# Effects of Phase-Forming Cations and Anions on the Partition of Ionic Solutes in Aqueous Polyethylene Glycol-Inorganic Salt Two-Phase Systems

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The partition bahavior of inorganic ions in aqueous polyethylene glycol (PEG)-salt two-phase systems of different polymer and salt concentrations has been studied. $Na_2SO_4$, $Na_2HPO_4$ and $K_2HPO_4$ were used as phase-forming salts. Phase diagrams for the three aqueous PEG-salt two-phase systems were determined, and the partition coefficients for the inorganic ions in each of the systems were investigated as a function of the difference in the concentration of PEG in the two phases, $\Delta w_{PEG}$. It was found from the phase diagrams that the salting-out abilities of $K^+$ and $SO_4^{2-}$ were lower than those of $Na^+$ and $HPO_4^{2-}$, respectively. However the former ions were more effective for the extraction of oppositely charged analyte ions to the PEG-rich phase than the latter ions when they were compared with each other under a constant $\Delta w_{PEG}$. On the other hand, $Na^+$ and $HPO_4^{2-}$ were more effective than $K^+$ and $SO_4^{2-}$ for the extraction of the ions which have the same charge sign as theirs. These counter-ion and co-ion effects of the phase-forming ions on the partition of ionic solutes are interpreted by a model regarding the partition mechanism of ions in the presence of excess amounts of co-existing salts.

When a certain water-soluble polymer is dissolved in water together with another kind of hydrophilic polymer or with a certain inorganic salt at specific concentrations, two aqueous phases can be formed. These aqueous two-phase systems or aqueous biphasic systems have been successfully used for the separation of biological materials, such as cells, organelles, enzymes and proteins, because they essentially have a non-denaturing environment.[1,2] It has also been shown that the aqueous two-phase partition technique can be efficient for the separation of inorganic compounds[2-5] and small organic molecules.[6]

Although a number of different water-soluble polymers may be utilized to form aqueous two-phase systems, polyethylene glycols (PEGs) are mainly used in combination with dextrans or inorganic salts. Because PEGs are nontoxic, nonflammable and nonvolatile the PEG-based aqueous two-phase systems cause less environmental problems compared to conventional solvent-extraction systems utilizing water-immiscible organic solvents.

The partition of solute compounds in aqueous two-phase systems depends on numerous factors originating from the polymers and inorganic salts to be used for forming the two phases. As well as the variables concerning the polymers, the type and concentration of the salts are among the most important factors. It has been demonstrated that the ionic composition in aqueous two-phase systems exerts pronounced effects on the partition of solute compounds, particularly on that of ionic solutes.[4,5,7,8] Johansson[7] has indicated that the partition coefficients of ionic solutes in a PEG-dextran two-phase system depends both on the type of salt added to the system as well as the net charge of the solute. Rogers *et al*.[5] investigated the partition behavior of pertechnetate ion in PEG-alkali metal and ammonium sulfate salt systems, and revealed that the trends in the partition coefficients follow from the relative salting-out ability of the salts used.

However, the effects of the salts added upon the partition of solute compounds are rather complex because the concentrations of the polymers in the coexisting phases depend on the type and concentration of the salts. Zaslavsky *et al*.[9,10] and Bamberger *et al*.[11] have shown that the salt additives alter the polymer composition of the coexisting phases in the aqueous two-phase systems formed by two different polymers, such as PEG-dextran and Ficoll-dextran. This means that the ionic composition in the aqueous two-phase systems influences the partition of solutes not only by its own effect, but also by the effect on the polymer composition of the two phases. Therefore, the inherent effect of ions on the solute partitioning in aqueous two-phase systems should be evaluated under the conditions where the effect of the polymer concentrations can be regarded as being constant.

In the present study, we chose PEG-salt ($Na_2SO_4$, $K_2HPO_4$ and $Na_2HPO_4$) two-phase systems and investigated the partition bahavior of inorganic cations and anions as a function of the difference in the concentration of PEG in the two phases. The obtained results have been discussed on the basis of a model that we presented for the partition of ionic solutes.[12] It will be shown that the effect of the type of phase-forming salts on the partition of ionic solutes can be interpreted by the ion partition model.
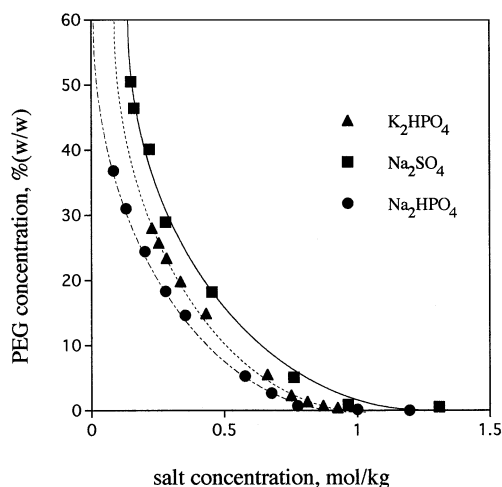
Fig. 1   Phase diagrams for aqueous PEG–$Na_2SO_4$, $Na_2HPO_4$ and $K_2HPO_4$ two-phase systems.  The weight percent of PEG#4000 in each phase is plotted as the ordinate and the molality of the salt as the abscissa.  Temperature: 25°C.



Fig. 2   ln $D$ values of inorganic anions in an aqueous PEG–$Na_2SO_4$ system as a function of $\Delta w_{PEG}$.

## Experimental

### Materials

The polyethylene glycol used in this study was PEG#4000 (mean molecular weight, 2700 – 3400) purchased from Kanto Chemicals (Tokyo, Japan).  Standard stock solutions of $Li^+$ and $Mg^{2+}$ for atomic-absorption use (1000 ppm) were also obtained from Kanto Chemicals.  All of the inorganic salts used were of analytical-reagent grade quality, and were used without further purification.  Distilled and deionized water was further purified *via* passage through an Organo (Tokyo, Japan) Puric-Z water purification system.

### Methods

Phase diagrams were constructed by preparing the two-phase systems and determining the concentrations of PEG and the salts in the coexisting phases in the following manner.  Aqueous two-phase systems of varied PEG and salt compositions were prepared by mixing appropriate amounts of a 50% (w/w) PEG#4000 solution, a salt solution (20% (w/w) for $Na_2SO_4$ and $K_2HPO_4$, 12% (w/w) for $Na_2HPO_4$) and water by mass in glass centrifuge tubes with ground-glass stoppers.  The water content in the PEG#4000 used was determined to be 0.844% (w/w) by the Karl Fischer method, and thus neglected in calculating the PEG concentration in the stock solution.  The total amounts of the resulting phase systems were adjusted to about 9.0 g.  The systems were mechanically shaken for 15 min after pre-equilibration at 25.0 ± 0.1°C overnight in a thermostated water bath and then centrifuged for 10 min at 2000 rpm.  The phases were again allowed to stand overnight in a water bath thermostated at 25.0°C.  Immediately before analysis, about 1 g of the top and bottom phases were carefully weighed out and diluted to 25 ml with water, respectively.  The concentrations of PEG and the phase-forming inorganic salts in both phases were determined by liquid chromatography with refractometric detection.  The column (500 mm × 8 mm i.d.) was a Pyrex tube packed with water-swollen Sephadex G-15 (40 – 120 μm, Pharmacia Fine Chemicals, Uppsala, Sweden).  The mobile phase used was an aqueous solution containing a salt (10 mM) which was the same as that to be determined; for example, we
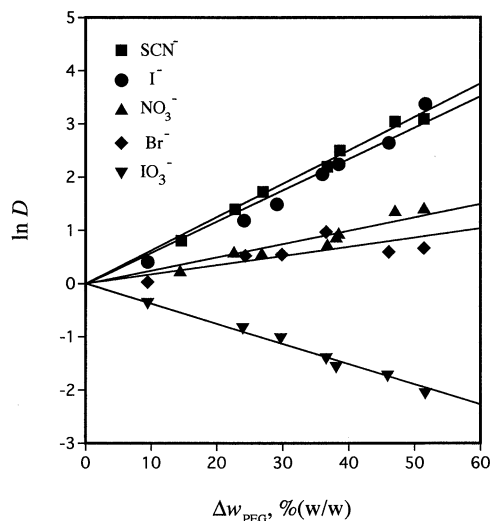
used a $Na_2SO_4$ solution to determine the concentration of $Na_2SO_4$ and PEG in the two phases of PEG-$Na_2SO_4$ system. This avoids errors in measurements of the peak areas caused by an overlap of the peaks for the sample salt and the mobile phase salt, the latter of which corresponds to the system peak.

The partition coefficients of inorganic cations and anions in the aqueous two-phase systems were determined as follows.  An aliquot of a stock solution of an analyte salt (KIO3, KBr, KNO3, KI, KSCN, LiNO3 or MgSO4·6H2O) was added to the prepared aqueous two-phase systems.  The final concentration of the added analyte salt was adjusted to *ca.* 1 mM in the whole system.  The procedures for equilibration, centrifugation and preparation of the sample solutions for analysis were the same as those described above.  The concentrations of inorganic anions were determined by liquid chromatography with UV spectrophotometric detection.  The column and the mobile phases used were the same as described above for the determination of PEG and the phase-forming salts.  The concentrations of metal ions were determined by means of a Shimadzu (Kyoto, Japan) atomic absorption spectrophotometer Model AA-646 with a $C_2H_2$–air flame.

## Results and Discussion

### Phase diagrams

Figure 1 shows phase diagrams for the PEG–$Na_2SO_4$, $Na_2HPO_4$ and $K_2HPO_4$ systems.  The concentration of PEG is expressed in weight percentage, whereas those of the salts are given in molality.  For each salt, the region below the binodial curve represents a homogeneous solution and that above, a two-phase region.  It can be seen from Fig. 1 that the effectiveness of salts to form aqueous two-phase systems with PEG depends on their constituent cations and anions; $Na^+$ and $HPO_4^{2-}$ are more effective than $K^+$ and $SO_4^{2-}$, respectively.

Several investigators have studied the influence of the type of inorganic salts on the phase separation of aqueous PEG solutions,[13–18] and have suggested that the stronger is the hydration of the ion, the lower is the concentration required to form a two-phase system.  Ananthapadmanabhan and Goddard[17,18] have reported that the relative concentration of various salts to form aqueous two-phase systems with PEG
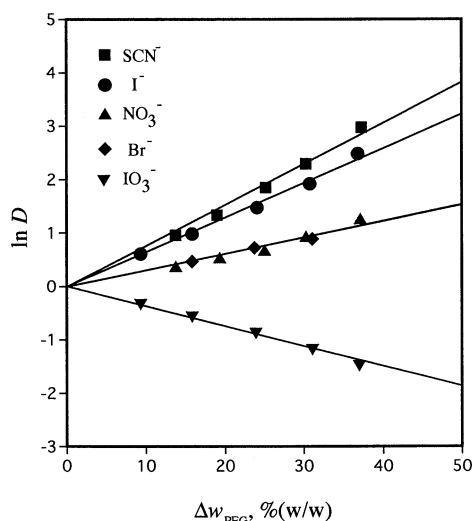
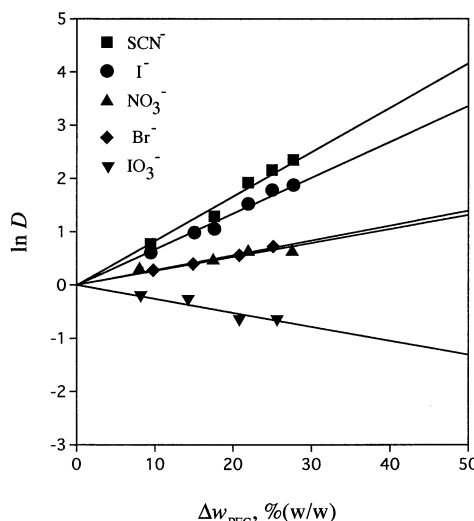Fig. 3   ln $D$ values of inorganic anions in an aqueous PEG–Na$_2$HPO$_4$ system as a function of $\Delta w_{PEG}$.



Fig. 4   ln $D$ values of inorganic anions in an aqueous PEG–K$_2$HPO$_4$ system as a function of $\Delta w_{PEG}$.

depends upon the valency and hydration of the ions as well as specific interactions of the ions with the ether oxygens of PEG. It is thus expected from the Gibbs free energy of hydration ($\Delta G_{hyd}$) that Na$^+$ has a salting-out effect stronger than that of K$^+$, although Florin *et al.*[16] have shown that the difference in the salting-out ability is very small between the two ions. The $\Delta G_{hyd}$ values for Na$^+$ and K$^+$ are –375 and –304 kJ/mol, respectively.[19] The difference in the salting-out ability between HPO$_4^{2-}$ and SO$_4^{2-}$ may be explained in a similar manner. The $\Delta G_{hyd}$ value for HPO$_4^{2-}$ ion is not available but the smaller standard partial entropy of aqueous HPO$_4^{2-}$ ion (10.9 J/K mol) compared to that of aqueous SO$_4^{2-}$ (64.5 J/K mol)[19] suggests that HPO$_4^{2-}$ attracts water molecules more strongly than SO$_4^{2-}$. Spivakov *et al.*[20] have also shown that the minimal molarity of (NH$_4$)$_2$HPO$_4$ solution necessary for phase separation in systems containing PEG is smaller than that of (NH$_4$)$_2$SO$_4$ solution.

*Effect of phase-forming ions on the partition of ionic solutes*

The partition of an analyte ion is described by the partition coefficient, $D$, defined as

$$D = \frac{C_T}{C_B} \qquad (1)$$

where $C_T$ is the concentration of the analyte ion in the top phase (PEG-rich phase) and $C_B$ is its concentration in the bottom phase (salt-rich phase). It has been shown that the ln $D$ values are well correlated to the difference, $\Delta w_i$, in the concentrations of the polymer i in the two phases of a given system by the following equation:[9,10,20]

$$\ln D = a\Delta w_i \qquad (2)$$

where $a$ is a proportionality constant. Figures 2 – 4 show ln $D$ of inorganic anions as a function of $\Delta w_{PEG}$, the difference in the % (w/w) PEG concentration between the top and bottom phases in the PEG–Na$_2$SO$_4$, Na$_2$HPO$_4$ and K$_2$HPO$_4$ systems, respectively. It can be seen from these figures that only IO$_3^-$, a water-structure making ion,[22] distributes into the bottom phase while Br$^-$, NO$_3^-$, I$^-$ and SCN$^-$, water-structure breaking ions,[22] favor the PEG-rich top phase. This means that the partition bahavior of the inorganic anions can be ascribed to a difference in the hydration of the anions. Rogers *et al.*[4] indicated that the

stronger is the hydration of the ion, or the more negative is the $\Delta G_{hyd}$ value, the smaller is its partition coefficient. The $\Delta D_{hyd}$ values for Br$^-$, NO$_3^-$, SCN$^-$ and I$^-$ are –321, –306, –287 and –283 J/K mol, respectively.[19] The order of the distribution coefficients of these anions, Br$^-$ ≈ NO$_3^-$ < I$^-$ ≈ SCN$^-$, approximately corresponds to that of the $\Delta G_{hyd}$ values.

Figures 5 – 7 show plots of ln $D$ of Li$^+$ and Mg$^{2+}$ against $\Delta w_{PEG}$ in the PEG–Na$_2$SO$_4$, Na$_2$HPO$_4$ and K$_2$HPO$_4$ systems, respectively. The cations, both of which are water-structure making ions,[22] preferred the salt-rich phases in all of the systems studied, and Eq. (2) also holds for the cations, although the data are slightly scattered.

The results shown in Figs. 2 – 7 indicate that the partition of inorganic ions in the PEG-salt aqueous two-phase systems is mainly governed by the difference in the polymer or salt concentrations between the two phases. However, it should be noted that the slope of a plot of ln $D$ *vs.* $\Delta w_{PEG}$ for an analyte ion obviously depends on the phase-forming cations and anions. The slopes of the ln $D$ *vs.* $\Delta w_{PEG}$ plots for the anionic analytes are larger in those systems containing HPO$_4^{2-}$ and K$^+$ than in the systems containing SO$_4^{2-}$ and Na$^+$, respectively, while for the cationic analytes the reverse tendency is observed. Figures 5 – 7 also show that the order of the ln $D$ values of Li$^+$ and Mg$^{2+}$ depends on the phase-forming salt used.

Shibukawa and Ohta[12,23,24] have presented a model for the partition of ionic solutes, and clarified the effect of an electrolyte in the mobile phase on the retention of analyte ions in partition chromatography. Based on this ion partition model, the partition coefficient of the analyte ion A$^{p+}$, $D_A^{YX}$, in a two-phase system containing cation Y$^{m+}$ and anion X$^{n-}$ is given by

$$\ln D_A^{YX} = \frac{1}{n} \ln K_{AX} - \frac{p}{n(m+n)} \ln K_{YX} + k_A^{YX} \qquad (3)$$

where $k_A^{YX}$ stands for a term comprising the activity coefficients of A$^{p+}$, Y$^{m+}$ and X$^{n-}$. $K_{AX}$ and $K_{YX}$ are the equilibrium constants for the following partition equilibria between the two phases, $\alpha$ and $\beta$:
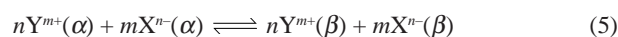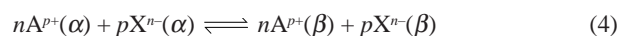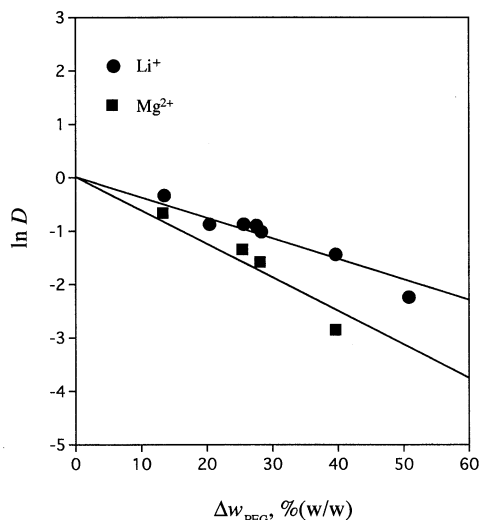
$$n\text{A}^{p+}(\alpha) + p\text{X}^{n-}(\alpha) \rightleftharpoons n\text{A}^{p+}(\beta) + p\text{X}^{n-}(\beta) \qquad (4)$$

$$n\text{Y}^{m+}(\alpha) + m\text{X}^{n-}(\alpha) \rightleftharpoons n\text{Y}^{m+}(\beta) + m\text{X}^{n-}(\beta) \qquad (5)$$

Fig. 5   ln $D$ values of lithium and magnesium ions in an aqueous PEG–Na$_2$SO$_4$ system as a function of $\Delta w_{PEG}$.
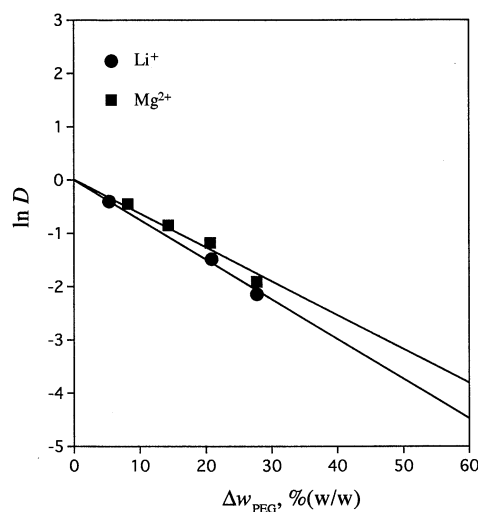


Fig. 7   ln $D$ values of lithium and magnesium ions in an aqueous PEG–K$_2$HPO$_4$ system as a function of $\Delta w_{PEG}$.
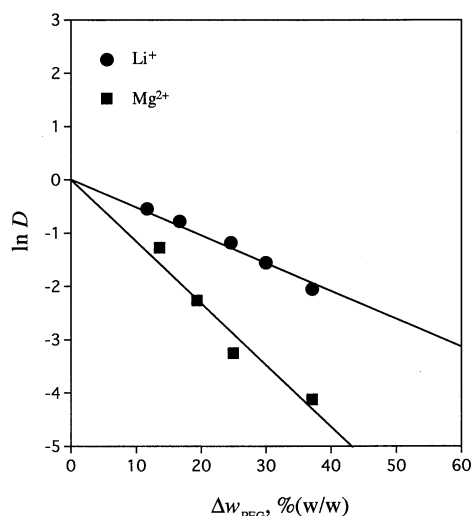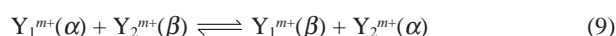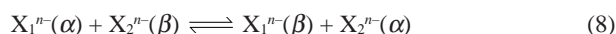


Fig. 6   ln $D$ values of lithium and magnesium ions in an aqueous PEG–Na$_2$HPO$_4$ system as a function of $\Delta w_{PEG}$.

From Eq. (3), the difference between ln $D_A^{YX_1}$ and ln $D_A^{YX_2}$, $\Delta \ln D_A^{(YX_1-YX_2)}$, and that between ln $D_A^{Y_1X}$ and ln $D_A^{Y_2X}$, $\Delta \ln D_A^{(Y_1X-Y_2X)}$ can be derived,[12] and are given by the following equations, respectively:

$$\Delta \ln D_A^{(YX_1-YX_2)} = \ln D_A^{YX_1} - \ln D_A^{YX_2} = \frac{p}{m+n} \ln K_{X_1/X_2} + k_A^{YX_1} - k_A^{YX_2} \quad (6)$$

$$\Delta \ln D_A^{(Y_1X-Y_2X)} = \ln D_A^{Y_1X} - \ln D_A^{Y_2X}$$

$$= -\frac{p}{m+n} \ln K_{Y_1/Y_2} + k_A^{Y_1X} - k_A^{Y_2X} \quad (7)$$

where $K_{X_1/X_2}$ and $K_{Y_1/Y_2}$ are the equilibrium constants for the following "ion-exchange" equilibria:

$$X_1^{n-}(\alpha) + X_2^{n-}(\beta) \rightleftharpoons X_1^{n-}(\beta) + X_2^{n-}(\alpha) \quad (8)$$

$$Y_1^{m+}(\alpha) + Y_2^{m+}(\beta) \rightleftharpoons Y_1^{m+}(\beta) + Y_2^{m+}(\alpha) \quad (9)$$

Equations (6) and (7) represent the effects of the counter-ion

and the co-ion in the system on the partition of an analyte cation, respectively. The equations concerning the counter-ion and co-ion effects on the partition coefficient of an anionic analyte, B$^{p-}$, can be also written as

$$\Delta \ln D_B^{(Y_1X-Y_2X)} = \ln D_B^{Y_1X} - \ln D_B^{Y_2X}$$

$$= \frac{p}{m+n} \ln K_{Y_1/Y_2} + k_B^{Y_1X} - k_B^{Y_2X} \quad (10)$$

$$\Delta \ln D_B^{(YX_1-YX_2)} = \ln D_B^{YX_1} - \ln D_B^{YX_2}$$

$$= -\frac{p}{m+n} \ln K_{X_1/X_2} + k_B^{YX_1} - k_B^{YX_2} \quad (11)$$

Equations (3), (6), (7), (10) and (11) can be applied to systems where the following requirements are satisfied:[12] (a) The equilibrium constants, such as $K_{AX}$ and $K_{YX}$, are independent of the ionic composition in the systems; (b) The concentration of the analyte ion is negligibly smaller than those of the component ions of the partition system; (c) The association of the analyte ion with counter-ions can be neglected. We tentatively tried to apply this ion partition model to the present aqueous two-phase systems assuming that they meet these requirements. It may be reasonable to assume that requirement (a) is satisfied because the affinity of each phase for the ions is essentially determined by the concentration of PEG. The concentrations of the analyte ions are also much lower than those of the phase-forming salts, not only in the salt-rich phases, but also in the PEG-rich phases of all of the systems studied. However, the concentrations of the phase-forming salts are so high that it may be difficult to meet requirement (c) in some cases.

We evaluated the effects of the phase-forming ions on the partition of the analyte ions by using the slopes of the ln $D$ vs. $\Delta w_{PEG}$ plots. The slopes of the plots represent the ln $D$ values at $\Delta w_{PEG}$ of 1%, ln $D$(1%). The ln $D$(1%) values for the analyte ions in the three PEG-salt systems are tabulated in Table 1.

Equations (6), (7), (10) and (11) indicate that the difference in the ln $D$ of ionic analytes in two different phase-forming salt systems is determined by the "ion exchange" equilibrium constants, $K_{X_1/X_2}$ and $K_{Y_1/Y_2}$. We estimated the $K_{SO_4/HPO_4}$ and $K_{K/Na}$ values at $\Delta w_{PEG}$ of 1%, $K_{SO_4/HPO_4}$(1%) and $K_{K/Na}$(1%), from

Table 1   ln $D$(1%) values for inorganic ions in aqueous PEG–salt two-phase systems

| Analyte ion | Phase-forming salt | | |
|---|---|---|---|
| | $Na_2SO_4$ | $Na_2HPO_4$ | $K_2HPO_4$ |
| $IO_3^-$ | –0.038 | –0.035 | –0.026 |
| $Br^-$ | 0.017 | 0.027 | 0.029 |
| $NO_3^-$ | 0.025 | 0.027 | 0.028 |
| $I^-$ | 0.059 | 0.064 | 0.067 |
| $SCN^-$ | 0.063 | 0.073 | 0.083 |
| $Li^+$ | –0.038 | –0.050 | 0.074 |
| $Mg^{2+}$ | –0.063 | –0.116 | –0.066 |

Table 2   Comparison of the calculated $\Delta \ln D_i^{(Na2SO4-Na2HPO4)}$ and $\Delta \ln D_i^{(K2HPO4-Na2HPO4)}$ values with the observed values for inorganic ions

| Analyte ion | $\Delta \ln D_i^{(Na2SO4-Na2HPO4)}$ | | $\Delta \ln D_i^{(K2HPO4-Na2HPO4)}$ | |
|---|---|---|---|---|
| | calcd | obsd | calcd | obsd |
| $IO_3^-$ | – 0.011 | – 0.003 | 0.005 | 0.009 |
| $Br^-$ | – 0.011 | – 0.010 | 0.005 | 0.002 |
| $NO_3^-$ | – 0.011 | – 0.003 | 0.005 | 0.001 |
| $I^-$ | – 0.011 | – 0.005 | 0.005 | 0.003 |
| $SCN^-$ | – 0.011 | – 0.010 | 0.005 | 0.010 |
| $Li^+$ | 0.011 | 0.012 | – 0.005 | – 0.024 |
| $Mg^{2+}$ | 0.022 | 0.053 | – 0.010 | – 0.050 |
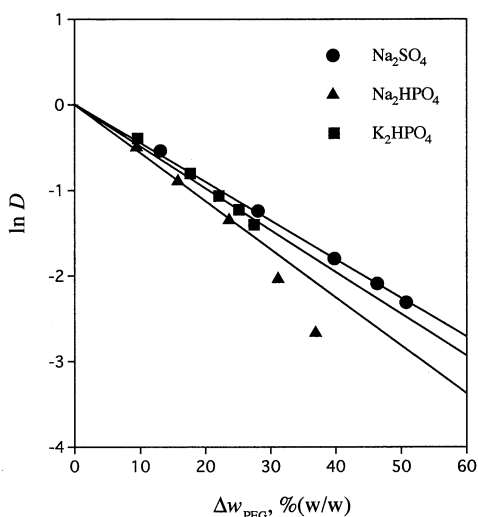


Fig. 8   ln $D$ values of the phase-forming salts in an aqueous PEG-$Na_2SO_4$, $Na_2HPO_4$ and $K_2HPO_4$ two-phase systems as a function of $\Delta w_{PEG}$.

the slopes of plots of ln $D$ for the phase-forming salts against $\Delta w_{PEG}$ in the following manner.  The obtained $D$ values for $Na_2SO_4$ in the PEG–$Na_2SO_4$ system, for example, represent the partition coefficients of $Na^+$ and $SO_4^{2-}$ in the system; the $D$ values for both ions are the same in this system.  Therefore, $K_{Na2SO4}$ is calculated as

$$K_{Na2SO4} = D_{Na}^2 D_{SO4} = D_{Na2SO4}^3 \qquad (12)$$

$K_{Na2HPO4}$ and $K_{K2HPO4}$ are obtained in a similar manner.  The $K_{SO4/HPO4}$(1%) and $K_{K/Na}$(1%) values can thus be estimated if the dependence of the ionic activity coefficients on the ionic strength and on the type of the coexisting ions is negligible, as follows:[12]

$$K_{SO4/HPO4}(1\%) = K_{Na2SO4}(1\%)/K_{Na2HPO4}(1\%)$$
$$= \{D_{Na2SO4}(1\%)/D_{Na2HPO4}(1\%)\}^3 \qquad (13)$$

$$K_{K/Na}(1\%) = \{K_{K2HPO4}(1\%)/K_{Na2HPO4}(1\%)\}^{1/2}$$
$$= \{D_{K2HPO4}(1\%)/D_{Na2HPO4}(1\%)\}^{3/2} \qquad (14)$$

ln $D$ *vs.* $\Delta w_{PEG}$ plots for $Na_2SO_4$, $Na_2HPO_4$ and $K_2HPO_4$ in their respective systems are shown in Fig. 8.  The plots for the PEG-$Na_2SO_4$ and $K_2HPO_4$ systems gave straight lines, whereas the data for the PEG–$Na_2HPO_4$ system form a convex curve. Because the cause of this result is not yet clear, the $D_{Na2HPO4}$(1%)

value was estimated from the slope of the plots in the low-$\Delta w_{PEG}$ range, where a linear relationship can be approximately obtained.  The ln $K_{SO4/HPO4}$(1%) and ln $K_{K/Na}$(1%) values calculated from the $D$ (1%) values of the phase-forming salts are 0.033 and 0.015, respectively.

The $\Delta \ln D_i^{(Na2SO4-Na2HPO4)}$ and $\Delta \ln D_i^{(K2HPO4-Na2HPO4)}$ for an analyte ion i can be calculated by substituting the ln $K_{SO4/HPO4}$(1%) and ln $K_{K/Na}$(1%) values into Eqs. (6), (7), (10) or (11), respectively. Table 2 gives the calculated $\Delta \ln D_i^{(Na2SO4-Na2HPO4)}$ and $\Delta \ln D_i^{(K2HPO4-Na2HPO4)}$ values together with the observed ones.  It can be seen from the table that the agreements of the calculated values with the experimental ones are relatively good, except in some cases.  The large difference between the two values for $Mg^{2+}$ may be attributed to the ion association with the anions. The dependence of the activity coefficients of the ions on the type of phase-forming salt may also be responsible for the discrepancy.  The ionic strength of each phase in the two-phase systems studied is so high that the activity coefficient of an ion is no longer independent of the ionic composition of the phase.[25] This means that the $k$ values vary with the composition of the systems.  However, the results obtained in this study indicate that the dependence of the partition of ionic solutes in the aqueous two-phase systems can be reasonably interpreted on the basis of the ion partition model.  Equations (6), (7), (10) and (11) are useful for predicting the effect of the phase-forming cation and anion on the partition coefficients of ionic solutes.  It should be noted that $K^+$ and $SO_4^{2-}$ are more effective for the extraction of oppositely charged ions to the PEG-rich phase than $Na^+$ and $HPO_4^{2-}$, respectively, when they are compared with each other under a constant $\Delta w_{PEG}$, although the former ions are less effective for salting out of PEG than the latter ions.

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