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Separation selectivity of aqueous polyethylene glycol-based separation systems: DSC, LC and aqueous two-phase extraction studies

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ABSTRACT

The distribution behavior of *n*-alcohols, ketones and nitroalkanes in aqueous liquid chromatography with a column packed with polyethylene glycol (PEG) gel, TSKgel Ether-250, was compared with that in aqueous two-phase systems (ATPSs) formed from PEG and Na_2SO_4 or $(NH_4)_2SO_4$. The plots of the distribution data obtained for the PEG gel system against those for the ATPS reveal that the separation selectivities exerted by the PEG gel system and the PEG-based ATPS are approximately the same. Differential scanning calorimetry studies on aqueous PEG solutions suggest that PEG polymer forms a hydration structure of which the composition is 50% (w/w) PEG or the hydration number per ethylene oxide is 2.4 and the separation selectivity of the PEG-water systems can be attributed to partition of solute compounds into the hydrated PEG polymer structure.

1. Introduction

When water-soluble polymers and certain inorganic salts, or two dissimilar water-soluble polymers are dissolved into water together, two phases can be formed [1]. This is known as an aqueous two-phase system (ATPS), and has been used for liquid–liquid extraction mainly of biomolecules owing to its gentle and non-denaturing properties [1,2]. In recent years, ATPSs have also been applied to separation or recovery of metal ions from aqueous solution instead of conventional liquid–liquid extraction with organic solvents due to the limitation of usage of toxic organic solvents [1,3–6]. Cross-linked hydrophilic polymer gels may also represent environmentally benign separation systems for extraction or permeation process and have found a wide range of applications for separation of organic and inorganic compounds in aqueous media. These polymer gels are used as separation membranes, column packings for liquid chromatography (LC), support matrices for gel electrophoresis, etc [7–9]. It is interesting to note that both ATPSs and hydrophilic polymer gel systems usually contain a substantial amount of water. In these separation systems, hence, separation selectivity is considered to depend not only on the water content but also on the states or structures of water in aqueous polymer solution or gel phases. Particularly, the gas permselectivity of water-swollen membranes has been extensively investigated in order to clarify the dependence of the permeability on the states of water in the membranes [10–16]. For example Hirata et al. [16] studied the dissolved oxygen permeability through different cation forms of Nafion membranes and concluded that the oxygen permeability was governed by the fraction of hydration water in the membrane and the mobility of the hydration water.

Polyethylene glycol (PEG) is a water-soluble and highly biocompatible non-toxic polymer and is often used as an ATPS phase-forming component in combination with another hydrophilic polymer such as dextran or inorganic salts such as $(NH_4)_2SO_4$ and Na_2SO_4 [1,2]. In most ATPSs, the distribution coefficient of a solute compound is relatively close to unity, whereas PEG–inorganic salt ATPS may exhibit high distribution coefficients comparable to those obtained with organic solvent/water extraction systems such as 1-octanol/water [17–21]; some solutes give distribution coefficients larger than 1000 [17,18]. Based on the study on ATPSs, Rogers et al. have synthesized cross-linked PEG gels as a packing material for LC or an extraction resin and investigated their physicochemical properties and extractability assuming that the PEG gels operate by a similar partition mechanism to that of ATPS [22].

Structures of aqueous PEG solutions and interaction between water and PEG molecules have been extensively studied by various experimental methods such as NMR [23,24], Raman spectroscopy [25–27], ultrasonic interferometry [28], differential scanning calorimetry (DSC) [23,29–31] and dielectric relaxation measurements [32,33]. Molecular dynamics (MD) simulations have also been conducted to gain atomistic level insight into thermodynamic

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structures and dynamic properties of PEG in aqueous solution [34–37]. The repeat unit of PEG contains a hydrophobic part (–CH_2–CH_2–) and a hydrophilic site (–O–) capable of forming hydrogen bonds with water molecules, which may lead to specific properties of aqueous solution of PEG such as phase separation. Matsuura et al. studied hydration of a short chain poly(oxyethylene) by analysis of the O–H Raman band and reported that different hydration structures are built up around the poly(oxyethylene) molecules owing to hydrogen bonding at the sites of the ether oxygens and the hydrophobic hydration around the ethylene groups [27]. It has been claimed that the former hydration structure is a consequence of the favorable incorporation of the oxyethylene groups with *gauche* conformation in the water network due to their similar O···O distances. Valuable information about the water-binding properties of PEG molecules has also been obtained by a time domain reflectometry (TDR) and DSC. Based on the results obtained by dielectric relaxation measurements using TDR, Sato et al. indicated that hydration complex of one ether oxygen and 1.7 water molecules is formed and the 1:1.7 complex behaves as one kind of component in the solution [33]. On the other hand, Huang and Nishinari estimated the maximum hydration number per ether oxygen as 1.6–3.3 depending on the molecular weight of PEG from the composition of eutectic mixture of PEG and water obtained by DSC measurements [30].

Only a few investigations on the structure of water swollen cross-linked PEG gels have been reported although the states or structures of water in other hydrophilic polymer gels such as poly(vinyl alcohol) and polyacrylamide have so far been extensively studied. Graham et al. [38,39] studied the association of water with cross-linked PEG gels by DSC and mechanical analysis and showed that the ether oxygen atom takes one to three molecules of water to form hydrates.

However, little study, if any, has been carried out on the relationship between the structure of PEG solution or gel and their separation selectivity. This may partly be attributed to the difficulty of determining which part or structure in a polymer solution or a polymer gel exerts separation selectivity to solute compounds. Several investigators discussed the distribution of solute compounds from aqueous solutions into water incorporated in polymer membranes assuming that the solute concentration in the water which shows DSC behaviour different from that of the bulk water is not equal to the concentration in the bulk water [40,41]. However, the water that shows thermal or spectrophotometric behaviour different from that of the bulk water does not necessarily have a different affinity to solutes from that of the bulk water. In recent years, we have demonstrated that the amount of water in the hydrogels which exhibits the affinity to solutes different from that of bulk water and functions as the stationary phase in LC can be determined by an LC method we presented [42–46]. This method makes it possible to compare the amount of water that exhibits a characteristic behaviour in DSC or other spectrophotometric measurements with that of water that shows a different affinity to solutes in the hydrogels [43–46].

In this study we investigated the distribution behaviour of some organic compounds in PEG–Na_2SO_4 and PEG–$(NH_4)_2SO_4$ ATPSs and a water-swollen PEG gel as well as the thermal phase transition behavior of these PEG-based aqueous separation systems. Based on the results obtained, the separation selectivity of these PEG-based aqueous separation systems is discussed in relation to the structures of aqueous PEG solution and gel phase.

2. Experimental

2.1. Materials

All chemicals were analytical reagent-grade unless otherwise stated. PEG used in this study was PEG #4000 (mean molecular weight, 2700–3400) purchased from Kanto Chemicals (Tokyo, Japan). Sodium sulfate, ammonium sulfate, methanol, ethanol, 1-butanol, 1-pentanol, acetone, 2-butanone, 2-pentanone, nitromethane, nitroethane and nitropropane were obtained from Kanto Chemicals (Tokyo, Japan). Water was purified with a Milli-Q system (Nihon Millipore, Tokyo, Japan) and used throughout the experiments.

2.2. ATPS extraction measurement

Aqueous two-phase systems of varied PEG and salt compositions were prepared by mixing appropriate amounts of a 50% (w/w) PEG #4000 solution, 20% (w/w) Na_2SO_4 or $(NH_4)_2SO_4$ solution and water to adjust to 30 g by mass in graduated glass centrifuge tubes with stoppers. Each of the organic compounds (less than 100 μL) was added to the two-phase systems. The systems were mechanically shaken for 10 min and equilibrated at 298 K overnight in a thermostated water bath. Immediately after centrifugation for 10 min at 3000 rpm, the volumes of two coexisting phases were measured. And then appropriate amounts of the top and bottom phases were carefully weighed out and diluted with water. The concentrations of the solutes were determined by liquid chromatography with UV spectrophotometric or refractometric detection. The separation column was a stainless steel column (100 mm × 8 mm i.d.) packed with TSKgel Toyopearl HW-40S (Tosoh, Japan) and the mobile phase was 0.01 mol/L Na_2SO_4 or $(NH_4)_2SO_4$ aqueous solution. The concentrations of PEG and the salt in the top and bottom phases were also determined by liquid chromatography with refractometric detection. Duplicates or triplicates agreed to less than 5% from the mean value of the partition coefficient.

2.3. LC measurement

A PEG gel, TSKgel Ehter-250 (5 μm, Tosoh, Japan) was slurry packed into a stainless steel column (150 × 4.6 mm i.d.) with water. The mobile phase used was water or aqueous solution of sodium chloride and sodium perchlorate with concentration of 0.1 mol/L, latter two of which were used only for the determination of the mobile phase volume of the column. The column was thermostated at 298 K using a GL Sciences (Tokyo, Japan) Model 556 LC column oven. The test solutions were prepared by dissolving solute compounds in the mobile phase to be used.

2.4. DSC measurement

About 2–8 mg samples of PEG solution for DSC measurements were placed in an aluminium sample vessel. The water content of the sample was adjusted by allowing water to vaporize from the sample in a desiccator containing silica gels at room temperature or in an oven. The sample vessel was then sealed hermetically to prevent the water loss. Any water leakage was not observed for weighing performed before and after DSC measurements. A Seiko Instruments Inc. (Chiba, Japan) DSC-120 differential scanning calorimeter equipped with a cooling device was used to measure thermal phase transition of the samples. DSC curves were obtained by cooling at the scanning rate of 2 K/min from 298 to 223 K and then heating at the same rate to 298 K after maintaining 223 K for 10 min. The temperatures were calibrated using the melting peaks of pure water and acetonitrile. After DSC measurements, the sample vessel was punctured with tweezers and placed in an oven at 363 K to dry samples for a night. Total water content of each sample, w_t (g/g dry polymer), was calculated as follows.

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

where W_w and W_g denote the weight of water in the polymer solution and that of dry polymer, respectively.

3. Results and discussion

3.1. Comparison of the partition of solute compounds in a PEG gel system with that in a PEG-based ATPS

We have previously reported that a PEG gel, TSKgel Ether-250, shows the separation ability for various organic and inorganic compounds [46]. Furthermore we have found out that water incorporated in the PEG gel exhibits different affinity to a solute compound from that of bulk water and functions as the stationary phase in LC [46,47]. These results suggest that comparison of the separation selectivity of the PEG gel system and that of the PEG-based ATPS may shed light on the structure of PEG-based aqueous separation systems exerting separation of solute compounds.

The partition coefficient, K_D, of a solute in a column packed with TSKgel Ether-250 was calculated as

$$K_D = \frac{V_R - V_m}{V_s} \tag{2}$$

where V_R, V_m and V_s are the retention volume of a solute compound, the mobile phase volume and the stationary phase water volume, respectively. Although the stationary phase in the hydrogels can be regarded as a mixture or solution of the polymer chain and water [46,47], we adopted the volume of water that functions as the stationary phase as the V_s value for calculating K_D because it is difficult to evaluate the fraction of the polymer which works as components of the actual stationary phase.

The mobile phase volume was calculated by the following equation [42]:

$$V_m = \frac{V_A^{YX}V_B^{WZ} - V_A^{WZ}V_B^{YX}}{V_A^{YX} + V_B^{WZ} - V_A^{WZ} - V_B^{YX}} \tag{3}$$

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^-, NO_3^-, I^- and SCN^- as probe analyte ions and NaCl and $NaClO_4$ as mobile phase electrolytes. On the other hand, the stationary phase volume was estimated as follows:

$$V_s = V_t - V_m \tag{4}$$

where V_t is the total liquid phase volume in the column. We determined the V_t value of the column according to the following equation:

$$V_t = \frac{W_t(c) - W_g(c)}{\rho} \tag{5}$$

where ρ is the density of water at 298 K, and $W_t(c)$ and $W_g(c)$ denote the total weight of the content in the column and that of the dry polymer gel, respectively.

The distribution of a solute in ATPS is described by the partition coefficient, D, defined as

$$D = C_T/C_B \tag{6}$$

where C_T and C_B are the concentrations of a solute in the top PEG-rich phase and the bottom salt-rich phase, respectively. It has been known that the ln D values are well correlated to the difference in the concentrations of PEG, ΔPEG ($=C_{T,PEG} - C_{B,PEG}$), expressed in weight percent in the two phases of a given system or in molality with respect to ethylene oxide monomers by the following equation [17,48–50]:

$$\ln D = a\,\Delta\mathrm{PEG} \tag{7}$$

where a is a proportionality constant. Guen et al. obtained the same relationship between the partition coefficient and the disparity in composition of the two phases as Eq. (7) using the form of the second virial expansions for the chemical potentials of the constituents of the ATPS [51].

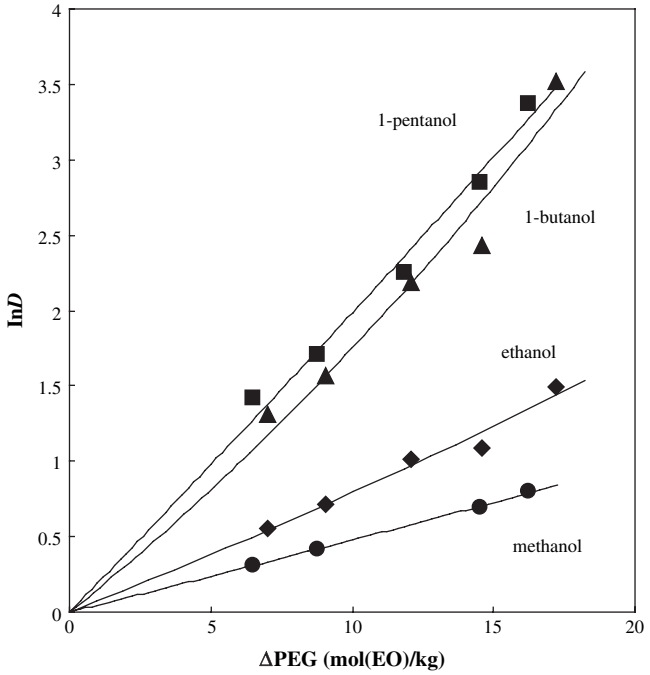

Fig. 1. ln D values of n-alcohols in PEG #4000–Na_2SO_4 ATPS as a function of ΔPEG.

On the other hand, deviation of the ln D vs. ΔPEG plots from Eq. (7) has been observed in some cases. In order to express the deviation of the plots for high-molecular weight proteins, Diamond and Hsu [52] derived the following empirical relationship introducing a second degree term into Eq. (7):

$$\ln D = A\,\Delta\mathrm{PEG} + B\,\Delta\mathrm{PEG}^2 \tag{8}$$

where the coefficients A and B are constants. We also observed slight deviation of the plots from Eq. (7) for some compounds as shown in Figs. 1–3. In these figures, the ΔPEG values were

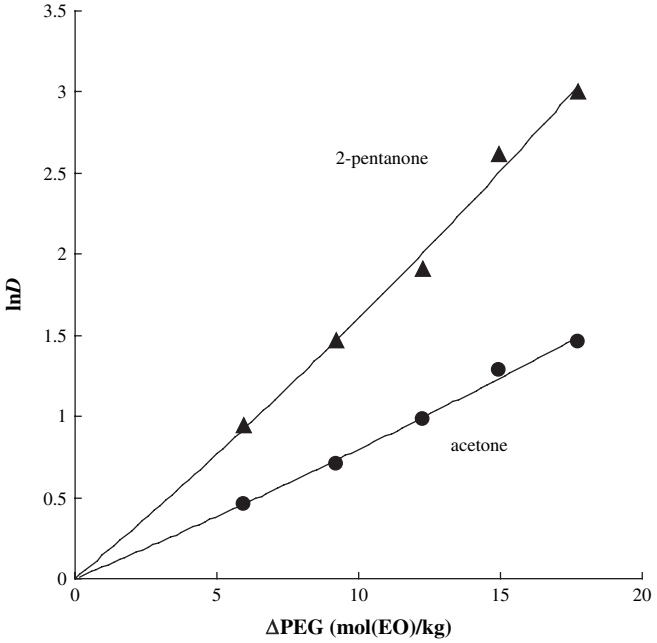

Fig. 2. ln D values of ketones in PEG #4000–Na_2SO_4 ATPS as a function of ΔPEG.

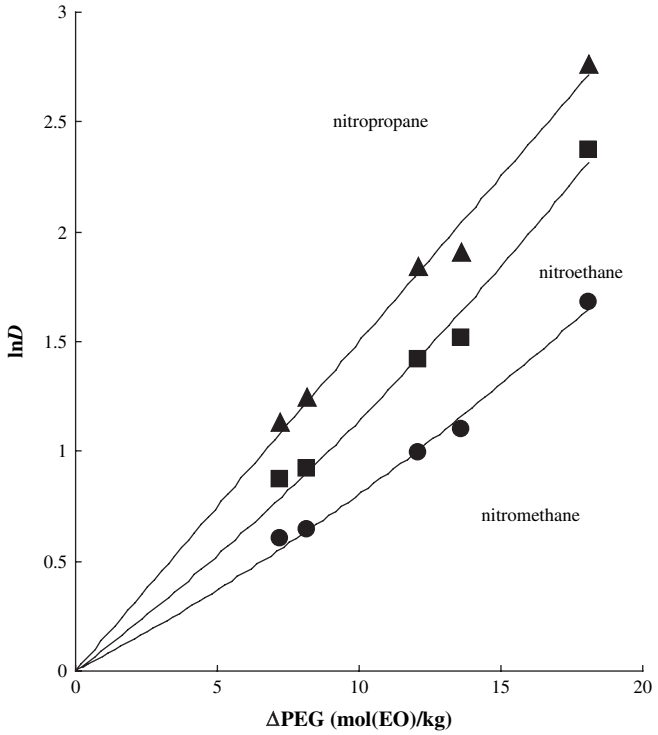

Fig. 3. ln D values of nitroalkanes in PEG #4000–Na_2SO_4 ATPS as a function of ΔPEG.

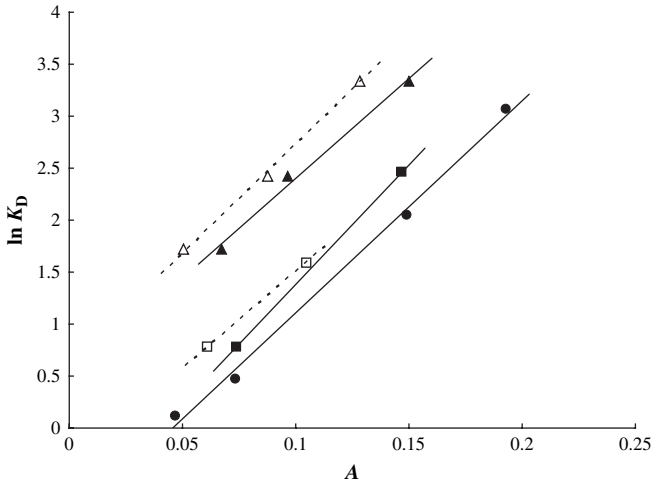

Fig. 4. Values of ln K_D for Ether-250 plotted against A values for PEG–Na_2SO_4 and PEG–$(NH_4)_2SO_4$ ATPSs. ● = methanol, ethanol, 1-butanol and 1-pentanol; ■, □ = acetone and 2-butanone or 2-pentanone; ▲, △ = nitromethane, nitroethane and nitropropane. Solid lines and dashed lines denote PEG–Na_2SO_4 and PEG–$(NH_4)_2SO_4$ ATPSs, respectively.

expressed in molality with respect to ethylene oxide (EO) monomers. The solid curves shown in Figs. 1–3 are the results from the curve fitting of the experimental data to a function expressed by Eq. (8) performed by Microsoft Excel®. Diamond and Hsu [52] have shown that the A and B values obtained for partitioning in PEG–dextran ATPS are positive for some proteins and negative for the others, while in PEG–potassium phosphate ATPS the A values are negative and the B values are positive for partitioning of all the proteins studied. It could be considered that the B values in PEG–salt ATPS reflect a certain salt effect on the partition of solute compounds such as salting-out or salting-in effect but the exact mechanism is not clear at present. According to Eq. (8), ln D approaches A as ΔPEG approaches zero or in a low ΔPEG range the ln D vs. ΔPEG plots can be assumed to be linear. We have thus calculated the coefficient A and used it as the counterpart of ln K_D for comparison of the partition in PEG–Na_2SO_4 and PEG–$(NH_4)_2SO_4$ ATPSs with that in the PEG gel system.

Fig. 4 shows the plots of ln K_D against the A value for n-alcohols, ketones and nitroalkanes. It should be noted that all the plots yield straight lines with the slope of 21 ± 2 mol(EO)/kg not only for PEG–Na_2SO_4 but also for PEG–$(NH_4)_2SO_4$ ATPS, while the intercepts depend on the nature of hydrophilic functional groups. The slope of the plots indicates the ratio of the separation factor of two compounds having different alkyl chain lengths obtained for the PEG gel system to that for the PEG–salt ATPS. Therefore it suggests that the water-swollen PEG gel exhibits the same separation selectivity for alkyl groups as that of the aqueous PEG solution with EG concentration of 21 mol/kg, or PEG weight fraction of 48% (w/w).

On the other hand, we reported that the amount of the stationary phase water incorporated in TSKgel Ether-250 is 0.49 ± 0.02 g/g dry gel [46]. This means that the PEG concentration in the PEG gel system is $67 \pm 1\%$ (w/w) provided that the stationary phase can be regarded as a homogeneous phase consisting of all the PEG matrices and water. Apparently this value does not agree with the value estimated from the slopes of the plots shown in Fig. 4. Thus, it seems reasonable to assume that Ether-250 does not have a homogeneous gel phase but a heterogeneous one as suggested in the previous work [46,47]. It is plausible that a part of the PEG polymers may not be fully hydrated but constitutes the rigid structure of the gel.

3.2. Thermal phase transition behavior of aqueous PEG solutions

We have demonstrated that water sorbed in hydrogels which exhibits the affinity to solutes different from that of free water or bulk water gives a characteristic thermal phase transition behavior in DSC measurements [43–47]. For the samples of TSKgel Ether-250, two peaks were observed in DSC heating curve, one of which was attributed to melting of free water and the other to melting of water compartmentalized in small pores of the relatively rigid gel matrix. From comparison of the amount of the stationary phase water determined by the LC method with the amount of nonfreezing water as well as those of free water and compartmentalized water, it has been revealed that only nonfreezing water functions as the stationary phase in the PEG gel system.

Fig. 5 shows the DSC heating curves of aqueous PEG solutions with various weight fractions of PEG, w_{PEG}. Only one endothermic peak was observed when $w_{PEG} > 0.5$, while two peaks were observed when $w_{PEG} < 0.5$. Similar thermal phase transition behaviour of aqueous PEG solutions has been reported and the peak at a higher temperature is attributed to the melting of free water. However, the lower-temperature peak is claimed to be due to PEG–water eutectic mixture by some investigators [23,29,30], whereas the other attributes it to the melting of freezable bound water, which may be water enclosed by the entangled PEG chains [31]. We have tentatively estimated the amount of free water, w_f, and of water which accounts for the lower-temperature peak, w_{fb}, assuming that the lower-temperature peak is attributed to the melting of freezable water. The w_f and w_{fb} values, expressed in g/g dry gel, were thus estimated by the following equations, respectively.

$$w_f = Q_H/\Delta H \times W_g \quad (9)$$

$$w_{fb} = Q_L/\Delta H \times W_g \quad (10)$$

where Q_H and Q_L are the heats absorbed in the heating process, which are calculated from areas of the peaks at higher and lower

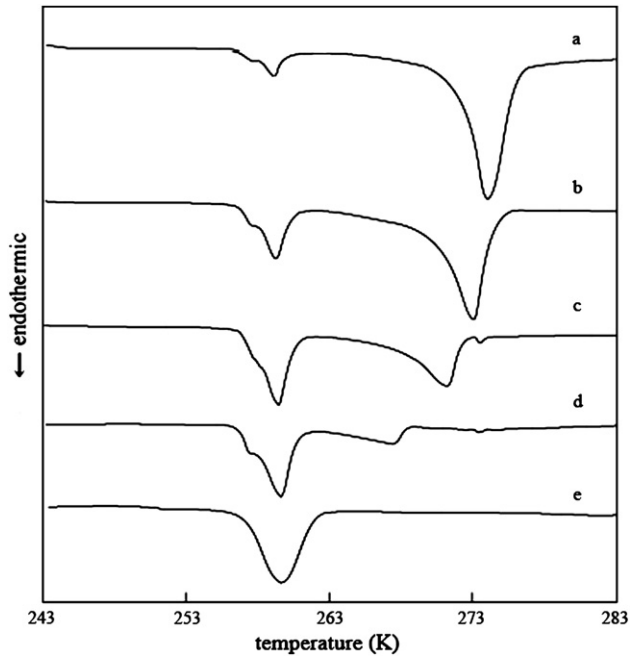

Fig. 5. DSC heating curves of aqueous PEG solutions. Total water content, w_t (g/g dry polymer) is (a) 8.94; (b) 3.93; (c) 2.32; (d) 1.50; (e) 0.44.

temperatures than 273 K in the DSC heating curve, respectively, and ΔH is enthalpy for the melting of water calculated at various temperatures [53].

Fig. 6 shows dependence of the w_f and w_{fb} values on the total content of water, w_t, for the sample of aqueous PEG solutions. It should be noted that the w_f value decreases as w_t decreases, while w_{fb} is constant until the free water vanishes at $w_t = 1.0$, i.e., at PEG weight fraction of 50% (w/w). This value agrees well with that obtained for the PEG–water solution exhibiting the same separation selectivity of the PEG gel, i.e., 48% (w/w). Based on these results, it can be assumed that PEG polymer forms a hydration structure of which the composition is 50% (w/w) PEG in its aqueous solution and the separation selectivity of the PEG–water systems is ascribed to the interaction of solute compounds with this specific structure. The hydration number per ethylene oxide was then estimated to be 2.4 from the value of the weight fraction of PEG in this hydration structure. In the region in which $w_f = 0$, w_{fb} linearly decreases with decreasing in w_t and vanishes at $w_t = 0.1$. This apparently indicates that the hydration structure of PEG may contain another type of water, that shows no phase transition over 223 K.

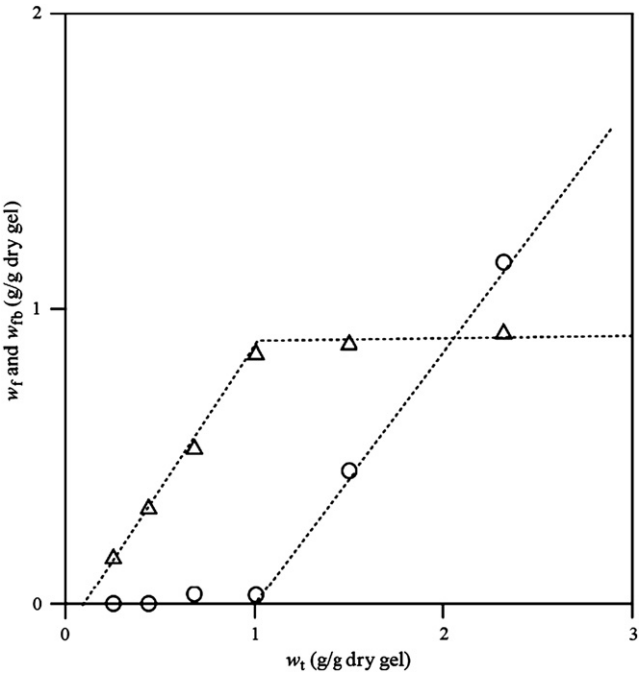

Fig. 6. Dependence of w_f and w_{fb} on w_t for aqueous PEG solutions. Symbols: ○ = w_f; △ = w_{fb}.

4. Conclusions

The distribution behaviour of *n*-alcohols, ketones and nitroalkanes in PEG–Na_2SO_4 and PEG–$(NH_4)_2SO_4$ ATPSs was compared with that in a PEG gel system, TSKgel Ether-250. The plots of logarithmic distribution coefficient obtained for a PEG gel system determined by liquid chromatography against the coefficient A in Eq. (8) for PEG–salt ATPS reveal that the separation selectivity of the PEG gel system is approximately the same as that of the PEG-based ATPS.

DSC was also used to investigate the thermal phase transition behaviour of aqueous PEG solution samples. The results obtained on dependence of the amounts of free water, freezable bound water and nonfreezing water on total water content in the sample suggest that PEG forms a hydration structure of which the composition is 50% (w/w) PEG in its aqueous solution. This value for the PEG concentration agrees well with that obtained from the plots of ln K_D vs. A. We have thus concluded that the separation selectivity of the PEG–water systems is attributable to partition of solute compounds into the hydrated PEG polymer structure. The hydration number per ethylene oxide in this structure was estimated to be 2.4.

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