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INTERFACIAL TENSION OF POLYETHYLENE GLYCOL/POTASSIUM PHOSPHATE AQUEOUS TWO-PHASE SYSTEMS

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The interfacial tension of Polyethylene glycol (PEG)/potassium phosphate two-phase systems was measured by the rotating drop method. The interfacial tension was as low as 0.001 dyne/cm and increased with increases in the total concentrations of both PEG and potassium phosphate in two-phase systems. The increase in the interfacial tension was a function of the concentration differences of PEG and potassium phosphate between the top and the bottom phases which was confirmed by the tie line analysis. The interfacial tension was affected also by the molecular weight of PEG. At low PEG molecular weights, the increase in the molecular weight greatly increased the interfacial tension, but at high molecular weights, the interfacial tension varied less with the molecular weight.

Keywords: Aqueous two-phase system; interfacial tension; PEG

1. INTRODUCTION

Aqueous two-phase systems can be prepared by mixing two different water soluble polymers in water exceeding certain threshold concentrations. The interfacial tension between two phases is as low as 5×10^{-4} dyne/cm [1]. This low interfacial tension was considered to be one of the reasons that the liquid–liquid extraction with aqueous two-phase systems provides mild separation conditions to preserve

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biological activities without detectable interfacial denaturation of proteins or other biological materials [2].

The aqueous two-phase systems which have received the most attention are polyethylene (PEG)/dextran systems. Recently, more interest has been shown in PEG/salt aqueous two-phase systems. [3–5]. The PEG/salt two-phase systems contain PEG and salt instead of another water soluble polymer. These systems are advantageous because they are cost less and have lower viscosity, leading to lower operating cost (pumping, centrifugation etc.). The interfacial tension of PEG/salt two-phase systems, however, is expected to be higher than that of PEG/dextran two-phase systems due to the presence of high salt concentrations. Therefore, in this article the interfacial tension of PEG/potassium phosphate two-phase systems, as the representative of PEG/salt two-phase systems, was determined to analyze the parameters governing the interfacial tension between two phases.

2. MATERIALS AND METHODS

Stock solutions for Two-Phase Systems

Polyethylene glycol (PEG) solutions with various molecular weights, 300 (PEG300), 600 (PEG600), 1,450 (PEG1450), 3,350 (PEG3350), and 8,000 (PEG8000) (Sigma Chem. Co., St. Louis, MO) were prepared in deionized water. The concentrations of the stock solutions for PEG1450, PEG3350, and PEG8000 were 50% (w/v). PEG300 (3.3 M) and PEG600 (1.7M) are liquid at room temperature, so they were used directly without preparing stock solutions (100%, w/v). All PEG solutions were kept at room temperature.

Potassium phosphate stock solutions were prepared by adding equal moles of monobasic and dibasic in deionized water.

Construction of Phase Diagrams

Phase diagrams of PEG/potassium phosphate two-phase systems were constructed by titration according to the method of Albdertsson [6]. Ten ml of a PEG stock solution was placed into a flask (50 ml). A potassium phosphate stock solution was then titrated into the flask,

stirring constantly. First a homogeneous solution appeared, but after a certain amount of salt had been added, one further drop of the potassium phosphate solution caused turbidity creating a two-phase system. The composition of this mixture was noted. When 1 ml of deionized water was added, the mixture became a clear homogeneous solution again. The potassium phosphate solution was then added dropwise until a turbid two-phase system was formed again. The composition of this mixture was noted and more deionized water was added to get a one-phase system and so forth. The titration was continued until 25 to 50 ml of the salt solution was used.

Ten ml of the potassium phosphate solution was then titrated with a PEG solution in the same manner as above. In this way a series of compositions were obtained and the concentration of PEG was plotted against that of potassium phosphate for these compositions.

Volume Fraction

The volume ratio of the top to the bottom phases was determined by measuring the volumes of the top and the bottom phases in a 10 ml graduated cylinder. The volume fractions of the top and the bottom phases were determined from the volume ratio.

Tie Line

The tie lines were determined by the volume ratio of the top to the bottom phases [6]. The length of the tie lines was calculated from the top and bottom phase compositions according to the equation [7]:

$$\text{Length of Tie Line} = [(m_{p,t} - m_{p,b})^2 + (m_{s,b} - m_{s,t})^2]^{1/2} \quad (1)$$

where m_p and m_s are the concentration of PEG and potassium phosphate, respectively, and the subscripts refer to the PEG and potassium phosphate contained in the top (t) and the bottom (b) phases.

Interfacial Tension of Two-Phase Systems

Interfacial tension of PEG/potassium phosphate two-phase systems was determined with the rotating drop method [7–11].

A piece of cylindrical glass tubing (length = 10 cm; inner diameter = 0.7 cm) was attached to a variable speed motor (Model 2T60-100, Gerald K. Heller Co., Las Vegas, NV) so that it could be rotated along its axis. The rotational velocity of the drop was measured with a Strobotac Type 1531 (General Radio Co., Concord, MA). One end of the tube was plugged with a cork and the other end with a rubber stopper. Each time a two-phase system was tested, the tube was filled with bottom (heavier, potassium phosphate-rich) phase and a known volume of top (lighter, PEG-rich) phase was injected through the rubber stopper with a 0.1 ml syringe. The volume of the top phase was small enough (≤ 0.03 ml) so that it formed a drop inside the bottom phase.

The tube was then rotated with the motor and the length of the drop was measured with a ruler (± 0.1 mm).

The interfacial tension, γ , was calculated by [11]:

$$\gamma = (\omega^2 \Delta \rho r^3) / 4 \quad (2)$$

where ω is rotational velocity, $\Delta \rho$ is difference in densities between the top and bottom phases, and r is bubble radius. The density of each phase was determined with pycnometers. Toluene was used as a standard.

The radius, r , was calculated by approximating the shape of the drop as a cylinder and measuring the axial length of the drop. Then

$$r = (V / \pi L)^{1/2} \quad (3)$$

where V is drop volume and L is axial length.

This is a good approximation since in all cases $V/L < 0.025$ cm² and, by calculation, in all cases $L/r > 12$.

The rotating drop method gave consistent results for the systems of interest at different rotational speeds, provided the RPM was high enough (Fig. 1). Figure 1 shows that at sufficiently high RPMs ($> 2,500$ RPM) the effects of RPM on interfacial tension is negligible. At all measurements for this study the rotational speed was 2,500 RPM or 3,000 RPM and the temperature was 25 °C.

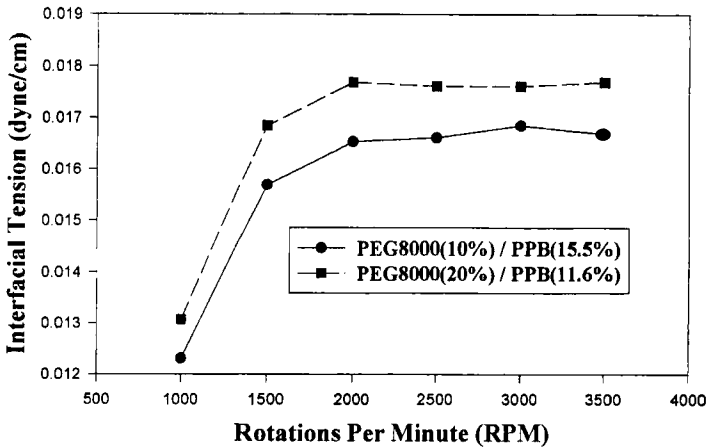


FIGURE 1 Effects of rotational speeds on the interfacial tension.

3. RESULTS AND DISCUSSION

Interfacial tension of PEG/potassium phosphate two-phase systems was measured. The effects of the total concentrations of PEG and potassium phosphate in the two-phase system and the molecular weight of PEG were studied.

Concentrations of PEG and Potassium Phosphate

Figure 2 shows the effect of the total concentration of potassium phosphate on the interfacial tension of PEG/potassium phosphate two-phase systems at given concentrations of PEG (10%, 15% and 20%, w/v). The interfacial tension between phases was as low as 0.004 dyne/cm and less than 0.08 dyne/cm at the concentrations of PEG and potassium phosphate tested. The range of the interfacial tension of PEG/dextran two-phase systems reported is 0.0005 to 0.07 dyne/cm [10, 12]. Therefore, product proteins may be partitioned in PEG/potassium phosphate two-phase systems as safely as in PEG/dextran two-phase systems without interfacial denaturation.

The interfacial tension between two phases increased with increases in the total concentrations of both potassium phosphate and PEG in

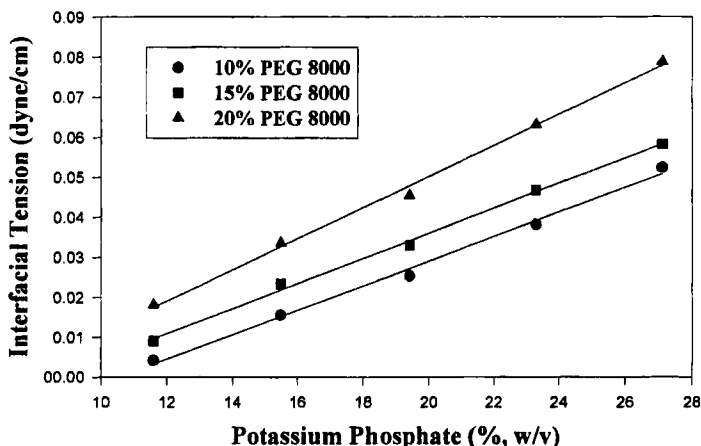


FIGURE 2 Interfacial tension of PEG8000/potassium phosphate two-phase systems increases linearly with increases in the total concentration of potassium phosphate in two-phase systems.

the two-phase systems (Fig. 2). The increment of the interfacial tension was linear with increases in the concentration of potassium phosphate at given concentrations of PEG.

The length of the tie line is the concentration difference between the top and the bottom phases. The length of the tie lines was calculated with Eq. (1) and plotted with the logarithm of the interfacial tension (Fig. 3). A slight exponential relationship between the logarithm of the interfacial tension and the length of the tie line was obtained. Regardless of the concentrations of PEG8000 in two-phase systems, all the points were on the same line. Therefore, the interfacial tension of the PEG/potassium phosphate two-phase systems could be explained with a function of the length of the tie line. This explained that the increment of the interfacial tension (with increases in the total concentrations of PEG and potassium phosphate) was due to the increases in the concentration difference of PEG and potassium phosphate between the top and the bottom phases.

As the total concentration of PEG in the two-phase system increased at given concentrations of potassium phosphate (11.6%, 15.5% and 19.4%, w/v), the interfacial tension increased exponentially (Fig. 4). The interfacial tension was lower than 0.07 dyne/cm and as low as

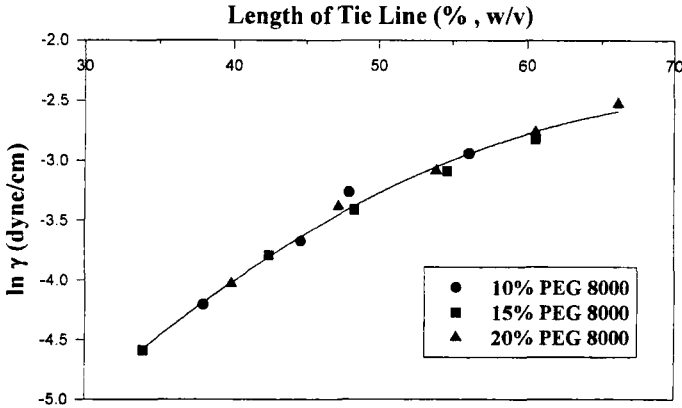


FIGURE 3 Logarithm of the interfacial tension of PEG8000/potassium phosphate two-phase systems increases with increases in the length of the tie line.

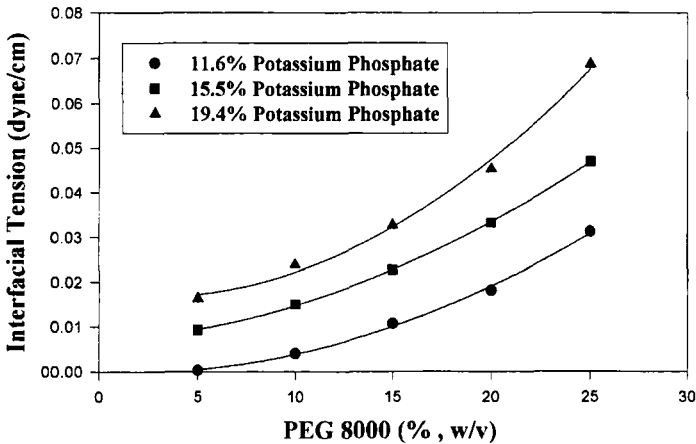


FIGURE 4 Interfacial tension of PEG8000/potassium phosphate two-phase systems increases exponentially with increases in the total concentration of PEG in two-phase systems.

0.001 dyne/cm. A slight exponential relationship between the logarithm of the interfacial tension and the length of the tie line was obtained again (Fig. 5). This confirmed that the interfacial tension between two phases increased with increases in the concentration difference of PEG and potassium phosphate between the top and the bottom phases.

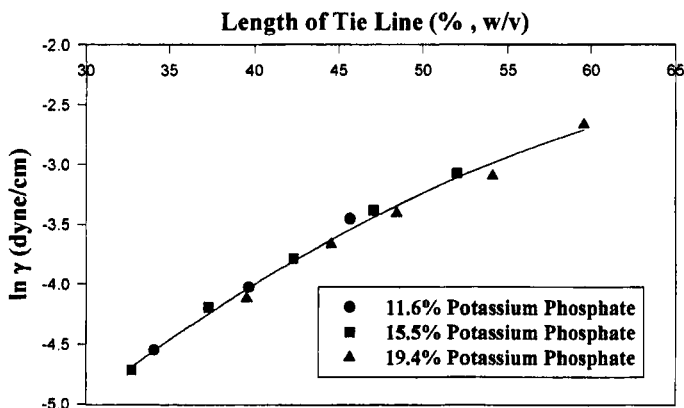


FIGURE 5 Logarithm of the interfacial tension of PEG8000/potassium phosphate two-phase systems increases with increases in the length of the tie line.

Molecular Weight of PEG

In order to study the effects of the molecular weight of PEG on the interfacial tension between two phases, two-phase systems with various molecular weights of PEG were constructed at a given concentration of PEG (20%, w/v) and various concentrations of potassium phosphate (15.5%, 17.5%, 19.4% and 23.3%). The interfacial tensions measured were plotted against the molecular weight of PEG in Figure 6. The interfacial tension increased with increases in the molecular weight of PEG. At low PEG molecular weights, a molecular weight change greatly affected the interfacial tension, but at high molecular weights, the interfacial tension varied less with the molecular weight.

The logarithm of the interfacial tension was plotted with the length of the tie line in Figure 7. The logarithm of the interfacial tension increased exponentially with increased in the length of the tie line. However, points were not on the same line. This explained that the interfacial tension between two phases was determined by also the molecular weight of PEG.

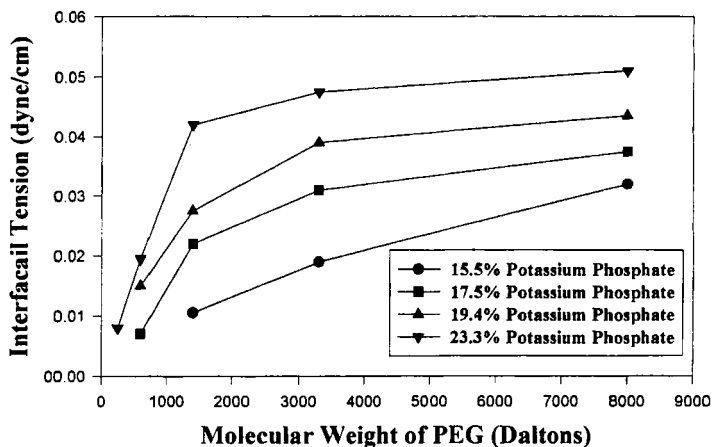


FIGURE 6 Interfacial tension of PEG(20%)/potassium phosphate two-phase systems increases with increases in the molecular weight of PEG.

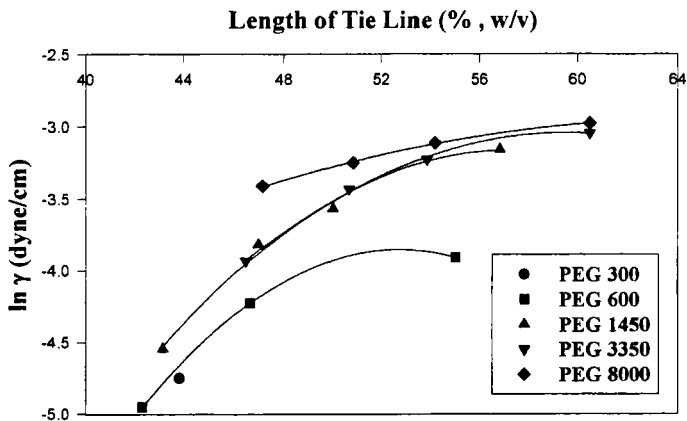


FIGURE 7 Logarithm of the interfacial tension of PEG(20%)/potassium phosphate two-phase systems increases with increases in the length of the tie line.

4. CONCLUSIONS

The interfacial tension of PEG/potassium phosphate two-phase systems was as low as 0.001 dyne/cm and increased with increases in the total concentrations of PEG and potassium phosphate in the two-phase systems. The interfacial tension was lower than 0.08 dyne/cm

within the practical concentration ranges of PEG and potassium phosphate. This suggested that PEG/potassium phosphate two-phase systems may be used as safely as PEG/dextran two-phase systems without interfacial denaturation of product proteins.

The interfacial tension of PEG/potassium phosphate two-phase systems was a function of the concentration differences of PEG and potassium phosphate between the top and the bottom phases. In addition, the interfacial tension increased with increases in the molecular weight of PEG at the same concentration of PEG and potassium phosphate. At high molecular weights of PEG, the interfacial tension, however, varied less with the molecular weight of PEG.

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