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Phase behavior of aqueous two-phase systems of cationic and anionic surfactants and their application to theanine extraction

Junwei Zhang[†], Yan Wang, and Qijun Peng[†]

The Key Laboratory of Food Colloids and Biotechnology, Ministry of Education,
School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, P. R. China

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Abstract−Phase behavior of aqueous two-phase systems (ATPS) containing cationic (SDS) and anionic (CTAB) surfactants and its application to theanine extraction was studied. Results indicated the ATPS could form under the certain SDS/CTAB molar ratio; there was a reasonable consistency between the conductivity and the formation region of ATPS, and the viscosity was higher in the formation region of ATPS. Additionally, the phase ratio increased with increase of CATB concentration, and the interfacial film between the top phase and the bottom phase was resilient. Moreover, the theanine extraction with ATPS was realized in the waste liquid of tea polyphenol production (WLTPP), and the partition coefficient of theanine decreased with increase of WLTPP concentration, whereas the extraction rate of theanine increased. The partition coefficient decreased with increasing SDS/CTAB molar ratio, and the extraction rate of theanine increased with increase of SDS/CTAB molar ratio.

Key words: Phase Behavior, Aqueous Two-phase Systems, Surfactant, Extraction, Theanine

INTRODUCTION

Ionic surfactants are among the most widely used surfactants in industrial practice [1,2]. The solutions of two oppositely charged ionic surfactants are mixed at a certain concentration and molar ratio, and the solution separates spontaneously into two immiscible aqueous phases with a clear interfacial boundary, which is a kind of aqueous two-phase system (ATPS) [3-5]. This phenomenon is helpful for separation of organic compounds due to the solubility difference of compounds between two phases [6-8]. The ATPS separation not only removes organic compound from solution but also preconcentrates target compound [4,9,10].

In the last decades, ATPS has been successfully applied to separation and purification of various species such as biomaterials, organic compounds and metal chalets [11-14]. What we know is that the formation of ATPS shows an enthalpy increase, and the enthalpy is a reflection of the nature of intermolecular interactions. Generally, the van't Hoff equation and Flory-Huggins equation have been used to obtain the apparent molar enthalpy of solution [15-18]. Filho and Mohamed indicated that the partitioning of biomolecules in the ATPS was predicted by the thermodynamic model of modified Flory-Huggins [12]. Nordström and Rasmuson proposed a semiempirical model for prediction of the temperature dependence of solid-liquid solubility [19]. However, it is not enough to study only the change of enthalpy; a systematic study of various properties, such as viscosity, conductivity, phase ratio and rheology is very important in order to analyze the formation of ATPS.

Theanine (γ-glutamylethylamide) is a special amino acid and an important flavor substance in tea plants [20]. Various health effects have been associated with theanine, such as neuroprotective, hypotensive, antitumor and inhibiting caffeine's negative effect [21,22]. The production process of tea polyphenols produces considerable quantities of waste liquid, which contains a certain amount of theanine. Thus, extraction of theanine from the waste liquid of tea polyphenols production (WLTPP) can raise the economic value of waste liquid.

The most common anionic surfactant is sodium alkyl sulfates such as sodium dodecyl sulfate (SDS); meanwhile the cationic surfactant cetyl trimethyl ammonium bromide (CTAB) is widely used in different fields. Thus the research about phase behavior SDS/CTAB systems is of great and practical significance. In this work, phase behavior of aqueous two-phase systems of cationic and anionic surfactants and its application to extraction of theanine from WLTPP were investigated in detail. This research not only provides information for phase behavior of aqueous two-phase systems of CTAB-SDS, but also provides an alternative means of obtaining theanine.

EXPERIMENTAL

1. Materials

Cationic surfactant CTAB and anionic surfactant SDS were two analytical grade reagents (purity 99%, Shanghai Reagent Company, China). Theanine with 99% purity was from Jiangnan University Biotech Co. Ltd. (China). WLTPP (insoluble substance 8.4%, theanine 2.3%, saccharide 42.1%, protein 4.3%, tea polyphenols 1.8% and the others were inorganic salt, pectin and impurities) was obtained from Green Separation Institute of Wuxi (China). The other chemicals were analytical reagent grade chemicals (purity greater than 95% in mass fraction, Shanghai Reagent Company, China). Deionized water was used to prepare the solution in the experiments.

2. Procedures

Samples were prepared by mixing stock solution of CATB and SDS at the desired concentration and the molar ratio in the test flask.

[†]To whom correspondence should be addressed.
E-mail: zjwseu@126.com, 13906170335@163.com

After shaking, all samples were sealed and equilibrated to phase separation in a thermostatic bath at 40±1 °C for at least 100 h or longer until equilibrium state was attained. The phase equilibrium was deemed to be reached when a clear interfacial boundary vertical to the wall was formed, and the concentrations of top phase and bottom phase were no longer changed with time (100 h). The time from the shaking to the appearance of clear interfacial boundary between top phase and bottom phase was taken as the phase separation time.

Moreover, the WLTPP was pretreated by filtration, the impurities were removed from the liquid, and then the filtrate containing theanine was obtained. The 0.1 mol/L SDS and the 0.1 mol/L CATB were prepared by addition of SDS and CATB to the filtrate, and the two solutions completely mixed according to the certain molar ratio. The system was shaken at the given time, allowing systems to stand for phase separation. Aliquots of samples were withdrawn and then used in the analysis.

3. Analysis

The phase ratio R was determined by calculating the volume ratio of top phase (V_1) to bottom phase (V_2). The viscosity and conductivity of solution were analyzed by a rheometer (RVCVIII, Brookfield Co., USA) and a conductivity meter (DDS307, Shanghai Eastsen Analytical Instrument Co. Ltd., China) respectively.

The concentrations of theanine in the top phase and the bottom phase were determined by HPLC (HP1100, C18, Agilent Co., America) at 210 nm. 0.08% trifluoroacetic acid (mass fraction) was used as a mobile phase with flow rate of 1.0 mL/min. The partition coefficient of theanine (K) was calculated with the ratio of concentration of theanine in the top phase (C_1) to concentration of theanine in the bottom phase (C_2). The extraction rate (Y) was determined by calculating the ratio of mass of theanine in the bottom phase (M) to total mass of theanine (M_{total}).

RESULTS ANS DISCUSSION

1. Formation of ATPS

Fig. 1 shows the ternary phase diagram of CTAB/SDS/water mixtures at 40 °C. It was found that there were three regions (homogeneous region (L region), the turbid region (L+S region) and the ATPS region) with change of CTAB/SDS molar ratio. The mixtures of SDS and CTAB formed a clear solution at very low concentration, which donated the L region, the range of SDS/CTAB molar ratio of L region was between 7.4/2.6 and 2.5/7.5 (experimental data). The mixtures of SDS and CTAB formed a turbid solution containing precipitate, which was the L+S region and had a disadvantage of formation of ATPS. The SDS/CTAB molar ratio of L+S region was between 6.7/3.3 and 3.3/6.7 (experimental data). Moreover, the mixtures of SDS and CTAB could form the ATPS in the certain range of SDS/CTAB molar ratio, which was between 7.4/2.6 and 6.8/3.2 or between 3.2/6.8 and 2.6/7.4.

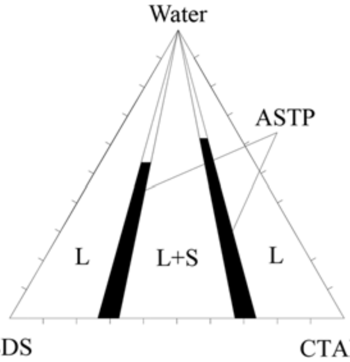

Fig. 1. Ternary phase diagram of CTAB/SDS/water where C_{SDS}=0.1 mol/L, C_{CTAB}=0.1 mol/L.

Fig. 2. Phenomenon of different regions.

Moreover, the L region was a clear solution without precipitate (Fig. 2). The L+S region showed turbidity with precipitate and appeared light blue, which was considered as the unstable region. Additionally, there could observe a clear interface between the two immiscible aqueous phases in the ATPS region (Fig. 2), and the interface between the two phases could recover after remixing. The top phase of ATPS appeared bright blue; this phenomenon could arise from the scattering of liquid crystal or micelles. In terms of size scale of liquid crystal or micelles, it is very small, and thus the top phase may be considered as a phase.

2. Conductivity of ATPS

As shown in Fig. 3, the curve of conductivity was fluctuating in the experimental range, and there were three peaks located in the anionic region of ATPS, the cationic region of ATPS and the equimolar region of SDS/CTAB, respectively. The white precipitate appeared in the equimolar region of SDS/CTAB (Fig. 2), which was produced by interaction effect of cationic surfactant and anionic surfactant. Lam et al. indicated that the equimolar mixture of cationic surfactant and anionic surfactant was similar to the nonionic sur-

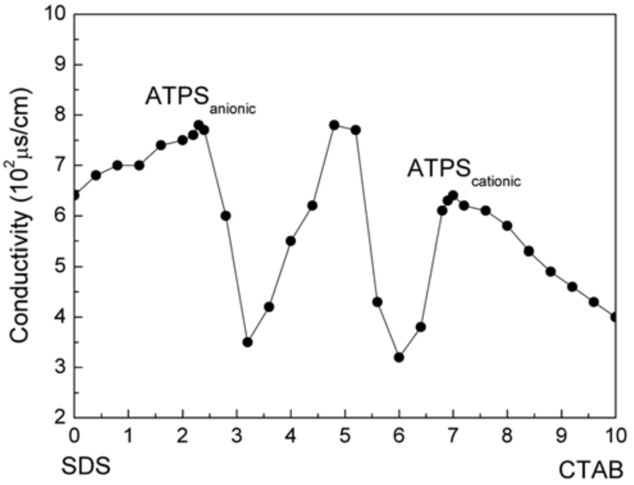

Fig. 3. Relationship between conductivity and solution composition where C_{SDS}=0.1 mol/L, C_{CTAB}=0.1 mol/L.

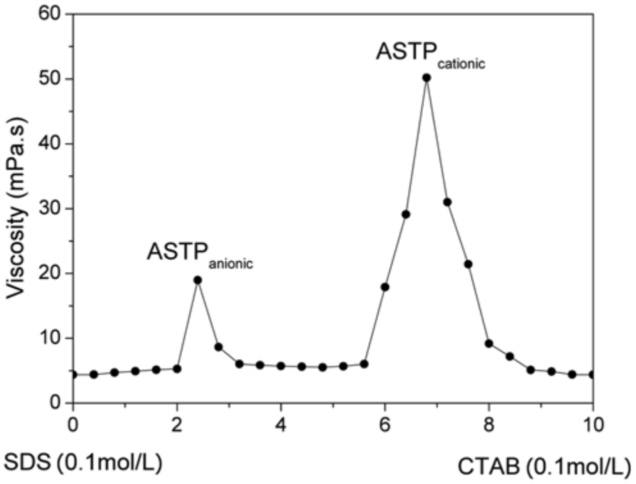

Fig. 4. Relationship between viscosity and composition of solution.

factant and not influenced by inorganic salts [23]. The results shown in Fig. 3 indicated that the counter-ion layer could disappear in the equimolar region of SDS/CTAB, and the ions became the free state.

The peaks of ATPS region may be different from the peak of equimolar region of SDS/CTAB. Zhang et al. indicated that the ATPS region had the liquid crystal structure [24]. The conductivity of liquid crystalline is similar to the conductivity of crystalline, which is due to the transfer of electrons. However, the conductivity of solution in the non ATPS region may depend on the transfer of charged micelle, and the transfer rate of charged micelle may be lower than that of liquid crystalline. Additionally, there were two regions of low conductivity indicated in the curve of conductivity. All phenomena indicate that the change of conductivity is reasonably consistent with the change of ATPS formation region.

3. Viscosity of ATPS

It is important to study the viscosity of solution since it may imply the region of ATPS formation. As shown in Fig. 4, there were two peak values of viscosity with change of SDS/CTAB molar ratio, which corresponded to the formation region of ATPS. In a sense, the formation region of ATPS may be approximately determined by the viscosity value of solution. Generally, the repulsive force is predominant when the solution contains only one surfactant, which is disadvantageous for micelle formation, and consequently the viscosity value of solution is very small. However, the formation of liquid crystalline may result in higher viscosity in the ATPS region. Additionally, the higher the concentration of CTAB in the region of ATPS, the higher value of viscosity, and this phenomenon may arise from highly ordered structure of micelle.

4. Phase Ratio of ATPS

Phase ratio is an important factor since it influences the extraction rate of ATPS. As shown in Fig. 5, phase ratio of ATPS increased with change of CATB concentration. A tiny change of SDS/CTAB ratio could result in great change of phase ratio despite the narrow formation region of ATPS (Fig. 1), which indicated that the increase of CTAB concentration was beneficial to enhancement of phase ratio of ATPS. Moreover, the phase ratio of ATPS in the cationic region was higher than that in the anionic region, and the increased amplitude of the phase ratio of ATPS in the cationic region was faster with increase of CTAB concentration. This phenomenon may arise from the volume increase of top phase in higher concentration of CTAB, and it is beneficial to the extraction of the top phase. Additionally, the results (Fig. 5) indicated that the formation of ATPS was a collaborative effect of SDS and CTAB, which could lead to the dense structure of liquid crystalline.

5. Interfacial Property of ATPS

The interfacial film between two phases, very clear and resilient, is a special membrane (Fig. 2). The interfacial film could be composed of bilayer liquid surfactant membranes; the polar group of surfactant extended toward the bilateral aqueous phase, and the nonpolar group of surfactant held together due to the hydrophobic interaction. The volumes of top phase and bottom phase were constant at a certain temperature, and the interfacial film was vertical to the wall of the bottle (Fig. 6). However, the interfacial film became concave at the higher temperature, and then transformed into a flat interface, whereas the interfacial film first became convex, and then transformed into flat interface at the lower temperature. Moreover, the change of interfacial film could arise from the interphase mass transfer and the tension change of interfacial film. The regular interface indicated that the tension of top phase was same as that of bottom phase. Additionally, the interfacial film was resilient (Fig. 7)

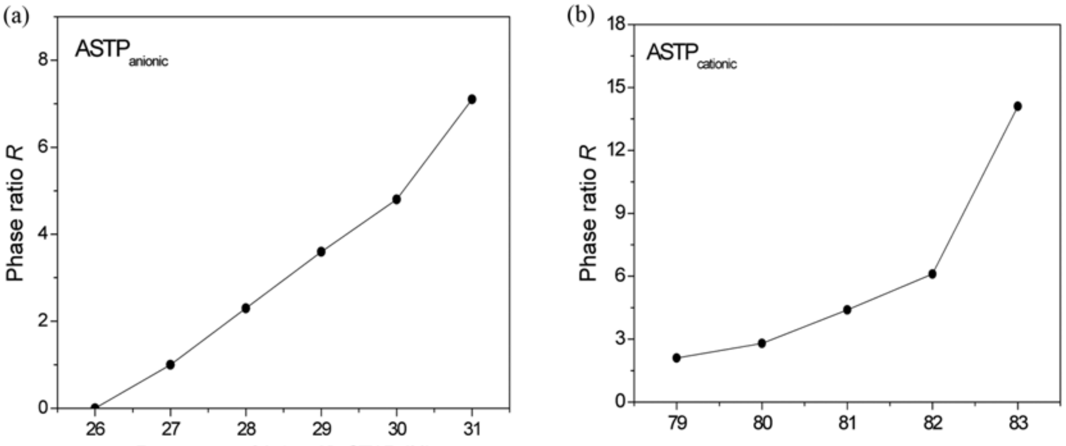

Fig. 5. Relationship between concentration of CTAB and phase ratio of ATPS.

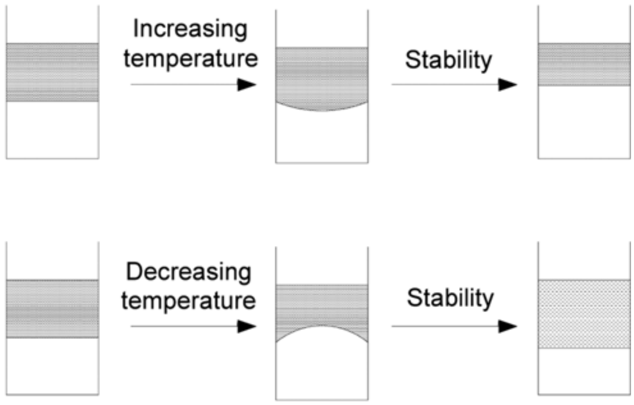

Fig. 6. Effect of temperature on the interfacial film.

Fig. 7. Elasticity of interfacial film.

Table 1. Extraction of theanine with ATPS in different concentrations of WLTPP

	WLTPP concentrations (mg/mL)			
	12	20	28	36
R	0.953	0.390	0.367	0.269
C_2 (mg/mL)	0.298	0.633	0.683	0.832
K	0.931	0.056	0.036	0
Y (%)	53.8	95.8	97.8	100

and could be greatly depressed.

6. Extraction of Theanine with ATPS

Extraction of theanine with ATPS could be realized in different concentrations of WLTPP (Table 1), and the phase ratio R and the partition coefficient of theanine K gradually decreased with increasing WLTPP concentration, whereas the concentration of bottom phase C_2 and the extraction rate Y gradually increased with increasing WLTPP concentration. This phenomenon indicates that the volume of bottom phase increases with increase of WLTPP concentration, and more theanine molecules enter into the bottom phase. Interestingly, the partition coefficient K was equal to zero when concentration of WLTPP was 36 mg/mL, which indicated that proper enhancement of WLTPP concentration could promote the transfer of theanine molecule into the extraction phase. This process can achieve extraction of theanine and is favorable to the subsequent separation.

Table 2. Effect of SDS/CTAB molar ratio on extraction of theanine with ATPS

	SDS/CTAB ratio				
	2.4/7.6	2.5/7.5	2.6/7.4	2.7/7.3	2.8/7.2
R	1.270	0.867	0.674	0.491	0.390
C_2 (mg/mL)	0.630	0.682	0.620	0.641	0.653
K	0.574	0.362	0.439	0.273	0.056
Y (%)	57.8	76.1	77.2	89.6	95.2

Fig. 8. Actual photographs of effect of SDS/CTAB molar ratio.

SDS/CTAB molar ratio, an important factor, may influence the phase ratio and extraction rate of theanine. As listed in Table 2, the increase of SDS concentration resulted in the decrease of phase ratio R, indicating that volume of bottom phase gradually increased with the increase of SDS concentration (Fig. 8). However, the volume increase of bottom phase did not mean the increase of theanine concentration in the bottom phase since the C_2 value had a little change. Moreover, the partition coefficient K gradually decreased with increase of SDS concentration, which indicated that the concentration of theanine C_1 in top phase gradually decreased since the C_2 value had little change. Additionally, the extraction rate of theanine Y gradually increased with the increase of SDS concentration, and the Y value reached to 95.2% when SDS/CTAB ratio was 2.8/7.2. However, high SDS concentration had a disadvantage of formation of ATPS since the mixtures of SDS and CTAB formed a clear solution (Fig. 1 and Fig. 2).

CONCLUSION

There was a homogeneous region, a turbid region and an ATPS region with change of SDS/CTAB molar ratio. The changes of conductivity and viscosity were reasonably consistent with the formation region of ATPS, and the phase ratio of ATPS increased with change of SDS/CTAB molar ratio. The interfacial film between top phase and bottom phase was resilient and could be greatly depressed. Moreover, extraction of theanine with ATPS was realized in the WLTPP; phase ratio R and partition coefficient of theanine K gradually decreased with increase of WLTPP concentration, whereas concentration of theanine C_2 and extraction rate Y gradually increased. Phase ratio R and partition coefficient K gradually decreased with increase of SDS concentration, whereas extraction rate Y gradually increased.

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