Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Predicting Partitioning in Aqueous Two-Phase Systems and the Effects of Temperature Changes

Larry M. Cohen[a]; John L. Gainer[a]
[a] DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VIRGINIA

To cite this Article Cohen, Larry M. and Gainer, John L.(1994) 'Predicting Partitioning in Aqueous Two-Phase Systems and the Effects of Temperature Changes', Separation Science and Technology, 29: 15, 1925 — 1942
To link to this Article: DOI: 10.1080/01496399408002181
URL: http://dx.doi.org/10.1080/01496399408002181

SEPARATION SCIENCE AND TECHNOLOGY, **29**(15), pp. 1925–1942, 1994

Predicting Partitioning in Aqueous Two-Phase Systems and the Effects of Temperature Changes

LARRY M. COHEN* and JOHN L. GAINER†
DEPARTMENT OF CHEMICAL ENGINEERING
UNIVERSITY OF VIRGINIA
CHARLOTTESVILLE, VIRGINIA 22903-2442

ABSTRACT

The use of aqueous two-phase extraction can be attractive for some separations, but the choice of such systems is frequently done in an empirical manner. Predictive models are needed for optimal design, but previous ones appear to have limitations. In addition, these systems have usually been used previously at temperatures around 25°C; however, it is possible that better separations may be achieved at other temperatures. An equation is developed here which can predict partitioning for a wide range of solutes. The effect of temperature on partitioning has been determined, and this can also be predicted by using the equation. Thus, this model may prove quite useful in designing and optimizing extractive separations.

Key Words. Two aqueous phase separations; Partitioning; Hydrophobicity

INTRODUCTION

As biotechnology continues to grow, more selective and economical separation processes are continuously being sought. Chromatography and centrifugation currently play the most significant roles in scaled-up sys-

* Current address: Abbott Laboratories, North Chicago, Illinois.
† To whom correspondence should be addressed at Department of Chemical Engineering, University of Virginia, Thornton Hall, Charlottesville, VA 22903-2442. Phone: (804) 924-6277. FAX: (804) 982-2658.

tems; however, the use of aqueous two-phase extraction may be attractive for some separations needs. Depending on the overall goal of the separation, aqueous two-phase extraction can be quite selective in partitioning desired solutes away from their contaminants, and strategies concerning the development of these systems have been outlined previously (1, 2). Another reason for using aqueous two-phase extraction is that it is fairly easy to scale up. Thus, selection of a system to meet design needs for larger volumes can be done in the laboratory.

Most aqueous two-phase systems described in the literature use solutions of polyethylene glycol (PEG) and dextrans. There are, however, problems with the use of dextran solutions on a larger scale, such as their considerable cost and high viscosities. Although there are other polymers that can be used instead of dextrans, a simpler system is possible through replacement of the dextran with a salt solution (3). The typical PEG/salt systems which have been used, such as PEG/ammonium sulfate, PEG/phosphate, PEG/magnesium sulfate, and PEG/citrate, are less expensive and generally not as viscous as dextrans (4–6).

The understanding of aqueous two-phase separations has evolved greatly since the initial experiments of Albertsson (1), although a complete explanation for solute partitioning is still not available. There have been a number of empirical studies in which aqueous two-phase extraction has been successfully used to partition a solute, and these are reviewed elsewhere (1, 2, 7). Interest has also has been directed toward the molecular and thermodynamic parameters of phase formation (8, 9). However, successful implementation of aqueous two-phase extraction on a larger scale also requires the development of design methods which allow one to predict the type of system needed for a given separation as well as the effect of certain operating parameters, such as temperature. In that respect, semiempirical models, or equations, should to be quite useful. An example of this is a model by Diamond and Hsu (10) to describe partitioning of proteins in various PEG/dextran and PEG/potassium phosphate systems.

Eiteman and Gainer (5, 11) have also developed an equation which appears to predict partitioning of low molecular weight solutes (both uncharged molecules and amino acids) as well as peptides and proteins:

$$\ln K = D\Delta W_2 \log\left(\frac{P}{P_0}\right) \tag{1}$$

In this equation D is a constant, called the discrimination factor (Eiteman), and it is related to the type of system used. For example, if PEG of a

certain molecular weight is used with a given salt at concentrations capable of forming two phases, then the discrimination factor found at one concentration of the two phase-forming components should also be valid for other concentrations. The term ΔW_2 is the difference in the concentration of the PEG between the top and bottom phases, and it essentially describes the location of the particular system on a two-phase diagram. P stands for the solute hydrophobicity (although sometimes log P is used for this quantity). A value for this parameter, which is based on the molecular structure of the solute, can be estimated using a method proposed by Rekker and de Kort (12). Finally, the value of P_0 is the hydrophobicity of a compound which will partition equally into both phases. It has been shown previously (5) that P_0 is intrinsic to the phase system and does not vary with the solutes used.

This equation has been shown to correlate partitioning data well; however, it has not been tested over a wide range of hydrophobicity values. Thus, it is not known if ln K is also a linear function of log P at higher values of P. This could be especially important in the design of separations involving very hydrophobic molecules, such as many pharmaceutical products. Nevertheless, this equation appears to be a good starting point for the development of a more comprehensive model for partitioning. In addition, it is not known if the parameters in Eq. (1) vary with temperature. In fact, most of the studies involving aqueous two-phase partitioning have focused on separations occurring around 20–25°C, and only a few have investigated the role of temperature. However, partitioning of proteins at higher temperatures may be of interest due to an apparent increase in resistance to denaturation in the presence of PEG (13). In addition, systems of PEG and NaCl have been shown to form two phases at elevated temperatures, and it has been suggested that this PEG/salt system may be useful in the food industry for separating food flavors, peptides, etc. (14).

One previous study concerning the influence of temperature changes involved the partitioning of myosin containing palmitate-bound PEG, which showed an increased affinity toward the palmitate when the temperature of the system was increased from 4 to 20°C (15). Johansson and Andersson (16) showed that increasing the temperature increases the partitioning of several yeast enzymes in an affinity partitioning system. Forciniti et al. (17) demonstrated, in systems containing 16 possible molecular weight combinations of PEG and dextran, that temperature affects phase diagrams, changing the tie line length, position of the binodal curve, and distribution of polymers in each phase. This means that, for Eq. (1), there should be an effect of temperature on ΔW_2.

The objective of this study, then, was to test the model for partitioning proposed by Eiteman and Gainer (Eq. 1) over a wider range of solute hydrophobicities. In addition, the temperature dependency of Eq. (1) is examined in order that partitioning might be predicted and optimized.

MATERIALS AND METHODS

The solutes used for these experiments were a series of *n*-alcohols, ranging from methanol to *n*-octanol (Aldrich, Milwaukee, Wisconsin). Two salt/PEG systems (PEG obtained from Sigma Chemical Co., St. Louis, Missouri) were used: ammonium sulfate/PEG-8000 and potassium phosphate/PEG-8000 (where the ratio 0.65 K_2HPO_4:0.35 KH_2PO_4 was kept constant). The preparation of the phase systems began by making a 40% (w/w) stock concentrate of both a salt solution and a PEG solution. Water was later added and the solutions mixed so as to produce the desired concentrations. The concentrations of the *n*-alcohol (methanol through *n*-hexanol) solutes did not exceed 0.1% (v/v).

Each experimental group was analyzed using either duplicate or triplicate samples. After proper dilution, replicates of an experimental condition were placed into conical tubes. Each tube was shaken vigorously and placed in a temperature bath to allow the system to come to thermal equilibrium. Once thermal equilibrium was achieved, the tubes were vigorously shaken again and returned to the bath for at least 18 hours.

The phase systems were carefully separated using a 100-μL lambda pipet attached to a 5-mL disposable syringe. This allowed for a clean piercing of the interface in order to recover the lower phase with minimal cross contamination from the upper phase. The higher viscosity of the PEG-rich phase sometimes necessitated that it be diluted prior to analysis by gas chromatography, which utilized a glass column containing Supelco Chromosorb 101 resin and flame ionization detection. All samples were chromatographed either two or three times in order to minimize errors.

The PEG concentrations in the solutions were measured using a modification of the method of Skoog (18). This was done using solutions not containing the alcohol solutes, and it was assumed that the alcohols did not affect the PEG concentrations since the solutes were present in such dilute concentrations. The concentration of PEG in these phase systems was many orders of magnitude greater than the assay could measure, however, so it required considerable sample dilutions. The assay consisted of adding 1 mL of the diluted sample containing PEG to 0.5 mL of 0.1 N perchloric acid and 0.250 mL of 5% barium chloride. The reaction, which produced a color, was initiated by the addition of 0.1 mL of 1% Titrasol (E. M. Merck). The absorbance of the sample at 535 nm was

linearly related to the PEG concentration over a narrow concentration range. The color formed after the addition of the Titrasol was immediate and remained stable for 20 minutes, although readings were taken after 10 minutes.

RESULTS AND DISCUSSION

The results from partitioning the series of *n*-alcohols is shown in Fig. 1, where the partition coefficient (ln K) is plotted versus the solute hydrophobicity (log P). These latter values were calculated using the method of Rekker and de Kort (12). Figure 1 indicates that, as the solute hydrophobicity is increased, the relationship between ln K and log P no longer displays the linear behavior that would be predicted by Eq. (1). Equation (1) was therefore modified in an attempt to predict the behavior at greater solute hydrophobicities using the following reasoning. It appears in Fig. 1 that the relationship between ln K and log P is linear up to a certain value of log P (i.e., it follows Eq. 1 over that range). Then there is a departure from linearity, suggesting that some "critical" hydrophobicity has been reached. When such behavior is seen, it can be modeled mathematically by dividing the right-hand side of Eq. (1) by the difference between this critical hydrophobicity (here denoted as a) and the hydrophobicity ratio P/P_0 as follows:

$$\ln K = \frac{D\Delta W_2 \log\left(\frac{P}{P_0}\right)}{a - \log\left(\frac{P}{P_0}\right)} \tag{2}$$

In order to determine if this modified equation would provide a better fit to the data, the results for the partitioning of the alcohols were analyzed again. First Eq. (2) was rearranged into a linear form (Eq. 3) in order to more easily determine the values of a and D to be used:

$$\frac{\Delta W_2}{\ln K} = \left(\frac{a}{D}\right)\left(\frac{1}{\log\left(\frac{P}{P_0}\right)}\right) - \left(\frac{1}{D}\right) \tag{3}$$

The data were then plotted as shown in Fig. 2. It can be seen that an excellent linear fit results, indicating that the modified equation describes the partitioning well. The terms a and D, for each system partitioned, can be determined from the slope and y-intercept of the plot. All such plots

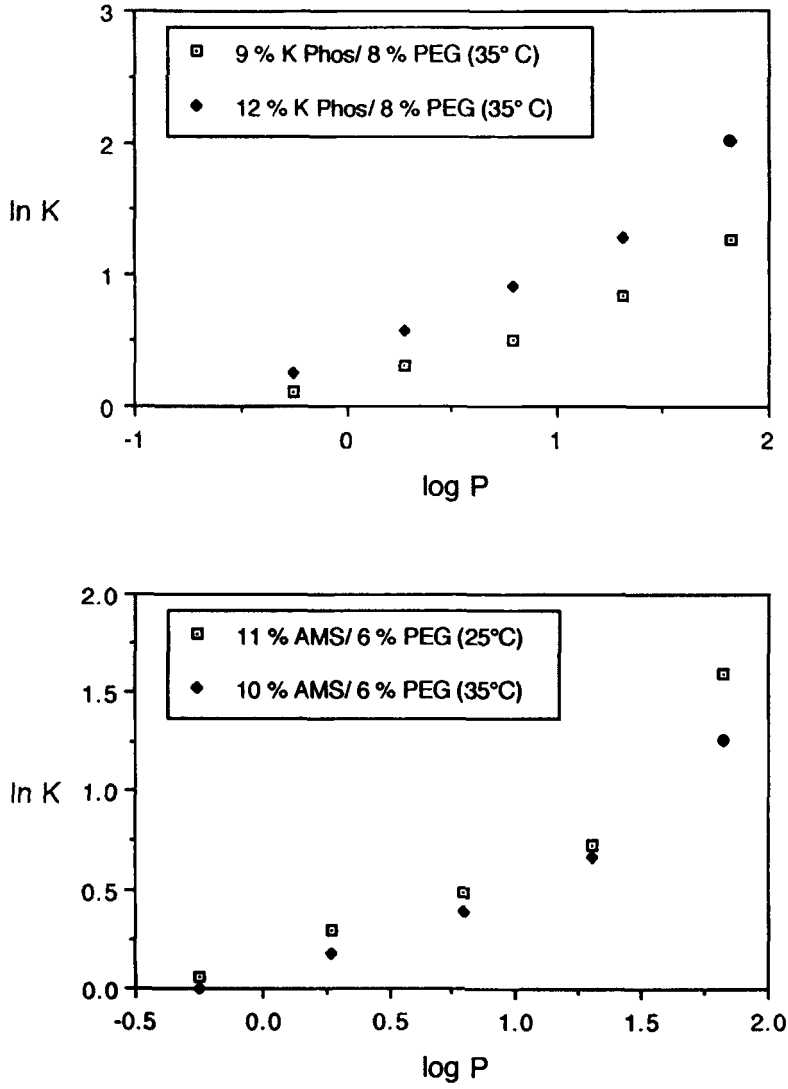

FIG. 1 Measured partition coefficients (ln K) of n-alcohols in several potassium phosphate/PEG-8000 and ammonium sulfate/PEG-8000 systems versus calculated solute hydrophobicity (log P). Solutes were n-alcohols, with hydrophobicities increasing from that of methanol to that of n-hexanol.

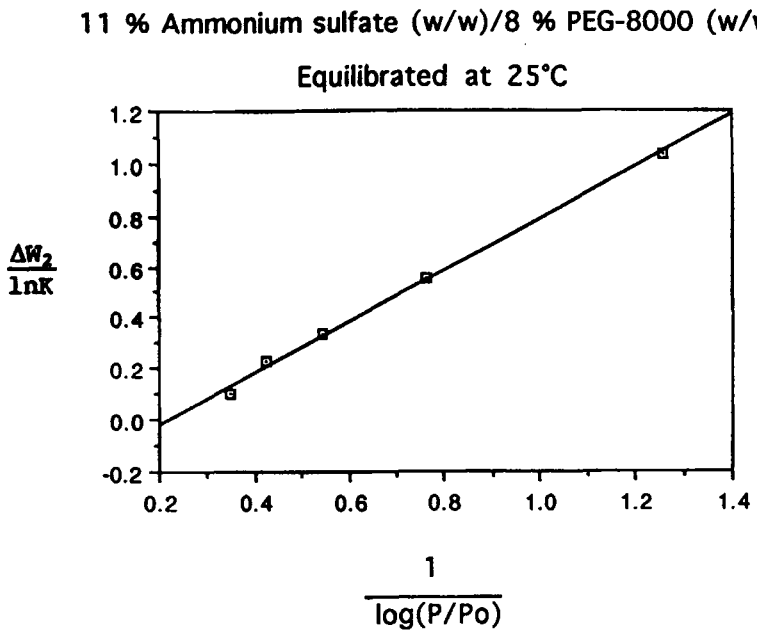

FIG. 2 Typical plot used to determine values of the parameters a and D of Eq. (2).

using the data for the partitioning of the alcohols resulted in excellent straight lines.

Using average values of a and D, the data are plotted in Fig. 3 for two of the systems studied. It would appear that the modified equation fits reasonably well over the entire hydrophobicity range. To better show that, a comparison was made between the Eiteman equation (Eq. 1) and the modified one (Eq. 2), and this is shown in Fig. 4. As can be seen, the difference between these two models is evident at the higher values of hydrophobicity, as expected.

Table 1 lists the parameters a and D for the several systems studied. An examination of those values suggests that small changes in the composition can have significant effects on partitioning in some cases. For example, an increase in the ammonium sulfate composition from 10 to 11%, while holding the PEG composition constant, primarily affects the P_0 term. If the ammonium sulfate concentration is held constant while increasing the PEG concentration, the effect is to reduce the discrimination factor, D, while the value of a is increased. When potassium phosphate/PEG systems were partitioned, increasing the salt from 9 to 12% caused little change in either term, though. It should also be noted that the values

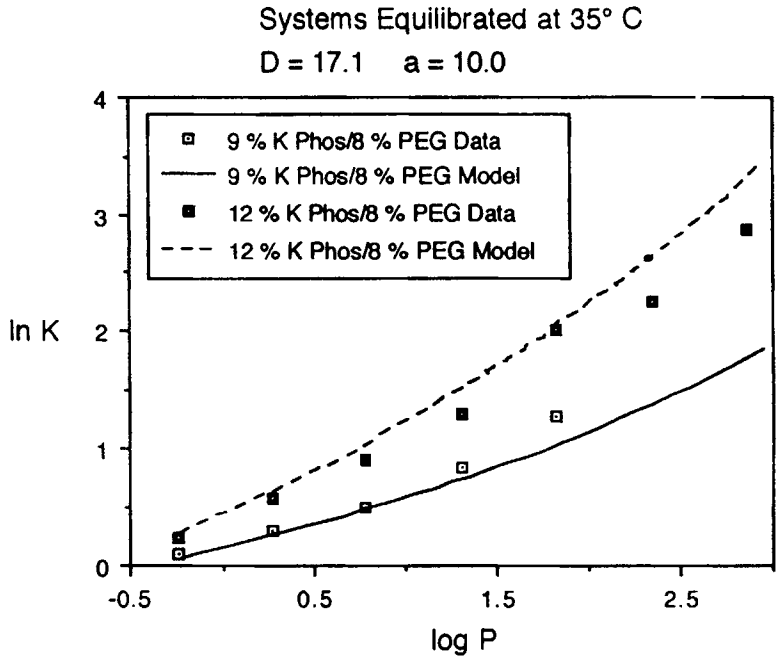

FIG. 3 Measured partition coefficients (ln K) in potassium phosphate/PEG-8000 systems versus calculated solute hydrophobicity (log P). Solutes were n-alcohols, with hydrophobicities increasing from that of methanol to that of n-heptanol. The lines represent the model predictions using average values of D and a.

of a and D do not depend on the solute being partitioned, but are only related to the compositions of the phase systems.

This same series of n-alcohols was also chosen as solutes to be partitioned at different temperatures. In particular, their partitioning in a 9% potassium phosphate/8% PEG-8000 system was determined over a 30° range of temperatures (from 30 to 60°C), and these results are shown in Fig. 5. Again, as log P increased, there was a deviation from linearity. When the temperature was raised from 30 to 45 or 60°C, the partitioning into the upper PEG-rich phase was greater, with the largest increases occurring with the more hydrophobic solutes.

Since the partition coefficient obviously varies with temperature, this may indicate that there is some sort of "activation" energy involved. To gain more insight into this, the data were plotted as ln K vs the reciprocal of temperature (Fig. 6) for a given component (i.e., a constant value of log P). As can be seen, the data appear to be correlated by a straight line

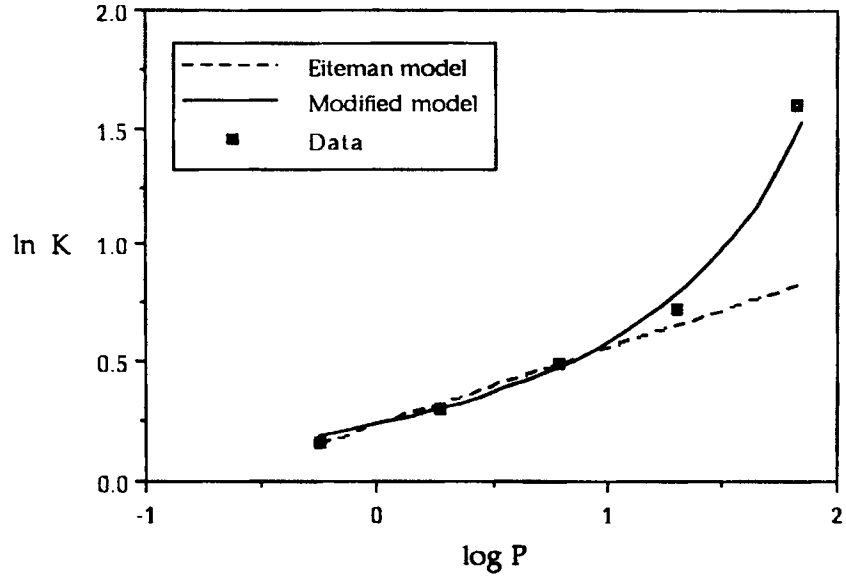

FIG. 4 Comparison of data to both the Eiteman model and the modified model for an 11% ammonium sulfate/6% PEG-8000 system equilibrated at 25°C. Solutes were *n*-alcohols, with hydrophobicities increasing from that of methanol to that of *n*-hexanol.

TABLE 1
Values of Constants in Equation (2) for Various Systems near Room Temperature

Salt	% Salt (w/w)	%PEG-8000 (w/w)	Equilibrium temperature (°C)	P_0	D	a
Ammonium sulfate	10	6	35	0.47	4.78	3.75
	11	6	25	0.09	4.1	4.36
	11	9	25	0.04	3.47	5.13
	10	12	25	0.20	16.34	7.56
Potassium phosphate,	9	8	35	0.38	18.20	9.61
0.65 K_2HPO_4:0.35 KH_2PO_4	12	8	35	0.20	16.06	10.41

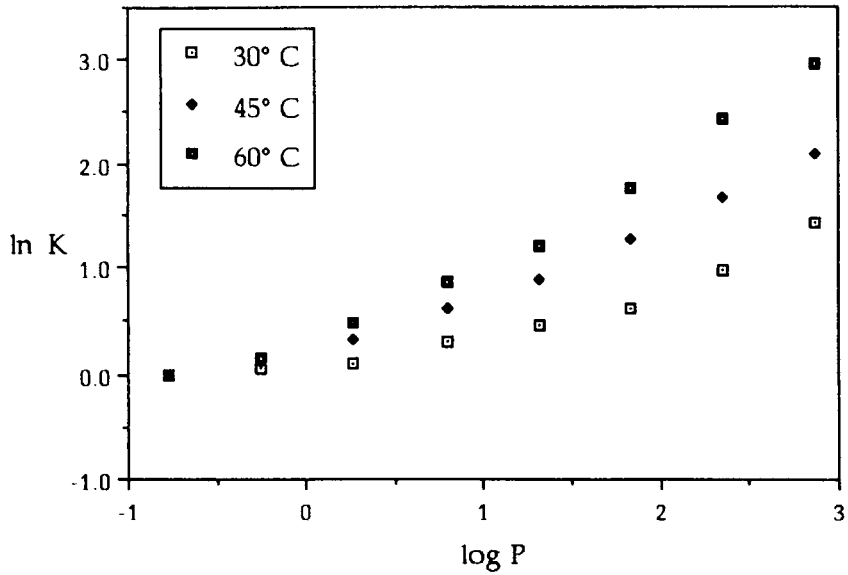

FIG. 5 Measured partition coefficients (ln K) in a 9% potassium phosphate/8% PEG-8000 (w/w) system versus calculated solute hydrophobicity (log P). Solutes were n-alcohols, with hydrophobicities increasing from that of methanol to that of n-octanol.

for each solute. At first glance the data for heptanol and octanol don't appear to be as linear as the others; however, the nonlinearities are probably due to the analytical procedures used since those alcohols are much less soluble in the aqueous phases than the others. From this plot it would appear that the partitioning data follow a pattern similar to the familiar can't Hoff relationship seen for chemical equilibria. For chemical equilibria, the slope of a plot of the logarithm of the equilibrium constant versus the reciprocal temperature is related to the enthalpy of the reaction. Similarly, we might assume that the slopes of the lines shown in Fig. 6 are proportional to a "partial molar enthalpy of partitioning." Since the lines are straight, this would mean that this quantity does not vary with temperature. Thus, using the product of the slope multiplied by the gas constant, R, for the value of the partial molar enthalpy of partitioning for each solute, it was found these values ranged from 640 cal/g-mol for ethanol to 11,135 cal/g-mol for n-octanol.

Since these partial molar enthalpies appear to increase with the carbon chain length of the alcohol, they are shown in Fig. 7 as a function of the hydrophobicities of the various compounds. The point at which the partial molar enthalpy, $\Delta H'$, equals zero should represent the "intrinsic hydro-

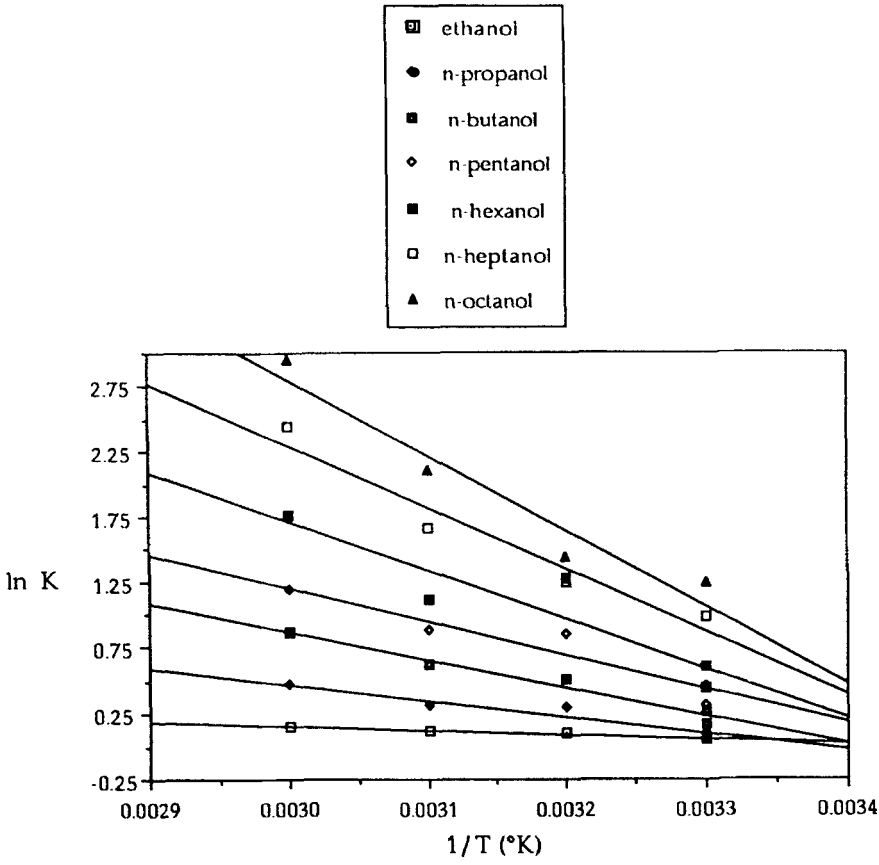

FIG. 6 Partition coefficients (ln K) of n-alcohols in a 9% potassium phosphate/8% PEG-8000 (w/w) system versus the reciprocal of temperature ($°K^{-1}$).

phobicity," P_0, of the 9% potassium phosphate/8% PEG-8000 system, and these data suggest that P_0 for a given system is not a function of temperature.

Additional experiments were performed using a different two-phase system, one composed of PEG/ammonium sulfate solutions. Figure 8 shows the relationship between partitioning and solute hydrophobicity for two ammonium sulfate/PEG systems equilibrated at different temperatures, and the data resemble those for the PEG/potassium phosphate system shown in Figure 5. However, a different composition of the PEG/ammonium sulfate system resulted in an apparent inversion of the partitioning (i.e., ln K changed from positive to negative values) when the temperature

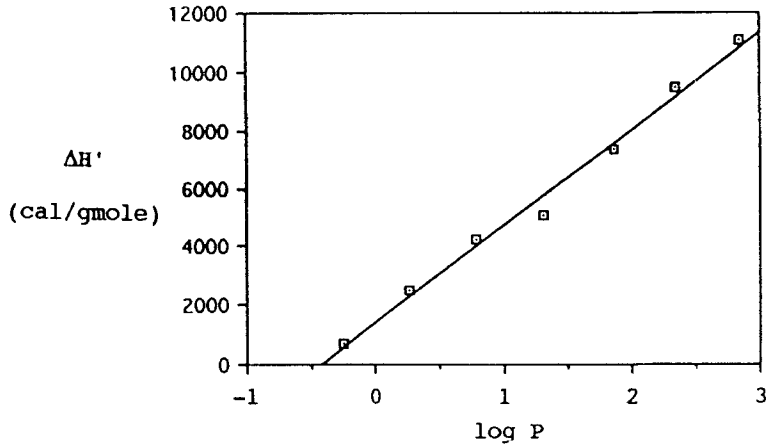

FIG. 7 Plot of calculated partial molar enthalpy of partitioning, $\Delta H'$ (cal/g-mol), for the partitioning in a 9% potassium phosphate/8% PEG-8000 (w/w) system versus the solute hydrophobicity (log P). Solutes were n-alcohols, with hydrophobicities increasing from that of methanol to that of n-heptanol.

was increased (Fig. 9A). This behavior was not seen, however, with the same concentration of that salt and a higher PEG concentration (Fig. 9B). In another experiment, a similar inversion was found when n-pentanol was partitioned in the 10% ammonium sulfate/6% PEG-8000 system for temperatures between 35 and 65°C. Figure 10 shows the results of that study. To our knowledge, inversions similar to this have not been reported previously.

Equation (2) should be valid for the prediction of the effect of temperature on partitioning as well as the apparent inversions seen (as in Figs. 9A and 10). For this, it is necessary to know which parameters in that equation are affected by temperature. As noted previously, P_0 does not appear to vary with temperature, nor does P. Thus, ΔW_2 values (the differences in PEG concentration between phases) were determined for the ammonium sulfate systems. Since ΔW_2 is defined as the concentration of PEG in the top phase minus the PEG concentration in the bottom phase, positive values mean that there is a higher concentration of PEG in the upper phase, and negative values for ΔW_2 indicate that there is a higher concentration of PEG in the lower one. Figure 11 shows the results of those experiments, and it can be seen that the systems exhibiting an inversion of partitioning at increased temperatures also showed negative values of ΔW_2 at those temperatures. Thus, it appears that this behavior is actually a result of the temperature dependency of ΔW_2, and suggests that Eq. (2) can still be used to predict the changes in partitioning. This is

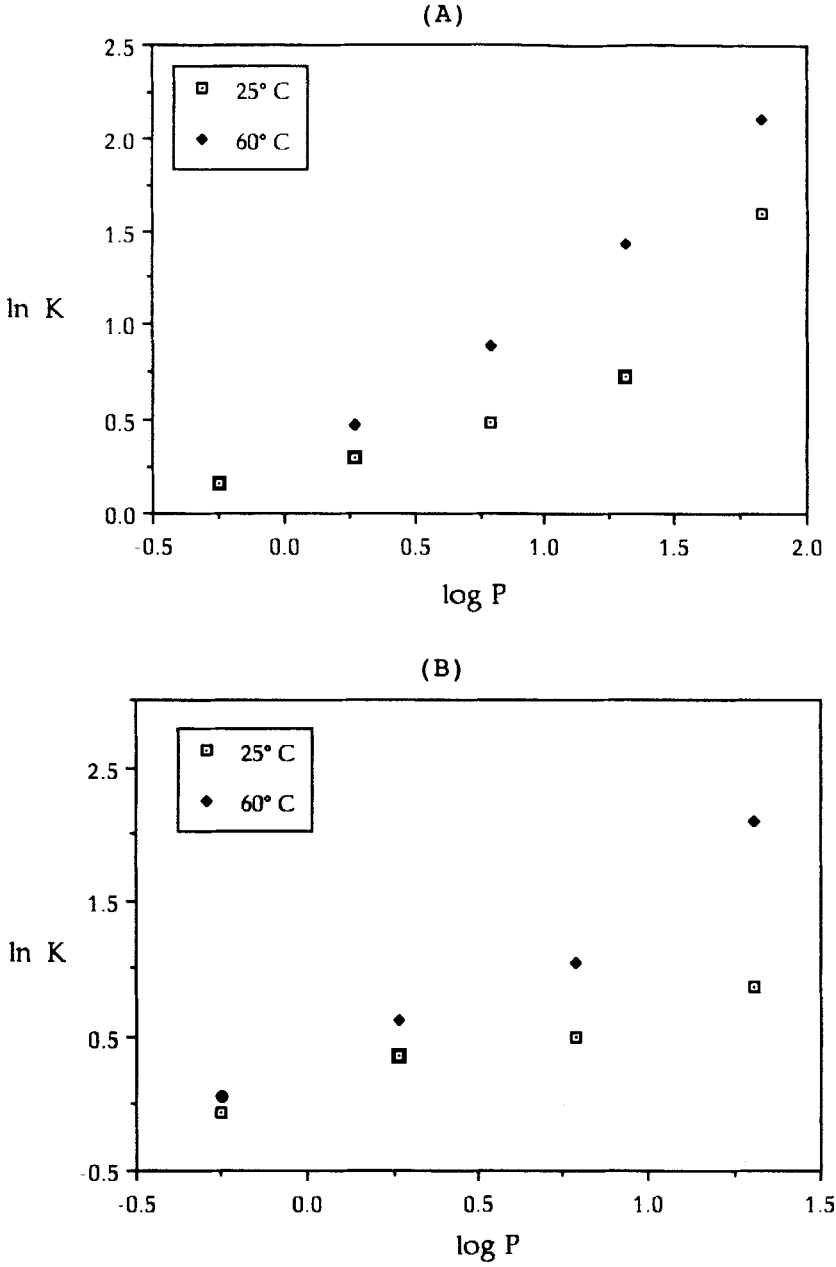

FIG. 8 Measured partition coefficients (ln K) of n-alcohols in an 11% ammonium sulfate/PEG-8000 system versus calculated solute hydrophobicity (log P) equilibrated at either 25 or 60°C: (A) 6% PEG, (B) 9% PEG.

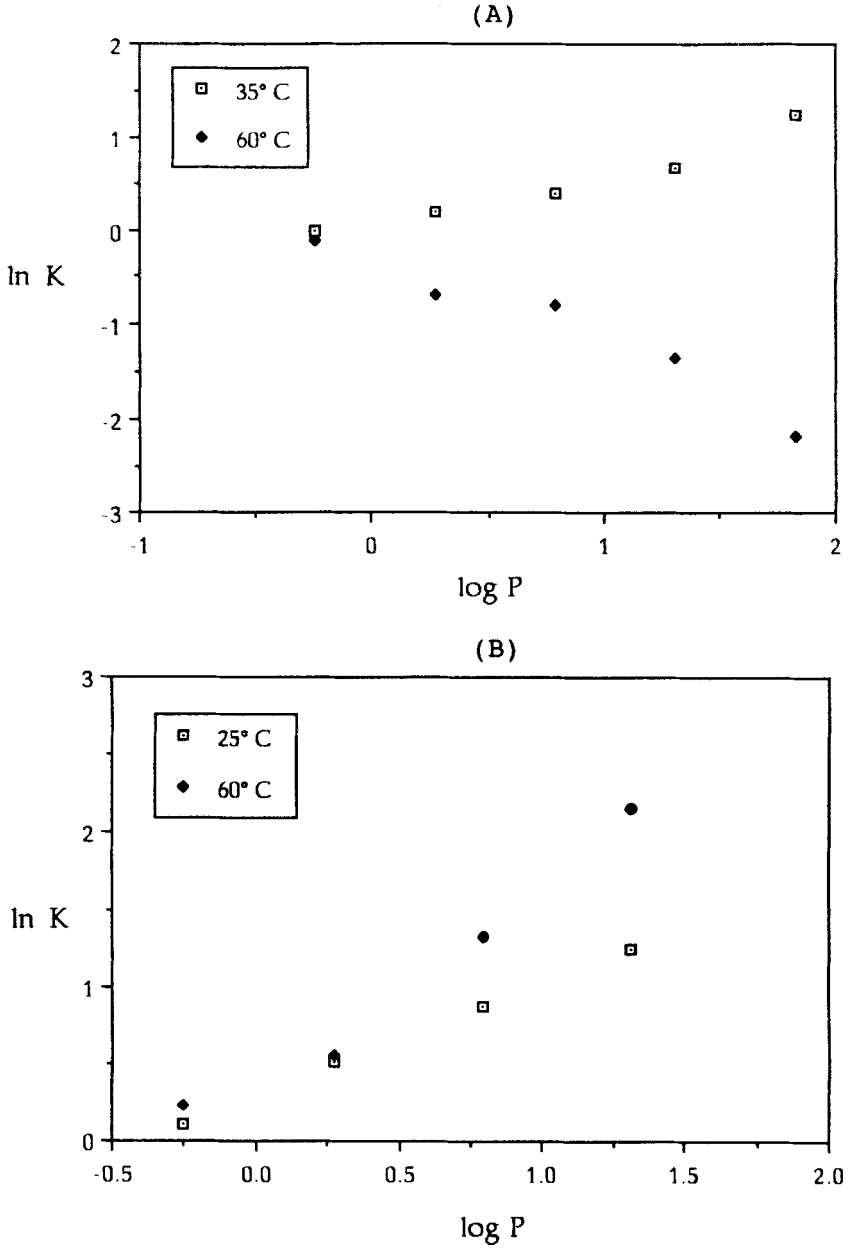

FIG. 9 Measured partition coefficients (ln K) of n-alcohols in a 10% ammonium sulfate/PEG-8000 (w/w) system versus calculated solute hydrophobicity (log P) equilibrated at either 35 or 60°C: (A) 8% PEG, (B) 12% PEG.

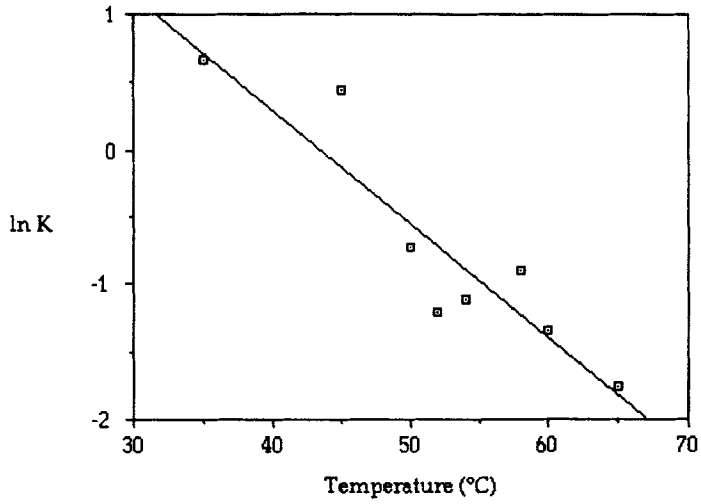

FIG. 10 Measured partition coefficients (ln K) of n-pentanol in 10% ammonium sulfate/6% PEG-8000 system versus temperature.

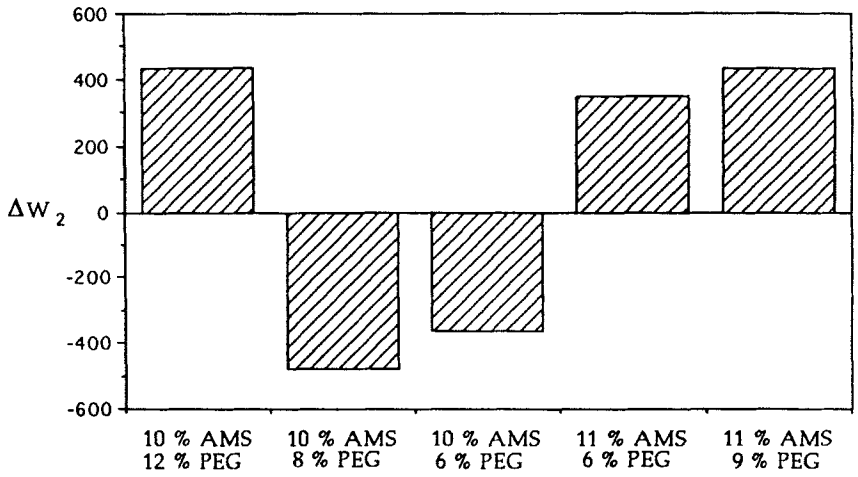

FIG. 11 The difference in PEG concentration between phases, ΔW_2, in mg/mL, for various ammonium sulfate/PEG-8000 systems equilibrated at 60°C.

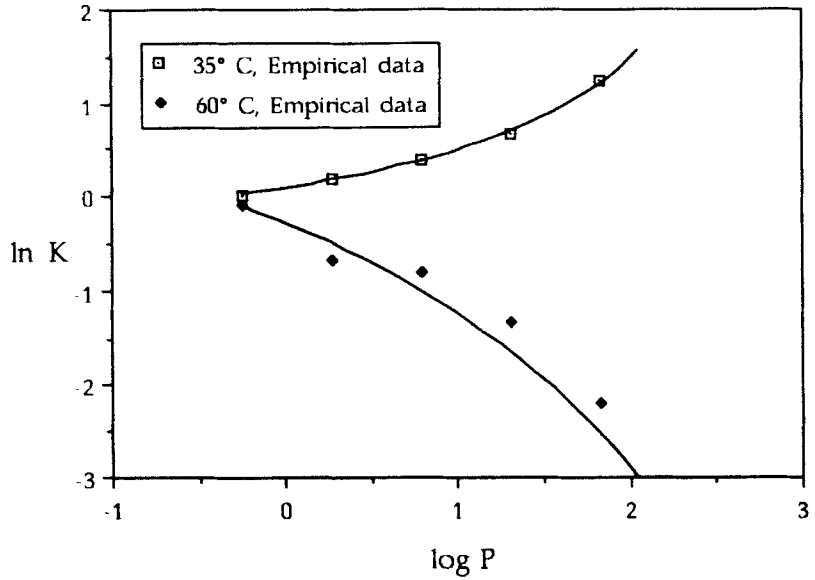

FIG. 12 Measured partition coefficients (ln K) in a 10% ammonium sulfate/6% PEG-8000 (w/w) system versus calculated solute hydrophobicity (log P) equilibrated at either 35 or 60°C. Solutes were n-alcohols, with hydrophobicities increasing from that of methanol to that of n-hexanol. The lines drawn through the points represent Eq. (1).

shown to be so in Fig. 12 where the points represent the data and the lines are for Eq. (2).

SUMMARY

It appears that this modified Eiteman equation (Eq. 2) allows one to predict partitioning over a broader range of hydrophobicities. Since it has been shown previously that Eq. (1) is valid for many types of solutes, it is expected that Eq. (2) is also, although these experiments utilized only n-alcohols. This modified equation may also provide further insight into an understanding of the partitioning behavior in aqueous two-phase systems in that it introduces a term for the "critical" hydrophobicity.

It appears that Eq. (2) is also valid for temperatures other than 25°C. Values for a and D in that equation are given in Table 2 for several two-phase systems, and it can be seen that those for the potassium phosphate system do not vary greatly with changes in temperature. Since P_0 is independent of temperature, it is suggested that ΔW_2 is the major factor which changes with temperature. In fact, although the partitioning in PEG/am-

TABLE 2
Values of Constants in Equation (2) for Various Systems at Higher Temperatures

Salt	% Salt (w/w)	%PEG-8000 (w/w)	Equilibrium temperature (°C)	P_0	D	a
Ammonium Sulfate	10	6	60	0.47	9.51	5.24
	11	6	60	0.09	5.42	4.13
	11	9	60	0.04	3.75	3.70
	10	12	60	0.20	4.92	2.99
Potassium phosphate,	9	8	35	0.38	18.2	9.61
0.65 K_2HPO_4:0.35	9	8	45	0.38	14.8	11.6
KH_2PO_4	9	8	60	0.38	20.6	11.5
	12	8	60	0.2	14.1	9.15

monium sulfate systems at elevated temperatures exhibits unusual behavior, Eq. (2) may be used to describe this by taking into account the changes in ΔW_2 with temperature.

Since the increase in partitioning with temperature is more marked with the more hydrophobic solutes, our data suggest that increasing the temperature may be advantageous for the separation of such compounds when using aqueous two-phase extraction. Thus, this equation may prove useful for specifying and designing larger scale systems.

REFERENCES

1. P.-Å. Albertsson, *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley, New York, 1986.
2. H. Walter, W. H. Brooks, and D. E. Fisher, *Partitioning in Aqueous Two Phase Systems—Theory, Methods, Uses, and Applications to Biotechnology*, Academic Press, New York, 1985.
3. S. Sturesson, F. Tjerneld, and G. Johansson, *Appl. Biochem. Biotechnol., 26*, 281 (1990).
4. J. G. Huddelston and A. Lyddiatt, *Biochem. Biotechnol., 26*, 249 (1990).
5. M. A. Eiteman and J. L. Gainer, *Biotechnol. Prog., 6*, 479 (1990).
6. J. Vernau and M.-R. Kula, *Biotech. Appl. Biochem., 12*, 397 (1990).
7. H. Walter, G. Johansson, and D. E. Brooks, *Anal. Biochem., 197*, 1 (1991).
8. Y.-L. Gao, Q.-H. Peng, Z.-C. Li, and Y.-G. Li, *Fluid Phase Equil., 63*, 157 (1991).
9. R. S. King, H. W. Blanch, and J. M. Prausnitz, *AIChE J., 34*, 1585 (1988).
10. A. D. Diamond and J. T. Hsu, *J. Chromatogr., 513*, 137 (1990).
11. M. A. Eiteman and J. L. Gainer, *Bioseparations, 2*, 31 (1991).
12. R. F. Rekker and H. M. de Kort, *Eur. J. Med. Chem.—Chim. Ther., 14*, 479 (1979).
13. G. Johansson, in *Partitioning in Aqueous Two Phase Systems—Theory, Methods, Uses, and Applications to Biotechnology* (H. Walter, D. E. Brooks, and D. Fisher, Eds.), Academic Press, New York, 1985.

14. S. K. Sikdar, K. D. Cole, R. M. Stewart, D. C. Szlag, P. Todd, and H. Cabezas Jr., *Bio/Technology*, *9*, 253 (1991).
15. G. Pinaev, A. Tartakovskii, V. P. Shanbhag, G. Johansson, and L. Backman, *Mol. Cell. Biochem.*, *48*, 65 (1982).
16. G. Johansson and M. Anderson, *J. Chromatogr.*, *303*, 39 (1984).
17. D. Forciniti, C. K. Hall, and M.-R. Kula, *Fluid Phase Equil.*, *61*, 243 (1991).
18. B. Skoog, *Vox Sang.*, *37*, 345 (1970).

Received by editor June 18, 1993
Revised January 23, 1994