AN INVESTIGATION OF THE PHASE BEHAVIOR OF AN AQUEOUS SYSTEM THAT CONTAINS A POLYAMPHOLYTE AND POLY(ETHYLENE GLYCOL)

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ABSTRACT

The phase behavior of aqueous two-phase polymer system containing poly(ethylene glycol) and a synthetic polyampholyte in 0.1 N KCl was studied as a function of pH. The top phase was poly(ethylene glycol)-rich and the bottom phase was polyampholyte-rich. The binodal curve was found to move towards lower concentrations of polymers with increasing pH as a result of decreased solubility of the polyampholyte. Phase compositions were correlated using a model based on Flory-Huggins theory. Also, a model based on excluded volume theory was used to correlate the binodal curve.

Keywords: Aqueous Two-Phase System, Phase Behavior, Polyampholytes, Poly(ethylene glycol), Polymers.

1. INTRODUCTION

Traditional techniques used for protein purification, for example precipitation and column chromatography, are not only expensive and difficult to scale-up but also result in lower yields. Thus, in recent years there has been an on-going interest for the development of innovative and efficient separation and purification methods in the biotechnology industry.
Aqueous two-phase system has gained a significant interest as a method of protein purification [Albertsson, 1986; Walter et al., 1985]. This novel separation methodology exploits the remarkable fact that many aqueous polymer-polymer systems, under appropriate solution conditions, can spontaneously separate into two water-based, yet immiscible, phases. The phases offer different physical and chemical environments, which allow for the selective partitioning of proteins and other biomolecules while maintaining their native conformations and biological activities. The attractiveness of the technique is due to its simplicity, amenability to linear scale-up and potential for continuous operation [Patrickios et al., 1992]. However, industrial application of the method is still not widespread. One of the main reasons for the reluctance of the industry to incorporate the technique is the cost of the phase-forming polymers. In order to make the method attractive as well as respond to current environmental awareness, there is need to develop alternative phase-polymers that are capable of being recovered and recycled.

Introduction of stimuli-responsive polymers, such as polyampholytes, in aqueous two-phase systems is a new development in the search for alternative phase-forming polymers. Polyampholytes are polyelectrolytes containing both positive- and negative-charged units [Ohlemacher et al., 1996]. Depending on the pH of the medium, polyampholytes can exhibit the properties of either polyacids or polybases. What makes polyampholytes interesting is that they can be easily recovered by isoelectric precipitation and recycled. But, in spite of the promising features of polyampholytes as shown by the work of Hughes and Lowe [1988], there seems to be little effort in further investigations to assess the potential of these very interesting polymers for separation of proteins.

Recently, [Al-Muallem et al., 2002] reported the synthesis of new ionic polymers using cheap and commonly available chemicals. The polymers, just like proteins, contain two pH-triggerable functionalities (N and CO₂⁻) that make them exhibit pH responsive behavior. One of the polymers was found to be almost water-insoluble in acidic pH range. This behavior makes it a potential candidate for industrial applications since it can be effectively removed from solution by pH-controlled precipitation. Furthermore, in applications such as protein partitioning, the protein-like structure of the polymer is expected to enhance protein-polymer interactions.

In this paper, we present a study on the phase behavior of a polyampholyte with poly(ethylene glycol) (PEG) in water. The polyampholyte (PA), which is composed of an anionic polyelectrolyte (APE) and a polybetaine (PB) in the mole ratio APE:PB=0.71:0.29, is a product of [Al-Muallem et al. research, 2002]. Our focus here is on determining the effect of pH on the phase behavior of the polyampholyte-PEG system as a preliminary to investigating the potential of this polymer in protein partitioning. PEG, a commonly studied water-soluble polymer, was selected as the second polymer for this study because of its low cost and availability in different molecular weights. In addition, the phase behavior of the polymer system was correlated using the correlation proposed by Diamond and Hsu [1998].
The binodal curve was also correlated using the model formulated by Guan et al [1993]. It is hoped that the experimental results from this study will help greatly in developing a cost-effective aqueous two-phase system for protein separation.

2. EXPERIMENTAL

2.1 Materials

Poly(ethylene glycol) (MW 35,000) was purchased from Mark-Schuchardt. Anionic polyelectrolyte (APE) (MW 33,000) was synthesized via Butler’s cyclopolymerization technique. Complete detail of the synthesis was described elsewhere [Al-Muallem et al, 2002]. The polyampholyte (PA) is composed of the anionic polyelectrolyte (APE) and a polybetaine (PB) in the mole ratio x:y=0.71:0.29. The structural formula of the polyampholyte is shown in fig. 1. Potassium chloride and other chemicals used are of analytical grade. All glassware were cleaned using deionized water.

![Chemical structure of the polyampholyte](image.png)

**Figure 1.** Chemical structure of the polyampholyte.

2.2 Phase behavior

A phase diagram graphically depicts the phase behavior, thereby delineating the potential working area, of an aqueous two-phase system. It provides information about the polymer compositions necessary to form a system with two phases that are in equilibrium, the resulting concentration of phase components in the top and bottom phases, and the ratio of phase volumes. These are vital information required for protein separation work. The binodal curve is a line that divides regions of component concentrations that will form two immiscible aqueous phases from those that will form one phase. Polymer concentrations of the two phases in equilibrium with each other are connected by tie lines. The binodal curve may be constructed using one of the following methods: turbidometric titration, cloud point method or node determination. Here, we use turbidometric titration method and node determination method to obtain the binodal curves and tie lines respectively.

Three stock solutions of PA (~19%) were prepared, each treated with a different amount of HCl; 0.25, 0.5 and 0.6 equiv. HCl. The corresponding pH values of the stock solutions, measured using a Corning pH meter, were found to be 7.89, 7.41 and 7.37. The concentration of the PEG stock solution was 25 %w/w. All the stock solutions were prepared in 0.1 N KCl. Known weights of the stock solutions of PA (pH 7.89) and PEG were added to a 10 mL magnetically stirred conical flask until the clear system became turbid. Then the system was...
titrated, dropwise, with 0.1 N KCl until it became clear, i.e., one phase is formed. The weight of KCl solution added just prior to one phase formation was noted. At this point, the final composition of the polymers calculated corresponds to a point on the binodal curve. After obtaining the first point, a concentrated solution of PEG was added again to obtain a turbid suspension, and dilution with 0.1 KCl solution was repeated to obtain a second point on the binodal. This procedure was continued until a sufficient number of points for the construction of the binodal curve were obtained. Similar experiments were conducted using the other two PA stock solutions.

Precise compositions of the phases were determined by analyzing the compositions of the top and bottom phases using $^1$H NMR technique. A detailed explanation of the method is available in the literature [Ali et al., 1997]. Phase densities were measured using a densimeter at 23 °C with water as reference fluid.

3. RESULTS AND DISCUSSIONS

3.1 Phase behavior

The new polymer used in this study is a polyampholyte (PA) having a mole ratio of 0.71:0.29 of APE:PB. The composition was determined using potentiometric titration [Al-Muallem et al., 2002]. In 0.1 KCl the polyampholyte precipitate out of solution upon addition of 0.62 equivalent HCl (pH 7.0). The addition of salt to the polymer solution screens electrostatic interactions and weakens attractions thereby enhancing the solubility of the polyelectrolyte. The PA was found to form two-phase system with PEG (mol wt 35 000). While the PEG showed greater preference to remain in the top phase, the PA displayed predominant presence in the bottom phase. Figure 2 shows the phase diagram obtained for the PA with PEG in water at various concentrations of HCl at 23°C. The figure suggests that the pH of the medium has a tremendous effect on the phase composition of the system. Compared to the binodals constructed at pH 7.41 and pH 7.37, the binodal at pH 7.89 require higher concentration of the phase polymers to form an aqueous two-phase system. This is probably due to decrease in hydrodynamic volume of the PA as the concentration of HCl increases. Previous study [Al-Muallem et al., 2002] on the effect of added HCl on the viscosity behavior of the PA has shown that increase in pH leads to reduction in intrinsic viscosity of the PA solutions. It is known that hydrodynamic volume is directly proportional to intrinsic viscosity. Hence, it can be deduced that increasing the pH reduces the net charge of the polymer chain with a consequent reduction of intramolecular electrostatic repulsions that cause the chain to assume a more coiled structure of smaller hydrodynamic size. The coiling of the PA results in less interaction with the PEG and as a result lower concentration of polymers are required for phase separation. An overlap was observed between the binodal at pH 7.41 and pH 7.37. It is important to note that, very close to the critical point lesser amounts of the phase-forming polymers are required at pH 7.37, while at polymer concentrations far away from the critical point smaller amounts of the polymers are required to form a two-phase system at pH 7.41.
Because the operating point of aqueous phase-systems used in protein partitioning is usually selected far away from the critical point, the system at pH 7.41 will probably be more suitable than the pH 7.37 system in such applications.

Figure 2: Binodal curves formed by PEG and PA at different pH values

Figure 3 shows the phase diagram for a system composed of PEG-35,000 and PA (pH 7.41) in 0.1 N KCl. The binodal curve was constructed by turbidometric titration as explained in the experimental section. The tie lines are constructed by $^1$H NMR technique [Ali et al, 1997]. The systems used to construct the lines are given in Table 1. The tie lines are helpful in constructing two-phase systems with similar volume ratio of the top and bottom phases.

Figure 3. Phase diagram for a system composed of PEG-35,000 and PA (pH 7.41) in 0.1 N KCl.
Table 1. Phase composition of the PEG-PA-water (0.1 KCl) system at 23°C (pH 7.41)

<table>
<thead>
<tr>
<th>System</th>
<th>Total system</th>
<th>Top phase</th>
<th>Bottom phase</th>
<th>Vol ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PA (% w/w)</td>
<td>PEG (% w/w)</td>
<td>HCl (equiv.)</td>
<td>PA (% w/w)</td>
</tr>
<tr>
<td>1</td>
<td>7.18</td>
<td>3.46</td>
<td>0.50</td>
<td>2.08</td>
</tr>
<tr>
<td>2</td>
<td>13.43</td>
<td>6.49</td>
<td>0.50</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>9.30</td>
<td>6.10</td>
<td>0.50</td>
<td>0.90</td>
</tr>
<tr>
<td>4</td>
<td>8.45</td>
<td>4.00</td>
<td>0.50</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* Volume ratio of top and bottom phase.

Phase separation in the system under study takes place at a relatively low total polymer concentration. This can be seen from a comparison of our system with a commonly studied two-phase system formed by PEG and dextran as presented in figure 4. The data for the PEG3400-DexT70 system was reported by Diamond and Hsu [1989].

Figure 4. Comparison of phase diagrams of PEG-Dex-water and PEG-PA-water (pH 7.41; 0.1N KCl) systems. Data for the PEG-Dex-Water system was from Diamond and Hsu [1998].

3.2 Phase diagram correlation

To aid equipment design and optimization in the use of aqueous two-phase systems, several theories have been proposed for predicting thermodynamic and phase behavior of phase-forming systems. [Cabezas, 1996] presented a comprehensive review of the principal mathematical models and the relevant theories of phase formation in aqueous two-phase systems. He pointed out that there are roughly three schools of thought in the area of modeling of phase separation: (i) models based on extensions of lattice theories such as the Flory-Huggins theory; (ii) models based on osmotic virial expansions descended from the original work of Edmond and Ogston; (iii) models incorporating integral equation theory as a major
element; and (iv) models that do not fall into the above categories, such as group contribution schemes and excluded volume approximations.

In order to correlate the tie lines, we employed the empirically extended relationship of Diamond and Hsu [1992] that is based on Flory-Huggins theory:

\[
\ln(K_1) = A_1 \left( w^*_1 - w^*_1 \right) \\
\ln(K_2) = A_2 \left( w^*_1 - w^*_1 \right)
\]

where \( w^*_1 \) and \( w^*_2 \) represent the polymer weight fractions in the upper and lower phase and A is function of the polymer molecular weight and the interactions between the polymers and water. The correlation result is shown in figure 5. It can be observed that the correlation of the type described above can be used to qualitatively describe the phase behavior of the two polymers system although it has been developed for nonelectrolyte systems. However, at pH 7.51, the PA is close to the isoelectric point (pH 7.0) and its behavior and chain conformation are fairly similar to the uncharged polymer leading to the observed correlation. Development of a more accurate model for phase behavior of a polyelectrolyte and an uncharged polymer is needed.

We used a modified form of the “effective excluded volume (EEV)” model formulated by Guan et al [1993] to reproduce the experimental results of the binodal curve. The binodal model is given in equation 3.

![Figure 5. Correlation of the phase diagram of PEG with PA (pH 7.41) in 0.1N KCl using the method of Diamond and Hsu(1992).](image-url)
\[
\ln \left( \frac{\langle V^* \rangle_{120}}{\langle M_{\text{rms}} \rangle_2} \right) + \frac{w_2}{\langle M_{\text{rms}} \rangle_2} = 0
\]  \tag{3}

where \( \langle V^* \rangle \) is the EEV, \( \langle M_{\text{rms}} \rangle \) is root-mean square average molecular weight and \( w \) is weight fraction. Subscripts 1 and 2 refer to PEG and PA, respectively, while 0 refers to the solvent.

Since \( \langle V^* \rangle_{120} / \langle M_{\text{rms}} \rangle_1 \) and \( \langle V^* \rangle_{120} / \langle M_{\text{rms}} \rangle_2 \) are constants for a given system, then equation 3 can be rewritten as

\[
\ln \left( B_2 w_2 \right) + B_1 w_1 = 0
\]  \tag{4}

In figure 6 the data points are the experimental results for the PEG-PA-water system at 23 °C and pH 7.41 and the curve is the binodal obtained by correlation using the modified binodal model (equation 4). The correlation coefficient for the model was found to be 0.997 while \( B_1 \) and \( B_2 \) are equal to 0.273 and 0.091 respectively. This shows that the binodal model correlates well with the experimental data within the limits of the accuracy of the experiments.

**Figure 6.** Comparison of experimental and predicted PEG-PA-water (0.1 N KCl) binodal curve at 23°C (pH 7.41).

4. **CONCLUSION**

The phase behavior of two-phase aqueous polymer system containing PEG and PA was studied under different conditions of pH. The binodal curve was found to move towards lower concentrations of polymers with increasing pH as a result of decreased solubility of the PA
with pH. A correlation of type proposed by Diamond and Hsu was found to correlate the phase behavior with acceptable accuracy. The binodal was also correlated accurately with a relationship of the type proposed by Guan et al. The two-phase shows good promise for protein partitioning because of small amounts of polymers required for preparing the two-phase system.

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