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Liquid chromatography and differential scanning calorimetry studies on the states of water in hydrophilic polymer gel packings in relation to retention selectivity

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Abstract

The amounts of water which exhibit selectivity to solutes in water-swollen hydrophilic polymer gel packings were determined by a liquid chromatographic method designed on the basis of the mobile phase electrolyte effects on the retention of ionic solutes. The estimated amounts of the water in three types of water-swollen hydrophilic polymer gels, a cross-linked dextran, poly(vinyl alcohol) and polyacrylamide, agree well with the sum of the amount of freezable bound water and that of non-freezing water determined by means of differential scanning calorimetry. Retention selectivities of these packings were evaluated based on the plots of logarithmic distribution coefficients, $\ln K_D$, of various organic compounds obtained on one packing versus those on another with the same mobile phase, water. It was found that the $\ln K_D$ vs. $\ln K_D$ plots between two packings of the same polymer matrix with different degrees of cross-linking were linear and the slopes of the plots depended on the fractions of the freezable bound water and the non-freezing water in the stationary phase; the water-swollen hydrophilic polymer packings which contained a smaller fraction of the freezable bound water exhibited larger retention selectivities. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

It is well known that water-swollen hydrophilic polymer gels exhibit selectivity to various kinds of inorganic and organic compounds. They are thus used as efficient separation materials such as those for separation membranes and LC column packings. A number of investigators have assumed that water molecules sorbed in polymer networks should play an important role in separation processes and therefore an understanding of the states of water in the polymer systems is of great importance in the elucidation of the mechanism of separation. Various methods have been used for investigation of the properties of water in polymer gels: NMR [1–4], IR [5], differential scanning calorimetry (DSC) [4,6–20] and other techniques [21–24]. Among them, DSC has been used extensively to obtain quantitative information on the different states of water in water-swollen polymers. The studies which have so far been carried out indicate that water usually exists in

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three different states within the polymer, which can be defined as follows [16,25]: free water, which undergoes similar thermal phase transitions to those of bulk water; freezable bound water, which exhibits a melting/crystallization temperature shifted with respect to that of bulk water; and non-freezing water, that does not exhibit a detectable phase transition over the range of temperatures normally associated with bulk water. The latter two states of water are considered to result from interactions with the polymer chains [6–13,15,16], capillary condensation in the gel [23,24], or compartmentalization of the water by the cross-linked network of the polymer [14].

Several researchers have dealt with the distribution of some solutes into water incorporated in polymer membranes [8,11,13,26–29]. They measured the distribution coefficients of the solute compounds by batch methods and discussed the obtained values using three or four-state models for water in polymer gel. Higuchi and Iijima [11] estimated the solubilities of urea and sodium chloride in both the freezable bound water and the non-freezing water in water-swollen poly(vinyl alcohol-co-itaconic acid) membranes. Based on the DSC measurements of the melting point depression of the free water and the freezable bound water in the membranes immersed in the solute solution, they concluded that the freezable bound water is almost identical with the free water with respect to the solubilities for urea and sodium chloride. Most of the other investigators have also discussed the solute distribution on the basis of the assumption that only non-freezing water has a different affinity to solute compounds from that of bulk free water.

However, the sensitivity of the method using melting point depression does not seem to be high enough to determine the solute concentration in the individual water phases in hydrogels; the melting point depression was observed only for the solution with the concentration of 2 mol/l but not for those with 0.2 and 0.02 mol/l. In addition, the batchwise determination of the distribution coefficients does not give precise results especially for weakly sorbed compounds.

Compared to batch methods, LC may give more reliable results with respect to the measurement of the distribution of solutes. In order to determine the distribution coefficients of solute compounds by LC, the amount of water which differs from bulk water in the affinity to solute compounds must first be determined accurately and precisely since the water which exhibits the different affinity from that of the bulk water should not be taken to be a part of the mobile phase or the solution phase but as a part of the stationary phase or the polymer phase. We have proposed a method for the determination of the mobile phase volume, V_m, in LC 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 [30]. We successfully applied this LC method to estimate the amount of water which functions as the stationary phase in hydrophilic polymer packings and showed that, as well as non-freezing water, freezable bound water takes an important role in the separation of inorganic anions [31].

In this study, we have determined the distribution coefficients of various organic compounds on the columns packed with several hydrophilic polymer gels with different degrees of cross-linking by using water as eluent. The distribution coefficients were calculated from the values of V_m and stationary phase volume, V_s, obtained by the proposed method. Thermal analysis of these water-swollen hydrophilic polymer gels was also carried out by DSC in order to clarify the states of the water in these systems. On the basis of the results obtained, the retention selectivity of solutes on the hydrophilic polymer gel column packing materials was discussed in relation to the relative amounts of water fractions in different states.

2. Experimental

2.1. Materials

All chemicals used were of analytical reagent grade quality and they were used without further purification unless otherwise stated. Acetone, 3-methyl-2-butanone, 3,3-dimethyl-2-butanone, 3-pentanone, 4-methyl-2-pentanone, nitromethane, nitroethane, nitropropane, nitrobenzene, acetonitrile, butyronitrile, benzonitrile, phenylacetonitrile, methanol, ethanol, 1-propanol, 1-pentanol, 1-hexanol, benzyl alcohol and phenethyl alcohol obtained from

Kanto Chemicals (Tokyo, Japan), 2-pentanone, 2,4-dimethyl-3-pentanone, propionitrile and 1-butanol from Wako Chemicals (Osaka, Japan) and 2-butanone from Tokyo Kasei (Tokyo, Japan) were used as test compounds. Deionized and distilled water was further purified via passage through an Organo (Tokyo, Japan) Puric-Z water purification system.

Cross-linked dextran gels, Sephadex G-10 and G-15 (40–120 μm) purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), cross-linked poly-(vinyl alcohol) gel, TSKgel Toyopearl HW-40S, HW-50S and HW-55S (20–40 μm) from Tosoh (Tokyo, Japan) and cross-linked polyacrylamide gel, Bio-Gel P-2 and P-4 (200–400 mesh) from Bio-Rad Laboratories (Richmond, CA, USA) were used in this study. All of the polymer gels were washed with water, ethanol and acetone in this order and dried at 90°C. Blue Dextran 2000 obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) was used as a reference material for evaluation of the interstitial volumes (interparticulate volume) in the LC columns used.

2.2. Chromatographic conditions

The liquid chromatographic system consisted of a Hitachi (Tokyo, Japan) L-6000 pump, a Hitachi L-4000 UV detector and an Erma Optical Works (Tokyo, Japan) ERC-7510 refractometric detector. A stainless steel column (100×8.0 mm I.D.) was packed with each polymer packing swollen by water. The column was water-jacketed and thermostated at 35°C. The detection signal was fed into a Hitachi L-2500 integrator. The eluents used were water or aqueous solutions of sodium chloride and sodium perchlorate with ionic strength of 0.1 *M*, the latter two of which were used only for the determination of the V_m values of the columns. Test solutions (ca. 1 m*M*) were prepared by dissolving analyte compounds in the eluent to be used. Elutions were carried out at a constant flow-rate; ca. 0.5 ml/min for a Toyopearl HW-50S column, ca. 0.6 ml/min for Toyopearl HW-55S and Sephadex G-10 columns, and ca. 0.8 ml/min for columns packed with Toyopearl HW-40S, Sephadex G-15, Bio-Gel P-2 and Bio-Gel P-4.

2.3. DSC measurements

The each polymer gel sample was left swell for over 1 day in water contained in a screw capped glass bottle. After the supernatant water was removed, the sample was stirred and 2 to 8 mg of sample was placed in an aluminum sample vessel used for volatile samples. The sample vessel was then sealed hermetically. Any water leakage was not observed for weighings performed before and after DSC measurements.

A Seiko Instruments (Chiba, Japan) DSC-120 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 scanning rate of 2°C/min from 25°C to −50°C and then heating to 25°C at the same rate after maintaining −50°C for 10 min. The temperatures of crystallization and melting of water sorbed in the polymer packings were calibrated using the melting peaks of pure water and HPLC grade acetonitrile (Kanto Chemicals).

After DSC measurements, the sample vessel was punctured with tweezers and placed in an oven at 90°C to dry samples. A constant weight was reached within 1 day. The total water content of each sample, w_t(g/g dry gel), was calculated as follows:

$$w_t = W_w / W_g \quad (1)$$

where W_w and W_g denoted the weight of water in the gel and that of dry polymer gel, respectively.

3. Results and discussion

3.1. Solute retention selectivities of hydrophilic polymer column packings

The polymer column packings used in this study are originally made for aqueous size-exclusion chromatography. However, the retention volumes of the test organic compounds used increased with increase in their molecular weights on all of the columns examined. This reveals that the size-exclusion effect is not a predominant factor in the separations of the solutes in these systems.

As described above, part of water sorbed in

hydrophilic polymer gels generally exhibits physical properties distinct from those of ordinary free water. 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. Therefore, the mobile phase volume, V_m, of the column packed with water-swollen gel beads should be represented as follows:

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

where V_{int} is the interstitial or interparticulate volume and V_α is the total volume of the water phase which functions as the mobile phase in the pores.

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. In a previous study, we found out that the V_m value can be calculated by substituting the retention volumes of two equally charged analyte ions determined in two mobile phase electrolyte systems into the following equation [30]:

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

where V_A^{YX} is the retention volume of analyte ion, A, when eluted with the solution of the electrolyte, YX. Eq. (3) can be applied to the systems where 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 and the association of analyte ions with counter ions can be neglected in both the mobile and the stationary phases [30].

The V_m value calculated from the experimental data of iodate, bromide, nitrate, iodide and thiocyanate ions and the V_{int} value for each column packing material are summarized in Table 1. In this study, V_{int} was assumed to be equal to the retention volume of Blue Dextran 2000 ($MW=2\times10^6$), which can be regarded as completely excluded from the pores of the packings. It can be seen that the V_m values are larger than the V_{int} values for all the gels. As the $(V_m - V_{int})$ value corresponds to V_α, this result reveals that a substantial amount of water in the water-swollen gel beads functions as the mobile phase.

Table 1
V_m and V_{int} values for the columns packed with water-swollen hydrophilic polymer gels

Polymer packing		V_m (ml)	V_{int} (ml)
Toyopearl	HW-40S	2.01±0.11	1.35
	HW-50S	2.90±0.09	1.43
	HW-55S	3.42±0.06	1.44
Bio-Gel	P-2	1.97±0.09	1.44
	P-4	2.87±0.44	1.88
Sephadex	G-10	2.29±0.13	1.90
	G-15	2.33±0.05	1.87

Now that the V_m values have been derived, we tentatively calculated V_s values as follows:

$$V_s = V_\beta = (W_t(c) - W_g(c))/\rho - V_m \quad (4)$$

where V_β is the total volume of water which functions as the stationary phase in the pores, ρ is the density of water at 35°C, and $W_t(c)$ and $W_g(c)$ denote the total weight of the contents in the column and that of the dry polymer gel, respectively. $W_t(c)$ was obtained by subtracting the weight of the empty column from that of the weight of the packed column. The $W_g(c)$ value was determined after the packing was quantitatively transferred into a glass filter and then dried at 90°C for 1 day. The distribution coefficient, denoted by K_D, is then calculated from the retention volume, V_R, by the following equation:

$$V_R = V_m + K_D V_s \quad (5)$$

In Fig. 1, $\ln K_D$ of alcohols, ketones, nitriles and nitro compounds obtained on Toyopearl HW-50S and Toyopearl HW-55S columns are plotted against those for a Toyopearl HW-40S column. As can be seen from the figure these plots give straight lines going through the origin.

Heitz [32,33] has claimed that all the solvent molecules sorbed in polymer gels have physicochemical properties different from those of bulk solvent due to the interaction with polymer matrix and function as the stationary phase. According to his model, the stationary phase volume is the volume of the internal solvent of the polymer gels, V_i, whereas the mobile phase volume is V_{int}. The retention volume of the solute compound is thus

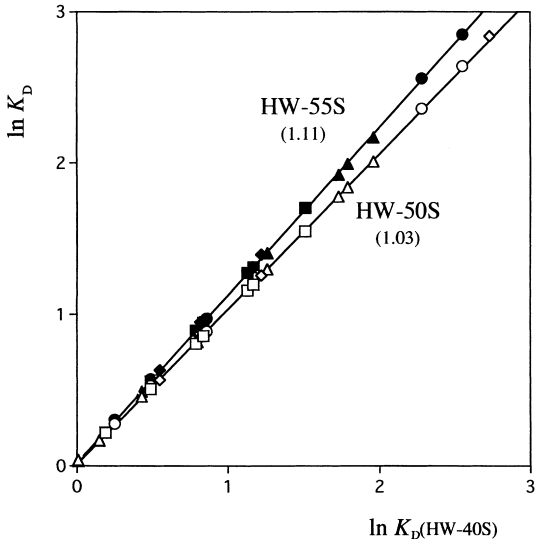

Fig. 1. Values of $\ln K_D$ for Toyopearl HW-50S and HW-55S plotted against $\ln K_D$ values for Toyopearl HW-40S. Values in parentheses give the slopes of the plots. Symbols: △, ▲= methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, benzyl alcohol and phenethyl alcohol; □, ■=acetone, 2-butanone, 3-methyl-2-butanone, 3,3-dimethyl-2-butanone, 2-pentanone, 4-methyl-2-pentanone, 3-pentanone and 2,4-dimethyl-3-pentanone:, ○, ●= acetonitrile, propionitrile, butyronitrile, benzonitrile and phenylacetonitrile; ◇, ♦=nitromethane, nitroethane, nitropropane and nitrobenzene.

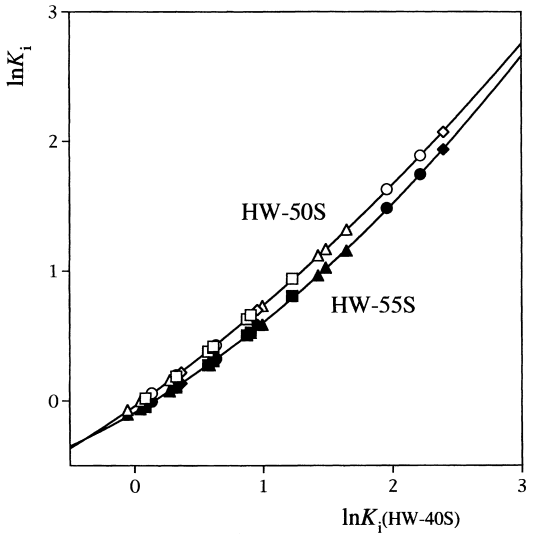

Fig. 2. Values of $\ln K_i$ for Toyopearl HW-50S and HW-55S plotted against $\ln K_i$ values for Toyopearl HW-40S. Symbols denote the same compounds as those shown in Fig. 1.

connected to another distribution coefficient, K_i, by the following equation:

$$V_R = V_{int} + K_i V_i \quad (6)$$

The V_i value can be estimated as follows:

$$V_i = (W_t(c) - W_g(c))/\rho - V_{int} \quad (7)$$

In Fig. 2, the $\ln K_i$ values for Toyopearl HW-50S and HW-55S are plotted against the values for Toyopearl HW-40S. These plots do not yield straight lines but concave ones. In general, the plots of the logarithm of the distribution coefficients of a homologous series of compounds against the carbon number in a biphasic distribution system yield straight lines [34]. It is thus expected that straight lines should be obtained for the logarithmic distribution coefficients measured on column pairs, at least for homologous compounds. Consequently, the result shown in Fig. 2 suggests that the estimation of the mobile phase, based on the assumption that all the water sorbed in the polymer gel beads functions as the stationary phase, is not correct. On the other hand, Fig. 1 shows that the plots based on the K_D values form straight lines going through the origin in contrast with those shown in Fig. 2, which suggests that the true V_m value can be estimated according to Eq. (3).

Fig. 1 also shows that both of the plots have slopes of approximately unity. This indicates that there is little difference in the solute retention selectivities of Toyopearl HW-40S, HW-50S and HW-55S regardless of the degree of cross-linking of the packing materials. In other words, the stationary phases in the three water-swollen Toyopearl HW packings are approximately identical with one another. Figs. 3 and 4 show the double $\ln K_D$ plots obtained for the pair of Sephadex G-10 and G-15 and for that of Bio-Gel P-2 and P-4, respectively. Both of the plots again give straight lines, while the slopes of the plots are not unity but 0.73 and 0.74, respectively. These results mean that the retention selectivities of Sephadex G-15 and Bio-Gel P-4 are lower than those of Sephadex G-10 and Bio-Gel P-2, respectively.

The content of the stationary phase water in each

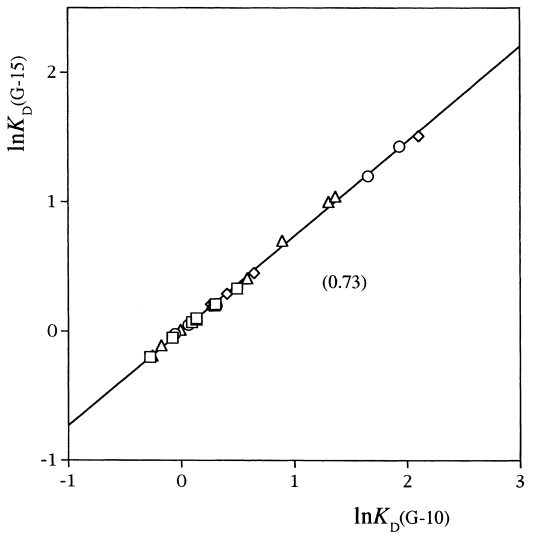

Fig. 3. Values of $\ln K_D$ for Sephadex G-15 plotted against $\ln K_D$ values for Sephadex G-10. See Fig. 1 for other details.

polymer gel, w_x, expressed in g/g dry gel, can be calculated as follows:

$$w_x = (W_t(c) - W_g(c) - \rho V_m)/W_g(c) \quad (8)$$

The w_x value is listed together with the water regain, S_r, for each polymer packing in Table 2, S_r being calculated by

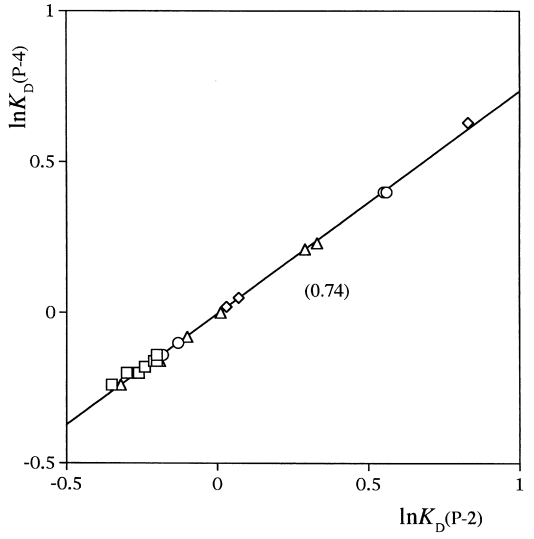

Fig. 4. Values of $\ln K_D$ for Bio-Gel P-4 plotted against $\ln K_D$ values for Bio-Gel P-2. See Fig. 1 for other details.

Table 2
w_x and S_r values for water-swollen hydrophilic polymer gels

Polymer packing		w_x (g/g dry gel)	S_r (g/g dry gel)
Toyopearl	HW-40S	1.02±0.06	1.40
	HW-50S	1.06±0.07	2.28
	HW-55S	0.96±0.06	2.93
Bio-Gel	P-2	1.13±0.05	1.43
	P-4	1.67±0.46	2.70
Sephadex	G-10	0.83±0.07	1.04
	G-15	1.05±0.03	1.34

$$S_r = (W_t(c) - W_g(c) - \rho V_{int})/W_g(c) \quad (9)$$

The w_x and S_r values for Sephadex G-10 and G-15 and Bio-Gel P-2 and P-4 shown in Table 2 are slightly different from the values previously reported [31]. This discrepancy can be probably attributed to differences in manufactured lots. Table 2 demonstrates that the polymer gel packing which has a smaller degree of cross-linking has a greater S_r value. On the other hand, the dependence of the w_x value on the degree of cross-linking is not identical for Toyopearl HW, Sephadex G and Bio-Gel P series. With respect to Toyopearl HW-40S, 50S and 55S, their w_x values are nearly the same, although their S_r values are quite different. However, for both of Sephadex and Bio-Gel, the packing which has a smaller degree of cross-linking shows a greater w_x value.

It is very interesting that all of the column packings of the Toyopearl HW series examined have approximately the same w_x values and also exhibit nearly the same solute retention selectivities in contrast to the packing materials of Sephadex G and Bio-Gel P series. For the latter two, the packing which has a smaller w_x value shows greater solute retention selectivity when compared with the column packing of the identical series. Melander et al. [35] pointed out that plots of logarithmic capacity factors, $\ln k'$, on one stationary phase, A, versus those obtained on another, B, with the same mobile phase can serve as a useful tool for comparing the energetics of solute retention on different stationary phases. If the difference in Gibbs retention energies of the two phases is zero for all solutes, $\ln k'_A$ and $\ln k'_B$ are related as:

$$\ln k'_A = \ln k'_B - \ln \phi_A + \ln \phi_B \quad (10)$$

where ϕ_A and ϕ_B are the phase ratios for the corresponding columns. They called this relationship homoenergetic retention. If the corresponding Gibbs energies for the two stationary phases are not identical but proportional, the following relationship, which they termed homeoenergetic retention, can be obtained:

$$\ln k'_A = \alpha \ln k'_B - \ln \phi_A + \alpha \ln \phi_B \quad (11)$$

where α is a constant. We can rewrite Eqs. (10) and (11) by replacing capacity factors with distribution coefficients as follows:

$$\ln K_{D,A} = \ln K_{D,B} \quad (12)$$

$$\ln K_{D,A} = \alpha \ln K_{D,B} \quad (13)$$

Collander [36] found out much earlier that a similar relationship to that represented by Eq. (13) exists between the partition coefficients observed in one liquid–liquid partition system and those observed in a second, provided the polar phase is water and the non-aqueous phases contain the same functional groups. Obviously, the $\ln K_D$ vs. $\ln K_D$ relationship obtained for Toyopearl HW packings and those obtained for Sephadex and Bio-Gel packings are expressed by Eqs. (12) and (13) and can be regarded as homoenergetic and homeoenergetic relations, respectively. The states of water which function as the stationary phase in water-swollen polymer gels are considered to be altered by the interaction between polymer matrix and water molecules. It can then be assumed that the larger the ratio of the weight of polymer matrix to that of the stationary phase water, which is given by $1/w_x$, the difference between the properties of stationary phase water and those of bulk water is greater.

As mentioned above, we tentatively calculated K_D values by assuming that the stationary phase involves only water but not the polymer matrices. If the polymer matrices are involved in the stationary phase, that is, the stationary phase is regarded as a mixture or solution of the polymer and water, the stationary phase volume is given by

$$V_s = V_\beta + V_g \quad (14)$$

where V_g is the volume of the polymer matrix in the column. In this case, the polymer concentration in the stationary phase increases with increase in the $1/w_x$ value. Eqs. (10)–(13) suggest that the slope of the $\ln K_D$ vs. $\ln K_D$ plot does not depend on the V_s value, while the intercept does; the plot must go through the origin if the stationary phase volume and then the distribution coefficients have been accurately determined. We compared the values of the intercepts of the plots obtained from the two V_s values, i.e., V_β and $V_\beta + V_g$. However, any intercept values deviated little from zero (between -0.1 and 0.1), and therefore the actual stationary phase volume could not be evaluated.

Figs. 5 and 6 show the plots of the $\ln K_D$ values obtained on Bio-Gel P-2 and on Toyopearl HW-40S against the values on Sephadex G-10, respectively. Each of the plots for a homologous series of compounds yields a straight line with the same slope. However, the intercepts of the lines are dependent upon the functional groups of the compounds. It has been known that the relationship represented by Eq. (11) or Eq. (13) breaks down when the chemical structure or functional groups of the stationary phases or organic phase solvents are significantly

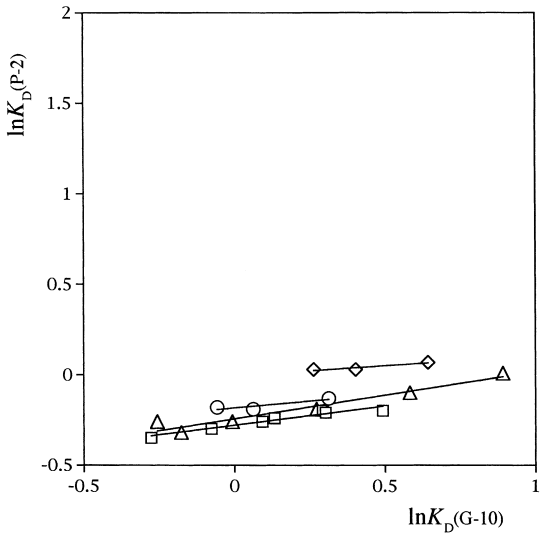

Fig. 5. Values of $\ln K_D$ for Bio-Gel P-2 plotted against $\ln K_D$ values for Sephadex G-10. Symbols: △ = methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol and 1-hexanol; □ = acetone, 2-butanone, 3-methyl-2-butanone, 3,3-dimethyl-2-butanone, 2-pentanone, 4-methyl-2-pentanone, 3-pentanone and 2,4-dimethyl-3-pentanone: ○ = acetonitrile, propionitrile and butyronitrile; ◇ = nitromethane, nitroethane and nitropropane.

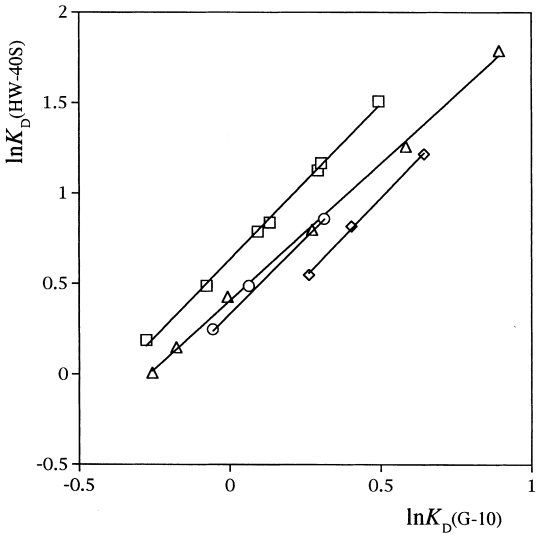

Fig. 6. Values of $\ln K_D$ for Toyopearl HW-40S plotted against $\ln K_D$ values for Sephadex G-10. Symbols denote the same compounds as those shown in Fig. 5.

different [37,38]. The results shown in Figs. 5 and 6 suggest that the difference in the interaction between the solutes and the polymer matrices among the three types of packings is not negligible. This should be noted when the molecular size is estimated by size-exclusion chromatography using these hydrophilic polymer packings.

3.2. DSC measurements of the states of water in polymer gel column packings

As described above, DSC enables one to classify the water in polymer gels into free, freezable bound and non-freezing water, and then evaluate the amounts of the individual water fractions. Hence we have determined the amounts of the individual water fractions in the water-swollen polymer packings in order to clarify the states of the stationary phase water. In this study, the scanning rate was set at 2°C/min, relatively lower than the rates usually adopted, e.g. 5 or 10°C/min, so that the measurements can be carried out under the conditions as close as possible to the equilibrium state [39]. Fig. 7 shows the DSC curves of water sorbed in Toyopearl HW-40S in heating and cooling processes. The enlarged heating DSC curve is shown in Fig. 8. The melting of sorbed water starts from a temperature lower than that of pure water, which is shown by the broken line. The water which melts below 0°C is regarded as freezable bound water. On the other hand, only one sharp peak was observed in the cooling curve for all polymer gel samples. It is

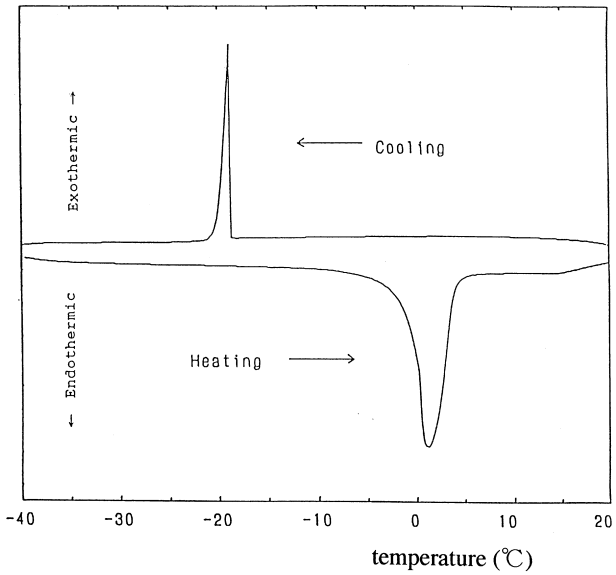

Fig. 7. DSC curves of water sorbed in Toyopearl HW-40S.

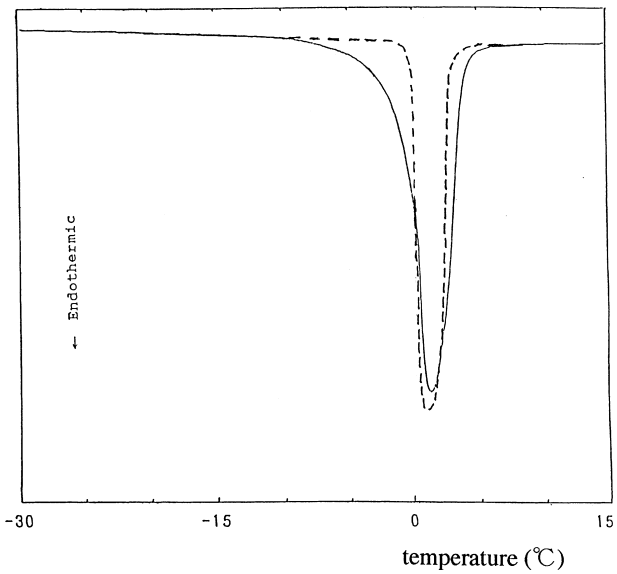

Fig. 8. DSC heating curve of water sorbed in Toyopearl HW-40S.

considered that the freezable bound water crystallizes together with the free water in the cooling process; the depression of the crystallization temperatures is attributed to the supercooling of the water. The DSC curves obtained for the packings of Sephadex and Bio-Gel were similar to that shown in Fig. 7.

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

$$w_f = Q(\geq 0°C)/\Delta H w_g \quad (15)$$

$$w_{fb} = Q(< 0°C)/\Delta H w_g \quad (16)$$

where Q is the heat absorbed in the heating process, which is calculated from the peak area on the DSC curve, and ΔH is the heat of fusion of water calculated at various temperatures [11]. w_f and w_{fb} were obtained from the areas of the peak above and below 0°C in the DSC heating curve, respectively. The content of non-freezing water, w_n, was calculated by subtracting w_f and w_{fb} from the total content of water, w_t, as follows:

$$w_n = w_t - w_f - w_{fb} \quad (17)$$

w_t was obtained from the weights of the gel sample before and after the drying.

w_n and w_{fb} values for the each water-swollen polymer gel are summarized in Table 3. It is noteworthy that the sum of w_n and w_{fb} is approximately equal to the w_x value given in Table 2 for the each polymer packing. This result suggests that both of the non-freezing water and the freezable bound water act as the stationary phase. It is also noted that the w_x and w_{fb} values scarcely depend on the degree of cross-linking or on the S_r values for the three Toyopearl HW packings, whereas the w_{fb} values for Sephadex G and Bio-Gel P gels are quite dependent on their degree of cross-linking, although the w_x values are approximately the same. This means that the composition of the stationary phase, i.e., the weight fractions of the non-freezing water, the

Table 3
w_{fb} and w_n values for water-swollen hydrophilic polymer gels

Polymer packing		w_{fb} (g/g dry gel)	w_n (g/g dry gel)
Toyopearl	HW-40S	0.61±0.01	0.40±0.02
	HW-50S	0.61±0.02	0.40±0.03
	HW-55S	0.56±0.01	0.37±0.00
Bio-Gel	P-2	0.61±0.01	0.64±0.01
	P-4	1.15±0.03	0.52±0.06
Sephadex	G-10	0.49±0.02	0.43±0.01
	G-15	0.71±0.01	0.47±0.02

freezable bound water and the polymer matrix, remains constant for Toyopearl HW packings, while the fraction of the freezable bound water in the stationary phase of the other two packings increases with decrease in the degree of cross-linking. The difference in the dependence of the solute retention selectivities of the three polymer packings on their degree of cross-linking may be interpreted with the difference in the fractions of the two water species in the stationary phase, as the difference from the bulk water in the affinity to solutes is probably less for the freezable bound water than for non-freezing water.

As already described, it has so far been considered that the freezable bound water is almost identical with the bulk water with respect to the dissolution of solute compounds and it exhibits little selectivity in the separation process. Several different models have been presented to explain the causes of the formation of non-freezing water and the freezable bound water as already mentioned [6–16,23,24]. The properties of these two types of water may depend on the physical and chemical structures of the polymer matrices and perhaps the behaviour of different kinds of water-swollen polymer gels cannot be fully interpreted with only one of the models which have so far been presented. Therefore water sorbed in polymer matrices may behave as a constituent of the stationary phase in some polymer systems but in the other ones it may not, even if the non-freezing and/or freezable bound water is observed. Further investigations on many different kinds of polymer gels including hydrophobic polymer systems are necessary to understand the effects of the states of water in the gels on the separation selectivity.

4. Conclusions

Part of water sorbed in three types of water-swollen hydrophilic polymer gel packings, i.e., a cross-linked dextran, poly(vinyl alcohol) and polyacrylamide, was ascertained to function as the stationary phase in the columns packed with the gels. The amount of the stationary phase water can be calculated from the mobile phase volume determined by an LC method designed on the basis of the mobile phase electrolyte effects on the retention of ionic solutes. The estimated amounts of the stationary phase water were in good agreement with the sum of the amount of freezable bound water and that of non-freezing water determined by means of DSC. The hydrophilic polymer packings which contains larger amount of the freezable bound water exhibit lower retention selectivities.

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