Liquid chromatography and differential scanning calorimetry studies on the states of water in polystyrene–divinylbenzene copolymer gels

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Abstract

The thermal phase transition behavior of water incorporated in crosslinked polystyrene–divinylbenzene copolymer (PS–DVB) gel packings for liquid chromatography was investigated by differential scanning calorimetry. Free or bulk water, freezable bound or intermediate water, and nonfreezing water were observed for TSKgel Styrene-250 samples, while only free water and nonfreezing water were observed for TSKgel Styrene-60 samples. Freezable bound water and nonfreezing water in these gel samples were considered to be water confined in pores of the polymer gels. A liquid chromatographic method for determination of the amounts of stationary phase water was applied to the characterization of water in the PS–DVB beads in the columns and it was found out that any water in the PS–DVB gels did not function as the stationary phase contrary to the water sorbed in hydrophilic polymer gels; not only freezable bound water but also nonfreezing water in hydrophobic PS–DVB gels are similar to bulk water with respect to the affinity to the solute compounds.
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1. Introduction

Various kinds of hydrogels have been used as efficient separation materials such as those for separation membranes and liquid chromatography column packings. Since these hydrogels contain water in their three-dimensional structures, it is highly probable that the physicochemical properties of water as well as those of polymer matrices determine the separation selectivity of the gels. In other words, the characterization of the states of water in the hydrogels is essential for understanding the mechanism of separation in the hydrogel media. Many investigations have been carried out on the states of water in polymer gels using differential scanning calorimetry (DSC) [1–16], NMR [14,15,17], IR [11,18], Raman spectrometry [19,20], and other techniques [16,21–23]. Most of them have demonstrated that the state of water incorporated in polymer gels is quite different from that of ordinary

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bulk water. For instance, three distinct states of water have been identified by DSC investigations [2,24]: free water, which shows similar temperature and enthalpy of melting/crystallization to those of bulk water; freezable bound water or intermediate water, which exhibits a melting/crystallization temperature shifted with respect to that of bulk water; and nonfreezing water, which shows no detectable phase transition over the range of temperatures normally associated with bulk water.

In earlier work [25,26], we investigated the thermal phase transition behavior of water in hydrophilic polymer gel beads, a crosslinked dextran, poly-(vinylalcohol) gel, and polyacrylamide gel, by DSC. We also packed the gels into chromatographic columns and estimated the amount of water in the gels which functions as a part of separation media, that is, the amount of the stationary phase water, using a liquid chromatographic method we presented. The estimated amount of the stationary phase water in the each type of hydrophilic polymer gels was in good agreement with the sum of the amount of freezable bound water and that of nonfreezing water determined by DSC. This means that freezable bound water as well as the nonfreezing water is not identical with the bulk water with respect to the affinity or solubility for solutes. We determined the distribution coefficients of various organic compounds between the bulk water and the stationary phase water and showed that the separation selectivity of the hydrophilic gels depend on the relative amounts of water fractions in different states.

Water species showing physicochemical properties different from those of bulk water are usually considered to interact with the polymer matrices. It has been reported that their properties depend on the nature of the polymer chains and/or functional groups on them. In particular, hydrophobic groups or hydrophobic polymer chains have been supposed to lead to water structuring in the gels totally different from that caused by hydrophilic counterparts [12,13,21,22]. However, almost all the studies on the states of water surrounding hydrophobic part of the polymers have been devoted to polymer gels containing hydrophilic polymer chains or groups as well as hydrophobic ones such as poly(*N*-iso-propylacrylamide) gels [12,21,22] or those consisting hydrophilic ionic groups embedded in a hydrophobic

phase such as sulfonated polystyrene–divinylbenzene copolymer gels [2–4]. In order to fully understand the effects of the states of water in hydrogels on the separation selectivity, investigations on hydrophobic polymer systems must be carried out.

The aim of this study is to clarify the state of water incorporated in totally hydrophobic crosslinked polymers and its effect on the separation selectivity of the gels. In this work, we adopted commercially available crosslinked polystyrene–divinylbenzene copolymer (PS–DVB) gels used as packing materials for liquid chromatography as model hydrophobic polymer gels. Although a few DSC studies on the thermal phase transition of water in sulfonated PS–DVB were reported, they were focused on the state of water around the fixed ions and their counterions. Here we tried to estimate the amount of water that exhibited separation selectivities for solutes by the liquid chromatographic method. The thermal phase transition behavior of water incorporated in the PS–DVB gels was also investigated by DSC and the states of water were discussed in relation to the separation selectivity of the gels in water.

2. Experimental

2.1. Materials

All chemicals were obtained from commercial sources and were of reagent-grade unless otherwise stated. Methanol, ethanol, 1-propanol, 1-butanol, acetone, 2-butanone, acetonitrile, phenylacetonitrile, nitromethane, nitroethane and nitropropane were obtained from Kanto (Tokyo, Japan), and propionitrile was obtained from Wako (Osaka, Japan). Water was purified with a Milli-Q system (Nihon Millipore, Tokyo, Japan) and used throughout the experiments.

Crosslinked PS–DVB gels, TSKgel Styrene-60 and Styrene-250 (Tosoh, Tokyo, Japan) were used in this study. According to the manufacturer, the pore diameters of Styrene-60 and Styrene-250 gels are 60 and 250 Å, respectively. Deuterium oxide from Merck (Darmstadt, Germany) and Dextran 40 (average molecular mass=$4\cdot10^4$) from Tokyo Kasei (Tokyo, Japan) were used as reference materials for evaluation of the liquid phase volumes and the

interstitial volumes (interparticulate volumes) in the each LC column, respectively.

2.2. Chromatographic conditions

Chromatographic measurements were performed on a liquid chromatographic system consisting of a Hitachi (Tokyo, Japan) L-6200 pump, an L-4000 UV-detector and an Erma Optical Works (Tokyo, Japan) ERC-7510 refractometric detector. Water or aqueous solutions of sodium chloride and sodium perchlorate with an ionic strength of 0.1 M were used as eluent and degassed through an Erma Optical Works (Tokyo, Japan) ERC-3325 on-line degasser. The columns were thermostated at 308 K using a GL Sciences (Tokyo, Japan) Model 556 LC column oven. Elutions were carried out at a constant flow-rate, 0.8 ml/min.

TSKgel Styrene-60 or Styrene-250 was slurry-packed into a stainless steel column (250×4.0 mm I.D.) using a 40% (v/v) aqueous methanol solution as a packing solvent. After the polymer gel particles were settled in the column, the packing solvent was replaced by 20% (v/v) and then 10% (v/v) methanol solutions in this order, and finally by water. Water was made to flow through the column and the packing reservoir attached to the column until methanol was completely washed off. It was confirmed by subjecting an aliquot of the effluent from the column to gas chromatographic analysis that the effluent contained no methanol. The analysis was performed by means of a gas chromatographic system Model 805 equipped with thermal conductivity detector (Ohkura Riken, Saitama, Japan). The packing reservoir was then removed and the inlet frit and endcap were attached.

2.3. DSC measurements

Samples of TSKgel Styrene-60 and Styrene-250 for DSC measurement were taken from the columns packed with these packing materials. The water content of the sample was adjusted by allowing water to vaporize from the sample in a desiccator containing silica gel at room temperature. About 3 mg of each sample was placed in an aluminum sample vessel to be used for volatile samples and the sample vessel was then sealed hermetically. After the

DSC measurement, the vessel was weighed in order to ensure that there is no water leakage.

A Seiko Instruments (Chiba, Japan) DSC-120 differential scanning calorimeter equipped with a cooling device was used to measure the phase transition of water in the PS–DVB polymer gels. DSC curves were obtained by cooling at the scanning rate of 2 K/min from 298 to 223 K and then heating at 1 K/min to 298 K after maintaining 223 K for 10 min. The temperatures of crystallization and melting of water in the polymer gels were calibrated using the melting peaks of pure water and HPLC-grade acetonitrile (Kanto Chemicals), while indium was used for enthalpy calibration.

After DSC measurements, the sample vessel was punctured with tweezers and placed in an oven at 363 K to dry samples. A constant mass was reached within 10 h. Total water content of each sample, w_t (g/g dry gel), was calculated as follows:

$$w_t = W_w/W_g \tag{1}$$

where W_w and W_g denote the mass of water in the sample and that of dry polymer gel, respectively.

3. Results and discussion

3.1. Estimation of the amount of stationary phase water in PS–DVB gels

If water sorbed in polymer gels has physico-chemical properties different from those of the ordinary bulk water, it can be expected that the water in the gels would exhibit the different affinity to solute compounds from that of the bulk water and play a role as a part of the separation media as reported in the previous study on water-swollen hydrophilic polymer gels [25,26]. In other words, the water phase distinct from the bulk water may function as the stationary phase when the gels are used as column packing materials for liquid chromatography with aqueous mobile phases.

The volume of the stationary phase water in a column, V_s, can be calculated as:

$$V_s = V_t - V_m \tag{2}$$

where V_t and V_m are the total volume of water in the

column and the mobile phase volume, respectively. In this study, the retention volume of deuterium oxide was used as the V_t value of the each column packed with the PS–DVB gel beads. The V_m value was calculated by the following equation [27]:

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

where V_A^{YX} is the retention volume of analyte ion, A, when eluted with the solution of the electrolyte, YX. We used inorganic anions, IO_3^-, Br^-, NO_3^-, I^- and SCN^- as probe analyte ions and NaCl and $NaClO_4$ as mobile phase electrolytes.

The V_t, V_m, and V_s values determined for TSKgel Styrene-60 and Styrene-250 columns are summarized in Table 1 together with the values of the interstitial or interparticulate volume, V_{int}. The V_{int} value was assumed to be equal to the retention volume of Dextran 40, which can be regarded as completely excluded from the pores of the gel beads. It can be seen from the table that the V_m values are approximately equal to V_t and thus the V_s values are almost zero for both of the two PS–DVB columns. This result reveals that almost all the water in the PS–DVB column functions as a constituent of the mobile phase and exhibit the same affinity as that of the bulk water to solute compounds. It should be noted that $V_t = V_{int}$ for the Styrene-60 column, whereas $V_t > V_{int}$ for the Styrene-250 column. This indicates that water molecules were squeezed out of the pores of Styrene-60 beads during the packing process due to shrinkage of the gel. On the other hand, water still remains in the pores of Styrene-250. However, the results shown in Table 1 suggest that the water phase in the pores of Styrene-250 does not function as the stationary phase. This is in remarkable contrast to the results obtained for hydrophilic polymer gels; a substantial amount of water sorbed in the hydrophilic polymer gels works as the stationary phase.

Table 1
V_t, V_m, V_s and V_{int} values (ml) for the columns packed with PS–DVB gels

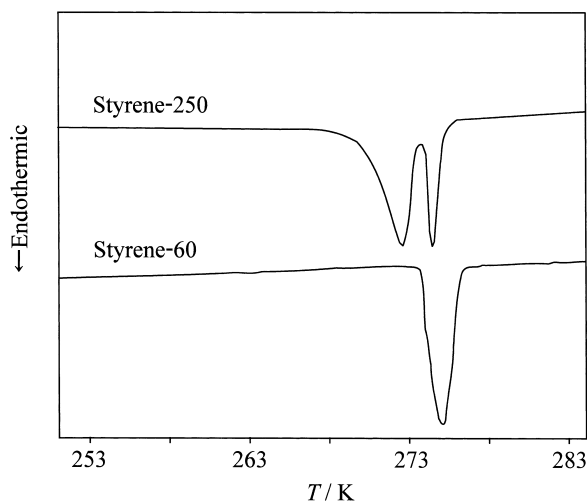| Polymer gel	V_t	V_m	V_s	V_{int}
TSKgel Styrene-60	1.32	1.25±0.05	0.06±0.05	1.34
TSKgel Styrene-250	2.39	2.42±0.09	−0.03±0.09	1.23

Fig. 1. DSC heating curves of water incorporated in TSKgel Styrene-60 and Styrene-250.

3.2. Thermal phase transition behavior of water in the PS–DVB gels

Fig. 1 shows DSC heating curves of water incorporated in TSKgel Styrene-60 and Styrene-250 samples. Two peaks were observed for TSKgel Styrene-250, while only one peak was observed for TSKgel Styrene-60. In the cooling curves, on the other hand, one sharp peak was observed below 260 K for both of the two samples; the depression of the freezing temperature may be ascribed to supercooling of the water. We used the heating curves to investigate the states of water as little information was obtainable from the cooling curves.

The melting temperatures for the peak for Styrene-60 sample and one of the two peaks for Styrene-250 sample are 273 K. Therefore, these peaks can be attributed to melting of bulk or free water in the gel samples. The observation that the Styrene-60 sample does not show any thermal phase transition except for that corresponding to the free water is in accord with the finding described in the preceding section that Styrene-60 contains little water in its pores.

The other peak observed for Styrene-250 sample has its melting temperature below 273 K so that the water for this peak may be called freezable bound water or intermediate water. This water exhibited very interesting DSC behavior, which has not been
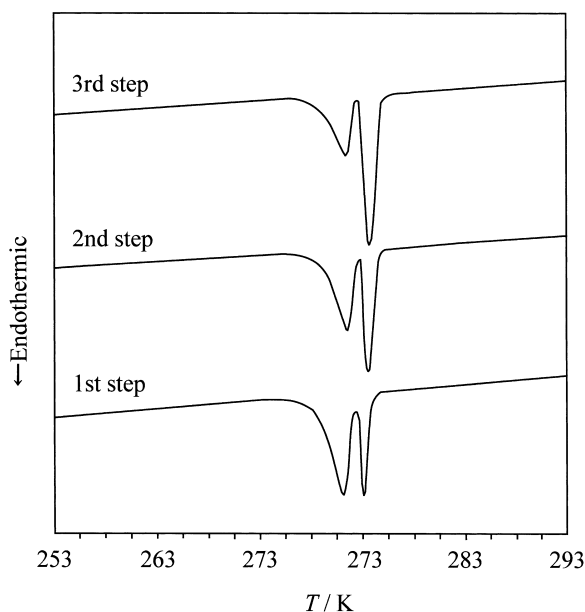
Fig. 2. DSC heating curves of water incorporated in TSKgel Styrene-250. $w_t = 0.60$ g/g dry gel.

observed for hydrophilic polymer gel samples. Fig. 2 shows DSC heating curves of water incorporated in TSKgel Styrene-250 obtained in three consecutive measurements for an identical sample. It can be seen from this figure that the peak area for freezable bound water gradually decreases, while that for free water increases. We presumed that the freezable bound water in this sample was water incorporated in the pores of Styrene-250 beads and it came out of the pores in the crystallization or melting process. In order to substantiate this presumption, we calculated the amounts of the free water, w_f, and freezable bound water, w_{fb}, from the DSC curves. w_{fb} and w_f expressed in g/g dry gel, were calculated as follows:

$$w_{fb} = Q \, (< 273 \text{ K})/\Delta H W_g \qquad (4)$$

$$w_f = Q \, (\geq 273 \text{ K})/\Delta H W_g \qquad (5)$$

where Q is the heat absorbed in the heating process, which is calculated from peak area on the DSC curve, and ΔH is the heat of fusion of water calculated at various temperatures [6]. The w_{fb} and w_f were obtained from the areas of the peaks below and above 273 K in the DSC heating curve, respec-

tively. The sums of w_f and w_{fb} for identical gel samples of various water contents obtained in the consecutive DSC measurements are shown in Table 2. Although the w_{fb} value becomes smaller and the w_f value becomes larger than the corresponding values obtained in the previous step as shown in Fig. 2, the sum of both values is approximately constant. These results support our assumption described above and reveal that the water incorporated in the pores of Styrene-250 is driven out of the pores during the freezing and/or melting processes in DSC measurements.

The content of nonfreezing water, w_n, can be calculated by subtracting w_f and w_{fb} from total content of water, w_t, as follows:

$$w_n = w_t - w_f - w_{fb} \qquad (6)$$

Dependences of the values of w_n and w_f or $w_f + w_{fb}$ on w_t for the Styrene-60 and Styrene-250 samples are shown in Figs. 3 and 4, respectively. These figures show that the amount of nonfreezing water is as small as ~0.1 g/g for all gel samples independent of the water content. The amount of water in the Styrene-250 pores, w_i, can be calculated by substituting the data shown in Table 1 into the following equation:

$$w_i = (V_t - V_{int})/\rho W_g(c) \qquad (7)$$

where ρ is the density of water at 308 K, the temperature of the liquid chromatographic column, and $W_g(c)$ is the total mass of the dry PS–DVB gel in the column. The w_i value thus obtained is 1.49 g/g and is considerably larger than the sum of the w_{fb} and the w_n values, 1.13 g/g, obtained from the first

Table 2
$w_f + w_{fb}$ values for TSKgel Styrene-250 samples obtained in three consecutive measurements

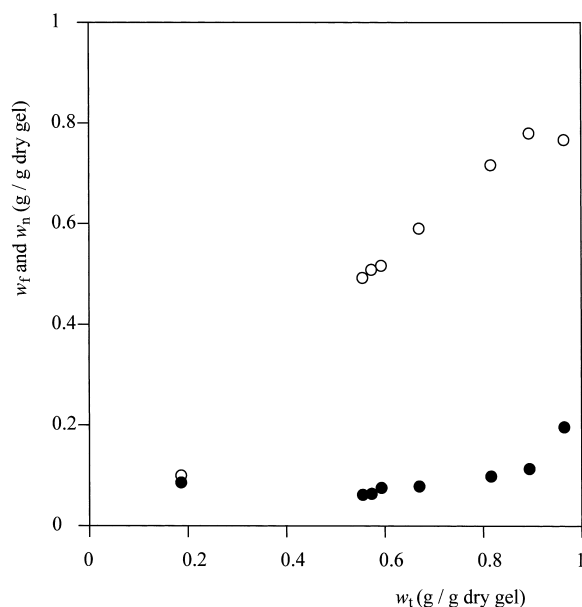| w_t	$w_f + w_{fb}$ (g/g dry gel)		
	1st	2nd	3rd
2.46	2.37	2.38	2.37
2.38	2.25	2.25	2.30
2.01	1.89	1.94	1.95
1.71	1.57	1.59	1.56
1.50	1.22	1.26	1.30
1.15	0.93	0.96	0.98
0.60	0.48	0.52	0.55
0.39	0.33	0.34	0.35

Fig. 3. Dependence of w_n and w_f on total content of water incorporated in TSKgel Styrene-60. $\bigcirc$, w_f; $\bullet$, w_n.
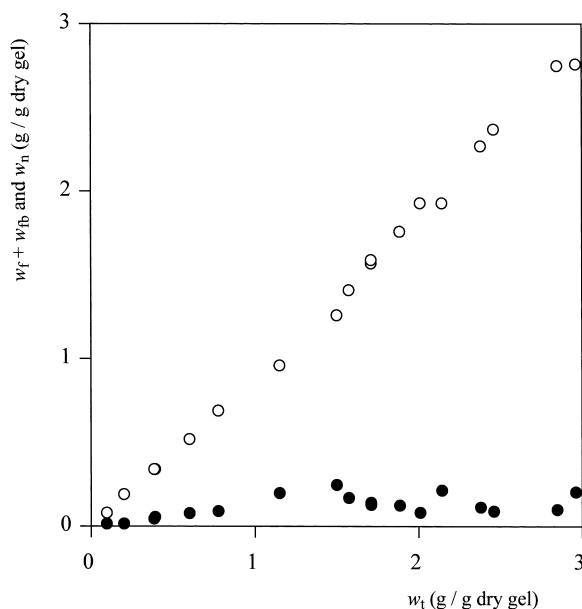

Fig. 4. Dependence of w_n and the sum of w_f and w_{fb} on total content of water incorporated in TSKgel Styrene-250. $\bigcirc$, $w_f + w_{fb}$; $\bullet$, w_n.

DSC curve for the Styrene-250 sample immediately taken out of the column. This indicates that a part of the water in the pores exhibits the thermal transition behavior of free water.

Contrary to the water incorporated in PS–DVB gels, water sorbed in hydrophilic polymer gels shows the reproducible thermal transition behavior on the DSC heating curves in the measurements performed repeatedly [26]. This means that freezable bound water observed for the PS–DVB gel sample exists in a different state from that for hydrophilic polymer gels. Ishikiriyama and Todoki [8] investigated the heat capacity of poly(methylmethacrylate) hydrogel using DSC and revealed that the mobility of the freezable water is similar to that of bulk water although the melting temperature is lower than that of bulk water. They concluded that the depression of the melting temperature was not caused by association of water molecules with the polymer matrix but by interfacial tension due to isolation of water in the small pores. Based on a relationship in which the melting and freezing temperatures of water confined in pores are lower than those of the bulk water, several methods of determining the pore size distribution have been proposed [28,29]. Murase et al. [7,30] also presented a concept of compartmentalized water in polymer gels, which may form a phase intermediate between continuous and discrete phases. They noted that the water which has melting or freezing temperature below 273 K may not necessarily be bound water and that compartmentalization of water due to the network of gels may be responsible for the depression of the phase transition temperature. The results obtained for the Styrene-250 samples by DSC and liquid chromatography indicate that the water molecules incorporated in Styrene-250 are not strongly bound to the matrix of PS–DVB although the water melts at temperatures lower than that of the bulk water. It was also found out that the water in the PS–DVB gel samples does not function as the stationary phase. This suggests that the water in pores of the PS–DVB gel has similar properties to those of the bulk water with respect to the interaction with solute compounds. The depression of the melting point of water in the pores of Styrene-250 may thus be attributed to isolation or compartmentalization of water phase in the small pores.

3.3. Solute retention selectivities of PS–DVB column packings

We also investigated retention behavior of some organic compounds on the columns packed with the PS–DVB polymer gel beads in order to substantiate the function of water concerning to the separation of solute compounds. We adopted the retention factor, denoted by k, calculated by the following equation as the retention parameter:

$$k = (V_R - V_m)/V_m \tag{8}$$

where V_R is the retention volume for a solute compound. Fig. 5 shows the log k values for Styrene-250 plotted against those for Styrene-60. The plots give a straight line with a slope of 1.09. This indicates that there are little difference in the solute retention selectivity between Styrene-250 and Styrene-60 because the slope of the plot is approximately unity. The fact that all results fall on a single straight line also reveals that the V_m value estimated
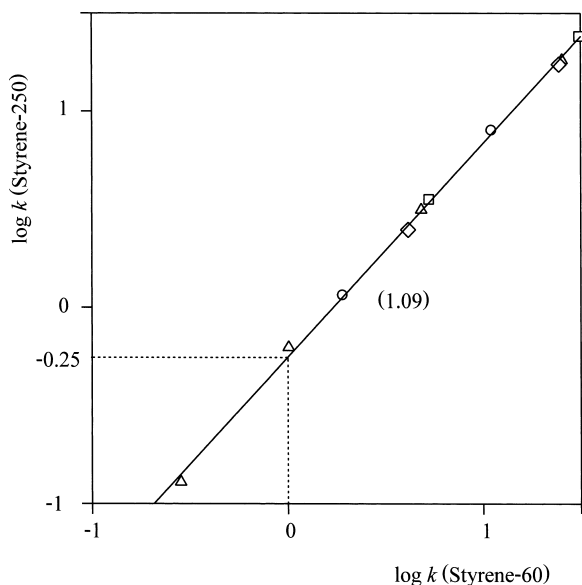

Fig. 5. Values of log k for Styrene-250 plotted against log k for Styrene-60. Value in parentheses gives the slope of the plots. △, methanol, ethanol, 1-propanol and 1-butanol; ○, acetone and 2-butanone; □, acetonitrile and propionitrile; ◇, nitromethane and nitroethane.

in this study is correct and thus all the water in the columns does not function as the stationary phase but as the mobile phase.

Based on the results obtained, the retention on the PS–DVB gels with aqueous mobile phase can be assumed to be caused by direct interaction of solute compounds with the polymer surfaces. The retention factor is thus related to the distribution coefficient, K, by the following equation:

$$k = K \cdot \frac{A}{V_m} \tag{9}$$

where A is the total surface area of the PS–DVB gel in the column accessible for solutes. Since the distribution coefficients for the Styrene-250 and the Styrene-60 columns can be assumed to be the same as described above, the relationship between the retention factor for Styrene-250, $k_{(Styrene-250)}$, and that for Styrene-60, $k_{(Styrene-60)}$, is given by:

$$\log k_{(Styrene-250)} = \log k_{(Styrene-60)}$$

$$+ \log \frac{V_{m(Styrene-60)}}{V_{m(Styrene-250)}}$$

$$+ \log \frac{A_{(Styrene-250)}}{A_{(Styrene-60)}} \tag{10}$$

The value of the intercept of the plot, -0.25, and the log $[V_{m(Styrene-60)}/V_{m(Styrene-250)}]$ value calculated from the data shown in Table 1, -0.29, indicate that the surface area or the number of adsorption sites of Styrene-250 is slightly larger than that of Styrene-60. The masses of the dry PS–DVB gels packed into the columns were 0.780 g and 1.235 g for Styrene-250 and Styrene-60, respectively. The Styrene-60 gel beads probably shrunk nearly completely in the column so that they may have had no open pores on their surface. These results again support our view that the water in PS–DVB gels is identical with the bulk free water with regard to the affinity to the solutes.

4. Conclusions

The thermal phase transition behavior of water

incorporated in PS–DVB gels was investigated by means of DSC. Three kinds of water, free or bulk water, freezable bound or intermediate water, and nonfreezing water were observed for TSKgel Styrene-250 samples, while only free water and nonfreezing water were observed for Styrene-60 samples. The freezable bound water observed for Styrene-250 samples is considered to be water incorporated in pores of the PS–DVB polymer gel; water in the pores of Styrene-60, of which the pore size is smaller than that of Styrene-250, may be squeezed out by shrinkage of the gel beads when the gel beads were packed into the column. The results of liquid chromatographic estimation of the amount of the stationary phase water and the investigation on retention behavior of some organic compounds show that both freezable bound water and nonfreezing water in the PS–DVB gels, as well as free water, function as the mobile phase. This is in striking contrast to the results we have obtained in our previous study on the water sorbed in water-swollen hydrophilic polymer gels. The mechanisms of generating freezable bound water and nonfreezing water incorporated in polymer gels may depend on the physicochemical structures of the polymer matrices. Water molecules incorporated in hydrophobic PS–DVB gels may not strongly interact with the polymer matrices contrary to them in hydrophilic polymer gels. It can be concluded that not only freezable bound water but also nonfreezing water in PS–DVB gels are similar to bulk water with respect to the affinity to the solute compounds. The depression of the melting point of water incorporated in these hydrophobic polymer gels may be due to isolation of water phase in the small pores.

Acknowledgements

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