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The pH Difference in Poly(Ethylene Glycol)/Citrate Aqueous Two-Phase Systems and the Influence of Sodium Chloride

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ABSTRACT

Eighteen different poly(ethylene glycol) (PEG)/citrate aqueous two-phase systems at 25.0°C were compared with otherwise identical systems containing 2% by mass sodium chloride. For all systems, the PEG concentration difference between the phases, a parameter proportional to tie-line length, increased with increasing total system PEG concentration. The PEG concentration difference was also greater in each system containing the sodium chloride than in identical systems without sodium chloride. In systems without sodium chloride, the pH difference between the phases increased with increasing PEG concentration difference from about 0.05 to 0.42 pH units. In the systems containing sodium chloride, the pH difference between the phases also increased with increasing PEG concentration difference, but only from 0.08 to 0.20 pH units. Indole 3-acetic acid and tryptamine, a model pair of oppositely charged compounds, were partitioned in the two-phase systems. In each system, partition coefficients were proportional to the concentration difference between the phases, irrespective of the proportion of sodium citrate and citric acid used. In the systems without sodium chloride, the partition coefficients of indole acetic acid were 1.5 to 4 times greater than the partition coefficients of tryptamine. The difference between the partition coefficients of these two solutes was greatest in phase systems with the greatest pH difference between the phases. In the systems containing sodium chloride, the partition coefficients of indole 3-acetic acid were only 1 to 2 times greater than the partition coefficients of tryptamine. The addition of sodium chloride to this phase system reduced the pH difference and concomitantly the difference between the partition coefficients of the oppositely charged solutes.

Key Words. Poly(ethylene glycol); Purification; Sodium citrate; Citric acid; Tryptamine; Indole acetic acid; Hydrophobicity; Charge; Partition coefficient; Extraction

INTRODUCTION

Two water-soluble but mutually incompatible components, such as poly(ethylene glycol) (PEG) and dextran, or PEG and sodium citrate, often form aqueous two-phase systems (1). Any solute such as a protein added to a two-phase system will distribute between the phases. The solute's partition coefficient (denoted K) is defined as its upper phase concentration divided by its lower phase concentration. Since such systems are composed primarily of water, they have received attention for the liquid–liquid extraction of biomaterials (2–6), and numerous studies have focused on the prediction of partition coefficients in aqueous two-phase systems. Partition coefficients depend on several factors including solute hydrophobicity (7–9), molecular weight (10, 11), temperature (2, 12), pH (13–17), solute charge (18, 19), and the presence of additional salts (20–22).

For over 20 years, solute or particle charge has been recognized as one important factor which influences the partition coefficient. Reitherman et al. (23) measured an electric potential in each phase and correlated the partitioning of negatively charged human erythrocytes with this potential difference. Johansson (24) correlated protein partitioning with salt partitioning. Theories have also been developed to relate protein partition coefficients to the protein's net charge and the difference in the electric potential between the phases (25, 26). In general, however, the mechanism and magnitude of charge effects have remained poorly understood.

A related observation has been made that the partition coefficients of negatively charged solutes decrease in PEG/phosphate or PEG/sulfate systems as the alkali halide concentration is increased. In separate studies, this observation has been made for thaumatin in PEG/phosphate and PEG/sulfate systems (27), and for ovalbumin (28) and α-amylase (29) in the PEG/phosphate system. Conversely, the partition coefficients of positively charged solutes, such as vancomycin (30) and lysozyme (28) in the PEG/phosphate system, increase with increasing alkali halide concentration. No satisfactory explanation has been advanced for these observations. Ideally, one would like to predict the concentration of a halide needed to afford a desired change in the partition coefficient of a particular solute.

Recently, a pH difference between the phases in the PEG/potassium phosphate aqueous two-phase system was shown to affect the partition coefficients of charged solutes (19, 22). Specifically, in systems in which the upper phase is the more basic (e.g., PEG/potassium phosphate), a negatively charged solute has a higher partition coefficient than an otherwise analogous positively charged solute. The addition of sodium chloride to PEG/potassium phosphate systems has also been investigated and observed to decrease the pH difference between the phases (22). At a particular pH, salt addition would hence serve to increase the partition coefficients of positively charged solutes and decrease those of negatively charged solutes. Whether this applies to other systems was not determined.

Because its components are readily biodegradable and nontoxic, the PEG/citrate phase system is of interest as a two-phase system (31). The objectives of this present work are to investigate any pH difference between the phases and its relationship to tie-line length in the PEG/citrate system, examine the partitioning of positively and negatively charged analogs in this system, and determine the effect of added sodium chloride on both the pH difference and the partition coefficients.

MATERIALS AND METHODS

Four different ratios of sodium citrate dihydrate and citric acid (Sigma Chemical Co., St. Louis, Missouri) were used for the preparation of the two-phase systems used in this study: 8.5% sodium citrate/0.5% citric acid, 10.0%/1.0%, 15.0%/1.0%, and 15.0%/1.5% (percentages indicate mass fractions). For each of these ratios, four or five actual 20 mL systems were prepared by varying the percentage of PEG-8000 (Sigma Chemical Co.) from 5.0 to 19.5%. The resulting 18 phases did not contain sodium chloride. A second group of 18 systems was similarly prepared with identical quantities of PEG, sodium citrate, and citric acid but with the addition of 2.0% sodium chloride. Hence, each of these systems contained 2.0% less water than the salt-free systems.

The 36 phase systems studied were agitated frequently for 2 days, equilibrated at 25.0°C for 5 days, then carefully separated with Pasteur pipets immediately before analysis. The pH of each phase was measured as reported previously (18), and the PEG concentration was measured by the method of Skoog (32).

Tryptamine and indole 3-acetic acid (Sigma Chemical Co.) were used for partitioning studies. In all the phase systems prepared, tryptamine is essentially positively charged while indole acetic acid is essentially negatively charged. Since these two small solutes have approximately identical hydrophobicities (33), any difference in their partition coefficients was assumed to be due solely to their opposite charges. Approximately 5 mg of each solute was added to 10 ml of each two-phase system studied. These phases were similarly agitated and equilibrated at 25.0°C, and the partition coefficients determined by absorbance at 270 nm.

RESULTS AND DISCUSSION

Figure 1 shows the measured PEG concentration difference in the 36 PEG/citrate phase systems studied. Increasing total PEG concentration for any set of systems resulted in a greater PEG concentration difference between the phases and hence a greater tie-line length. In general, the greater the total citrate concentration, the greater the PEG concentration difference between the phases. Adding sodium chloride to any two-phase system also increased the PEG concentration difference between the phases. Since the amount of water used decreased when sodium chloride was added, it is not surprizing that those phase systems with added salt showed a greater tie-line length. Note that since partition coefficients of neutral solutes are directly proportional to tie-line length (8), the partition

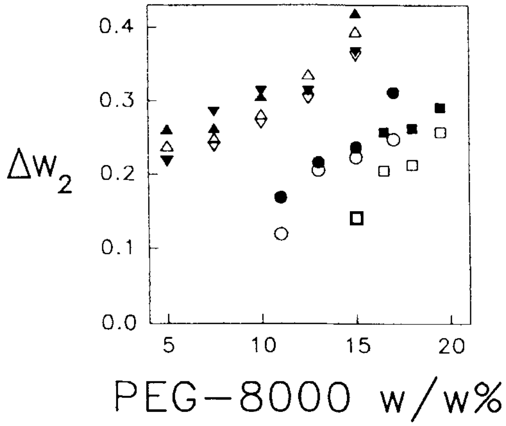

FIG. 1 Observed PEG concentration difference between the phases (Δw_2) at 25.0°C as a function of total weight percent of PEG. Phase systems used: 8.5% sodium citrate/0.5% citric acid (□, ■); 10.0% sodium citrate/1.0% citric acid: (○, ●); 15.0% sodium citrate/1.0% citric acid: (△, ▲); 15.0% sodium citrate/1.5% citric acid: (▽, ▼). Hollow symbols represent phase systems without sodium chloride. Filled symbols represent phase systems containing 2.0% sodium chloride.

coefficient of such a solute would be expected to increase by the addition of sodium chloride.

Next, the pH difference between the phases was measured. Figure 2 presents the results for the systems with and without 2% sodium chloride. In the PEG/citrate system without salt, increasing pH difference was directly correlated with increasing tie-line length. The measured pH difference increased particularly steeply between PEG concentration differences of 0.2 and 0.3. The pH difference did not depend on the ratio of sodium citrate to citric acid in the two-phase system.

The measured pH difference between the phases for those systems with 2% sodium chloride also increased with increasing tie-line length, irrespective of the ratio of sodium citrate to citric acid. However, the pH difference increased more slowly in systems with sodium chloride than in systems without this salt, particularly above a PEG concentration difference of 0.2. At a PEG concentration difference of 0.4, the pH difference in phase systems with added sodium chloride was less than half the pH difference in systems not containing this salt. In all systems, the pH difference between the phases was positive, as in the PEG/phosphate system observed previously (17).

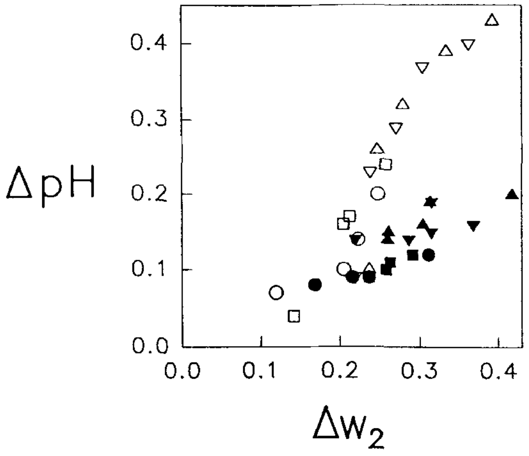

FIG. 2 Observed pH difference between the phases (ΔpH) at 25.0°C as a function of the observed PEG concentration difference between the phases (Δw_2). Phase systems used: 8.5% sodium citrate/0.5% citric acid (□, ■); 10.0% sodium citrate/1.0% citric acid (○, ●); 15.0% sodium citrate/1.0% citric acid (△, ▲); 15.0% sodium citrate/1.5% citric acid (▽, ▼). Hollow symbols represent phase systems without sodium chloride. Filled symbols represent phase systems containing 2.0% sodium chloride.

Figure 3 shows the results of partitioning a pair of oppositely charged analogs, indole 3-acetic acid and tryptamine, in the salt-free two-phase systems. The partition coefficients of both compounds increased with increasing PEG concentration difference. However, the partition coefficients of the negatively charged acid were 1.5 to 4 times greater than those of the positively charged amine. This difference increased with increasing tie-line length (and hence pH difference). The partition coefficients of neither solute depended on the ratio of sodium citrate to citric acid in the system.

Figure 4 shows the partition coefficients of indole 3-acetic acid and tryptamine in the two-phase systems containing 2.0% sodium chloride. The partition coefficients in these systems also increased with increasing tie-line length. For the systems containing sodium chloride, the partition coefficients of the negatively charged acid were only slightly greater than the partition coefficients of the positively charged amine. The partition coefficients of tryptamine, in particular, were observed to increase compared to those in the systems without sodium chloride. Again, the partition coefficients did not depend on the ratio of sodium citrate to citric acid in the phase system.

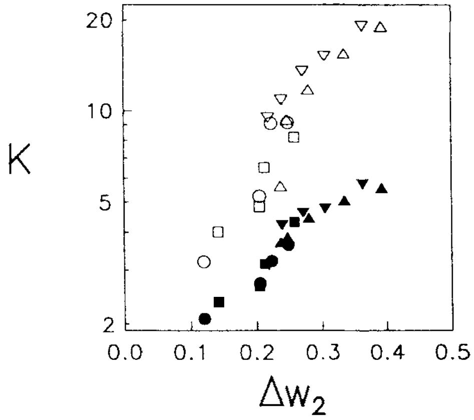

FIG. 3 Observed partition coefficients for indole acetic acid (hollow symbols) and tryptamine (filled symbols) versus the observed PEG concentration difference between the phases (Δw_2) for systems *without* sodium chloride. Phase systems used: 8.5% sodium citrate/0.5% citric acid (□, ■); 10.0% sodium citrate/1.0% citric acid (○, ●); 15.0% sodium citrate/1.0% citric acid (△, ▲); 15.0% sodium citrate/1.5% citric acid (▽, ▼).

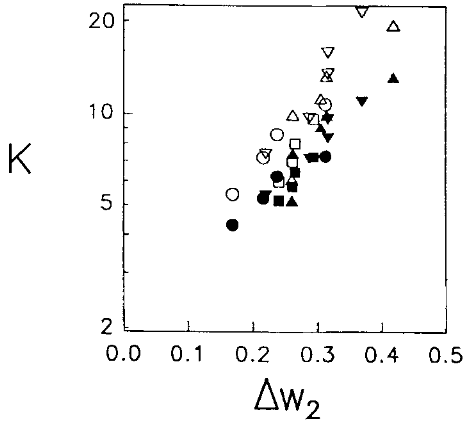

FIG. 4 Observed partition coefficients for indole acetic acid (hollow symbols) and tryptamine (filled symbols) versus the observed PEG concentration difference between the phases (Δw_2) for systems *containing* 2.0% sodium chloride. Phase systems used: 8.5% sodium citrate/0.5% citric acid (□, ■); 10.0% sodium citrate/1.0% citric acid (○, ●); 15.0% sodium citrate/1.0% citric acid (△, ▲); 15.0% sodium citrate/1.5% citric acid (▽, ▼).

Figures 3 and 4 indicate that both the tie-line length and pH difference appear to influence the partition coefficients of the two charged solutes. For either solute, increasing the tie-line length directly increased the partition coefficient. The pH difference, or some other effect that the presence of dilute sodium chloride caused, affected the partition coefficients of the two charged compounds relative to each other. One instructive method to show the charge effect is to find the ratio of the partition coefficient of the negatively charged solute to the partition coefficient of the positively charged solute, K^-/K^+. This partition ratio has been previously modeled (34). For otherwise identical oppositely charged solutes, and if the pH of the phase system is two or more pH units removed from the dissociation constants for both solutes, the partition ratio becomes (34):

$$K^-/K^+ \approx 10^{2\Delta \mathrm{pH}} \tag{1}$$

Figure 5 shows the results of determining this partition ratio by dividing the observed partition coefficients of indole 3-acetic acid by those of tryptamine in each of the 36 phase systems studied. The solid curve is the model prediction given by Eq. (1). The data show substantial scatter since both partition coefficients, the values of Δw_2, and the values of ΔpH were all measured quantities. Nevertheless, the ratio determined by this means does generally increase with increasing pH difference between the phases.

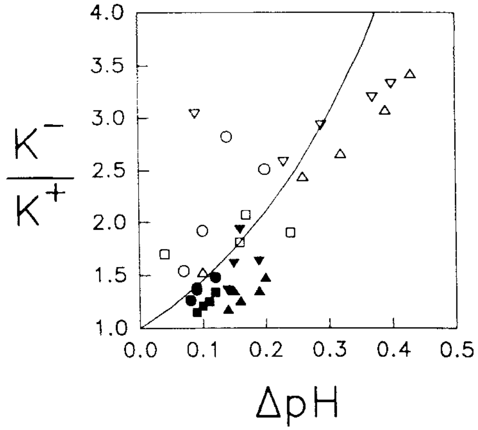

FIG. 5 Observed ratio of partition coefficient of indole acetic acid to partition coefficient of tryptamine versus the measured pH difference between the phases. Phase systems used: 8.5% sodium citrate/0.5% citric acid (□, ■); 10.0% sodium citrate/1.0% citric acid (○, ●); 15.0% sodium citrate/1.0% citric acid (△, ▲); 15.0% sodium citrate/1.5% citric acid (▽, ▼). Hollow symbols represent phase systems without sodium chloride. Filled symbols represent phase systems containing 2.0% sodium chloride. Solid curve indicates theoretical prediction $K^-/K^+ = 10^{2\Delta pH}$.

CONCLUSIONS

Partition coefficients of both tryptamine and indole 3-acetic acid depend on the PEG concentration difference between the phases, or tie-line length, in the PEG/citrate aqueous two-phase system. The relationship between the logarithm of the partition coefficient and the tie-line length is approximately linear, regardless of the ratio of sodium citrate to citric acid. The presence of sodium chloride serves to shift this linear relationship.

Indole 3-acetic acid and tryptamine do partition differently. The charge of one solute shifts its partition coefficient relative to the partition coefficient of the other analogous solute. The greater the tie-line length, the greater the observed pH difference between the phases, and the greater the difference between the partition coefficients of the oppositely charged solutes. Addition of sodium chloride to the PEG/citrate phase system decreased the positive pH difference between the phases, therefore decreasing the difference between the partition coefficients of these two solutes. These results suggest that proper selection of pH, phase system, and salt concentration could greatly enhance the separation of solutes.

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