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Hydration in hyaluronic acid and its esters using differential scanning calorimetry

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Summary

Differential scanning calorimetry (DSC) was used to distinguish three types of water of hydration in sodium hyaluronate and ethyl, benzyl, and partial benzyl esters of hyaluronic acid. These three types were defined as follows: type I: free, freezing water (freezing temperature ≈ 0 ° C); type II: weakly bound, freezing water (freezing temperature < 0 ° C); and type III: strongly bound, non-freezing water. Samples were scanned from (–) 50 to 20 ° C at a rate of 2.5 ° C/min. In the case of sodium hyaluronate, the onset temperature of melting deviated from that of pure water; such deviation was not observed for the esters of hyaluronic acid. 9–21 mol type III water were associated with each polymer repeat unit, with the value depending on the type of ester and degree of esterification. Hydrated sodium hyaluronate powder samples stored at (–) 50 ° C for up to 29 h did not show any changes in onset temperature and heat of fusion values, suggesting that the kinetics of freezing did not greatly influence the results. In hydrated benzyl ester and partial benzyl ester membranes, a good correlation was found between the percent of freezable water and permeability coefficients for various model compounds, suggesting that these solutes may be transported primarily in freezable water.

Introduction

Hyaluronic acid (HA) and its esters are being studied as possible drug delivery matrices; the characterization of membranes prepared from ester derivatives of HA has been described in a previous report (Hunt et al., 1990). Permeability and partition coefficient values of these membranes were reported for various charged and neutral solutes. The degree of hydration for membranes of various ester derivatives of HA was also determined, and found to be highly correlated to the permeability coefficient values for ethyl paraben. These results suggest that the degree of hydration of the polymer may be a key factor in drug release from these membranes. Similar findings have been reported for other polymers (Kanke et al., 1989; Bronsted and Kopecek, 1990).

This study is an attempt to probe the nature of water of hydration in HA and its esters using differential scanning calorimetry (DSC). DSC is

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one of the most widely used analytical methods for studying the water of hydration in various polymers and food products. It has been used to determine water of hydration in collagen (Hoeve and Tata, 1978), soy proteins (Muffett and Snyder, 1980), poly(acrylic acid) (Haldankar and Spencer, 1989), polycations and polyanions (Ohno et al., 1983), poly(vinyl alcohol) (Zhang et al., 1989), L-dipalmitoylphosphatidylcholine (Kodama et al., 1987), and carboxymethylcellulose (Nakamura et al., 1987). Three types of water of hydration have been identified and are designated as types I–III water. Type I water is free water which freezes at the normal freezing point of pure water (0 ° C). Type II water is weakly bound to macromolecules and displays a lower freezing point than that for pure water (< 0 ° C). Type III water is tightly bound to hydrophilic and ionic groups on the polymer and shows no freezing behavior (Muffett and Snyder, 1980; Ohno et al., 1983; Nakamura et al., 1987; Haldankar and Spencer, 1989; Zhang et al., 1989).

Hyaluronic acid is a linear polysaccharide. The samples of HA and its esters used in this study have an average molecular mass of approx. 150 kDa (FIDIA, S.p.A., personal communication). It has been reported that for polysaccharides the conformation of polymer chains, possible rotation about glycosidic bonds, intramolecular interactions, and the presence of hydrophobic groups on the polysaccharide may all affect the interaction with water (Suggett, 1972). While the determination of the glass transition temperature and of the different types of water in a sodium hyaluronate (Na-HA) gel have been reported previously (Hirohisa et al., 1989), no similar reports for esters of HA have been published.

Ultimately, it is not the hydration behavior itself that is of interest, but the relationship between hydration and the chemical composition of the polymer on the one hand and the release of drug on the other. Previous studies have shown that the transport properties of tightly bound water differ from those of free water. Higuchi et al. (1984, 1985) studied the permeation of gases through water-swollen gel cellophane and poly (vinyl alcohol-co-itaconic acid) membranes and estimated diffusion coefficients and solubilities in both non-freezing water and free water. An objective of this study is to develop similar relationships between different types of water and permeability coefficients for the hydrated hyaluronate polymers.

The specific objectives of this work therefore are: (1) to identify and to determine the percentage of types I–III water present in Na-HA powder and in membranes of several esters of HA; (2) to calculate the number of moles of tightly bound water per polymer repeat unit (PRU) for these esters; and (3) to relate the DSC results to permeability and partition coefficient values for various model penetrants in these membranes.

The presence of type III water is thought to be due to the equilibrium binding of water to the hydrophilic groups on the polymer chains. However, Roorda et al. (1988) and Bouwstra et al. (1990) proposed recently that the presence of non-freezing water was due to the inability of water to diffuse through the ice crystals in the gel which in turn was due to a glass-rubber transition on cooling. Their results suggest that the presence of different 'types' of water in hydrated polymers may be a time-dependent 'kinetic' effect rather than a true 'thermodynamic' effect. The last objective of this work is to test for these kinetic effects.

Materials and Methods

Materials

Na-HA powder and the polymers listed in Table 1 were used in this study; all polymers were supplied by FIDIA, S.p.A. (Abano Terme, Italy). All the polymers were dried in an oven at 50 ° C for 2 h to remove residual water prior to DSC experiments.

Hydrocortisone, hydrocortisone hemisuccinate sodium, benzyl alcohol, fluorescein sodium, and mafenide acetate were selected as the model compounds. The selection of these model compounds was based on the charge at neutral pH and their applicability as drugs. A sample of mafenide acetate was supplied by Sterling-Winthrop Research Institute (Rensselaer, NY). All other model drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as received.

Preparation of membranes

Membranes of the ethyl and benzyl esters of HA were supplied by FIDIA, S.p.A. Both types of membranes consisted of 100% esterified polymers, as determined by the manufacturer. Benzyl ester membranes with reduced degrees of esterification (50 and 75%) were prepared from 100% esterified membranes by subjecting the supplied membranes to hydrolysis in phosphate buffer, pH 9.0. The concentration of benzyl alcohol released was determined and the time to cleave 25 or 50% of the benzyl alcohol groups was calculated; these values were 8.9 and 19.1 h, respectively. After hydrolysis, the membranes were washed with water and stored in 0.05 M phosphate buffer, pH 4.0. In an earlier study, hydrocortisone esters of HA were reported to have maximum stability in acidic media (= pH 4–5; Goei et al., 1992).

Calibration of differential scanning calorimeter

A Perkin Elmer DSC-4 (Norwalk, CT) instrument was calibrated with indium ($\Delta H_{\text{fusion}} = 6.8$ cal/g, melting point $-156.3\,^\circ$C) (CRC Handbook, 1984/85a) for the melting point and heat of fusion. A second standard, cyclohexane ($\Delta H_{\text{fusion}} = 7.47$ cal/g, melting point $= 6.6\,^\circ$C) (CRC Handbook, 1984/85b), was also used because it has a melting point closer to that of water. The heat of fusion and melting point of ice were determined daily prior to experiments.

Preparation of samples

Standard aluminum sample pans (Perkin Elmer) for volatile liquids were used. The samples were weighed using a Cahn automatic electrobalance (model no. 21, Cahn Industries, Inc., Cerritos, CA). 1–4 mg of polymer were weighed into an empty pan. Various amounts of distilled water were then added to the polymer in the pan to obtain different ratios (R) of water to dry polymer (w/w). The pans were then capped, sealed, weighed and stored at room temperature overnight to allow equilibration between water and polymer. Prior to experiments, the pans were reweighed to check sealing; pans with faulty seals were discarded.

DSC experiments

DSC endotherms of the hydrated polymers. Thermograms of the samples were recorded in the temperature range (−) 50 to 20 °C at a scanning rate of 2.5 °C/min. The enthalpy of fusion was calculated by a computer (Perkin Elmer, Norwalk, CT) interfaced with the DSC from the peak area of the melting endotherm. The program also estimated the onset temperature (melting point) for each phase transition by extrapolating the leading edge of the peak back to the baseline. The reported enthalpy of fusion of the supercooled water was corrected for onset temperatures (T) less than 0 °C using the equation (Zhang et al., 1989):

$$\Delta H(T) = \Delta H(273\text{ K}) - \int_{T}^{273} \Delta C_{\mathrm{p}}\, \mathrm{d}T \qquad (1)$$

where ΔC_{p} is the difference between the heat capacities of liquid water and supercooled water at T K and ΔH is the heat of fusion value at temperatures T or 273 K. The same relationship may be applied to freezing bound water (Zhang et al., 1989). The maximum percent change in the enthalpy of fusion introduced by this correction was found to be 10%.

In the DSC experiments described above, all samples were cooled to (−) 50 °C and then the experiment was started immediately. In order to investigate the effect of freezing time, samples of hydrated Na-HA powder ($R = 8.63$) were cooled to (−) 50 °C and the temperature was maintained for different periods of time ranging from 0 to 29 h. The onset temperatures of the melting endotherms and heats of fusion values were then measured as described above.

Calculation of moles of type III (non-freezing) water. To determine the amount of type III water, the following procedure was used. A heat of fusion value for a polymer/water sample was reported with each endotherm. When this heat of fusion value was multiplied by the total amount of water in the sample, it yielded the total calories involved in the melting phase transition. This heat of fusion for pure water value was then corrected for sub-zero melting temperatures using Eqn 1. By dividing the total calories by the temperature-corrected heat of fusion, the total mass of freezable water (types I + II) in the sample was determined. From the amount of total water in the sample and the amount of freezable water, the mass of non-freezing water (type III) was calculated by difference. From the moles of non-freezing water and the number of polymer repeat units (total polymer mass/repeat unit molecular weight), the moles of tightly bound water per PRU and the mg type III water per 100 mg dry polymer were calculated. Using the percent hydration and mg type III water per 100 mg dry polymer values, the amount of freezing water (types I + II) in the fully hydrated membranes was estimated by difference.

Percent hydration

Percent hydration values for the polymer membranes were determined by modifying the procedure for asbestos fiber reported by the American Society for Testing and Materials (1989). The extent of hydration of the membranes under study was determined by placing small pieces (4–5 mg) of the membranes, dried in an oven at 50 ° C for 2 h, into distilled water maintained at approx. 0–2 ° C for 30 min. Preliminary experiments indicated that these membranes hydrate rapidly (< 10 min). Membranes were weighed before and after hydration on a Cahn Automatic Electrobalance (model no. 21, Cahn Instruments, Inc., Cerritos, CA) and the increase in weight was determined. The percent hydration values were calculated as:

$$\% \text{ hydration} = \frac{W_h - W_d}{W_d} \times 100 \qquad (2)$$

where W_h and W_d are the weights of the hydrated and dry membrane, respectively. The thickness values of these membranes were also measured before and after hydration, using a micrometer (Ames, Waltham, MA). A second type of hydration value was calculated so that the results could be compared with the 'free volume theory' developed by Yasuda and Lamaze (1971). In the free volume theory, hydration is defined as the ratio of the volume of water in the membrane to the total hydrated volume (Yasuda and Lamaze, 1971). The volume of water absorbed was calculated from the weight of water absorbed, assuming the density of water to be unity. The volume of the hydrated membrane was determined by measuring the thickness of a hydrated polymer disk of known diameter.

Permeability coefficient measurements

Permeability coefficient values for various membranes were determined using Side-Bi-Side® glass diffusion cells (model DC100-B, Crown Glass Co., Inc., Somerville, NJ). The detailed procedure has been reported in an earlier publication (Hunt et al., 1990). In all experiments, 0.05 M phosphate buffer, pH 7.00 was used as the donor and receptor fluid and the temperature was maintained at 32.0 ± 0.1 ° C by a circulating water-bath (VWR Scientific, Inc., San Francisco, CA).

Partition coefficient measurements

The membrane : solution partition coefficient values of the model drugs were measured using the 'solution-depletion' method (Hunt et al., 1990). In these experiments, disks (diameter 1.35 cm) of the membranes of polymers under study were presoaked in buffer solution (0.5 M phosphate buffer, pH 7.0, $\mu = 0.3$ M), blotted dry, and immersed in a drug solution for 3 h at 32 ° C. The loss of drug from the solution was evaluated by analyzing the concentration of drug before and after partitioning. The volume of the hydrated membrane was determined from the hydrated membrane thickness and the diameter of disk. In preliminary experiments, it was found that the radial expansion of membranes after hydration was minimal. The partition coefficient was then calculated as the ratio of the concentration of drug in the membrane to the concentration in the buffer (Hunt et al., 1990).

HPLC assay procedures

HPLC assays were developed for the model compounds; the detailed procedures are reported elsewhere (Joshi et al., 1992). In summary, the model compounds were analyzed using a C18 reversed-phase column (15 cm × 4 mm). In the case of hydrocortisone, mafenide acetate, benzyl alcohol and fluorescein sodium, a phosphate buffer : acetonitrile system was used as the mobile phase. For mafenide acetate, pentane- and octanesulfonic acids were added to the mobile phase to improve the peak shapes. An acetate buffer (pH 4.6) : acetonitrile system was used as the mobile phase for the hydrocortisone hemisuccinate assay.

Results and Discussion

Fig. 1 shows DSC heating curves for hydrated Na-HA powder. The hydrated powder has the appearance of a clear gel. For comparison, Fig. 1 also shows an endotherm for pure water. The onset temperature for the melting of pure water was found to be $0.5 \pm 0.3\,°C$ and the average value for the heat of fusion was 79.8 ± 2.19 mcal/mg ($n = 5$). Numbers to the right of each curve identify *R* values, the ratio of water to dry polymer (w/w) in each sample. For various *R* values, the onset temperatures were lower than that for pure water and shapes of the endotherms were asymmetrical. As the *R* value decreased, indicating a decreased water content, the onset temperature and the area of the phase transition peak decreased and the fronting of the peak became more pronounced. Two distinct peaks for type I (free water) and type II water (freezing bound water) were not observed for Na-HA/water endotherms. A similar observation was made by Ikada et al. (1980) in their study of Na-HA and other mucopolysaccharides. At the lowest *R* value of this report (0.824), a small peak was observed with onset temperature (−)

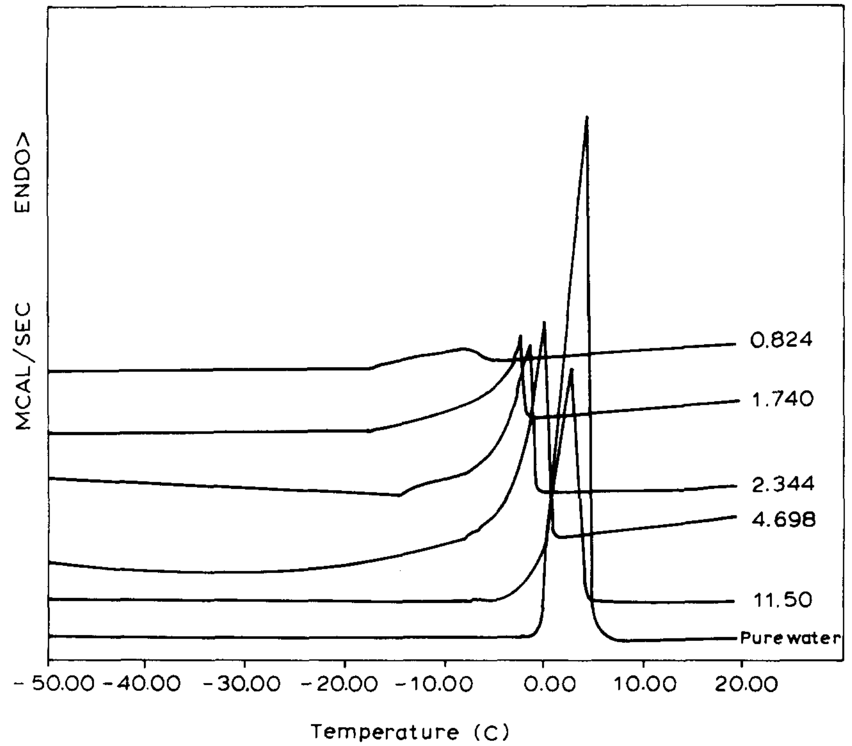

Fig. 1. Melting endotherms for pure water and water : sodium hyaluronate (Na-HA) samples. Temperature range: (−) 50 to 20 °C with a scanning rate of 2.5 °C/min. To the right of the endotherms are the values for the different ratios (*R*) of water to dry polymer (w/w).

16.3 ° C, which may be identified tentatively as type II water; type I (free water) was not present in this case. For all other *R* values studied, the fronting of the type I peak may be due to overlapping of the peaks for types I and II water. For a ratio of water to polymer of 0.46, Hirohisa et al. (1989) did not observe a melting endotherm, suggesting that all water was tightly bound to HA (type III). Although *R* values as low as 0.46 were not included in this study, the current data support the disappearance of freezing water with decreasing *R* values. In summary, the data suggest that types I–III water are present in hydrated Na-HA, although types I and II water could not be distinguished clearly due to overlapping of the melting endotherms. Types I and II water in Na-HA are grouped together as 'freezable water' in the remainder of this report.

Fig. 2 shows DSC heating curves for hydrated benzyl ester membranes having various ratios (*R*) of water to dry polymer. All the endotherms were symmetrical; the fronting observed in Na-HA samples was completely absent. The onset temperature was almost 0 ° C for all the samples, independent of the *R* value. Similar endotherms were observed for ethyl ester membranes. These results suggest that in these hydrated ester membranes, only two types of water were present: types I (free water) and III (non-freezing bound water).

Fig. 3 summarizes the onset temperatures for powders of Na-HA and for membranes of the esters of HA as a function of the water/polymer ratio (*R* value). As the *R* value increased for Na-HA powders, the onset temperature increased; when *R* was greater than 10, the onset temperature approached the value for pure water, 0 ° C. In contrast, the onset temperature for the ester membranes was approx. 0 ° C for all *R* values. HA and the esters of HA have similar chemical structures; water probably interacts with the hydroxyl, secondary amine and carboxylic acid

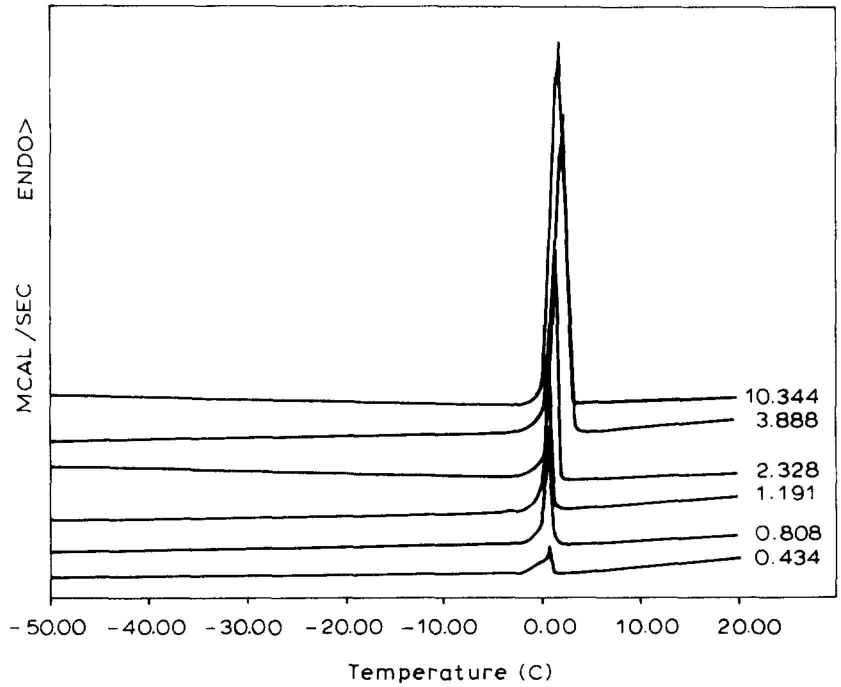

Fig. 2. Melting endotherms for water in hydrated membranes of the 100% benzyl ester of hyaluronic acid (HYAFF 11). Temperature range: (−) 50 to 20 ° C with a scanning rate of 2.5 ° C/min. The values for the different ratios (*R*) of water to dry membrane (w/w) are given on the right-hand side of the endotherms.

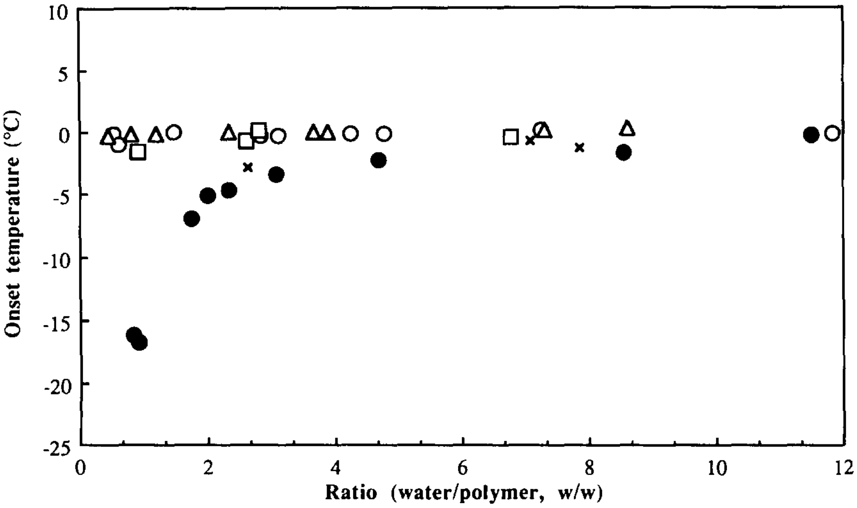

Fig. 3. Effect of water to polymer ratio, R (w/w), on the onset temperature (°C) of melting for water in powders of sodium hyaluronate and membranes of esters of hyaluronic acid. (●) Sodium hyaluronate, (○) ethyl ester, (△) benzyl ester, (□) 75% benzyl ester, (×) 50% benzyl ester. Temperature range in the DSC experiments was (−) 50 to 20 °C with a scanning rate of 2.5 °C/min.

groups on the polymer backbone. However, an important difference between the esters and HA is that the esters are uncharged while HA is negatively charged ($pK_a = 3.36$, Park and Chakrabarti, 1978) at the pH of these experiments. Thus, a charge effect may contribute to the interaction between the polymer and freezable water observed for HA. The reduced onset temperature for Na-HA powder at low R values suggests a stronger interaction between the polymer and freezable water, perhaps due to interactions between water and charged carboxyl groups on the polymer. The fact that this reduction is absent for the esters suggests a weaker interaction of freezable water with the polymer, perhaps due to the absence of charged sites on the polymer. The results are consistent with the findings of Ikada et al. (1980) in which a comparison of

TABLE 1

Hydration behavior of ethyl, benzyl and partial benzyl esters of hyaluronic acid

Polymer	% hydration [a]	Moles of type III [b] water per PRU	mg type III water [c,d] / 100 mg dry polymer	mg types I + II water [d,e] / 100 mg dry polymer
100% ethyl ester	341 ± 11	12.5 ± 1.9	55.1 ± 8.2	286 ± 7
100% benzyl ester	72 ± 19	9.1 ± 1.6	34.9 ± 6.2	37 ± 18
75% benzyl ester + 25% sodium salt	158 ± 16	13.4 ± 1.8	53.4 ± 7.2	105 ± 14
50% benzyl ester + 50% sodium salt	688 ± 22	20.6 ± 0.8	85.1 ± 3.4	603 ± 22
Sodium hyaluronate	dissolves	13.4 ± 1.7	60.1 ± 7.6	N.D. [f]

[a] Percent hydration at 0 °C, calculated using Eqn 2.

[b] Moles of type III (non-freezing) water per polymer repeating unit (PRU).

[c] Mass of type III (non-freezing) water per 100 mg dry polymer.

[d] Standard deviations were calculated by the method of propagation (Meyer, 1975).

[e] Mass of types I + II (freezing) water per 100 mg dry polymer.

[f] Since equilibrium percent hydration could not be calculated for Na-HA, this value was not determined.

sulfated and non-sulfated muocopolysaccharides was performed. In their study, sulfated mucopolysaccharides with more charged moieties (additional sulfate groups) showed a greater number of moles of water per polymer repeat unit, suggesting the importance of charged groups in mucopolysaccharide/water interactions.

Table 1 lists the percent hydration values, moles of type III (non-freezing) water per polymer repeat unit (PRU), and the masses of freezable water (types I + II) and non-freezable water (type III) per 100 mg dry polymer for the various membranes. Ethyl ester membranes are more hydrated than 100% benzyl ester membranes, consistent with the data reported by Hunt et al. (1990). Benzyl ester membranes with reduced esterification (50 and 75%) showed greater hydration than 100% esterified benzyl ester membranes. The values for moles of type III water per PRU ranged from 9.1 to 20.6. In a previous study, Nakamura et al. (1987) determined a value of 7–11 mol water per glucose residue in alkali metal salts of carboxymethylcellulose. The values obtained for the moles of type III water per PRU in this study were similar to those reported by Nakamura et al. (1987), suggesting a similar degree of interaction between water and the various polymers. Finally, the amount of type III water (mg) per 100 mg dry polymer ranged from 35 to 85; the amount of types (I + II) water per 100 mg dry polymer ranged from 37 to 603 mg (Table 1). These results indicate that the variation in the amount of non-freezing water for the different esters of HA was relatively small, whereas the amount of freezable water was more variable.

Analysis of variance showed significant differences in the values determined for moles of type III water per PRU for the different polymers. As benzyl ester membranes were hydrolyzed (50 and 75% esterification), the number of water molecules per PRU increased significantly. Values for benzyl ester membranes were significantly lower than those for partial benzyl ester membranes ($p < 0.05$). This may be due to binding of water to charged carboxylic acid groups formed by hydrolysis. However, Na-HA showed fewer moles of type III water per PRU than the 50% benzyl ester membranes, which are presumably more hydrophobic. The reason for this is not understood at this time, but may involve differences in polymer morphology in the powder and membrane forms. The value for the mean number of moles of type III water per PRU for ethyl ester membranes (12.5 ± 1.9) was significantly different ($p < 0.05$) from that for the benzyl ester membranes (9.1 ± 1.6). In addition, the hydration of the 100% ethyl ester membranes was significantly greater than that of the 100% benzyl ester membranes ($p < 0.05$).

TABLE 2

Effect of length of refrigeration at (−) 50 °C on the onset temperature and heat of fusion of water in hydrated sodium hyaluronate powder (weight of water / weight of polymer, (R) = 8.63)

Time of refrigeration (h)	Onset temperature (°C)	Heat of fusion of water (mcal/mg)
0	−1.29	71.38
0	−1.05	71.04
1	−1.05	71.30
3	−1.02	70.77
14	−0.98	70.70
29	−1.13	71.13

Table 2 lists onset temperature and heat of fusion values for a Na-HA/water sample subjected to freezing at (−) 50 °C for different lengths of time. There was no change in either the onset temperature or heat of fusion values even after freezing the sample for as long as 29 h. In contrast, Roorda et al. (1988) found that the melting enthalpy of water in acrylate gels was strongly dependent on the cooling time and the peak for type II water or ‘the double character of the melting peak’ was found to disappear. Both Roorda et al. (1988) and Bouwstra et al. (1990) argued that the rubber-to-glass transition of these gels in the temperature range of the studies prevented water molecules from diffusing to the ice crystals that were forming in the gel. Thus, the rate of water diffusion towards the nuclei of forming ice was substantially reduced because of the stiff, glassy structure of the gel. They also found that the glass transition temperature (T_g) of the acrylate was greater than 0 °C and was

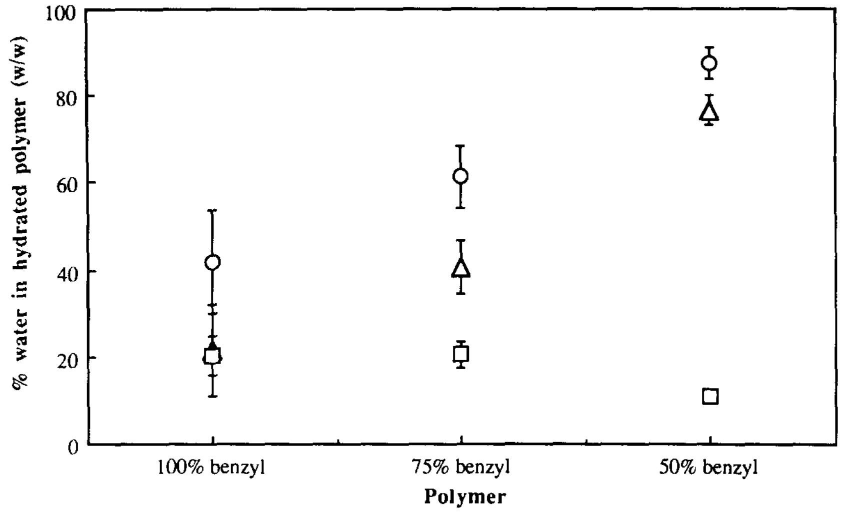

Fig. 4. Freezing behavior of water in a series of hydrated benzyl ester membranes. The hydration values are based on the percent of total hydrated polymer weight. Error bars represent standard deviations of the values ($n = 3$). (△) % types I + II water (freezing water), (○) % types I–III water (total water), (□) % type III water (non-freezing water). 100% benzyl = 100% esterified benzyl ester of hyaluronic acid (HA), 75% benzyl = 75% esterified partial benzyl ester of HA and 50% benzyl = 50% esterified partial benzyl ester of HA.

dependent on the ratio of water to polymer; a similar result has been reported for poly-HEMA (Sung et al., 1978). For Na-HA, Hirohisa et al. (1989) reported T_g values ranging between 150 and 190 K for hydrated polymer with R values between 0.5 and 2.0. Thus, given the temperature

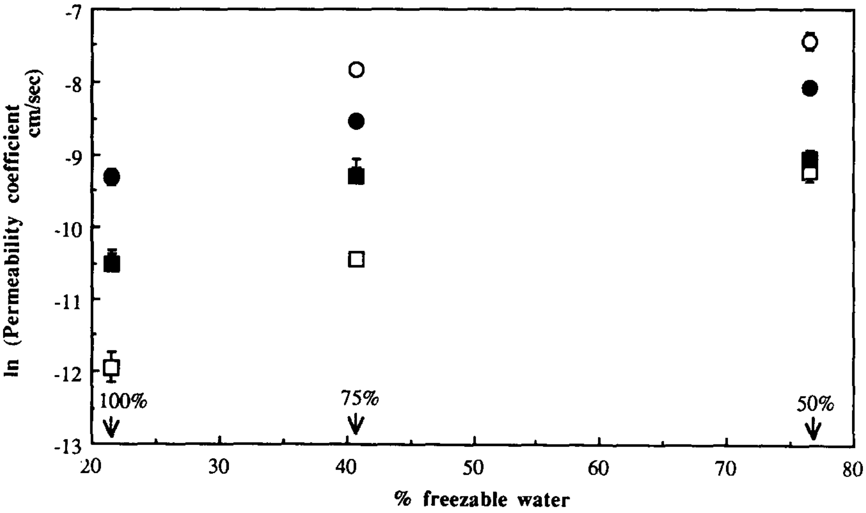

Fig. 5. Effect of percent freezable water (types I + II) on permeability coefficients for a series of hydrated benzyl ester membranes to various model drugs. The % freezable water values were based on the total hydrated polymer weight. Error bars represent standard deviations of the permeability coefficient values ($n = 3$ or 4). 100% = 100% esterified benzyl ester of HA, 75% = 75% esterified partial benzyl ester of HA and 50% = 50% esterified partial benzyl ester of HA. (X) Hydrocortisone, (□) hydrocortisone hemisuccinate, (●) benzyl alcohol, (○) mafenide acetate, (■) fluorescein sodium.

range of the present experiments (223–293 K) and the high R value studied ($R = 8.63$), the polymer was probably present in the rubbery state. The absence of cooling time effects and the rubbery state of the polymer in the current studies suggest that the types of water observed are a true thermodynamic phenomenon, and not a kinetic artifact. It is not possible to extrapolate the results of the freezing time studies to esters of HA.

Significance of DSC results for drug delivery

A detailed discussion of the permeability and partition coefficients of the model compounds has been reported elsewhere (Joshi et al., 1992). In summary, the ethyl ester membranes showed larger permeability values than those of the benzyl ester membranes for all the model compounds tested. In the benzyl ester series, the permeability values increased with de-esterification. The partition coefficients of all the model compounds in the ethyl ester membranes were small (< 5), while hydrocortisone and hydrocortisone hemisuccinate were found to have large partition coefficient values in benzyl ester membranes ($K = 43.4 \pm 3.6$ and 16.3 ± 3.0, respectively).

Fig. 4 shows the percentages of total (types I–III), freezing (types I + II), and non-freezing (type III) water in a series of benzyl ester membranes with varying degrees of esterification. The percentage values were calculated on the basis of the total weight of the hydrated polymer. The non-freezing water content was essentially constant, while the freezing and total water content increased with decreasing esterification. Thus, the increased total hydration of the partially esterified membranes is primarily due to an increase in the amount of freezable water (types I + II).

Fig. 5 shows the relationship between the permeability coefficient values and the amount of freezable water (Types I + II). The permeability coefficient values were related to the percent freezable water in the fully hydrated polymer for all compounds tested. An increase in the amount of freezable water was associated with an increase in the permeation rate of various model compounds. This suggests that solute permeation may have occurred primarily through the freezable water in the membranes. Diffusion coefficient values were calculated from permeability coefficient, partition coefficient, and membrane thickness values; a correlation between the diffusion coefficient values and percent freezable water was also observed (data not shown). In these calculations, the diffusion path length was assumed to be equal to the hydrated membrane thickness. This assumption is questionable, since porosity and tortuosity can affect the true diffusion path length. Since porosity and tortuosity may change in complex ways with changing membrane hydration, the calculated effective diffusion coefficients may contain this hidden bias, and so are not reported.

Kim et al. (1980) and Wisniewski and Kim (1980) studied the permeation of hydrophilic and hydrophobic solutes through poly(2-hydroxyethyl methacrylate) (HEMA). The authors described two domains in the hydrated membranes: domain A consisted of polymer, interfacial water, and bound water and domain B consisted of 'bulk-like' water. It was concluded that small, hydrophilic solutes like sucrose and lactose permeate through bulk water (domain B). Hydrophobic solutes permeated by a 'pore mechanism', i.e., via domain B in the case of hydrogels without cross-linking agent. Hydrophobic solutes permeated through cross-linked hydrogels by a 'partition' mechanism (i.e., partitioning or dissolution followed by diffusion in domain A; Kim et al., 1980) governed by the solubility of the solute in domain A. In the present study, a positive correlation was found between the permeability coefficients and the percent freezable water. This suggests that the transport of solutes occurs through freezable water (roughly analogous to domain B) and that little transport occurs in tightly bound water (domain A). The lack of domain A transport may indicate that this route is relatively unimportant in hyaluronate ester membranes, or that the compounds studied are too hydrophilic to permeate by this route.

Fig. 6 depicts the relationship between partition coefficient values (membrane : buffer) of various model drugs in a series of benzyl ester membranes and the domain A content of the polymer (i.e., type III water plus polymer). For mafenide

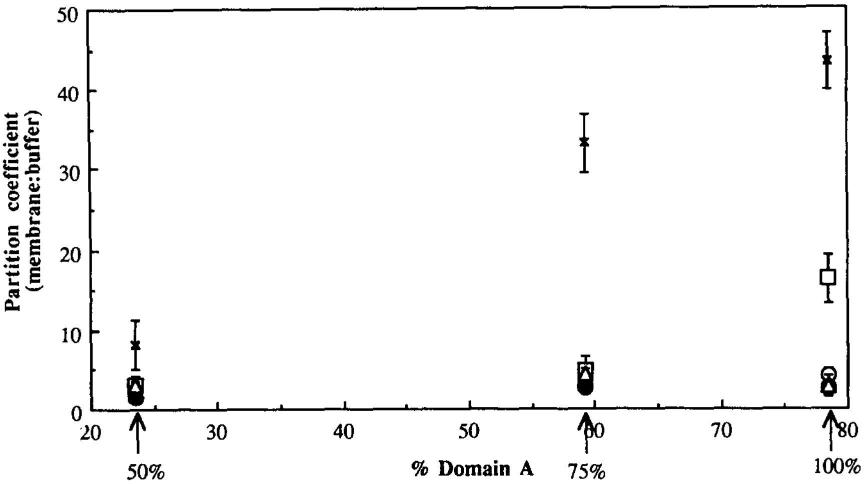

Fig. 6. Partition coefficients for various drugs in a series of benzyl ester membranes as a function of domain A (polymer plus type III water) content. The percent domain A values were based on the total hydrated polymer weight. 100% = 100% esterified benzyl ester of HA, 75% = 75% esterified partial benzyl ester of HA and 50% = 50% esterified partial benzyl ester of HA. Error bars represent standard deviations of the partition coefficient values ($n = 5$). (×) Hydrocortisone, (□) hydrocortisone hemisuccinate, (●) benzyl alcohol, (○) mafenide acetate, (△) sodium fluorescein.

acetate, benzyl alcohol and fluorescein sodium, the partition coefficient values did not vary with changes in polymer/bound water content. For hydrocortisone and hydrocortisone hemisuccinate, the partition coefficient values increased with an increase in polymer/bound water content. This suggests that the relatively large membrane:buffer partition coefficients reported for

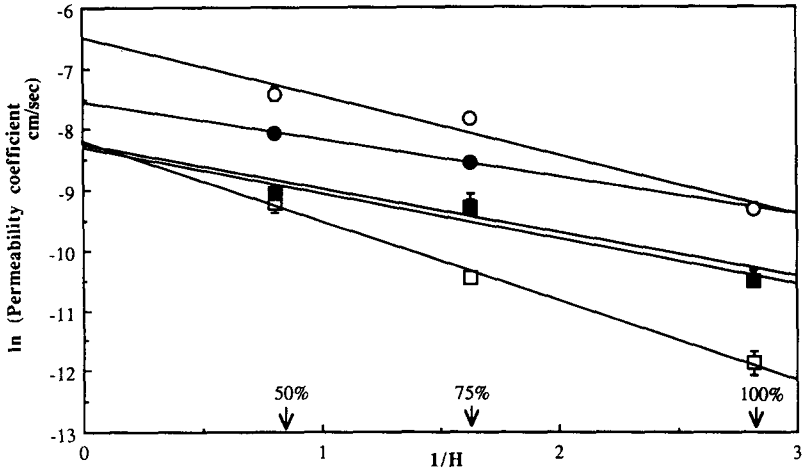

Fig. 7. A plot according to the free volume theory of Yasuda and Lamaze (1971), depicting a relationship between permeability coefficients for various model drugs in a series of hydrated benzyl ester membranes with various degrees of esterification (100, 75 and 50%) and the reciprocal of membrane hydration. The free volume theory defines hydration as the ratio of the volume of water in the membrane to the total hydrated membrane volume. Error bars represent standard deviations of the permeability coefficient values ($n = 3$ or 4). 100% = 100% esterified benzyl ester of HA, 75% = 75% esterified partial benzyl ester of HA and 50% = 50% esterified partial benzyl ester of HA. (× ——— ×) Hydrocortisone, (□ ——— □) hydrocortisone hemisuccinate, (● ——— ●) benzyl alcohol, (○ ——— ○) mafenide acetate, (■ ——— ■) sodium fluorescein.

these compounds (Joshi et al., 1992) may be related to their affinity for tightly bound water and/or the hydrated polymer.

It should be noted that in Figs 4–6, the freezable or non-freezing water content was calculated as a percentage of the total weight of the hydrated polymer (i.e., water plus polymer). Other methods for reporting freezing or non-freezing water content are possible, such as the ratio of freezing or non-freezing water mass to polymer mass, or as a percentage of the water content of the hydrated membrane (i.e., excluding the dry polymer mass). The method used in these correlations was selected because partition coefficients are calculated on the basis of the total volume of the hydrated membrane, and because permeation may involve the polymer chains as well as the water of hydration.

The free volume theory proposed by Yasuda and Lamaze (1971) relates the permeability coefficient in a swollen hydrogel to the degree of swelling and molecular size of the permeant. According to this theory, a plot of ln(permeability coefficient) vs $1/H$ should be linear, where H is the degree of hydration of the membrane. Fig. 7 is a plot of ln(permeability coefficient) vs $1/H$ for various model compounds through a series of benzyl ester membranes of varying H values. Linearity was observed for all the permeants, with the values of the correlation coefficient ranging from 0.965 to 0.999. The results are therefore consistent with the free volume theory. For the diffusion of ethyl paraben through a series of esters of HA, it was found in a previous report (Hunt et al., 1990) that the free volume theory of Yasuda and Lamaze (1971) did not hold. In that study, different types of esters of HA were used. In the present study, membranes with the same ester functional group, but varying only in the degree of esterification were used. This suggests that physical/chemical factors other than hydration were important in earlier report, and that these factors are absent in the present study, probably because a single type of ester was considered.

The applicability of the DSC results to drug transport is somewhat limited. The DSC studies were conducted near 0 °C while the drug permeation studies were performed at 32 °C. It may be argued that the DSC results cannot be correlated with those of drug transport because of this temperature difference. It is not possible with the present experimental methods to answer this question. Solid-state NMR studies, Raman scattering and X-ray diffraction measurements can be employed to study the interaction of water with the polymer at higher temperatures. It seems reasonable to assume that even at 32 °C, different types of water would be detected in the hydrated polymers, with only the relative fractions of these three types of water differing from those found here. Also, the mechanism of drug transport through the hydrated polymers is complex and thus, the DSC results alone cannot explain fully the process of drug transport. However, these results do provide insight into the mechanism of drug diffusion through the membranes of esters of HA, and the role of the water of hydration in drug transport.

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References

American Society for Testing and Materials, Standard test method for moisture content of asbestos fiber. Designation: D 2987-88. *Annual Book of ASTM Standards, Vol. 8.02, Plastics (II)*, American Society for Testing and Materials, Philadelphia, 1989, pp. 496–497.

Bronsted, H. and Kopecek, J., Hydrogels for site-specific oral drug delivery, *Proc. 17th Int. Symp. Control. Rel. Bioact. Mater.*, Controlled Release Society, Reno, NV, 1990, pp. 128–129.

Bouwstra, J.A., Peschier, L.J.C., de Vries, M.A., Van Miltenburg, J.C., Leyte, J. and Junginger, H.E., Is there evidence for different types of water in hydrogels? *Proc. 17th Int. Symp. Control. Rel. Bioact. Mater.*, Controlled Release Society, Reno, NV, 1990, pp. 120–121.

CRC Handbook of Chemistry and Physics, 65th Edn, Weast, R.C., Astle, M.C. and Beyer, W.H. (Eds), CRC Press, Boca Raton, FL, 1984/85a, p. B-231.

CRC Handbook of Chemistry and Physics, 65th Edn, Weast, R.C. Astle, M.J. and Beyer, W.H. (Eds), CRC Press, Boca Raton, FL, 1984/85b, p. C-691.

Fidia, S.p.A., Abano Terme, Italy, personal communication.

Goei, L., Benedetti, L., Biviano, F., Callegaro, L., Topp, E. and Stella, V., Drug release from hydrocortisone esters of hyaluronic acid: influence of ester hydrolysis rate on release rate, *Proc. Int. Conference on Polymers in Medicine: Biomedical and Pharmaceutical Applications* (1992) in press.

Haldankar, G.S. and Spencer, H.G., Properties of bound water in poly(acrylic acid) and its sodium and potassium salts determined by differential scanning calorimetry. *J. Appl. Polym. Sci.*, 37 (1989) 3137–3146.

Higuchi, A., Abe, M., Komiyama, H. and Iijima, T., Gas permeation through hydrogels. I. Gel cellophane membranes. *J. Membr. Sci.*, 21 (1984) 113–121.

Higuchi, A., Fushimi, H. and Iijima, T., Gas permeation through hydrogels. II. Poly(vinyl alcohol-co itaconic acid) membranes. *J. Membr. Sci.*, 25 (1985) 171–180.

Hirohisa, Y., Tatsuko, H., Kunio, N. and Hyoe, H., Glass transition of hyaluronic acid hydrogel. *Kobunshi Ronbunshu*, 46 (1989) 597–602.

Hoeve, C.A.J. and Tata, A.S., The structure of water absorbed in collagen. *J. Phys. Chem.*, 82 (1978) 1660–1663.

Hunt, J.A., Joshi, H.N., Stella, V.J. and Topp, E.M., Diffusion and drug release in polymer films prepared from ester derivatives of hyaluronic acid. *J. Controlled Release*, 12 (1990) 159–169.

Ikada, Y., Suzuki, M. and Iwata, H., Water in mucopolysaccharides. In Rowland, S.P. (Ed.), Chap. 17, *Water in Polymers*, ACS Symp. Ser. 127, American Chemical Society, Washington, DC, 1980, pp. 287–305.

Joshi, H.N., Stella, V.J. and Topp, E.M., Drug release from membranes of hyaluronic acid and its esters. *J. Controlled Release*, (1992) in press.

Kanke, M., Katayama, H., Tsuzuki, S. and Kuramoto, H., Application of chitin and chitosan to pharmaceutical preparations. I. Film preparation and in vitro evaluation. *Chem. Pharm. Bull.*, 37 (1989) 523–525.

Kim, S.W., Cardinal, J.R., Wisniewski, S. and Zentner, G.M., Solute permeation through hydrogel membranes. Hydrophilic vs. hydrophobic solutes. In Rowland, S.P. (Ed.), Chap. 20, *Water in Polymers*, ACS Symp. Ser. 127, American Chemical Society, Washington, DC, 1980, pp. 347–359.

Kodama, M., Hashigami, H. and Seki, S., Role of water molecules in the subtransition of the L-dipalmitoylphosphatidylcholine-water system as studied by differential scanning calorimetry. *J. Colloid Interface Sci.*, 117 (1987) 497–504.

Meyer, S.L., *Data Analysis for Scientists and Engineers*, Wiley, New York, 1975, p. 40.

Muffett, D.J. and Snyder, H.E., Measurement of unfrozen and free water in soy proteins by differential scanning calorimetry. *J. Agric. Food Chem.*, 28 (1980) 1303–1305.

Nakamura, K., Hatakeyama, T. and Hatakeyama, H., DSC studies on monovalent and divalent cation salts of carboxymethylcellulose in highly concentrated aqueous solutions. In Kennedy, J.F., Phillips, G.O. and Wiliams, P.A. (Eds), Chap. 10, *Wood and Cellulosics: Industrial Utilization, Biotechnology, Structure and Properties*, Wiley, New York, 1987, pp. 97–103.

Ohno, H., Shibayama, M. and Tsuchida, E., DSC analyses of bound water in the microdomains of interpolymer complexes. *Makromol. Chem.*, 184 (1983) 1017–1024.

Park, J.W. and Chakrabarti, B., Optical characteristics of carboxyl group in relation to the circular dichroic properties and dissociation constants of glycosaminoglycans. *Biochim. Biophys. Acta*, 544 (1978) 667–675.

Roorda, W.E., Bouwstra, J.A., De Vries, M.A. and Junginger, H.E., Thermal behaviour of poly hydroxy ethyl methacrylate (pHEMA) hydrogels. *Pharm. Res.*, 5 (1988) 722–725.

Suggett, A., Polysaccharides. In Franks, F. (Ed.), Chap. 6, *Water – A Comprehensive Treatise*, Vol. 4, *Aqueous solutions of amphiphiles and macromolecules*, Plenum, New York, 1972, pp. 519–567.

Sung, Y.K., Gregonis, D.E., Russell, G.A. and Adrade, J.D., Effect of water and tacticity on the glass transition temperature of poly(2-hydroxyethyl methacrylate). *Polymer*, 19 (1978) 1362–1363.

Wisniewski, S. and Kim, S.W., Permeation of water-soluble solutes through poly(2-hydroxyethyl methacrylate) and poly(2-hydroxyethyl methacrylate) crosslinked with ethylene glycol dimethacrylate. *J. Membr. Sci.*, 6 (1980) 299–308.

Yasuda, H. and Lamaze, D.E., Permselectivity of solutes in homogeneous water-swollen polymer membranes. *J. Macromol. Sci. Phys.*, 5 (1971) 111–134.

Zhang, W.Z., Satoh, M. and Komiyama, J., A differential scanning calorimetry study of the states of water in swollen poly (vinyl alcohol) membranes containing nonvolatile additives. *J. Membr. Sci.*, 42 (1989) 303–314.