Influence of Hydrophobe Content on Phase Coexistence Curves of Aqueous Two-Phase Solutions of Associative Polyacrylamide Copolymers and Poly(ethylene glycol)

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ABSTRACT: The influence of hydrophobe content and type on the phase coexistence curves of aqueous two-phase polymer systems containing poly(ethylene glycol) and associative polyacrylamide copolymers has been investigated. The top phase was poly(ethylene glycol)-rich while the bottom phase was rich in the copolymer. Increased intramolecular association resulting from increased hydrophobe content was found to increase compatibility of the two polymers in the bottom phase. Large size asymmetry was found to be an important factor in determining the binodal curve. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 1351–1355, 2003

Key words: aqueous two-phase system; hydrophobic modification; poly(ethylene glycol); phase coexistence, associative copolymers

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 ongoing interest in the development of innovative and efficient separation and purification methods in the biotechnology industry.[1–5]

Aqueous two-phase systems (ATP), in which two water-rich phases coexist in equilibrium, have gained significant interest as a method of protein purification.[1–7] This novel separation methodology exploits the 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 extraction of proteins and other biomolecules while maintaining their native conformations and biological activities. The most commonly used two-phase systems are aqueous solutions of poly(ethylene glycol) (PEG) and dextran or PEG and potassium phosphate. Aqueous two-phase partitioning (ATP) is attractive because of its simplicity, amenability to linear scale up, and potential for continuous operation.[8]

The partitioning of proteins in ATP systems is influenced by many factors—for example, molecular weights, concentrations, and structures of both the protein and the phase-forming polymers. An effective way of enhancing the separation in ATP is by the chemical modification of the phase forming polymers. Chemical modification can influence partitioning of proteins in two ways. First, it alters the phase behavior of the two-phase polymer system. Second, it changes interactions between polymers and protein in such ways that can lead to highly selective extraction.

In this article, we investigate the influence of hydrophobic modification on the phase behavior of PEG with associative polyacrylamides. The influence of incorporating different hydrophobes with different characteristics and in different fractions on phase coexistence is investigated. Hydrophobically modified polyacrylamides copolymerized with hydrophobic monomers of different characteristics are first synthesized. The hydrophobes used are N-phenylacrylamide, N-benzylacrylamide, and *para*-methoxy styrene (PMS) (Fig. 1). Influence of hydrophobe content and characteristics on phase behavior of the copolymers with PEG was then investigated. It is hoped that the experimental results from this study will help in developing cost-effective aqueous two-phase systems for protein separation.

EXPERIMENTAL

Materials

PEG (MW 35,000) was purchased from Merck-Schuchardt (Germany). N-phenyl acrylamide was pre-
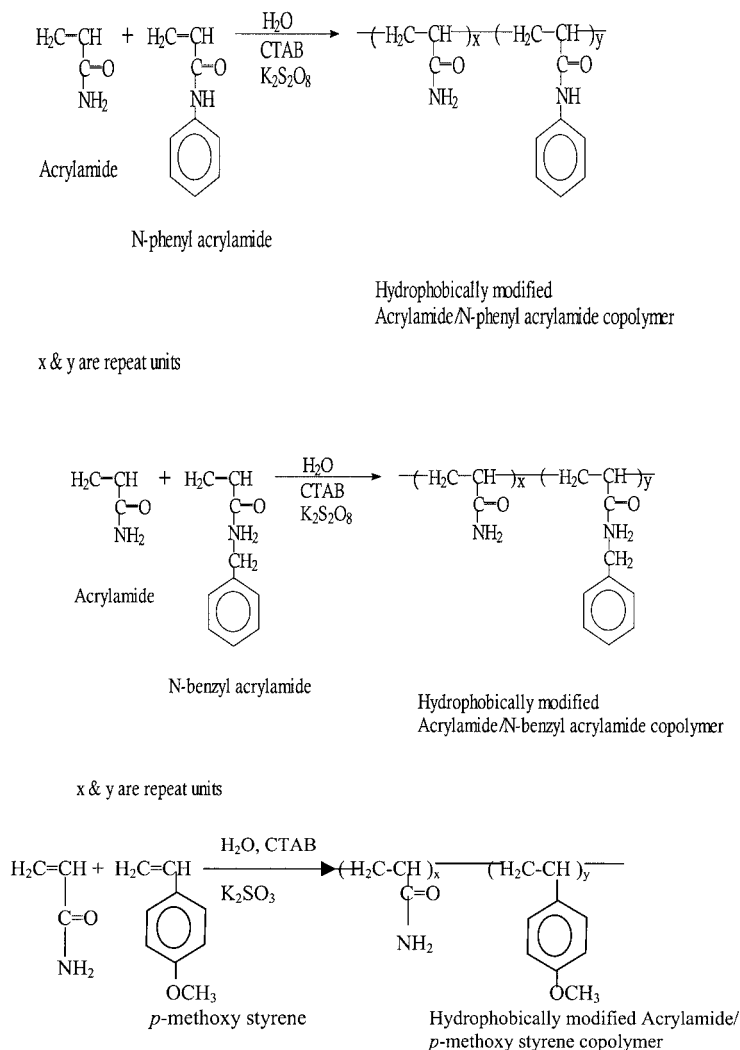
Figure 1 Reaction schemes and molecular structures of monomers and hydrophobically modified copolymers.

pared as described in the literature.[9] Micellar copolymerization was used to synthesize multiblock copolymers of acrylamide with various hydrophobes. Acrylamide/N-phenyl acrylamide multiblock copolymers containing 1.5 and 3 mol % of the hydrophobic monomer were prepared as described previously.[10] The source, purification and synthesis details of the poly(acrylamide/N-benzyl acrylamide) block copolymers were described elsewhere.[11,12] The synthesis details of the poly(acrylamide/p-methoxy styrene) block copolymer containing 0.76, 1.87, and 2.68 mol % hydrophobe are also described in the literature.[13]

Characterization of the copolymers

The hydrophobe content of the copolymers was determined using ^{1}H-NMR. The molecular weights of the copolymers were determined by measuring the intrinsic viscosities of the copolymers. Table I shows the characteristic of the polymers used in this study. Viscosity measurements were carried out using an Ost-

wald viscometer. The shear rates are difficult to determine in this type of viscometer, but will be low enough to avoid any polymer degradation. Table I shows the number average molecular weight (M_n) and weight average molecular weight (M_w) determined using the intrinsic viscosity and eqs. (1) and (2) given below.

$$[\eta] = 6.8 \times 10^{-4}[M_n]^{0.66} \tag{1}$$

$$[\eta] = 6.31 \times 10^{-5}[M_w]^{0.8} \tag{2}$$

The results are summarized in Table I for N-benzylacrylamide-AA, N-phenylacrylamide-AA, and p-methoxystyrene-AA copolymers. The average molecular weights of the resulting polymers were found to be of the order of magnitude of 10^5.

Phase behavior

A phase diagram graphically depicts the phase behavior, thereby delineating the potential working area, of

TABLE I
Characteristics of the Hydrophobically Modified Copolymers

Comonomer	Sample	% Hydrophobe	$[M_n] \times 10^{-5}$ (g/mol)	$[M_w] \times 10^{-5}$ (g/mol)
N-phenylacrylamide	1	3	2.92	6.29
N-phenylacrylamide	2	1.5	3.08	6.58
N-benzylacrylamide	1	1.5	4.70	9.34
N-benzylacrylamide	2	3	4.20	8.51
p-Methoxy styrene	1	2.18	2.93	6.33
p-Methoxy styrene	2	2.68	2.35	5.27
p-Methoxy styrene	3	1.87	2.17	4.93

$[M_n]$ = Number average molecular weight.
$[M_w]$ = Weight average molecular weight.

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.

Concentrated stock solutions of each of the copolymers were first prepared in deionized water. The concentration of the PEG stock solution was 25% w/w. Concentration of the paramethoxy styrene copolymer stock solution was 20% w/w. Concentration of the other copolymer stock solutions was 8% w/w. Known weights of the stock solutions of the copolymers 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 deionized water until it became clear, i.e., one phase is formed. The weight of deionized water 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 deionized water 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 all the copolymer stock solutions. Phase densities were measured using a densimeter at 23°C with water as reference fluid. For all copolymers, the upper phase was PEG rich and the

bottom phase was rich in the polyacrylamide copolymers.

RESULTS AND DISCUSSION

Figure 2 shows the phase diagram obtained for the PMS copolymer containing different amounts of hydrophobe with PEG in water at 23°C. The figure suggests that the hydrophobe content has substantial effect on the phase composition of the system. It is observed that increasing the hydrophobe content increases the PEG content of the hydrophobically modified copolymer phase. It is also observed that the PEG-rich phase contains very small amounts of the copolymer. Contrary to expectation, compatibility of the PEG and hydrophobically modified copolymers increased with increasing hydrophobe content. This behavior can be explained by considering the solution behavior of hydrophobically modified water-soluble copolymers. It is known that incorporating minor amounts of a hydrophobic moiety in a water-soluble polymer induces intermolecular aggregation as a result of association between the hydrophobic moieties from different molecules. However, increasing the hydrophobe content beyond a certain fraction enhances intramolecular interaction between the hydrophobic
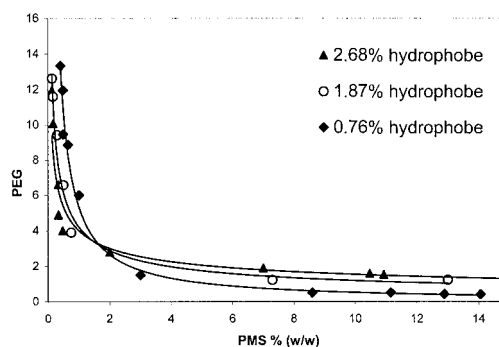

Figure 2 Phase coexistence of poly(acrylamide-*co*-paramethoxy styrene) copolymers with different hydrophobe contents.
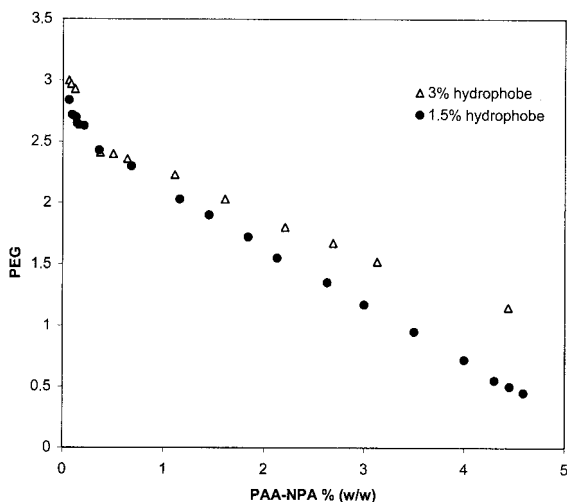
Figure 3 Phase coexistence of poly(acrylamide-*co*-N-phenyl acrylamide) copolymer with PEG.

moieties. The hydrophobic moieties within the same molecule associate in the aqueous solution, leading to collapse of the copolymer chain. The smaller size of the copolymer chain leads to availability of more free water for solubilizing the PEG molecules. In addition, the smaller size of the copolymer chains leads to higher compatibility with the small PEG molecules. Size asymmetry causes phase separation in systems containing molecules varying largely in size. At low hydrophobe content, intramolecular association dominates. The copolymer molecules form an extended structure of associated molecules with a large molecular size. The network formation eliminates the PEG molecules from the copolymer phase. A similar behavior is observed for the poly(acrylamide/N-phenylacrylamide) copolymer (Fig. 3) and for the poly(acrylamide/N-benzylacrylamide) copolymer (Fig. 4) aqueous two-phase systems with PEG. For both copolymers, it can be observed that increasing the
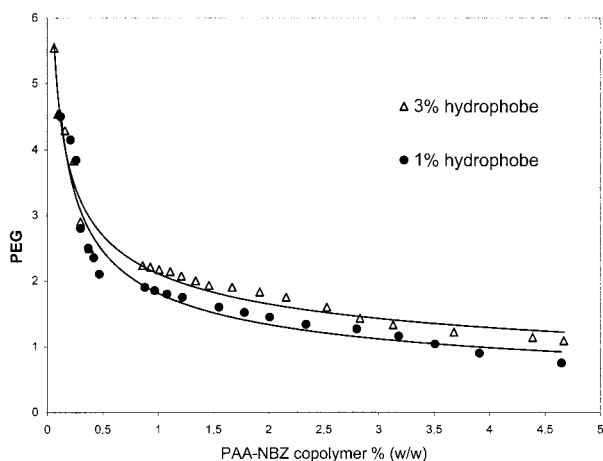

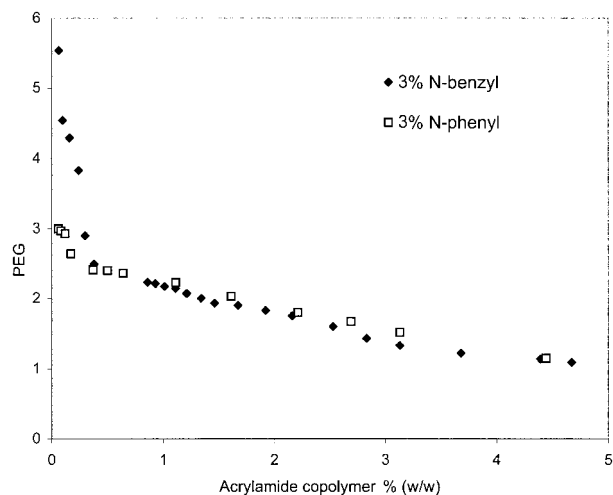**Figure 4** Phase coexistence of N-benzyl acrylamide copolymer with PEG.

hydrophobe content of the copolymer increases the PEG content of the copolymer phase.

Figure 5 shows a comparison of the phase behavior of two copolymers containing the same amount of hydrophobes with different sizes. The first copolymer contains 3 mol % N-benzylacrylamide and the second contains 3 mol % N-phenyl acrylamide. The phase coexistence curves of both polymers are very similar, especially in the copolymer-rich phase. The similar behavior might be a result of the similar structures of the two hydrophobes and the small difference in sizes between them. The PEG-rich phase contains less of the N-phenylacrylamide copolymer than the N-benzylacrylamide copolymer. A possible cause of the higher incompatibility of the N-phenylacrylamide copolymer is its lower hydrophobicity compared to the other comonomer. Lower hydrophobicity causes the copolymer to be less collapsed under intramolecular attractions and subsequently have a larger size. The larger size of the copolymer causes it to be less compatible with the smaller PEG.

Figure 5 Comparison of the phase coexistence curves for the 3% N-benzyl and 3% N-phenyl copolymers.

CONCLUSIONS

The phase behavior of two-phase aqueous polymer system containing PEG and hydrophobically modified polyacryalamide was studied under different conditions of hydrophobe type and content. The binodal curve was found to move toward lower concentrations of polymers with decreasing hydrophobe content as a result of the increase in intermolecular association vs intramolecular association with decreasing hydrophobe content. The hydrophobically modified two-phase formed by the *p*-methoxy styrene copolymer show good promise because of small amounts of polymers required for preparing the two-phase system.

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