Investigation of the States of Water in Water-Swollen Hydrogels by Liquid Chromatography and Differential Scanning Calorimetry

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A new liquid chromatographic method was proposed for the estimation of the amount of water which exhibits selectivity to solutes in water-swollen hydrogels based on the model regarding the retention of ions on the column packed with the hydrogels. The estimated amount of the water in polyacrylamide, dextran, and poly(vinyl alcohol) gels corresponds to the sum of the amount of freezable bound water and that of nonfreezing water determined by means of differential scanning calorimetry. This means that the freezable bound water takes an important role in the separation process using water-swollen hydrogels contrary to the conventional view of the freezable water.

Selectivity of water-swollen hydrophilic polymer gels to solutes has been noted owing to their applicability to efficient separations such as dialysis and liquid chromatography. A number of investigators have considered that an understanding of the properties of water in hydrogels is highly important for making clear the mechanism of the selectivity of hydrogels. It has so far been elucidated that there are at least one or two states of water in hydrogels which exhibits properties different from normal (bulk) water based on NMR,[1-3] IR,[4] differential scanning calorimetry (DSC),[5-13] and other investigations.[14-17]

Several workers have dealt with the selectivity of water incorporated in polymer gels.[7,10,12,18-21] However, the quantitative estimation of the amount of water which exhibits the selectivity to solutes has yet scarcely been made.

We have recently proposed a new method for the determination of the mobile phase volume (V_m) in liquid chromatography based on the distribution of ions and revealed that this method produces reasonable V_m values not only for binary solvent systems but also single solvent systems.[22] Stationary phase, which exhibits the selectivity to solutes, must have the physicochemical properties different from those of the mobile phase. The volume of the region which does not function as mobile phase (the stationary phase is contained in this region) can be estimated from the mobile phase volume.

This study was undertaken to apply this method to the estimation of the amount of water in various hydrophilic polymer gels which has properties different from those of free water as to the distribution of solutes. DSC analysis was also carried out in order to clarify the state of the water in the hydrogels.

Experimental

Materials. All chemicals used in this study were of reagent grade quality and they were used without further purification unless otherwise stated. Deionized and distilled water was used throughout the experiment.

Cross-linked polyacrylamide gels, Bio-Gel P-2 (200—400 mesh) and P-4 (200—400 mesh) purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.), cross-linked dextran gels, Sephadex G-10 (40—120 μm), G-15 (40—120 μm) and G-25 (10—40 μm) purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and a poly(vinyl alcohol) gel, TSK-GEL Toyopearl HW-40S (20—40 μm) purchased from Tosoh (Tokyo, Japan) were used in this experiment. All of the polymer gels were washed with water, ethanol, and acetone in this order and then dried at 90 °C.

Blue Dextran 2000 (Pharmacia) was used as a reference material for evaluating the interstitial volume (interparticulate volume) in the column used for liquid chromatographic experiment.

Chromatographic Conditions. The liquid chromatographic system consisted of a Kyowa Seimitsu (Tokyo, Japan) Model KHP-010 pump, a Model KHP-UI-130A injection valve (Kyowa), a Model KLC-800 UV-visible variable wavelength absorption detector (Kyowa) and a Tosoh (Tokyo, Japan) Model CM-8 conductivity detector. The solvent reservoir was a commercially available glass syringe with the 200 ml capacity.[23] A Pyrex column was packed with each polymer packing swollen by water, water-jacketed and thermostated at 25.0±0.1 °C. The detection signal was fed into a System Instruments (Tokyo, Japan) Intelligent Integrator Model 7000A.

The eluents used were aqueous solutions of the various sodium salts with ionic strength of 0.1, the Donnan exclusion effect of fixed ionic groups of the matrix of each polymer gel turning out negligible in these media.[24,25] Test solutions were prepared by dissolving the sodium salts of analyte anions in the eluent used. A 10 μl portion of a solution was introduced into the column.

Elutions were carried out at a constant flow rate of ca. 0.5 ml min^{-1}. Exact values of the volumetric flow rate were measured using the buret designed so as to prevent the vaporization of solvent.

The volume relating to the spaces between the sample injector and the column inlet, and between the column outlet and the detector, was corrected for in the determination of the retention volumes of analytes. All retention data were averaged from three consecutive measurements.

DSC Measurements. A Perkin-Elmer (CT, U.S.A.)

DSC-7 differential scanning calorimeter equipped with a cooling device was used to measure the phase transition of water sorbed in the polymer gels. DSC curves were obtained by cooling at the scannning rate of 5 °C min^{-1} from 20 °C to −40 °C and then heating to 20 °C at the same rate after maintaining −40 °C for 1 min. The temperatures of crystallization and melting of water sorbed in the polymer gel were calibrated using the melting peaks of pure water and indium.

The each polymer gel sample was left swell for over one day in distilled water contained in a screw capped glass bottle. After the supernatant water was removed, the sample was stirred and then 2 to 8 mg sample was quickly weighed in a small aluminium pan used for volatile samples on a microbalance. The sample pan was then sealed hermetically. Any water leakage was not observed for weighings performed before and after DSC measurements.

The remainder of the each sample in the glass bottle was weighed and washed with ethanol and acetone in this order after it was transfered into a glass filter and then dried at 90 °C in an oven until a constant weight was reached. The water content of each polymer gel sample, ϕ_w, was calculated as follows:

$$\phi_w = \frac{W_s(b) - W_g(b)}{W_s(b)}, \quad (1)$$

where $W_s(b)$ and $W_g(b)$ denote the total weight of the sample and the weight of dry gel in the bottle, respectively.

Results and Discussion

As described above, a part of water sorbed in hydrophilic polymer gels generally exhibits physical properties distinct from those of ordinary free water. Usually this phenomenon is surmised to result from specific interaction between water molecules and the polymer matrix. This means that part of the water sorbed in pores of the polymer beads may play the role of the stationary phase, and the other part that of the mobile phase.[22]

The partition of solute molecules in such a system was schematically illustrated in Fig. 1. The water phase sorbed in the pores is divided into phases α and β, the former corresponding to free water phase and the latter the stationary phase. Therefore, the real

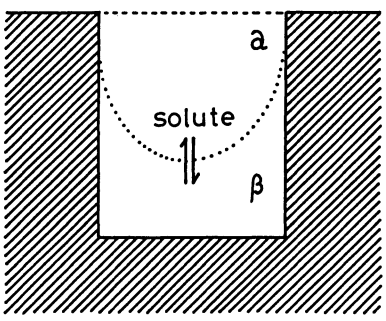

Fig. 1. Schematic illustration of the partition of solutes in pores of water-swollen hydrogels. See text for discussion.

mobile phase volume, V_m, of the column packed with water-swollen gel beads is represented as follows:

$$V_m = V_{int} + V_\alpha, \quad (2)$$

where V_{int} and V_α are the interstitial or interparticulate volume and the total volume of phase α in the column, respectively.

The V_{int} value can be easily determined by using a high molecular weight compound as a probe which is completely excluded from the pores. On the other hand, the determination of the V_m value is not easy because no ideal tracer compound is available, which explores the mobile phase but does not interact with the stationary phase. We have recently found out, however, that the V_m value can be calculated by substituting the retention volumes of two equally charged analyte ions determined in two eluent electrolyte systems into the following equation,[22]

$$V_m = \frac{V_A^{YX} V_B^{WZ} - V_A^{WZ} V_B^{YX}}{V_A^{YX} + V_B^{WZ} - V_A^{WZ} - V_B^{YX}}, \quad (3)$$

where V_i^{AB} is the retention volume of the analyte ion, i, when eluted with the solution of the electrolyte, AB. Eq. 3 can be applied to the systems where the following requirements are satisfied:[22,26] (a) The amount of ionic groups in the stationary phase is so small that their electrostatic effect on the retention of analyte ions can be suppressed by adding an electrolyte to the eluent; (b) The association of analyte ions with counter ions can be neglected in both the mobile and the stationary phases; (c) The size of the eluent ions is so small that size-exclusion effect on the retention of the ions is negligible; (d) The concentration of analyte ion is negligibly smaller than that of eluent electrolyte; (e) The stationary phase can be regarded as made up of only one homogeneous phase.

The requirements (a)—(d) can be satisfied by using aqueous solutions of suitable salts such as alkali halide with ionic strength of 0.1 as eluents and adopting small sample size.[22] The structure of water-swollen hydrogels, however, has not yet been elucidated so that we tentatively tried to determine the V_m value of the column packed with each polymer packing assuming that the requirement (e) is met. If there would be more than one region having selectivity to analyte ions in the water-swollen polymer gel, the V_m value calculated from Eq. 3 should depend on the kinds of analyte ions used.

The error of V_m value calculated by Eq. 3 is determined by the precision of the experimental values of retention volumes.[22] In order to minimize this error, the difference between V_A^{YX} and V_B^{YX} and between $V_A^{YX}$and V_A^{WZ} should be as large as possible. We adopted small inorganic anions as analyte ions because the hydrophilic polymer gels used in this experiment have been known to be considerably selective to these ions.[27]

The retention volumes of some univalent anions obtained on Bio-Gel P-2 column by elution with aqueous solution of CH_3COONa, NaCl, NaBr, or $NaClO_4$ with ionic strength of 0.10 are listed in Table 1. The V_m values were then calculated from the experimental data given in Table 1 using Eq. 3 and are listed in Table 2. Although the variance of the V_m values given in Table 2 seem not to be small, the dependence of the values on the kinds of analyte ions and eluent electrolytes cannot clearly be observed. This is also the case for the other polymer gels used. Therefore it is considered that the stationary phase in these polymer gels can roughly be regarded as composed of single homogeneous phase.

The mean V_m values obtained for all the gel columns used are summarized in Table 3 together with the V_{int} values. V_{int} was assumed to be equal to the retention volume of Blue Dextran 2000 by elution with water free of the salts.[22]

The content of water in each polymer gel which has properties different from those of free water and then does not function as mobile phase, w_x, expressed in g/g dry gel, can be calculated from the V_m value as follows:

$$w_x = (W_t(c) - \rho V_m)/W_g(c), \quad (4)$$

where $W_t(c)$ and ρ are the weight of water contained in the column and density of water at 25 °C. The dry weight of the polymer gel in the column, $W_g(c)$ was determined by the same way as described in the experimental section about DSC measurements after the gel was quantitatively transfered to a glass filter. $W_t(c)$ was then calculated from the following equation

$$W_t(c) = W_{pc} - W_{ec} - W_g(c), \quad (5)$$

where W_{pc} is the total column weight and W_{ec} the empty column weight. The w_x value is listed together with the water regain, S_r, for each polymer gel in Table 4, S_r being calculated by

$$S_r = (W_t(c) - \rho V_{int})/W_g(c), \quad (6)$$

Table 4 demonstrates that the polymer gel which has a smaller degree of cross-linking and greater S_r shows the greater difference between w_x and S_r compared with one another among polymer gels of the same type. This result apparently indicates that the amount of free water which exists in the polymer gels decreases with a decrease in total imbibed water amount.

In recent years, many workers have measured the states of water in various hydrogels by DSC. This

Table 3. V_m and V_{int} Values (ml) for the Columns Packed with Water-Swollen Hydrophilic Polymer Gels

Polymer gel	Column size	V_{int}	V_m
Sephadex G-10	8×300 mm	6.43	7.26±0.37
G-15	8×300 mm	6.12	7.62±0.41
G-25	5×500 mm	3.75	5.60±0.87
Bio-Gel P-2	5×500 mm	3.10	4.49±0.30
P-4	8×300 mm	7.00	10.94±0.42
Toyopearl HW-40S	8×300 mm	4.50	7.04±0.21

Table 1. Retention Volumes (ml) of Univalent Inorganic Anions on a Bio-Gel P-2 Column

Analyte ion	Eluent electrolyte			
	CH_3COONa	NaCl	NaBr	$NaClO_4$
CH_3COO^-		5.84		5.41
IO_3^-	10.69	8.56	8.06	7.37
Br^-		9.51		8.04
NO_3^-	12.46	9.79	9.12	8.24
I^-	14.90	11.40	10.52	9.34

Table 4. S_r and w_x Values (g/g dry gel) for Water-Swollen Hydrophilic Polymer Gels

Polymer gel	S_r	w_x
Sephadex G-10	0.91	0.78±0.05
G-15	1.34	1.06±0.07
G-25	2.40	1.52±0.41
Bio-Gel P-2	1.58	1.12±0.10
P-4	2.90	1.48±0.15
Toyopearl HW-40S	1.79	1.26±0.04

Table 2. V_m Values (ml) Calculated from Eq. 3 for a Bio-Gel P-2 Column

Analyte ion	Eluent electrolyte					
	CH_3COONa /NaCl	CH_3COONa /NaBr	CH_3COONa /$NaClO_4$	NaCl /NaBr	NaCl /$NaClO_4$	NaBr /$NaClO_4$
CH_3COO^-/IO_3^-					4.30	
CH_3COO^-/Br^-					4.32	
CH_3COO^-/NO_3^-					4.32	
CH_3COO^-/I^-					4.37	
IO_3^-/Br^-					4.52	
IO_3^-/NO_3^-	3.71	4.13	4.16	4.94	4.49	4.21
IO_3^-/I^-	4.14	4.36	4.45	4.82	4.68	4.60
Br^-/NO_3^-					4.37	
Br^-/I^-					4.80	
NO_3^-/I^-	4.61	4.62	4.78	4.65	4.90	5.01

technique enables us to classify the water in hydrogels into free, freezable bound, and nonfreezing water, and then estimate the amount of the each water.[5-7,9,10] Freezable bound water is the water having a phase transition temperature lower than 0 °C. This depression is ascribed to the weak interaction of the water with the polymer matrix,[7] the capillary condensation in the gel,[16,17] or the compartmentalization of the water by the cross-linked network of the gel.[13] Nonfreezing water is generally considered to be influenced by the strong interaction with the hydrophilic groups of the polymer chain. We have thus determined the amount of the each water in the water-swollen polymer gels in order to explore which water among them corresponds to the water functioning as the stationary phase.

Figure 2 shows the DSC heating curve of water sorbed in Bio-Gel P-2. The melting curve of sorbed water start from a temperature lower than that of pure water, which is shown by the broken line. The water which melts below 0 °C can be regarded as freezable bound water. On the other hand, only one sharp peak around −20 °C was observed in the cooling curve. The DSC curves for the other polymer gel samples were also similar to that shown in Fig. 2. It is considered that the freezable bound water crystallizes together with the free water in cooling process. The depression of the crystallization temperatures is due to the supercooling of the water.

The content of free water, w_f, and that of freezable bound water, w_{fb}, expressed in g/g dry gel, was estimated by the following equations, respectively

$$w_f = Q(\geq 0\,°C)/\Delta H W_s(1-\phi_w), \quad (7)$$

$$w_{fb} = Q(<0\,°C)/\Delta H W_s(1-\phi_w), \quad (8)$$

where Q is the heat absorbed in the heating process, which is calculated from the peak area on the DSC curve, ΔH is the heat of fusion calculated at various temperatures,[10] and W_s is the weight of the gel sample taken into the sample pan. w_f and w_{fb} were calculated from the areas of the peak above and below 0 °C in the DSC heating curve, respectively. The content of nonfreezing water, w_n, was calculated by subtracting w_f and w_{fb} from the total content of water as follows

$$w_n = \frac{\phi_w}{(1-\phi_w)} - w_f - w_{fb}. \quad (9)$$

w_n and w_{fb} for each water-swollen polymer gel except for Sephadex G-10 and G-15 are given in Table 5. It was impossible to obtain reliable data for the Sephadex G-10 and G-15 samples because these gel samples did not stick to the sample pan so that they gave distorted DSC curves.

It has so far been considered that the water sorbed in hydrogels which exhibits the selectivity to solutes such as salts and urea is not freezing water but nonfreezing one.[7,10,12] However, the w_n value is much smaller than w_x value given in Table 4 for the each polymer gel examined in this study. The sum of w_n and w_{fb} is approximately equal to w_x for each gel instead. This suggests that the following two cases are possible: (1) Both the freezable bound water and the nonfreezing water act as the stationary phase and they are not clearly distinguishable from each other with respect to the partition of inorganic ions; (2) Only the freezable bound water acts as the stationary phase. The latter case means that the nonfreezing water completely rejects all the inorganic ions examined. We presume that the former case is more plausible than the latter, because it has been reported that nonfreezing water can contain some salts.[7,10,12,21]

Higuchi and Iijima[10] estimated for the first time the partition coefficients of the solutes, i.e., urea and NaCl, between the free water and the freezable bound water as well as those between the free water and the nonfreezing water in water-swollen poly(vinyl alcohol-co-itaconic acid) membranes. Based on the measurements of DSC and the melting point depression of the free water and freezable bound water in the membranes immersed in the solute solution, they concluded that the freezable bound water is almost identical to free water with respect to the partition of urea and NaCl. We feel, however, that the sensitivity of the method they used does not seem to be highly enough to determine the solute concentration in the liquid phase in hydrogels because the melting point depression was observed only for the solution with the

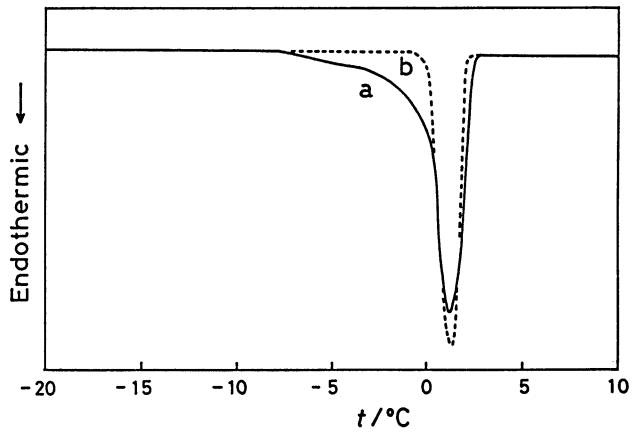

Fig. 2. DSC heating curve of water sorbed in Bio-Gel P-2 (a) and pure water (b).

Table 5. w_n and w_{fb} Values (g/g dry gel) for Water-Swollen Hydrophilic Polymer Gels

Polymer gel	w_n	w_{fb}
Sephadex G-25	0.63	0.82
Bio-Gel P-2	0.49	0.58
P-4	0.61	0.71
Toyopearl HW-40S	0.54	0.52

concentration of 2 M and not for those with 0.2 and 0.02 M.

On the other hand, the method proposed in this paper is more sensitive and reliable because the retention volumes of solutes in liquid chromatography, from which we have calculated the w_x values, can be precisely determined by the system used. It can then be concluded that the freezable bound water sorbed in hydrophilic polymer gels plays an important role in the separation process although the reason why the water is selective to inorganic ions has not yet been clarified.

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