

# The opposite effect of temperature on polyethylene glycol-based aqueous biphasic systems versus aqueous biphasic extraction chromatographic resins

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## Abstract

Variation in operational temperatures has revealed differences in the partitioning behavior of probe solutes between the phases in aqueous biphasic systems (ABS) and the related aqueous biphasic extraction chromatographic resin (ABEC). This difference has been studied using the hydrophobic anion,  $^{99}\text{TcO}_4^-$ , as a probe and  $(\text{NH}_4)_2\text{SO}_4$  as the kosmotropic salt. Distribution of the hydrophobic anion  $^{99}\text{TcO}_4^-$  to the PEG-rich phase in a MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  ABS increases with increasing temperature, but decreases are observed in batch uptakes of this anion to ABEC resins from  $(\text{NH}_4)_2\text{SO}_4$  solutions. Phase diagrams were constructed at five different temperatures from 10 to 50 °C using cloud point titration for the ABS and a correlation between the phase divergence, measured in terms of tie line length (TLL), and the temperature of the partitioning system was verified. Thermodynamic parameters ( $\Delta H^\circ$ ,  $\Delta S^\circ$ ,  $\Delta G^\circ$ ,  $\Delta C_p^\circ$ ) as a function of temperature were calculated for the various systems studied and the results imply thermodynamic differences between partitioning in ABS versus ABEC.

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## 1. Introduction

Aqueous biphasic systems (ABS), also known as aqueous two phase systems (ATPS), are formed when (a) two polymers (e.g., polyethylene glycol (PEG) and dextran) are combined with each other in aqueous solution [1], (b) a polymer and certain water structuring inorganic salts (e.g., PEG and  $(\text{NH}_4)_2\text{SO}_4$ ) are mixed [2], or (c) by mixing a chaotropic salt (including hydrophilic ionic liquids) and a kosmotropic salt [3] above a critical concentration or temperature. Here we will refer to these respectively, as: (a) polymer–polymer, (b) polymer–salt, and (c) salt–salt ABS. The driving force behind separation in a polymer–salt ABS is not completely understood, although phase separation may be due to water structuring and the partial dehydration of the polymer chain within the two phases [1,2,4]. Recently, a wide range of applications of the polymer–salt ABS in the separation of

organic molecules, metal ions, radiochemicals, and the recovery of nanoparticulate matter and minerals have been demonstrated [5–13]. ABS may be thought of as relatively benign separation media since they are made of water and rather innocuous polymers such as PEG. Thus, the use of ABS in place of traditional solvent extraction systems has attracted some attention in the field of “green chemistry” [4]. Unlike other “neoteric solvents” such as ionic liquids, the toxicity for a range of PEG molecular weights has been studied in more detail and indeed, many have been approved by the FDA for consumption [14–16].

In addition to volatile organic compound (VOC) replacements, ABS have been widely applied to the separation and characterization of biological macromolecules largely due of their gentle, non-denaturing properties [2,17,18]. Distribution coefficients of molecules in an ABS may also represent an alternative to  $K_{ow}$  (or  $\log P$ , the partition coefficient in an octanol–water system) which is often used to predict bioavailability, bioconcentration, and transport of pharmaceuticals in biological systems [19,20].

ABS may be characterized by a phase diagram constructed from the component compositions of PEG and salt required

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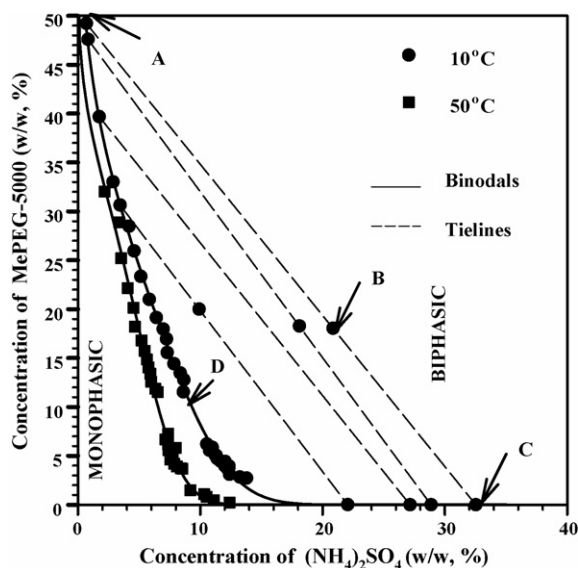


Fig. 1. Experimentally determined phase diagrams for the MePEG-5000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS at 10 °C: (●) binodal curve ADC and (- - -) tie line ABC. Biphasic regions are to the right of the binodal curve. The binodal at 50 °C (■) is also shown for comparison.

to induce biphasic formation. Typical phase diagrams for monomethyl polyethylene glycol (MePEG) with a molecular weight of 5000 (MePEG-5000) with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at 10 and 50 °C are shown in Fig. 1. The binodal curve (ADC in Fig. 1) marks the boundary between the one-phase region and the two-phase region and its location is temperature dependent [21]. System compositions above the binodal curve are biphasic, whereas system compositions below the binodal curve exist as a single homogeneous phase. Tie lines (e.g., ABC in Fig. 1) connect the mixture compositions to points on the binodal (the nodes A and C) representing the compositions of the upper (A) and lower (C) phases in equilibrium. The binodal for the 50 °C system (shown in Fig. 1 without tie lines) is shifted towards the left, indicating that it takes less salt at higher temperatures to form a biphasic. All mixture compositions lying on the same tie line give identical compositions of the equilibrium phases, but with variable phase ratios.

The tie line length (TLL) for PEG-based polymer–salt ABS, may be represented as in Eq. (1):

$$\text{TLL} = \sqrt{(\Delta\text{PEG})^2 + (\Delta\text{Salt})^2} \quad (1)$$

and has been shown to be a measure of phase divergence or the chemical potential difference between the phases [5]. TLL increases with temperature in the case of PEG–salt systems and decreases in the case of many polymer–polymer systems represented by a shift in the binodal towards higher or lower mixture compositions depending on the system under study [21–23].

The relative ability of various salts to salt-out PEG has been found to be dependent on such factors as ionic charge, hydration radius, ability to structure water, and specific interactions between the polymer and salt. Salting-out effects of ions can also be related to the ion's lyotropic number [22],

position in the Hofmeister series [24,25] or the Gibbs free energy of hydration ( $\Delta G_{\text{hyd}}$ ) of the ions and their salting-out ability [26].

The clouding of a PEG–salt solution, and therefore phase separation, can be said to result from the lowering of its lower critical solution temperature (LCST) by the addition of a kosmotropic salt [27]. We have previously observed that an increase in temperature in a PEG–salt ABS has the same effect as increasing the kosmotropic salt concentration, that is, an increase in phase divergence [28]. Thus, increasing the temperature of a PEG–salt system, shifts the binodal to lower mixture compositions, increasing the immiscibility of the phases and therefore increases divergence of the phase compositions.

In principle it is possible to raise the temperature even further to a point where the phases become miscible again, provided the temperature does not go above the liquid/gas boundary. This represents an UCST (upper critical solution temperature) arising from the increased entropy, which favors a homogeneous mixing of the components. The presence of both a LCST and UCST exemplifies a closed-loop coexistence curve and has been observed in many binary systems [29–33]. Polymer–polymer systems, however, lie closer to an UCST and exhibit the opposite effect of a PEG–salt ABS. In this case, increasing the temperature increases phase miscibility due to the binodal moving closer to the UCST. As the UCST is approached, increased entropic contributions to the free energy of mixing overcome the unfavorable enthalpic contributions and thus, the biphasic system eventually becomes monophasic.

To overcome the loss of PEG in practical applications of ABS, aqueous biphasic extraction chromatographic (ABEC) resins were developed by covalently attaching PEG to a polystyrene co-divinylbenzene bead. ABEC resins are effectively an immobilization of one of the ABS forming components and have been shown to have analogous application as ABS in a variety of applications [34–38]. ABEC resins and PEG–salt ABS were shown to have several similarities and these have been discussed in detail in previous publications [37–41].

One interesting finding is that solutes can partition to the ABEC resin at salt concentrations well below those typically required for biphasic formation in ABS, suggesting an ordering effect on PEG well below the critical point (where phase separation is visually observed) [42]. This may also suggest a difference in the component mixtures of the micro-domains found in the PEG surface of the resin beads versus the macro-domain of the bulk salt solution [43].

In addition, ABS and ABEC differ in respect to the effect of temperature on solute partitioning. In a PEG–salt ABS, temperature increases solute partitioning to the PEG-rich phase at fixed polymer and salt concentration, which follows from the increase in TLL with temperature as discussed above [42]. The effect of temperature on an ABEC system at fixed salt concentration, however, has been found to decrease solute partitioning to the ABEC resin. Here we report a comparative study of the effect of temperature on the distribution of a probe (the pertechnetate anion) in MePEG-5000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS and the analogous ABEC-5000 resin.

## 2. Experimental

### 2.1. Chemicals

Ammonium sulfate and MePEG-5000 were obtained from Aldrich (Milwaukee, WI, USA). One percent crosslinked Merrifield's peptide resin (200–400 mesh) containing approximately 1.7 milliequivalence Cl/g was also obtained from Aldrich. All chemicals were of reagent grade and used as received. The ABEC resin, ABEC-5000 (200–300 mesh) was obtained from EICRoM Industries (Darien, IL, USA). The numeric identifier refers to the molecular weight of monomethyl PEG grafted onto Merrifield's resin.

All water was deionized using commercial deionization and polishing systems. The technetium tracer (as  $\text{NH}_4^{99}\text{TcO}_4$  in 0.1 M  $\text{NH}_4\text{OH}$ ) was obtained from Amersham Life Science Inc. (Arlington Heights, IL, USA) and used as received. Technetium-99 activities were determined using Ultima Gold Scintillation cocktail and a Packard Tri-Carb 1900 TR Scintillation Analyzer (Packard Instrument Co. Inc., Meriden, CT, USA).

### 2.2. Phase diagrams

The phase diagrams were constructed using turbidimetric titration, also known as cloud point titration, described in detail elsewhere [44]. For the phase diagrams at various temperatures, the solutions were equilibrated in a water bath maintained at the desired temperature and the titration was performed in the water bath as well. Care was taken to ensure that the temperature was held constant throughout the experiment. The binodals were fitted using an empirical equation derived by Merchuk and the tie lines were determined by assigning a relationship between mass phase ratio and system composition using mathematical methods [45].

### 2.3. Liquid/liquid partitioning studies of $^{99}\text{TcO}_4^-$ in ABS

MePEG-5000 stock solutions were prepared on a weight/weight percent (w/w) basis, while the salt stock solutions were prepared on a molar (M) basis. Equal volumes of these stock solutions were added and equilibrated to form each ABS studied.

Partitioning of pertechnetate in liquid/liquid ABS systems was determined using the following procedure. Equal aliquots of 40% (w/w) MePEG-5000 and a salt stock solution of varying molar concentration were mixed. Each system was vortexed for 2 min to mix the phases followed by 2 min of centrifugation ( $2000 \times g$ ) to allow the system to phase separate. A tracer quantity of  $\text{NH}_4^{99}\text{TcO}_4$  (10  $\mu\text{L}$ , ca. 0.035  $\mu\text{Ci}/\mu\text{L}$ ) was added to the system. The system was again vortexed for 2 min and centrifuged ( $2000 \times g$ ) for 2 min. All systems were mixed and allowed to equilibrate at defined experimental temperatures using a Neslab RTE-110 water bath (Neslab Instruments Inc., Newington, NH, USA).

Equal aliquots of the top and bottom phase were drawn using a pipette for radioanalysis. Since equal aliquots of each phase were analyzed and the activity of the tracer is directly proportional to

its concentration, the distribution ratios were determined as in Eq. (2):

$$D = \left( \frac{\text{Activity of top PEG-rich phase (counts per minute, cpm)}}{\text{Activity of bottom salt-rich phase (cpm)}} \right) \quad (2)$$

### 2.4. Batch partitioning studies of pertechnetate onto ABEC resins

ABEC resins contain a large amount of water (60–85% w/w) and the water content varies with temperature, humidity, and treatment of the resin before use. Therefore, the ABEC resins were pre-conditioned before each use, by washing the resin with deionized water followed by filtration using a 2 in. diameter Büchner funnel with a water aspirator (Whatman #2 qualitative filter disk). The resins were dried for 10 min by passing air first bubbled through a 500 mL Erlenmeyer flask containing 200 mL of deionized water through the resin bed. This ensures that the water content of the resins is constant from batch to batch.

Solute distributions to ABEC resin were determined using a batch procedure in which a measured quantity of resin (0.015–0.025 g) was contacted with the salt solution of appropriate concentration and then placed into the temperature bath. The solution was stirred for 30 min followed by centrifugation for 2 min. Thereafter, it was allowed to equilibrate at the given temperature for 45 min with stirring. The solution was then filtered to remove the resin by inserting a pipette tip with a 0.045  $\mu\text{m}$  filter attached to the end directly into the solution in the temperature bath. After the solution was filtered, samples were withdrawn for radioanalysis.

The dry weight distribution ratios were calculated as in Eq. (3):

$$D_w = \left( \frac{A_i - A_f}{A_f} \right) \times \frac{\text{Contact volume (mL)}}{\text{Mass of resin (g)} \times \text{dwcf}} \quad (3)$$

where  $A_i$  is the activity of the solution before contact with the resin,  $A_f$  is the activity of the solution after contact, and dwcf is the dry weight conversion factor relating the mass of the hydrated resin to its dry weight. The distribution ratios ( $D$  and  $D_w$ ) reported here are the average of at least two measurements and are typically accurate to  $\pm 5\%$ .

For ease of comparison, plots of  $D$  and  $D_w$  have been plotted against the stock salt solution concentration used to prepare the samples. It should be noted, however, that an ABS system is more accurately described in terms of TLL, because the overall system concentrations are less than that of the stock solution concentrations since equal volumes of both components (MePEG and  $(\text{NH}_4)_2\text{SO}_4$ ) are mixed to generate each system. In an ABEC system, there is little to no dilution from the small amount of water contained in the hydrated resin. However, the local concentration of each component *at the resin bead* is difficult to estimate. Thus, for ABEC, the exact amount of PEG on the beads that will *effectively* be available for partitioning is unknown. Since the PEG polymer is bound to a resin bead, the local concentration of PEG may be quite high or, the PEG may be bound in such a way as to make it inaccessible for partitioning. (An empirical assay is currently being developed in our labs for quantifying

the amount of PEG that is available for the partitioning process in ABEC.)

### 2.5. Thermodynamic study: theory

The distribution of a solute, e.g.,  $\text{TcO}_4^-$ , between phases in ABS and partitioning onto ABEC resins may be thermodynamically represented as in Eq. (4):

$$K = e^{(-\Delta G^\circ/RT)} \quad (4)$$

where  $K$  is the equilibrium constant,  $\Delta G^\circ$  the standard free energy change associated with the transfer of  $\text{TcO}_4^-$  from the salt-rich phase to the PEG-rich phase or from the salt solution onto the ABEC resin, respectively. This is equivalent to distribution ratios as described if the phase ratio is unity for all experiments.

According to Brønsted partition theory, the distribution ratio ( $C_1/C_2$ ) has a temperature dependence according to Eq. (5) [46]:

$$\frac{C_1}{C_2} = e^{(\lambda M/kT)} \quad (5)$$

where  $C_1$  and  $C_2$  are the concentrations of the solute in phases 1 and 2, respectively,  $\lambda$  a potential energy term relating to the interaction of the solute to its environment,  $M$  the molar mass of the solute,  $k$  the Boltzmann's constant, and  $T$  is the absolute temperature.

We must be careful to distinguish between temperature effects on the phase diagram that lead to increase in  $K$  through a TLL effect and effects of temperature on  $K$  *per se*. In addition, it is interesting to note that an increase in system temperature can lead to a phase inversion where the lower phase becomes the PEG-rich phase and the top phase, the salt-rich phase, due to the changing relative densities of the phases [18]. No temperature inversion was noticed in the concentration and temperature range studied in the present research.

The Gibbs free energy of transfer of a solute between two phases can be expressed by Eq. (6):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

in which  $\Delta H^\circ$  denotes the standard enthalpy change ( $\text{J mol}^{-1}$ ) and  $\Delta S^\circ$  ( $\text{J mol}^{-1} \text{K}^{-1}$ ) denotes the standard entropy change accompanying the partitioning process. Combining Eqs. (4) and (6) yields Eq. (7).

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (7)$$

When  $\Delta H^\circ$  is invariant to temperature a linear van't Hoff plot of  $\ln k$  versus  $1/T$  can be obtained to evaluate the thermodynamic constants  $\Delta H^\circ$  and  $\Delta S^\circ$  from the slope ( $-\Delta H^\circ/R$ ) and the intercept ( $\Delta S^\circ/R$ ), respectively.

Nonlinear van't Hoff plots have been observed for temperature studies of reversed-phase liquid chromatography [47,48] and in hydrophobic interaction chromatography (HIC) and indicate a temperature dependence [49–51]. The temperature dependant thermodynamic parameters can be obtained by fitting the temperature dependant data to the quadratic equation below (Eq.

(8)) to obtain the parameters  $a$ ,  $b$ , and  $c$ .

$$\ln k = a + \frac{b}{T} + \frac{c}{T^2} \quad (8)$$

The three parameters  $a$ ,  $b$ , and  $c$  can then be used in Eqs. (9)–(11) that are derived from Kirchoff's relations:

$$\Delta H^\circ = -R \frac{\partial \ln k}{\partial (1/T)} = -R \left( b + \frac{2c}{T} \right) \quad (9)$$

$$\Delta S^\circ = R \left( a - \frac{c}{T^2} \right) \quad (10)$$

$$\Delta C_p^\circ = \frac{2Rc}{T^2} \quad (11)$$

Notice that in Eq. (11) the heat capacity is also dependant upon temperature.

## 3. Results and discussion

### 3.1. The effect of temperature on ABS

Phase diagrams for the MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  ABS system were constructed using cloud point titration at five temperatures ranging from 10 to 50 °C (Fig. 2). As observed for many PEG–salt ABS [28,52–56], binodals shift to the left meaning that a lower salt concentration is needed to form a biphasic system with a given amount of PEG at higher temperatures. Thus, for a given system composition, tie line length increases with increasing temperature as also with increasing concentration of salt (Table 1). Thus, the effect of increasing temperature in an ABS may be considered similar to increasing the salt concentration, as both methods result in increasing tie line length [26].

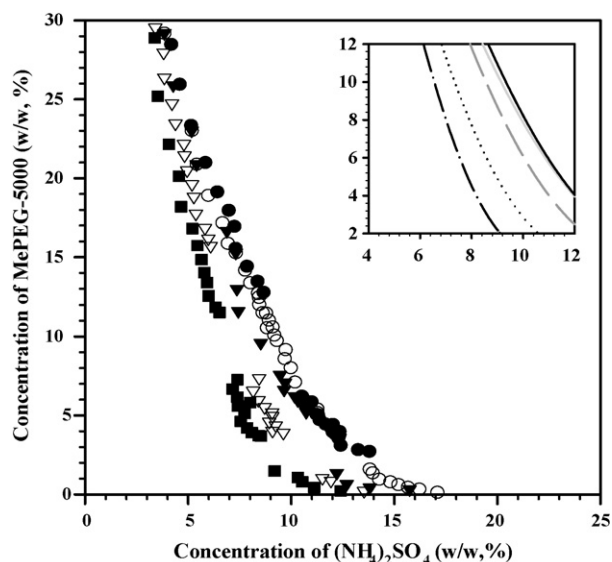


Fig. 2. Experimentally determined phase diagrams for MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  ABS at (●) 10 °C; (○) 20 °C; (▼) 30 °C; (▽) 40 °C; and (■) 50 °C. The inset shows a blow up of a section of the binodals fitted to Merchuck's equation (—) 10 °C; (---) 20 °C; (· · ·) 30 °C; (- · - ·) 40 °C; (- - - -) 50 °C.



Table 1  
Tie line lengths obtained for MePEG-5000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS at various temperatures

System concentration of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> in ABS (M)	Tie line length at given temperature (w/w, %)				
	10 °C	20 °C	30 °C	40 °C	50 °C
0.75	35.8	36.8	39.6	34.1	41.9
1.25	47.1	48.7	49.1	49.7	53.6
1.5	55.2	58.5	52.8	58.8	59.1
1.75	58.6	59.4	59.5	60.1	63.0

The liquid/liquid (*l/l*) distribution ratios (*D*) for <sup>99</sup>TcO<sub>4</sub><sup>-</sup> have been previously studied in the PEG-2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS system [28] and here, we present similar results for the MePEG-5000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS system (Fig. 3). As a probe used to characterize solute partition in ABS, pertechnetate has a number of advantages; TcO<sub>4</sub><sup>-</sup> is robust, chemically stable, unreactive, invariant to temperature and pH, and partitions to the PEG-rich phase in an ABS without the need of an extractant. Pertechnetate also has the advantage (over non-radioactive probe molecules) in that it can be easily analyzed using radiometric analysis. The distribution ratios increase with increasing temperature and with increasing salt concentration, in accordance with increased phase divergence [28,57]. This point is illustrated in Fig. 4 where the partitioning of <sup>99</sup>TcO<sub>4</sub><sup>-</sup> in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> is plotted versus temperature in terms of the phase divergence, that is, versus the TLL. The fact that all the data points appear to fall along a common line indicates that the effects of temperature and salt concentration are almost identical. The differences in the distribution ratios appear to depend almost entirely upon the degree of phase divergence and that increasing the salt concentration has the same affect as increasing the temperature for a PEG–salt ABS.

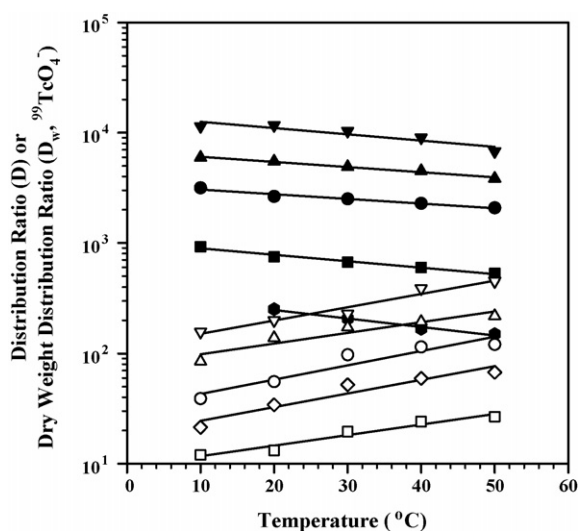


Fig. 3. Distribution ratios (*D*) or dry-weight distribution ratios (*D<sub>w</sub>*) for <sup>99</sup>TcO<sub>4</sub><sup>-</sup> vs. temperature with ABEC-5000 (closed symbols) and ABS (open symbols). The symbols correspond to the molarity of stock (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution: (●) 0.5 M; (■) 1.5 M; (◆) 2.0 M; (●) 2.5 M; (▲) 3.0 M; (▼) 3.5 M used to prepare ABS (by mixing with equal volumes of 40% MePEG-5000) or contacted with ABEC resin.

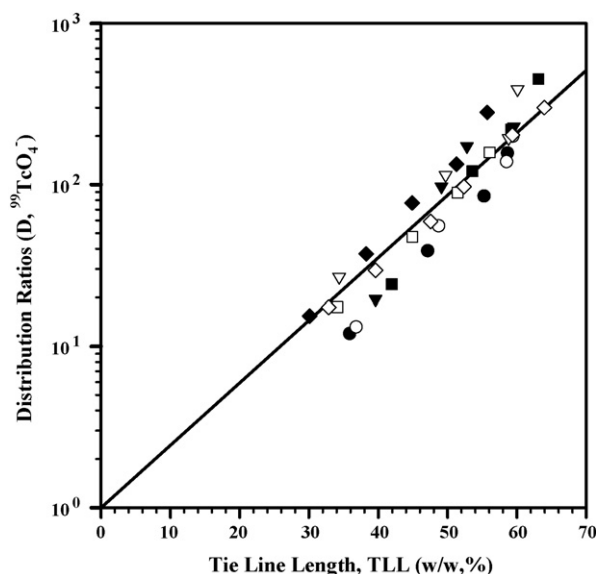


Fig. 4. Distribution ratios (*D*) for <sup>99</sup>TcO<sub>4</sub><sup>-</sup> vs. the TLL for 40% (w/w) PEG-2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS [42]: (◆) 10 °C; (□) 25 °C; (◇) 55 °C; 40% (w/w) MePEG-5000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ABS (●) 10 °C; (○) 20 °C; (▼) 30 °C; (▽) 40 °C; (■) 50 °C.

### 3.2. The effect of temperature on ABEC

Now we turn our attention to uptakes of <sup>99</sup>TcO<sub>4</sub><sup>-</sup> with ABEC-5000 from 0 to 3.5 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> versus temperature, also shown in Fig. 3. Here competing effects are observed: as the salt concentration increases, *D<sub>w</sub>* increases (as observed for ABS), however, as the temperature is increased, the uptake of <sup>99</sup>TcO<sub>4</sub><sup>-</sup> decreases. This is one of the first observable differences in the behavior of the *l/l* ABS and PEG-supported ABEC systems.

ABEC may be considered similar to absorption chromatography in that the resin may be considered a solid stationary phase while the kosmotropic salt solution is the liquid mobile phase. However, the exact mechanism for ABEC partitioning is not entirely known. It is generally understood that in solid–liquid absorption chromatography, very strict temperature control is required because there is a 2% decrease in retention time for every degree increase in temperature [58]. Here, we observe an average decrease of about 1.2% per °C for ABEC-5000 and a 2% per °C increase for *D<sub>s</sub>* in the ABS. One would expect competing forces in play for the ABEC system, one tending towards increased *D<sub>w</sub>*s with temperature (similar to liquid–liquid phase separation forces) and the other tending to decrease *D<sub>w</sub>*s with increase in temperature (similar to bonded phase and absorption chromatography). (It should be noted that at any given temperature the *D<sub>w</sub>*s still increase with increasing salt concentration.)

As stated earlier, when enthalpy and entropy are invariant to temperature, linear van't Hoff plots (ln *K* versus 1/*T*) can be constructed and the slope and intercept of the line can be used to directly evaluate Δ*H*<sup>o</sup> and Δ*S*<sup>o</sup> (Eq. (7)). The van't Hoff plots for the partitioning of pertechnetate in ABS and ABEC with respect to temperature are shown in Fig. 5. It can be observed that except for the ABEC-5000 uptakes from water, the van't Hoff plots are

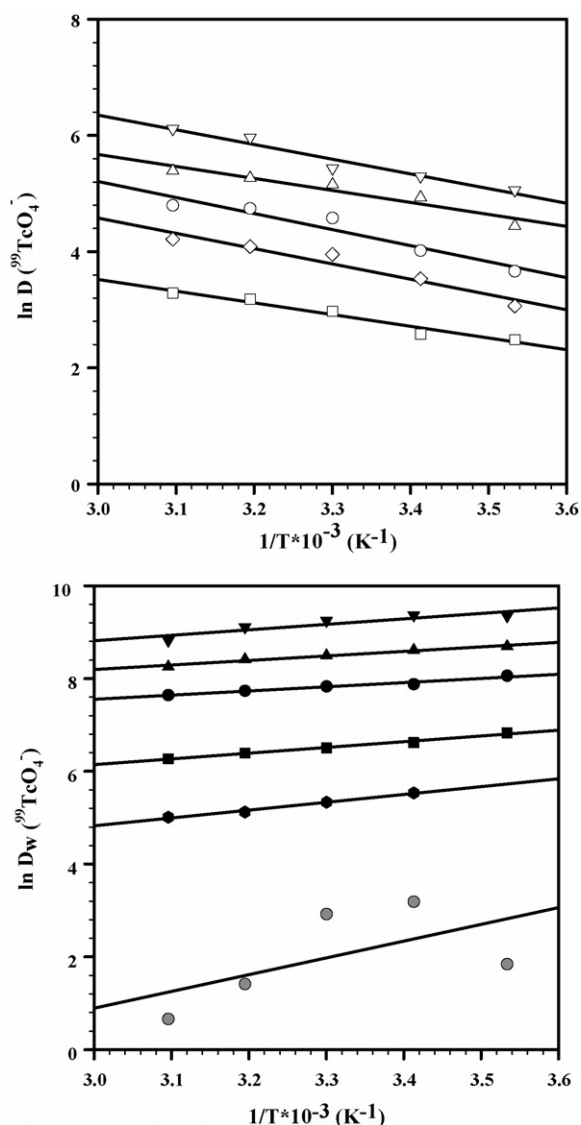


Fig. 5. van't Hoff plots ( $\ln D$  vs.  $1/T$ ) for (top, open symbols) ABS (MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  system) and (bottom, filled symbols) ABEC-5000 (using  $(\text{NH}_4)_2\text{SO}_4$  as equilibration salt): symbols represent molarity of  $(\text{NH}_4)_2\text{SO}_4$  (●) 0 M; (●) 0.5 M; (■) 1.5 M; (◆) 2.0 M; (●) 2.5 M; (▲) 3.0 M; (▼) 3.5 M.

nearly linear and thus, at high enough  $(\text{NH}_4)_2\text{SO}_4$  concentrations,  $\Delta H^\circ$  and  $\Delta S^\circ$  are invariant to temperature allowing us to evaluate the data directly using Eq. (7).

The thermodynamic parameters derived following the procedure described above are provided in Table 2. Inspection of the data shows that at least for the partitioning of the pertechnetate anion, the process must be entropy-driven since the enthalpy is unfavorable ( $\Delta H^\circ > 0$ ). Additionally, the same conclusion can be inferred from the data derived from Fig. 4, that is that the differences in the distribution ratios depend almost entirely upon the degree of phase divergence so that Fig. 5 (van't Hoff plot of  $\ln k$  versus  $1/T$ ) is essentially a plot of  $\ln \text{TLL}$  versus  $1/T$ . A plot of  $\ln D/\text{TLL}$  versus  $1/T$  would show no temperature effect as exemplified by the Brønsted relationship (Eq. (5)). Thus, the conclusion that  $\ln K$  is entropically driven is really to say that phase separation is entropically driven, which has been stated previously in literature [59].

The enthalpic contributions average  $20.0 \pm 2.8$  kJ/mol for the range of salt concentrations studied, while the entropy values increase from 0.080 to 0.116 kJ/mol K with increased salt concentration. For the ABEC system, both enthalpy and entropy are favorable at salt concentrations at or above 1.5 M. However, below 1.5 M the entropy becomes unfavorable ( $< 0$ ), but  $\Delta H^\circ$  jumps to a larger negative value indicating a change to an enthalpy-driven process. These observations indicate a change of mechanism in the partitioning onto ABEC resin depending upon the salt concentration.

While using Eq. (7) it is assumed that  $\Delta H^\circ$  and  $\Delta S^\circ$  are invariant to temperature however, this assumption may not be entirely correct. For this reason, we also evaluated the thermodynamic quantities by fitting the experimental data to Eq. (8) in order to derive the temperature dependent thermodynamic parameters. Thus, in this case not only are  $\Delta H^\circ$  and  $\Delta S^\circ$  temperature dependent but the equation also allows us to calculate the change in heat capacity using Eqs. (9)–(11).

Fig. 6 shows the plots of  $\Delta H^\circ$ ,  $\Delta S^\circ$ ,  $\Delta G^\circ$ , and  $\Delta C_p^\circ$  (obtained by using Eqs. (9)–(11);  $\Delta G^\circ$  was obtained indirectly using Eq. (6)) versus temperature for MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  ABS (I in Fig. 6) and ABEC-5000/ $(\text{NH}_4)_2\text{SO}_4$  (II in Fig. 6). These plots show almost no temperature dependence on  $\Delta H^\circ$  and  $\Delta S^\circ$  for the ABS data, indicating that the linear van't Hoff approach is valid for this system. There is also a similar trend when comparing  $\Delta H^\circ$  and  $\Delta S^\circ$  based on salt concentration. The enthalpy values

Table 2  
Thermodynamic quantities associated with the distribution of pertechnetate on ABS/ABEC as evaluated by fitting the van't Hoff plots to a linear equation

Stock concentration of $(\text{NH}_4)_2\text{SO}_4$ used <sup>a</sup>	MePEG-5000 ABS		ABEC-5000	
	$\Delta H^\circ$ (kJ/mol)	$\Delta S^\circ$ (kJ/mol K)	$\Delta H^\circ$ (kJ/mol)	$\Delta S^\circ$ (kJ/mol K)
Water	–	–	–30.0	–0.0826
0.5 M	–	–	–14.0	–0.00192
1.5 M	16.8	0.080	–10.3	0.0203
2.0 M	21.9	0.104	–	–
2.5 M	22.9	0.099	–7.47	0.0404
3.0 M	17.2	0.116	–8.13	0.0437
3.5 M	21.0	0.112	–9.80	0.0439

<sup>a</sup> The system concentration of  $(\text{NH}_4)_2\text{SO}_4$  for the ABS system would be approximately half the value of the stock salt solution used (when equal volumes of PEG and  $(\text{NH}_4)_2\text{SO}_4$  are used) due to dilution. While for ABEC, the stock salt concentration is essentially the system concentration.

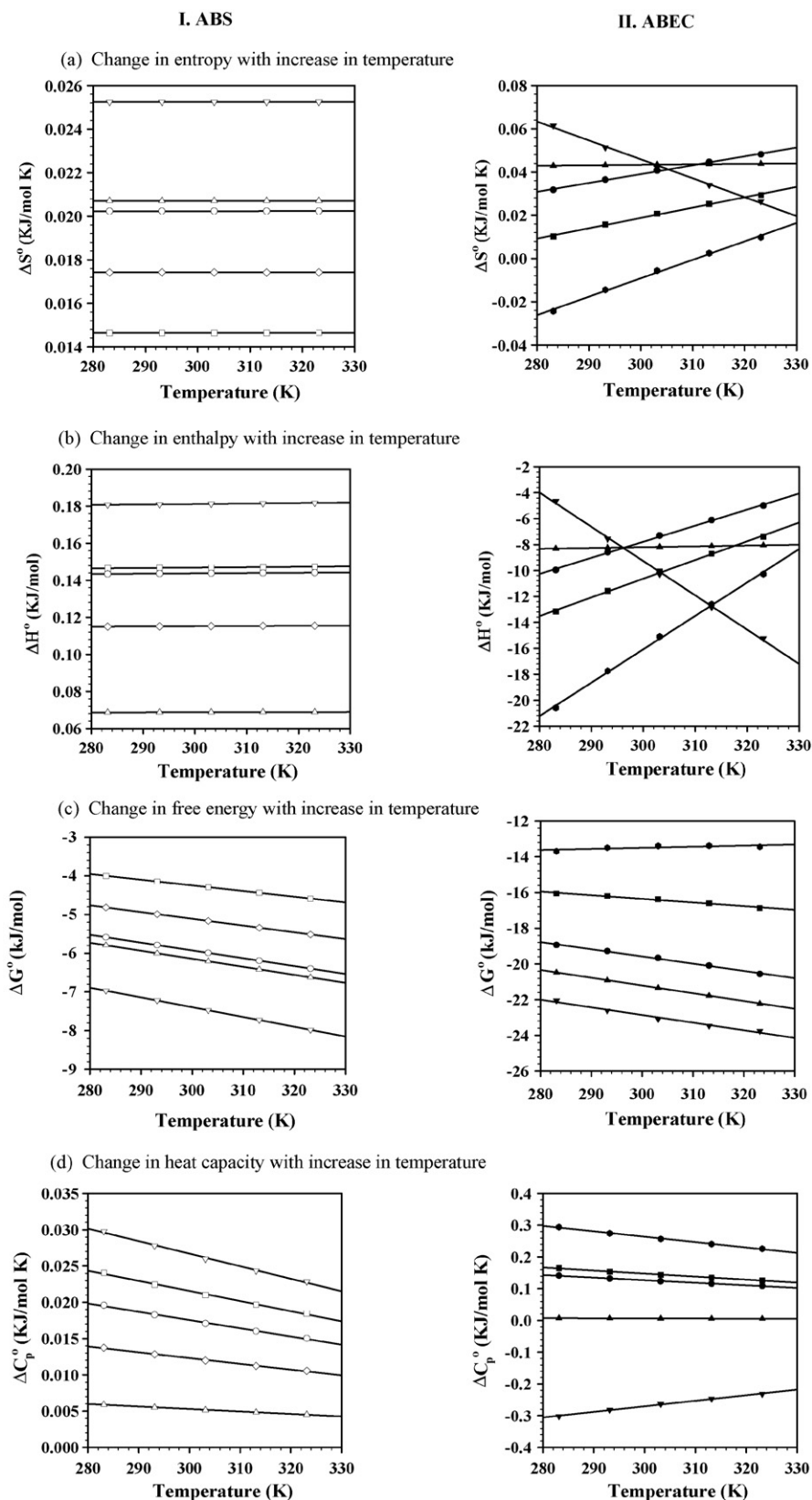


Fig. 6. Temperature dependence of change in (a) entropy, (b) enthalpy, (c) free energy, and (d) heat capacity with partitioning of pertechnetate in (I) MePEG-5000/ $(\text{NH}_4)_2\text{SO}_4$  ABS (open symbols) and (II) ABEC-5000 (filled symbols) resin using  $(\text{NH}_4)_2\text{SO}_4$  as equilibration salt. Symbols represent molarity of  $(\text{NH}_4)_2\text{SO}_4$ : (○) 0.5 M; (□) 1.5 M; (◇) 2.0 M; (○) 2.5 M; (△) 3.0 M; (▽) 3.5 M.

are positive as before, but appear to have no trend with salt concentration. The entropy values are lowest at 1.5 M salt and increase with increasing salt.

Examining the plots of  $\Delta H^\circ$  and  $\Delta S^\circ$  versus temperature for ABEC, we notice a temperature dependence for each salt concentration *except at 3.0 M*  $(\text{NH}_4)_2\text{SO}_4$  which is fairly linear. It was also noted that at the 3.5 M salt concentration, the slope has switched from positive to negative. (*Note:* Plots for water in these figures have been omitted for clarity.) A very strong temperature dependence for  $\Delta H^\circ$  of several orders of magnitude and an order of magnitude for  $\Delta S^\circ$  over the temperature range studied is observed. A similar trend with respect to salt concentration is seen for both  $\Delta H^\circ$  and  $\Delta S^\circ$  at low temperature as discussed above. At high temperature and salt concentration (3.5 M), the 3.5 M line drops, i.e., becomes more enthalpy-driven and less entropy-driven.

Merrifield's resin provides a very hydrophobic backbone and may contribute to the observed behavior of ABEC resins. Batch uptakes performed with Merrifield's resin alone showed no uptake of pertechnetate at any salt concentration or temperature (data not shown). Therefore, the increases observed in the partitioning with ABEC resins are not due to favorable interactions with the backbone. However, since there was no uptake, we cannot conclude with certainty if there are any changes that may be decreasing the uptake.

The change in Gibbs free energy in all cases is negative (indicative of a spontaneous process) and in all cases becomes more negative as temperature is increased. This is easily understood in the ABS systems, but for ABEC where the distributions actually decrease with increasing temperature, the reason for the more negative  $\Delta G^\circ$  is not completely understood. However, the average slope for the plots of free energy change versus temperature is certainly smaller in the case of ABEC than ABS.

Partitioning using ABEC has been previously likened to that in hydrophobic interaction chromatography. Generally, in processes driven by the hydrophobic effect, one can expect nonlinear van't Hoff plots, negative heat capacities, entropy driven at low temperatures and enthalpy driven at high temperatures [50]. The van't Hoff plots for ABEC in Fig. 5 are predominately linear and enthalpy-driven at low temperatures and entropic-driven at high temperatures (the opposite of HIC), and mostly positive heat capacities (Fig. 6). Therefore, the ABEC resins do not appear to be solely driven by the hydrophobic effect, but may in fact be favorably based on concentration of salt and temperature of study.

#### 4. Conclusions

ABEC resins appear in most cases to be analogous to ABS, without some of the problems one faces in an ABS process (e.g., stripping and loss of phase forming components). Here, however, ABEC resins were found to behave differently than ABS with regard to the variable of temperature, implying thermodynamic differences between partitioning in ABS versus ABEC.

One possible explanation for the observed differences could be that by anchoring PEG molecules to the surface of a resin bead, the local concentrations of PEG and salt may be much

higher than in an ABS PEG-rich phase. These biphasic micro-domains of high component concentrations may in fact lie closer to the UCST than the LCST found for PEG–salt ABS. If this is true, raising the temperature in the ABEC system, and thus the entropic contribution promoting mixing, would cause these micro-domains to become monophasic and thus reduce partitioning. The solvent properties of these micro-domains may also be considerably different than an ABS PEG-rich phase.

Further studies are called for if either system is to find real world utility. We are currently attempting to clarify the differences in partitioning process, including removing any backbone effects by utilizing PEG hydrogels [42] and investigating the role of the PEG polymer itself with more hydrophobic modifications, such as polyethylene/polypropylene glycol copolymers. While operationally, ABEC resins and ABS may afford useful, potentially 'green' applications, it is quite clear that there is a need for a more fundamental understanding of the partitioning mechanism in these "alternative" separations systems.

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