

Two-phase aqueous systems of cetyltrimethylammonium bromide/sodium dodecyl sulfate with and without polyethylene glycol

Fei Wang, Ting Chen, Yazhuo Shang[†], and Honglai Liu

Key Laboratory for Advanced Materials, East China University of Science and Technology, Shanghai 200237, China
(Received 15 April 2010 • accepted 13 October 2010)

Abstract—Aqueous two-phase systems of cetyltrimethylammonium bromide and sodium dodecyl sulfate mixtures without and with polyethylene glycol (PEG) added have been studied at 313.15 K. The results have shown that PEG has a strong effect on the phase diagram of ATPS and microstructures of surfactants aggregates. The addition of PEG leads to the formation of bigger surfactant aggregates, which can be attributed to both the screening effect and the connection effect of PEG. All ATPS, especially those with PEG added, may have potential applications in separation and purification of biomaterials.

Key words: Aqueous Two-phase System, CTAB, SDS, Polyethylene Glycol

INTRODUCTION

An aqueous two-phase system (ATPS) is conventionally composed of a water solution of two structurally distinct hydrophilic polymers or of one polymer and certain salts. Aqueous polymer two phase systems for the partitioning of biological macromolecules were first established by Albertsson in the 1950s [1]. Usually, the polymer-polymer type of system consists of fractionated dextran and poly (ethylene glycol). The drawback of dextran as a phase forming polymer is, however, the high viscosity of the lower phase [2] as well as the higher cost of it. To avoid these problems, Tjerneld [3] used the hydroxypropyl derivative of starch as a substitute for dextran to purify enzyme and made great progress. After that, the ATPS formed in systems of PEG-dextran-water and PEG-salt-water is commonly used for protein separation. However, studies have shown that the ATPS formed in the PEG-salt-water system has certain advantages over that formed in PEG-dextran-water systems, such as low viscosity, significantly lower cost, and higher density difference between the two phases. [4]. Therefore, aqueous PEG-salt systems have been used in large-scale protein separation. The physicochemical properties of aqueous two-phase polymer-salt or polymer-polymer systems have been investigated quite extensively [5-8] because of the potential utilization as novel solvent phases capable of solubilizing labile biological molecules in large-scale purification processes.

In recent years, with the growing demand for novel solvent systems for the large-scale purification and separation of biomaterials, many different types of ATPS have been exploited, such as the ATPS formed by aqueous nonionic micellar solutions, ATPS obtained by mixing a polymer and a cationic-anionic surfactant. The ATPS that formed in mixed cationic and anionic surfactant systems have attracted much attention [9,10]. It is found that the solution separates spontaneously into two immiscible aqueous phases with a clear interfacial boundary at certain experimental condition. Once the ATPS is formed, one phase is rich and the other phase is poor in surfactants.

Many studies have shown that almost all aqueous cationic and anionic surfactants can form one or two narrow ATPS regions above critical micelle concentrations (CMC) of mixed solutions [11-19]. In these ATPS, both phases are dilute and the surfactant in one phase is significantly different from that in the other. Therefore, biomaterials can be efficiently isolated and purified in ATPS without loss of activity.

Since the ATPS formed both in systems containing polymers and in systems containing anionic and cationic surfactants have their own advantages in purifying and separating particles, proteins and cells. The research about the ATPS formed in systems containing polymer, cationic and anionic surfactants is rarely done. In this work, we studied the effect of the polymer-poly ethylene glycol (PEG) on the properties including phase diagram and surfactant aggregates morphologies of ATPS containing cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS). The results may be useful for separation processes in biotechnology.

EXPERIMENTAL SECTION

1. Materials

Sodium dodecyl sulfate (SDS) was purchased from Shanghai Chemical Reagent Company (purity-97%). It was recrystallized three times by ethanol, washed by ether, and then dried in a vacuum oven for 8 hours at 323 K and the pressure of 13.33 kPa. Cetyltrimethylammonium bromide (CTAB) was purchased from Shanghai Runjie Chemical Reagent Limited Corporation (purity>99%). Polyethylene glycol with an average molecular weight of 6000 was obtained from the Research Institute of Xinhua in Shanghai. All water used was redistilled water.

2. Methods

First we prepared the 0.2 M cationic surfactant aqueous solution (0%PEG, 1%PEG, 2%PEG, and 5% PEG), 0.2 M anionic surfactant solution (0%PEG, 1%PEG, 2%PEG, and 5% PEG). Samples were prepared by mixing stock-solutions of SDS and CTAB in test tubes at different ratios. The tubes were then immersed in a water bath at given temperature for about 48 hours or longer until equilibrium state was attained. Phase equilibrium is deemed to be attained

[†]To whom correspondence should be addressed.
E-mail: shangyazhuo@ecust.edu.cn

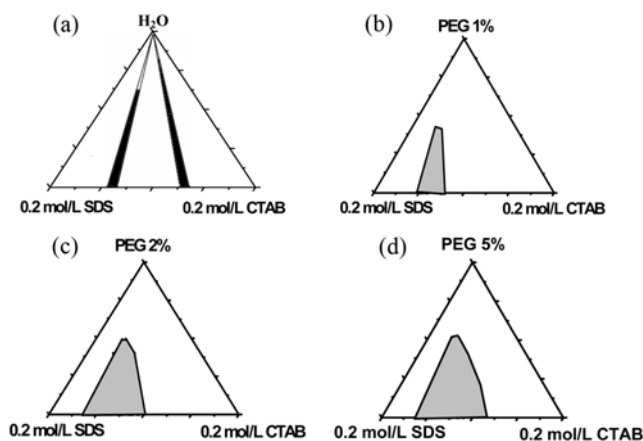


Fig. 1. Pseudo ternary ATPS diagram of CTAB/SDS/H₂O (PEG) systems at 313.15 K.

when a clear interfacial boundary vertical to the test tube wall and the volumes of the upper and bottom phases do not change with time.

The microstructures of both phases were obtained by negative-staining and transmission electron microscope (JEM-100CX II). A drop of the sample was first spread on a 200-mesh copper grid coated with a carbon film; another drop of the staining solution (2.0 wt% of uranyl acetate in ethanol solvent) was then added. The excess solution was removed immediately using filter paper. The sample was then air dried before the TEM observation.

RESULTS AND DISCUSSION

1. Phase Behavior

The pseudo ternary phase diagram of CTAB/SDS/H₂O mixture at 313.15 K is presented in Fig. 1(a). The three apexes are pure water, CTAB aqueous solution and SDS aqueous solution, respectively. The phase behavior of cationic-anionic surfactant aqueous mixture is quite different from that of common aqueous solutions of surfactants and polymers. Usually, the phase diagram of the traditional cationic-anionic surfactant system can be distinguished into seven regions including two aqueous two-phase regions ATPS-A (anionic surfactant is in excess), and ATPS-C (cationic surfactant is in excess), two liquid crystal regions, two transparent homogeneous solution regions as well as a lightly bluish or turbid liquid phase, or a region of precipitate coexisting with liquid [19]. During the experiments we found that the addition of PEG changes the phase behavior of mixed solution dramatically. When the PEG concentration is kept constant and the molar ratio of anionic to cationic surfactants (SDS/CTAB) is gradually varied under certain total surfactant concentration, the appearance of the mixed solutions changes regularly from transparent homogeneous phase to another homogeneous phase through a complex course. For example, when the PEG concentration is 1% and the total surfactant concentration is $0.16 \text{ mol} \cdot \text{L}^{-1}$, the optical properties of mixed solution change with the molar ratio of two surfactants as follows:

SDS+PEG		CTAB+PEG	
Transparent	ATPS	Opalescence, precipitate, turbid and liquid crystal	Transparent

Generally, when the molar ratio of cationic to anionic surfactant is near 1 : 1, opalescence, white flocculation and even precipitation appears because of the neutralization of positive and negative charges, leading to new surfactant aggregates dispersed in solution. ATPS appears only in one range where the anionic surfactant SDS is in excess. The single ATPS-A is located on the boundary between the homogeneous region and complex course (turbid, liquid crystal, precipitate and opalescence) region. Bigger alkyl groups around the positively charged center (i.e., in the case of CTAB the nitrogen atom in the quaternary ammonium salt) and the long molecule of polyethylene glycol weakened the interaction between CTAB and the negatively charged sulfate group; thus, precipitate could be avoided and a homogeneous solution or an aqueous two-phase system could form under certain conditions.

2. Phase Separation Time

Phase separation time refers to the time needed by the two phases to reach equilibrium. Before the equilibrium, the interface is either convex or concave, indicating that a difference exists between the interfacial tension of the upper phase to the tube wall and that of the bottom phase to the tube wall. In general, phase separation of ATPS in this system is a fast process. The phase separation time varies with total concentration and molar ratio of surfactants. Furthermore, if we keep the molar ratio constant, the phase separation time decreases with the increase of surfactant concentration for ATPS formed in the system of SDS/CTAB/water at 313.15 K. And if we keep the total surfactant concentration constant, the phase separation time also decreases with increasing molar ratio of two surfactants. The phase separation time is longer for the system containing PEG compared with that of the system without PEG. This can be attributed to the higher viscosity of the new system resulting from the long-chain of PEG molecules, as well as the bigger aggregates formed due to the strong electrostatic interaction between the oppositely charged head groups of the anionic and cationic surfactants.

3. Effect of Polymer on Phase Diagram of ATPS

The pseudo ternary phase diagrams of CTAB/SDS mixtures in 1%, 2%, and 5% PEG aqueous solutions at 313.15 K are presented in Fig. 1(b)-(d), respectively, and the three apexes are PEG aqueous solution, CTAB and SDS dissolved in PEG aqueous solution, respectively. The concentration of the surfactant solution is represented by mole fraction in these figures. The phase behavior of aqueous mixtures of cationic-anionic surfactants is quite different from that of aqueous solutions of surfactants and polymer. For the system without PEG, aqueous two-phase system regions were observed, the one in the left side symbolized as ATPS-A, in which the anionic surfactant SDS is in excess, while the other in the right side symbolized as ATPS-C in which the cationic surfactant CTAB is in excess (shown in Fig. 1(a)). In our experiments we found that the two regions located in the far right and left are transparent homogeneous liquid single-phase regions where SDS or CTAB is in excess, respectively. While the region located between the ATPS-A and ATPS-C different phenomena appear, including liquid crystal, slightly bluish, opalescent, turbid, and precipitate. In this diagram we can see the prolonged lines of each ATPS region almost across the acme of the water. To form ATPS, the cationic and anionic surfactants almost mixed in fixed molar ratio regardless of the concentration of solutions.

Compared with Fig. 1(a), when PEG aqueous solution substitutes

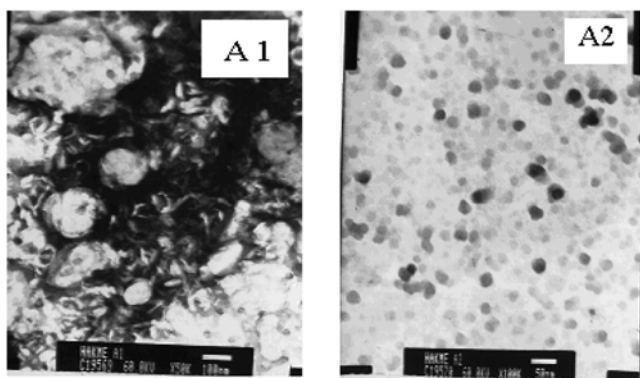


Fig. 2. TEM micrograph of the upper phase A1 and the bottom phase A2 of the ATPS formed by CTAB/SDS/H₂O mixture at 313.15 K. $C_T=0.18 \text{ mol}\cdot\text{L}^{-1}$, molar ratio $R=M_{CTAB}/M_{SDS}=0.4:1$.

water as solvent, only one region of ATPS appears in the phase diagrams; it is seen in Fig. 1(b)-(d). The ATPS region becomes wider and wider with the increase of the PEG concentration. From Fig. 1 we also can see that the total surfactant concentration needed for formation of ATPS is higher for the system containing PEG than that for system without PEG. Compared with the traditional cationic-anionic surfactant system, ATPS-C disappears in the system containing PEG. The ATPS-C region was supposed to exist in the Fig. 1(b)-(d), appearing as turbid and liquid crystal in the mixed solutions because the PEG participates the formation of aggregates.

4. Microstructures of the Two Coexisting Phases

4-1. ATPS of CTAB/SDS/H₂O System

The formation of ATPS usually is attributed to the difference in the aggregate morphology of the two coexisting phases. First, we show microstructures of the two coexisting phases of the aqueous system CTAB/SDS without PEG as a reference. Fig. 2A1 is for the upper phase of ATPS with a total concentration $C_T=0.18 \text{ mol}\cdot\text{L}^{-1}$, and a molar ratio $R=M_{CTAB}/M_{SDS}=0.4:1$ at 313.15 K. Many big vesicles appear in this phase, with a diameter of about 60 nm. Then the vesicles seem to tend to aggregate and thus form bigger aggregates

(the biggest of the aggregates can reach 800 nm), which may be the reason for the appearance of a slightly bluish phase. Fig. 2A2 presents the TEM micrograph of the corresponding bottom phase of ATPS. Vesicles and micelles appear in this phase, with a diameter of about 25 nm and the solution looks transparent.

4-2. ATPS of CTAB/SDS/PEG System

The addition of PEG affects the phase behavior of the CTAB/SDS/H₂O system. The difference of the behavior for the system with and without PEG should be attributed to the difference of the aggregate microstructures. Fig. 3 provides the aggregate morphology of the upper phase of ATPS for the system of CTAB/SDS/PEG mixture with a total concentration $C_T=0.18 \text{ mol}\cdot\text{L}^{-1}$ and a molar ratio $R=M_{CTAB}/M_{SDS}=0.4:1$ at 313.15 K when PEG concentration is 1%, 2% and 5%, respectively. It can be seen that the opalescence upper phase is composed of densely packed irregular aggregates with diameter of 500 nm to 1,500 nm. For the upper phase, the concentration of surfactant and PEG is higher; therefore, the micelles and spherical aggregates are formed easily. When the concentration of PEG is increased, the aggregates become bigger and bigger. That means the higher concentration of PEG is responsible for the formation of bigger aggregates because of the screening effect and the connection effect (it seems that the PEG can connect the aggregates with each other) of PEG. Fig. 4(d), (e) and (f) present the TEM micrograph of the corresponding bottom phase of ATPS. The bottom phase of ATPS is composed of irregular aggregates, spheroid aggregates as well as some vesicles; diameters of these aggregates are different from about 50 nm to 400 nm.

CONCLUSION

Polyethylene glycol has a significant effect on the properties of ATPS of the SDS/CTAB/H₂O system. The addition of polyethylene glycol enlarges the region of ATPS-A, diminishes the region of ATPS-C, prolongs the phase separation time and changes the microstructures of surfactant aggregates. The higher the PEG concentration, the bigger the size of the surfactant aggregates. This can be attributed to both the screening effect and the connection effect of PEG. All ATPS, especially those with PEG added, may have potential

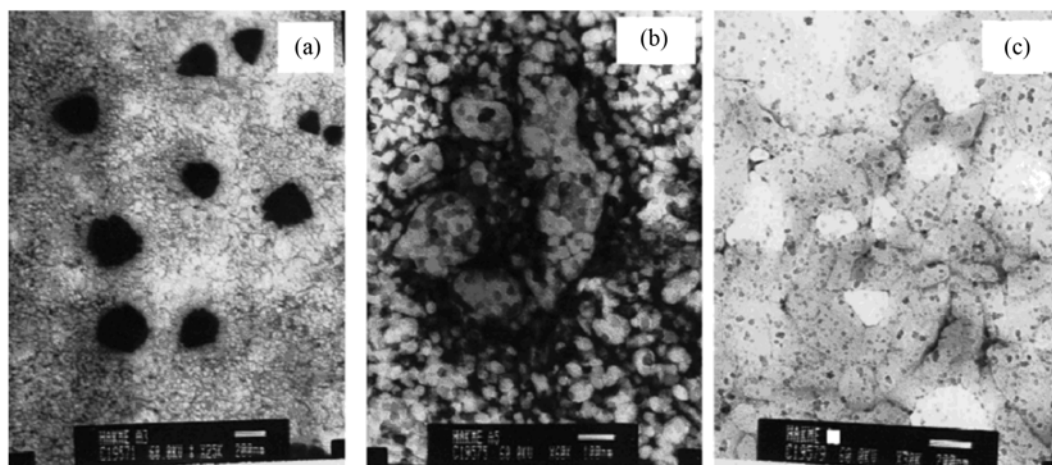


Fig. 3. TEM micrograph of the upper phase of ATPS formed by CTAB/SDS/PEG mixture at 313.15 K. 1% (a), 2% (b) and 5% (c) PEG $C_T=0.18 \text{ mol}\cdot\text{L}^{-1}$, molar ratio $R=M_{CTAB}/M_{SDS}=0.4:1$.

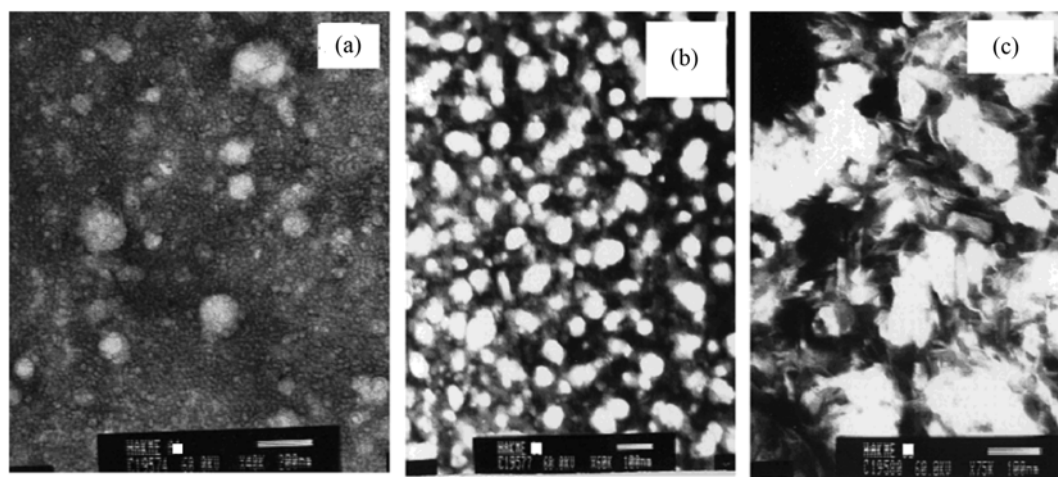


Fig. 4. TEM micrograph of the bottom phase of ATPS formed by CTAB/SDS/PEG mixture at 313.15 K. 1% (a), 2% (b) and 5% (c) PEG $C_T=0.18 \text{ mol}\cdot\text{L}^{-1}$, molar ratio $R=M_{CTAB}/M_{SDS}=0.4:1$.

applications as partition systems.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Projects No. 20706013, 20736002), Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0721) and the 111 Project of Ministry of Education of China (No. B08021).

REFERENCES

1. P. A. Albertsson, *Partition of cell particles and macromolecules*, 3rd edition, Wiley, New York (1986).
2. H. Hustedt, K. H. Kroner and M. R. Kula, *Trend in Biotechnology*, **3**, 139 (1985).
3. F. Tjerneld, S. Berner, A. Cajarville and G. Johansson, *Enzym. Microb. Technol.*, **8**, 417 (1986).
4. A. F. Bückmann, M. Michael and M. R. Kula, *Biotechnol. Appl. Biochem.*, **9**, 258 (1987).
5. F. Tjerneld, G. Johansson and M. Joelsson, *Biotechnol. Bioeng.*, **30**, 809 (1987).
6. H. Walter, G. Johanson and D. E. Brooks, *Anal. Biochem.*, **197**, 1 (1991).
7. Y. J. Nikas, C. L. Liu, T. Srivastava, N. L. Abbott and D. Blank-shtein, *Macromolecules*, **25**, 4797 (1992).
8. Z. J. Yu and G. X. Zhao, *J. Colloid Interf. Sci.*, **130**, 421 (1989).
9. Y. Z. Shang, H. L. Liu, Y. Hu and J. M. Prausnitz, *J. Disp. Sci. Technol.*, **27**, 335 (2006).
10. Y. Q. Nan, H. L. Liu and Y. Hu, *J. Disp. Sci. Technol.*, **28**, 255 (2007).
11. L. Liu and M. J. Rosen, *J. Colloid Interf. Sci.*, **179**, 454 (1996).
12. M. T. Yacilla, K. L. Herrington and L. L. Brasher, *J. Phys. Chem.*, **100**, 5874 (1996).
13. J. X. Xiao, U. Sivars and F. Tjerneld, *J. Chromatogr. B*, **743**, 327 (2000).
14. Y. Z. Shang, H. L. Liu, Y. Hu and J. M. Prausnitz, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **302**, 58 (2007).
15. R. E. H. Lucassen, J. Lucassen and D. Giles, *J. Colloid Interf. Sci.*, **81**, 150 (1981).
16. E. W. Kaler, K. L. Herrington and A. K. Murthy, *J. Phys. Chem.*, **96**, 6698 (1992).
17. C. L. Liu, Y. J. Nikas and D. Blankshtein, *J. Biotechnol. Bioeng.*, **52**, 185 (1996).
18. Z. J. Yu and G. X. Zhao, *J. Colloid Interf. Sci.*, **421**, 130 (1989).
19. Y. Q. Nan, H. L. Liu and Y. Hu, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **269**, 101 (2005).
20. H. N. Teng, PhD thesis, East China University of Science and Technology, 13 (2001).