDMA was used as the crosslinking agent. Photopolymerization has been shown to be an adequate method of crosslinking PAA, but it was not utilized because PVA did not crosslink in either the homopolymer or the IPNs.

The preparation of IPNs was first investigated using photopolymerization. The PVA and AA solutions were prepared as described before. The two solutions were combined to produce the desired VA:AA ratio. Glutaraldehyde and EGDMA were used as crosslinking agents, and after 1 h of photopolymerization, a crosslinked membrane was produced. To determine if PAA and PVA had crosslinked in the IPNs, the homopolymers were separately photopolymerized under the same conditions as the IPNs. It was observed that PAA crosslinked while PVA remained in solution. It was concluded that the desired IPNs were not successfully crosslinked under these conditions using photopolymerization. As a result of the above study, chemical crosslinking was performed for 3 h in a 60°C oven.

3.2. Swelling parameters of membranes

Swelling studies in distilled water, and pH 3 and pH 6 buffer solutions were performed to characterize the membranes at 37°C. The technique used was the same as that used by Peppas and Barr-Howell [34]. The membranes were prepared and their polymer volume fraction in the relaxed state was calculated using Eq. (2). After each membrane had swollen to equilibrium at 37°C, the polymer volume fraction of the swollen polymer was calculated using Eq. (3).

$$\upsilon_{2,r} = \frac{V_d}{V_r} \tag{2}$$

$$\upsilon_{2,s} = \frac{V_d}{V_s} \tag{3}$$

Here, V_d, V_r and V_s are the volumes of the polymer sam-

Table 2
The crosslinking ratio and polymer volume fractions of PVA, PAA, and PVA/PAA hydrogels in pH 3 buffer at 37°C

Hydrogel code	PAA content (%)	Solvent pH	Nominal crosslinking ratio ($X \times 10^3$)	Polymer volume fraction, $v_{2,r}$	Polymer volume fraction, $v_{2,s}$
PVA200	0	6.7	10.0	0.1094	0.1053
PP18	40	3	6.7	0.2575	0.0361
PP22	75	3	3.9	0.3937	0.0350
PAA001	100	3	1.8	0.5919	0.0085

Glutaraldehyde and EGDMA were both used as crosslinking agents.

ple in the dry, relaxed, and swollen states, respectively; and $v_{2,r}$ and $v_{2,s}$ are the polymer volume fractions of the relaxed and swollen polymer gel, respectively. The volumes were calculated using Eqs. (4)–(6) which utilize the weights of the dry polymer, W_d, the relaxed polymer, W_r, and the swollen polymer, W_s, in air and heptane.

$$V_d = \frac{W_{a,d} - W_{h,d}}{\rho_h} \quad (4)$$

$$V_r = \frac{W_{a,r} - W_{h,r}}{\rho_h} \quad (5)$$

$$V_s = \frac{W_{a,s} - W_{h,s}}{\rho_h} \quad (6)$$

Here r_h is the density of heptane. Tables 2 and 3 list the nominal crosslinking ratio and the polymer volume fraction of the relaxed and swollen polymer samples at pH 3 and pH 6. The polymer volume fraction of the relaxed polymer increased as the PAA content increased while the polymer volume fraction of the swollen polymer decreased as the PAA content increased.

The density is swollen and dry polymer could also be calculated using Eqs. (7) and (8).

$$\rho_{swollen} = \frac{W_{a,s}}{V_s} \quad (7)$$

$$\rho_{dry} = \frac{W_{a,d}}{V_d} \quad (8)$$

The equilibrium swelling ratio of a hydrogel sample can be affected by the ionic strength, temperature, and pH of the swelling agent. In these studies the ionic strength and temperature were kept constant at 0.2 N and 37°C, respectively. The pH of the swelling agent was 6.7 for the PVA homopolymer due to its neutral nature. For the studies of the PAA homopolymer and the IPNs, the pH values of the swelling agents used were 3 and 6, in order to be able to observe the significant changes of the hydrogel swelling ratio above and below the pK_a of PAA (pK_a = 4.7). At pH 3 the hydrogels were not ionized while at pH 6 they were in the ionized form. The data taken from the swelling studies were used to calculate the equilibrium swelling ratio which is defined as:

$$Q = \frac{1}{v_{2,s}} \quad (9)$$

Table 4 lists the equilibrium swelling ratios for PAA and PVA/PAA hydrogels swollen in pH 3 buffer solution, whereas Table 5 lists the equilibrium swelling ratios for PAA and PVA/PAA hydrogels swollen in pH 6 buffer solution. The results indicate that swelling increased as the pH increased. They also indicate that as the percentage of PAA increased in the membranes the equilibrium swelling ratio increased. Due to the ionic nature of PAA, the IPNs and PAA membranes swelled much more than that PVA which is neutral. Khare and Peppas [5] and Hariharan and Peppas [4] studied the effects of pH on the swelling ratio and observed the same behavior as above.

The molecular weights between crosslinks, $\bar{M}_c$, was calculated from the swelling data using Eq. (10) as discussed by Peppas and Merrill [35].

$$\frac{1}{\bar{M}_{c,e}} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{v}}{\bar{V}_1}\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s^2}\right]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \quad (10)$$

Here, $\bar{M}_n$ is the number-average molecular weight of the polymer before crosslinking, $\bar{v}$ is the specific volume of the polymer, $\bar{V}_1$ is the molar volume of the solvent, $v_{2,r}$ is the volume fraction of the polymer in the relaxed state, $v_{2,s}$ is

Table 3
The crosslinking ratio and polymer volume fractions of PAA, and PVA/PAA hydrogels in pH 6 buffer at 37°C

Hydrogel code	PAA content (%)	Solvent pH	Nominal crosslinking ratio ($X \times 10^3$)	Polymer volume fraction, $v_{2,r}$	Polymer volume fraction, $v_{2,s}$
PP19	40	6	6.7	0.2655	0.0110
PP23	75	6	3.9	0.4025	0.0158
PAA002	100	6	1.8	0.6105	0.0085

Glutaraldehyde and EGDMA were both used as crosslinking agents.

Table 4

The effects of pH 3 buffer on the equilibrium swelling ratio of PVA, PAA, and PVA/PAA hydrogels at 37°C

Hydrogel code	PAA content (%)	Nominal crosslinking ratio ($X \times 10^3$)	Equilibrium swelling ratio at pH 3 (Q)
PVA200	0	10.0	9.52
PP18	40	6.7	17.73
PP22	75	3.9	28.57
PAA001	100	1.8	36.21

the volume fraction of polymer in the swollen state, and c is the interaction parameter of the polymer-solvent system in water. The Flory polymer-solvent interaction parameters for PVA/water and PAA/water are 0.494 and 0.500, respectively [36]. Table 6 gives details of all the values of the parameters used in calculating $\bar{M}_c$.

A theoretical $\bar{M}_c$ value could be calculated from knowledge of the nominal crosslinking ratio X as follows:

$$\bar{M}_{c,t} = \bar{M}_r / 2X \tag{11}$$

Here, $\bar{M}_r$ is the molecular weight of the repeating unit of PVA ($\bar{M}_r$ = 44) or PAA ($\bar{M}_r$ = 72).

Eq. (10) was used to calculate the experimental $\bar{M}_c$ for neutral homopolymers such as PVA. For PAA-based gels, an equation was needed to account for their ionic nature in the homopolymer and IPNs. Brannon-Peppas [37] developed equations to calculate $\bar{M}_c$ for cationic and anionic membranes. Starting from the Peppas–Merrill equation she accounted for ionization of the polymers. In our work, Eq. (12) was used to calculate $\bar{M}_c$ for PAA homopolymer and PVA/PAA membranes.

$$\bar{V}_1 / 4I\,\bar{M}_r \left[\frac{K_a}{10^{-pH} + K_a}\right]^2 \left[\frac{v_{2,s}}{\bar{v}}\right]^2 = [\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s^2}] + \left(\bar{V}_1 / v\bar{M}_c\right)\left(1 - 2\bar{M}_c / \bar{M}_n\right) v_{2,r} \left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right] \tag{12}$$

Here, I is the ionic strength, pH is the pH of the buffer solution in which the membrane was swollen, and K_a is the dissociation constant.

The theoretical and experimental values of $\bar{M}_c$ are listed in Table 7. In general the presence of PAA led to a more open network structure with higher $\bar{M}_c$ values. The data indicate that the experimental values of $\bar{M}_c$ were the same as the theoretical values for the PVA homopolymers tested.

For the PAA homopolymers and the PVA/PAA hydrogels, the experimental values $\bar{M}_c$ were significantly larger than the theoretical values. The values of $\bar{M}_c$ for the PVA/PAA hydrogels which contained 40 and 75% PAA were two to three times larger than the theoretical values, respectively. This discrepancy is probably due to partial reaction of the crosslinking agent in the short reaction time (3 h). Obviously, the theoretical values of $\bar{M}_c$ are calculated under the assumption of complete reaction. Unlike the homopolymers, PAA was crosslinked in the presence of PVA network which acted as a barrier for the crosslinking of AA monomers and PAA. Because the hydrogels were only crosslinked for 3 h, the crosslinking process was not completed, resulting in a large $\bar{M}_c$.

Table 5

The effects of pH 6 buffer on the equilibrium swelling ratio of PVA, PAA and PVA/PAA hydrogels at 37°C

Hydrogel code	PAA content (%)	Nominal crosslinking ratio ($X \times 10^3$)	Equilibrium swelling ratio at pH 6 (Q)
PP19	40	6.7	39.48
PP23	75	3.9	63.49
PAA002	100	1.8	118.12

Table 6

Values used in the calculations of the characterization of the prepared hydrogels

Parameter	Component	Value	Reference
$\bar{M}_n$	PVA	48237	–
$\bar{M}_n$	PAA	75000	[37]
$\bar{V}_1$	Water	18 cm^3/mol	–
$\bar{v}$	PVA	0.788 cm^3/g	[26]
$\bar{v}$	PAA	0.951 cm^3/g	–
χ	PVA	0.494	[36]
χ	PAA	0.500	[36]
$\bar{M}_r$	PVA	44	–
$\bar{M}_r$	PAA	72	–
C_n	PVA	8.3	[23]
C_n	PAA	6.7	[23]

3.3. Determination of mesh size and crosslinking density

The hydrogel mesh size, ξ, defines the linear distance between consecutive crosslinks. Indirectly, it indicates diffusional space available for solute transport and can be calculated using Eq. (13).

$$\xi = v_{2,s}^{-1/3}\left[C_n\left(2\bar{M}_c / \bar{M}_r\right)\right]^{1/2} l \tag{13}$$

Here, C_n is the Flory characteristic ratio and l is the carbon–carbon bond length (Table 6).

The crosslinking density of the hydrogels was calculated using Eq. (14).

$$\rho_x = \frac{1}{v\bar{M}_c} \tag{14}$$

Table 8 lists the calculated values of the mesh size and the crosslinking density for each hydrogel as a function of crosslinking ratio and pH. The data indicate that as the crosslinking ratio decreased in the hydrogels, the mesh size increased. Thus, as the amount of crosslinking agent decreased the space between the crosslinks became larger.

Table 7

The calculated theoretical and experimental molecular weight between crosslinks

Hydrogel code	PAA content (%)	Nominal crosslinking ratio ($X \times 10^3$)	Molecular weight between crosslinks, $\bar{M}_{c,t}$ (g/mol)	Molecular weight between crosslinks, $\bar{M}_{c,e}$ (g/mol)
PVA200	0	10.0	2200	2500
PP18	40	6.7	9320	29380
PP19	40	6.7	9320	29380
PP22	75	3.9	15550	31700
PP23	75	3.9	15550	31700
PAA001	100	1.8	20000	35000
PAA002	100	1.8	20000	35000

This space is defined as the diffusional space or mesh size. The data also indicate that the crosslinking density decreased with decreasing crosslinking ratio meaning that there is more space between the crosslinks, and the gel is less dense. It was also noted that the mesh size is dependent on the pH of the swelling solution for ionic membranes.

3.4. Diffusion analysis

Once the hydrogels were characterized through swelling studies, their permeability coefficients were determined. The solutes used varied in molecular weight and degree of ionization and included theophylline (mol. wt. = 180, pK_a = 8.6), vitamin B_{12} (mol. wt. = 1355, neutral solute), and myoglobin (mol. wt. = 17 200, pK_a = 7.0). Because of the ionic nature of theophylline and myoglobin, polymer/solute interactions were also investigated.

Solute size was important because the permeability coefficients of the solute decreased as its hydrodynamic radius, d_h, approached the size of the diffusional space or mesh size. Another important parameter was the polymer/solute interaction. This parameter had a profound impact on the drug diffusion depending on whether the drug charge was the same or opposite to the charge of the hydrogel.

The drug diffusion through each hydrogel was determined from the drug permeation studies. Fig. 5 presents typical data of the drug permeated through PVA/PAA IPN hydrogels as a function of normalized time, dividing by the square of the hydrogel thickness. The drug size, hydrogel mesh size, pH, and degree of ionization were determined to investigate their effects on drug diffusion. Permeation of theophylline through the membranes at both pH 3 and pH 6 was linear, and the amount permeated in the pH 6 was greater than the amount permeated at pH 3. As the size of theophylline was much smaller than the mesh size of the IPN in both pH 3 and pH 6, these data cannot be explained in terms of drug size exclusion only. At pH 6 both theophylline and the hydrogel were ionized. Due to their opposite charges, ionic interaction and binding may be occurring, thus hindering the permeation of theophylline in the membrane at pH 6. At pH 3, theophylline was not ionized and binding did not occur. However, due to the increased mesh size of the hydrogels at pH 6 and the small solute size compared to the pore size of the hydrogel, drug binding did not dominate the permeation of theophylline.

Permeation of vitamin B_{12} showed a short lag period at the beginning of each permeation study which was attributed to low vitamin B_{12} permeation due to size exclusion phenomena. This was indicated in the figures by the curvature of the line at the beginning of each study. Afterwards, permeation proceeded linearly. The molecular diameter (d = 16.6 Å) of vitamin B_{12} is smaller than the mesh sizes of these hydrogels. At pH 6 solute permeation was greater because of the more space available for diffusion. Since vitamin B_{12} is a neutral solute, there was no polymer/solute interactions.

Fig. 6 shows typical data of the permeation of theophylline through each hydrogel membrane at pH 3 while Fig. 7

Table 8

The crosslinking densities and mesh sizes of the PVA, PAA and PVA/PAA hydrogels

Hydrogel code	PAA content (%)	Swelling pH	Nominal crosslinking ratio ($X \times 10^3$)	Mesh size (Å)	Crosslinking density (mol/cm^3) $\rho \times 10^4$
PVA200	0	–	10.0	98	4.16
PP18	40	3	6.7	319	2.88
PP19	40	6	6.7	420	2.88
PP22	75	3	3.9	341	2.87
PP23	75	6	3.9	502	2.87
PAA001	100	3	1.8	398	2.36
PAA002	100	6	1.8	589	2.36

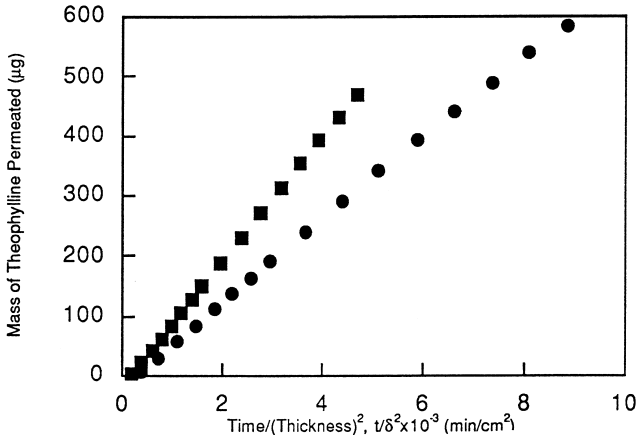

Fig. 5. Diffusion of vitamin B_{12} through PVA/PAA IPN hydrogels containing 40% PAA and with a mesh size, ξ, of 319 Å at pH 3 (●) and 420 Å at pH 6 (■).

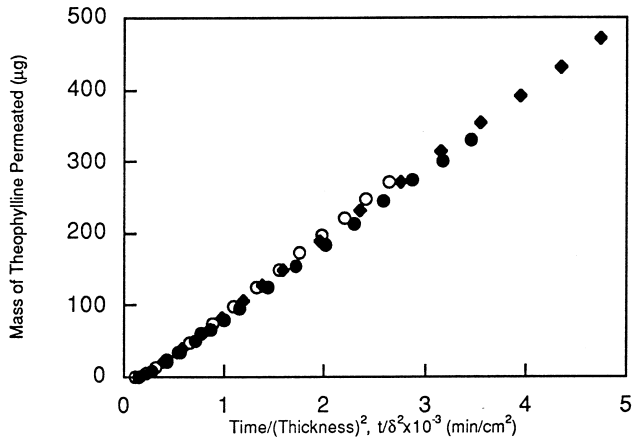

Fig. 7. Diffusion of theophylline through PAA, and PVA/PAA hydrogels at pH 6: 40% PAA, mesh size, ξ, of 420 Å (♦), 75% PAA, mesh size, ξ, of 502 Å (●), and 100% PAA, mesh size, ξ, of 589 Å (○).

shows the permeation of theophylline at pH 6. It can be seen that permeation of theophylline through the membrane containing 40 and 75% PAA (mesh size, ξ = 319 and 341 Å, respectively) was the same. Permeation was fast in the PAA hydrogel (mesh size, ξ = 398 Å). However, at pH 6 (Fig. 7) the permeation of theophylline was essentially the same for all of the hydrogels. At pH 6, polymer/solute interactions took place, but because of the size of theophylline was very small compared with the mesh sizes of the membranes, these interactions did not dominate the diffusion of theophylline.

In the permeation of vitamin B_{12} at pH 3 and pH 6 polymer/solute interactions do not occur; therefore, size exclusion phenomena dominate the diffusion process. At pH 3, vitamin B was shown to have permeated more slowly through PVA/PAA membrane containing 40% PAA (mesh size, ξ = 319 Å). As the mesh size increased, the permeation of vitamin B_{12} increased.

3.5. Determination of permeability, partition and diffusion coefficients

Permeability coefficients were calculated from the data obtained in the permeation experiments using the following equation:

$$\ln\left(\frac{2c_t}{c_0}-1\right)=\frac{2A}{V}Pt \tag{15}$$

Here, c_t, is the drug concentration in the receptor cell at time t; c_0 is the initial drug concentration of the donor cell; V is the volume of each half cell; A is the effective area of permeation; and P is the membrane permeability coefficient. To determine the permeability coefficient, P, a plot of $-(V/2A)\ln[1-2(c_t/c_0)]$ versus time, t, was constructed and yielded a slope of the permeability coefficient, P. Fig. 8 shows a typical plot for myoglobin through a PVA/PAA membrane containing 75% PAA at pH 6. The permeability

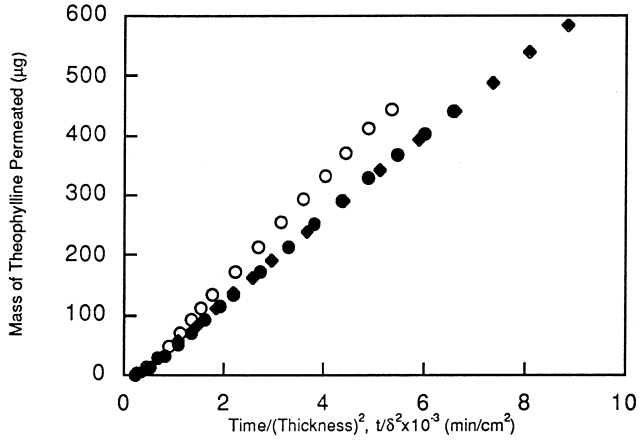

Fig. 6. Diffusion of theophylline through PVA/PAA IPN hydrogels containing 40% PAA and with a mesh size, ξ, of 319 Å at pH 3 (●) and 420 Å at pH 6 (■).

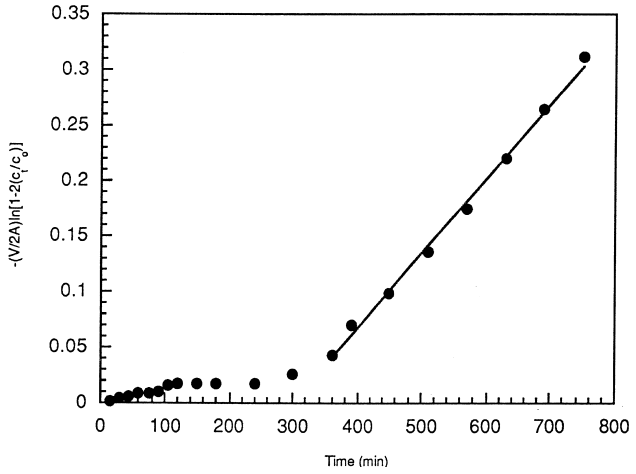

Fig. 8. Determination of the permeability coefficient for myoglobin through PVA/PAA hydrogels containing 75% PAA at pH 6.

Table 9

The partition coefficients, K_d, at pH 3 and pH 6 for solutes in PAA, and PVA/PAA hydrogels at 37°C

Hydrogel	Swelling pH	PAA content	Solute	Partition coefficient, K_d
PP18	3	40	Theophylline	25.14
			Vitamin B_{12}	17.67
			Myoglobin	–
PP19	6	40	Theophylline	32.57
			Vitamin B_{12}	19.10
			Myoglobin	11295.7
PP22	3	75	Theophylline	27.73
			Vitamin B_{12}	15.61
			Myoglobin	–
PP23	6	75	Theophylline	31.38
			Vitamin B_{12}	16.21
			Myoglobin	12319.2
PAA001	3	100	Theophylline	36.46
			Vitamin B_{12}	9.22
			Myoglobin	–
PAA002	6	100	Theophylline	31.87
			Vitamin B_{12}	16.98
			Myoglobin	4653.7

coefficient was found to be 6.72×10^{-4} cm/s. Only the late time data were selected to be used because, as seen earlier, it is only after 350 min that myoglobin permeates freely.

The diffusion coefficient was calculated from the permeability coefficient, P, and the partition coefficient, K_d, as shown in the following equation:

$$D_m = \frac{P\,l}{K_d} \tag{16}$$

Here, l is the membrane thickness in the swollen state at constant pH, and K_d is the ratio of the drug concentration in the membrane, c_m, to the drug concentration in the solution at equilibrium, c_s. Eq. (17) was used to calculate the partition coefficient.

$$K_d = \frac{c_m}{c_s} = \frac{V_s(c_0 - c_e)}{V_m c_0} \tag{17}$$

Here, V_s and V_m are the volume of the drug in solution and the hydrogel, respectively; c_0 is the initial concentration of the drug in solution, and c_e is the drug concentration in the solution at equilibrium. Table 9 lists the partition coefficients for each drug and hydrogel. At pH 6, the partition coefficients were found to be higher for all solutes, indicating a higher affinity for the membranes than at pH 3. In the case of theophylline and myoglobin, this was due to the binding of the solute to the hydrogels. This would be correct, as at pH 6 there is complete ionization of the hydrogel. However, the values of K_d for myoglobin are extremely high, indicating an excessive binding in the membrane.

Vitamin B_{12} was found to have the smallest partition coefficients, which may be attributed to its neutrality and consequent lack of interactions with the membranes. At pH 6 the partition coefficients of theophylline and vitamin B_{12} were similar for the hydrogels that contained 75 and 100% PVA.

The highest partition coefficients were observed for myoglobin at pH 6 due to the size and shape of myoglobin as well as the permanent binding between myoglobin and the hydrogels. These partition coefficients and data from the permeability studies were used to determine the diffusion coefficients of each drug through the hydrogels which are listed in Table 10.

3.6. Analysis of permeation studies using the Peppas–Reinhart theory

The Peppas–Reinhart theory (Eq. (1)) can be used to describe the permeation of solutes through highly swollen non-porous hydrogels. The Peppas–Reinhart equation relates these parameters as:

$$\frac{D_{SM}}{D_{SW}} = k_1\left(\bar{M}_c - \bar{M}_c^*\middle/\bar{M}_n - \bar{M}_c\right)\exp\left(-k_2 r_s^2\middle/Q_{m-1}\right) \tag{18}$$

Thus, a plot of $\ln(D_{SM}/D_{SW})$ versus (r^2/Q_m^{-1}) yields a straight line, showing the dependence on the diffusion coefficient on the equilibrium swelling ratio. The experimental results were plotted for PVA/PAA membranes containing 40, 75 and 100% PAA using both methods, as shown in Fig. 9. A straight line is produced for each membrane as indicated by the Peppas–Reinhart model. However, according to the theory, the lines of the three membranes should collapse into one line. Yet, this does not happen, indicating that although the membranes are highly swollen, ionic interactions between the solute and the membrane lead to

Table 10

The diffusion coefficients of the solutes through the hydrogels and water at 37°C

Solute	PAA content	Swelling pH	Mesh size (Å)	Diffusion coefficient $D_m \times 10^7$	Diffusion coefficient in water $D_{water} \times 10^7$ (cm^2/s)
Theophylline	40	3	319	49.6	
		6	420	60.2	
	75	3	341	47.8	
		6	502	58.4	$87.4_{(37°C)}$ [38]
	100	3	398	45.3	
		6	589	59.8	
Vitamin B_{12}	40	3	319	28.7	
		6	420	31.8	
	75	3	341	27.6	
		6	502	34.3	$37.9_{(37°C)}$ [27]
	100	3	398	31.9	
		6	589	35.7	
Myoglobin	40	3	319	–	
		6	420	0.17	
	75	3	341	–	
		6	502	0.16	$12.8_{(25°C)}$ [27]
	100	3	398	–	
		6	589	0.16	

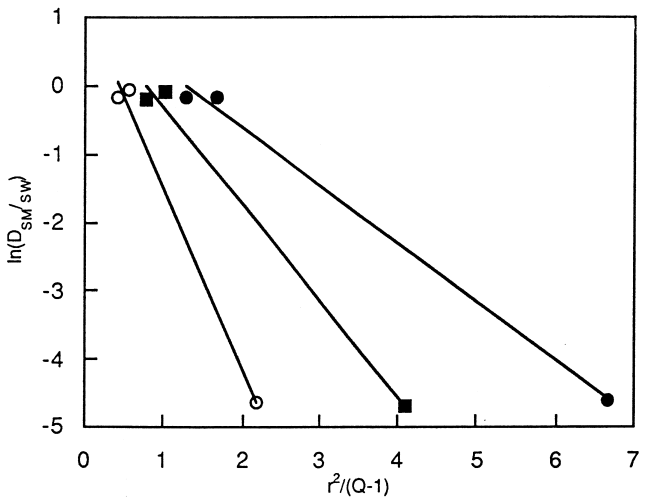

Fig. 9. Diffusion data for PVA/PAA hydrogels containing 40% PAA (●), 75% PAA (■), and 100% PAA (○) fitted to the Peppas–Reinhart model.

derivations from the theory. As the amount of PAA increased in the membrane, the swelling ratio and polymer/solute interactions increased.

3.7. ATR-FTIR spectroscopy

ATR-FTIR spectroscopy was used to study the interactions between PAA hydrogel and myoglobin. A spectrum of the clean germanium crystal was taken and set as the background. A spectrum of the hydrogel swollen at pH 6 in the absence of solute was taken and compared with the spectra of myoglobin and a swollen hydrogel loaded with myoglobin. The background was subtracted from each spectrum to eliminate noise. Figs. 10–12 show the ATR-FTIR spectra of the hydrogel, myoglobin, and the system and identify the bands at which stretching and vibration may be occurring. Table 11 defines each band and lists the relevant wavenumber in the membrane, myoglobin and membrane loaded with myoglobin. Peaks between 1000 and 2000 cm^{-1} which were observed in myoglobin and in the loaded hydrogel spectra were not observed in the hydrogel without myoglobin. However, the peaks that were seen in the loaded hydrogel spectrum were also observed in the myoglobin spectrum with shifting of some of the peaks. Each sample exhibited OH^- stretching due to hydrogen bonding and carboxylic vibrations and CO^- due to intramolecular bonding in the same regions. Peak E represents C=O stretching at 1734 cm^{-1}, but is seen at 1728 cm^{-1} in the loaded hydrogel. Peak G represents the ionized carbonyl group at 1558 cm^{-1} but is seen at 1550 cm^{-1} in the loaded hydrogel. Peaks H and J, which represent $C(=O)–O^-$ symmetric stretching and C–O stretching respectively, also were shifted in the loaded hydrogel. Each of these shifts can be attributed to the binding of myoglobin to the membrane. Therefore, these ATR-FTIR studies indicate a significant binding of myoglobin on PAA hydrogels at the pH values relevant to the diffusion experiments. This binding would explain the significant reduction of myoglobin transport in these gels.

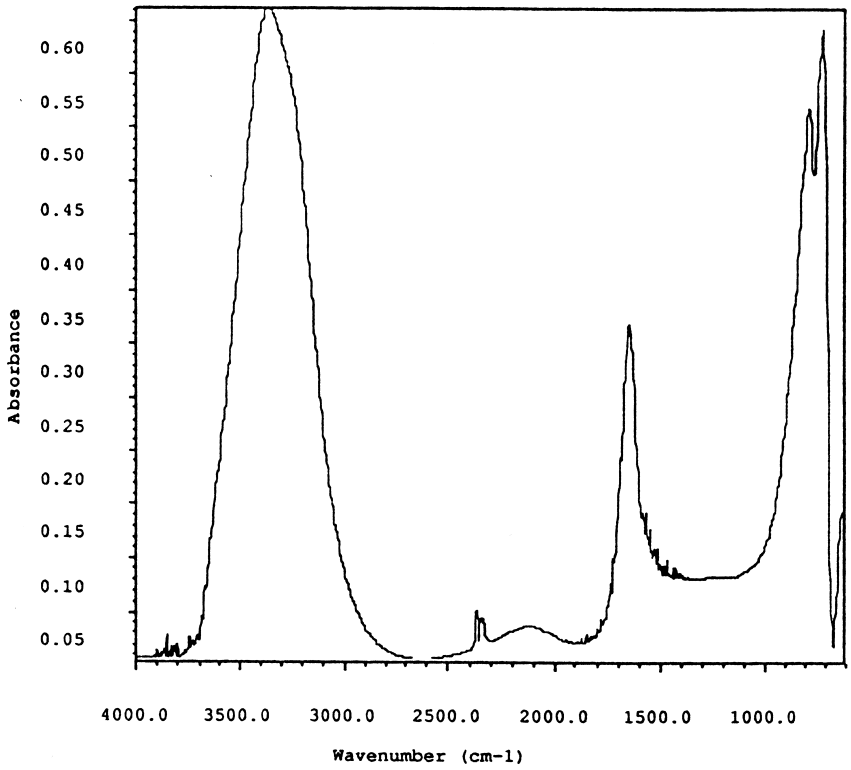

Fig. 10. ATR-FTIR spectrum of a swollen PAA hydrogel at pH 6.

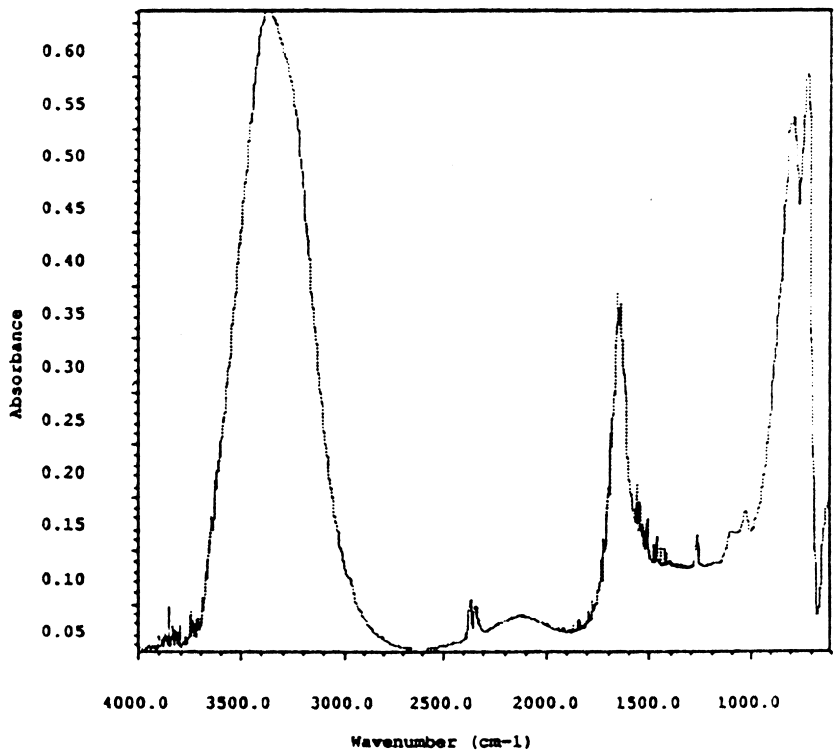

Fig. 11. ATR-FTIR spectrum of myoglobin solution.

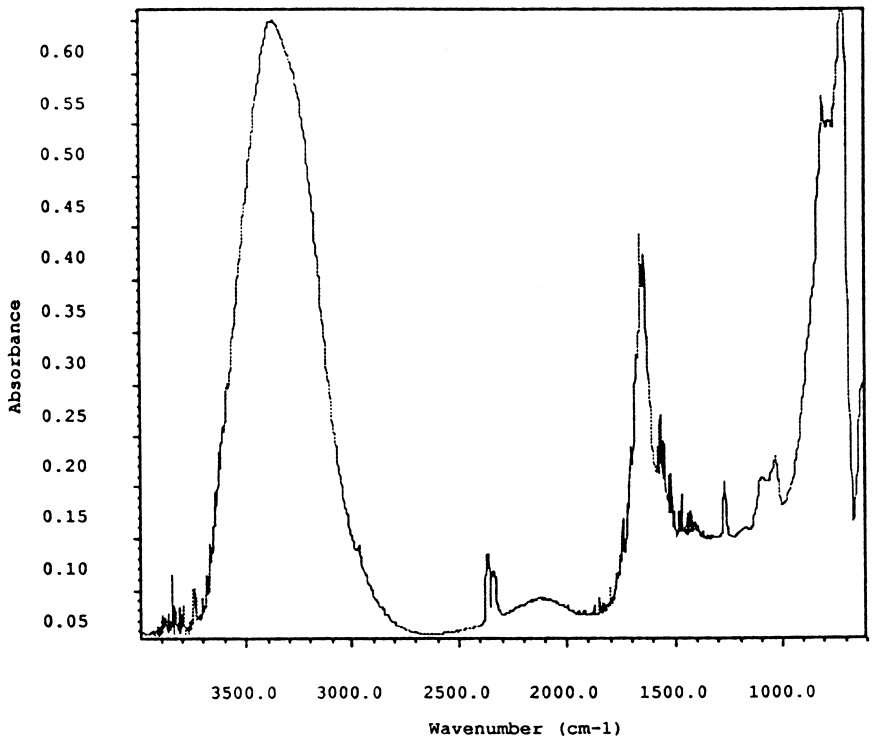

Fig. 12. ATR-FTIR spectrum of a swollen PAA hydrogel loaded with myoglobin at pH 6.

Table 11

FTIR data of PAA hydrogel, myoglobin, and PAA hydrogel loaded with myoglobin at pH 6

Peak	Functional vibration	Wavenumbers (cm^{-1})		
		PAA hydrogel	Myoglobin	Hydrogel loaded with myoglobin
A	OH^-	3370	3370	3370
B	OH^- stretching, hydrogen bonding	–	2964	2964
C	OH^- (associated carboxylic acid vibration)	2363	2363	2363
D	OH^- (associated carboxylic acid vibration)	2336	2338	2338
E	C=O stretching	1734	1734	1728
F	C=O stretching, intramolecular hydrogen bonding	1635	1635	1635
G	$(C=O)^-$	1558	1558	1550
H	$C(=O)–O^-$ symmetric stretch	–	1400	1393
I	C–O stretching (dimer)	–	1262	1262
J	C–O stretching (monomer)	–	1069	1083

3.8. Implications of binding on solute transport theory

The previous conclusions can be used in a somewhat quantitative way to identify the shift in diffusional binding. For example, recently am Ende and Peppas [22] proposed a modification of the Peppas–Reinhart theory which takes into consideration the binding (and the associated shifts of the IR spectra). According to this theory:

$$\frac{D_{SM}}{D_{SW}} = k_1\left(\bar{M}_c - \bar{M}_c^*/\bar{M}_n - \bar{M}_c\right)\exp\left(-k_2 r_s^2/Q_{m-1}\right) - k_3\exp\left(\frac{\Delta H^{ab}}{kT}\right) \quad (19)$$

Here, k_3 is a constant dependent on solute and polymer system and DH^{ab} is proportional to the shift in frequency. An inspection of the data of Fig. 9 indicates that the solute diffusion coefficient decreases significantly (in some cases by 4–5 orders of magnitude) as the PAA content of the PVA/PAA content increases from 40 to 100%. Thus, if one considers the data of Fig. 9 represented by the symbol (●), as the ones observed under the lowest binding, the further reduction of the diffusion coefficient is due to binding and can be calculated from the last term on the right-hand-side of Eq. (19).

For example, the diffusion data through a membrane with 40% PAA can be written as:

Table 12

Analysis of solute transport

PVA/PAA hydrogel (PAA content %)	Intercept ln k_{40}	Values k_{40}	Slope
40	1.07	2.90	0.85
75	1.12	3.06	1.42
100	1.15	3.16	2.64

$$\frac{D_{SM}}{D_{SW}} = k_{40}\exp\left(-\frac{\lambda_{40} r_s^2}{Q_{m-1}}\right) \quad (20)$$

This equation is a simple expression of the data of Fig. 10. The term k_{40} and λ_{40} are shown in Table 12.

The diffusion data through a membrane with 75% PAA can be written as:

$$\frac{D_{SM}}{D_{SW}} = k_{75}\exp\left(-\frac{\lambda_{75} r_s^2}{Q_{m-1}}\right) \quad (21)$$

and are shown in Table 12. Of course, this last curve could be represented also as:

$$\frac{D_{SM}}{D_{SW}} = k_{40}\exp\left(-\frac{\lambda_{40} r_s^2}{Q_{m-1}}\right) - K_{40} \quad (22)$$

Here, k_{40} is the binding term (diffusional reduction or decrease) which corresponds to the last term of the right-hand-side of Eq. (19). Similar analysis is done for the membrane containing 100% PAA.

4. Conclusions

PVA, PAA, and IPNs hydrogels were prepared varying the contents of PAA to study the effect of ionization on drug diffusion at pH 3 and pH 6 (above and below the pK_a of PAA). Each hydrogel was well characterized through swelling studies, and the molecular weight between crosslinks, the mesh size, and the crosslinking density were calculated. The molecular weight between crosslinks was found to be greater than the theoretical values due to the short reaction times of the polymers. The mesh size of the networks was greater at pH 6.

Drug diffusion was studied as a function of pH, mesh size, and PAA content. The results indicated that for vitamin B_{12}, a neutral solute with mol. wt. = 1355, permeation was greater at pH 6 at which the hydrogel was expanded and the mesh size was greater. The permeation of theophylline, mol.

wt. = 180 with pK_a of 8.6, was greater at pH 6, although the hydrogel and theophylline were ionized. Polymer/solute interactions may have occurred but because the mesh size of the hydrogel was significantly larger than the drug size, the interactions did not dominate diffusion. Myoglobin, mol. wt. = 17 200 with pK_a of 7.0, did not permeate through the hydrogels at pH 3 within 18 h. This is due to the immediate binding of myoglobin as it is placed in contact with the hydrogel. Because the mesh size is smaller at pH 3, the bound myoglobin may have reduced the space available for transport. At pH 6, permeation of myoglobin did occur. For the hydrogel that contained the least amount of PAA (40%), there was no permeation of myoglobin for 3.5 h afterwhich permeation proceeded at a linear rate. Myoglobin permeated through the hydrogels that contained higher amounts of PAA (75 and 100%), immediately at a linear rate, but the rate changed after 2.5 h due the polymer/solute interactions occurring between the hydrogels and myoglobin.

The Peppas–Reinhart theory was used to predict the drug transport through the membranes. The data did not fit the theoretical predictions due to the polymer/drug interactions that occurred between the membrane and the solutes. To further support the polymer/drug interactions, ATR-FTIR quantitatively confirmed the polymer/drug interactions by showing a shift in the carbonyl region of the spectra. These data could be explained by a modification of the Peppas–Reinhart theory by including a binding term for reduction of the drug diffusion.

Acknowledgements

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