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ORIGINAL CONTRIBUTION

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Hydrotrope and hydrotrope-solubilization action of cephanone in CTAB/n-$C_5H_{11}OH/H_2O$ system

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Abstract Cephanone is found to show the hydrotrope and hydrotrope-solubilization action in CTAB/n-$C_5H_{11}OH/H_2O$ system. Cephanone can increase the solubilities of cationic surfactant CTAB or n-$C_5H_{11}OH$ in water and water in n-$C_5H_{11}OH$. It can also increase the solubilization amount of n-$C_5H_{11}OH$ in O/W microemulsion and that of water in W/O microemulsion, which makes the two regions of O/W and W/O microemulsion larger, and even linked together. The mechanism of the hydrotrope-solubilization action of cephanone is related to the location of cephanone in the palisade of microemulsion which causes the stability of O/W and W/O microemulsion to be enhanced and that of lamellar liquid crystal to be reduced. Therefore, the mechanism of hydrotrope-solubilization is the structural transition from lamellar liquid crystal to the bicontinuous structure.

Keywords Cephanone · CTAB · Hydrotrope · Hydrotrope-solubilization · Microemulsion · Lamellar liquid crystal

Introduction

Microemulsion is a thermodynamic stable system, which is composed of surfactant, cosurfactant, oil and water (or brine) [1]. Considering the wide application of hydrotrope-solubilization action in the fields of detergents and cosmetic, medicine preparation and oil recovery for its higher solubilization amount [2], it is very important to seek better hydrotropic agent to further enhance the solubilization amount of microemulsion [3, 4].

The phenomenon of increasing the aqueous solubility of substances normally insoluble or sparingly soluble in water by the third compound or additive without surfactant aggregation, is termed hydrotropy or hydrotropism. In contrast, the phenomenon of enhancing the solubility of some water-insoluble substance by the surfactant aggregation, such as micelles, microemulsion, liquid crystal, is called solubilization. As opposed to surfactant solubilization, hydrotrope-solubilization is achieved by combining hydrotropy with solubilization [5, 6, 7, 8, 9], and the mechanism of hydrotrope-solubilization action is that the O/W and W/O microemulsions are stabilized and the lamellar liquid crystal is destabilized, which results in the ‘phase transition’ from lamellar liquid crystal phase to bicontinuous structure.

Our past work found that vitamin C, penicillin G potassium salt, and urea show hydrotrope-solubilization action in CTAB/n-$C_5H_{11}OH/H_2O$ system [7, 8, 9]. Cephanone, which can inhibit the synthesis of mucopeptide of cell walls of Gram-positive bacterial, is a β-lactam with high medicinal value [10, 11]. There are few reports of the interaction between cephanone and surfactants. In the present paper, we find that cephanone shows hydrotrope-solubilization action in the CTAB/n-$C_5H_{11}OH/H_2O$ system. The results of hydrotrope-solubilization action of cephanone will broaden the field of its application.

Experiments

Materials Hexadecyltrimethylammonium bromide (CTAB, Aldrich, 99 + %), *n*-pentanol (n-$C_5H_{11}OH$, Aldrich, 99 + %), pyrene (Aldrich, 99 + %), and cephanone (The Pharmaceutical Factory of ChongQing, 1,000,000 Units) were used. The water used was distilled twice.

Determination of partial phase diagram The isotropic regions of CTAB/n-$C_5H_{11}OH/H_2O$ system with or without cephanone were determined by observing the changing point from clarity to cloudiness when CTAB was added to the samples with various weight ratios of n-$C_5H_{11}OH/H_2O$ (or n-$C_5H_{11}OH$/cephanone (aq)), or when n-$C_5H_{11}OH$ was titrated into the samples with various weight ratios of CTAB/H_2O (or CTAB/cephanone (aq)). Samples were placed in the thermostat at 25 ± 0.1 °C for at least 2 h for phase equilibrium. The phase boundaries of lamellar liquid crystal were determined by a Polaroid or a polarizing microscope. (59x, Shanghai Photology Apparatus Co.)

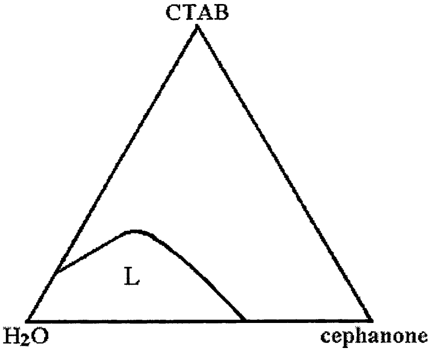


Fig. 1 The partial phase diagram of CTAB/cephanone/H_2O system: L—the isotropic region

Measurement of IR spectra The IR absorption spectra of lamellar liquid crystal with different cephanone contents were observed by liquid film method (CaF_2), by which the oriented angle of hydrocarbon chain in CTAB molecule of the lamellar liquid crystal was determined (740 FT-IR Nicolet Co.).

Measurement of electric conductivity The electric conductivity was measured with a conductometer (DDS-11A, Shanghai Second Analytical Instruments Co., China) at 25 ± 0.1 °C. The structure of microemulsion could be determined according to the measurements of electric conductivity [12].

Freeze-fracture measurement of the microemulsion structure The microemulsion structure images were observed with electronic microscope (TECNAI 12 Philip Apparatus Co., USA.) by the freeze-fracture method (Balzers BAF 400 D).

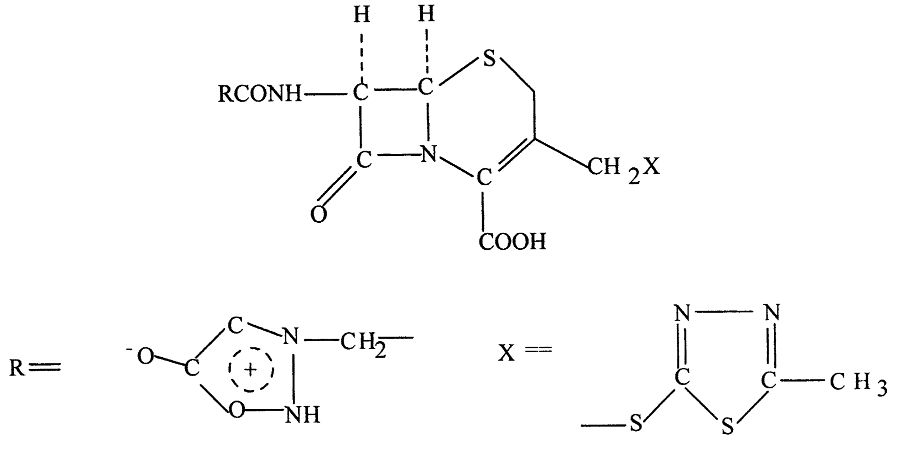
Measurement of micropolarity in microemulsion The stable-state fluorescence spectra were measured by using pyrene as probe with the excitation wavelength being 338 nm and the emission wavelength being 384 nm, respectively. The intensity ratio of the first peak (near 373 nm) to the third peak (near 384 nm) (I_1/I_3) of the fluorescence spectra can show the micropolarity of the microenvironment where pyrene exists[13]. The pyrene concentration is 1×10^{-6} mol/L. The experimental temperature is 25 ± 0.1 °C.

Results and discussions

The hydrotrope action of cephanone

Figure 1 is the partial phase diagram of cationic surfactant (CTAB)/cephanone/H_2O system, in which L shows the isotropic region. Figure 1 reveals that the solubility of the cationic surfactant CTAB in water increases with the addition of cephanone, which shows that the hydrotrope action of cephanone is probably related to the molecular interaction between the cepha-

Fig. 2 The molecular structure of cephanone



none molecule and the surfactant. Cephanone is an electron-rich molecule with carbonyl group, amino-group and carboxy group (Fig. 2). It can easily be combined with electron-defect center CTA^+ through electrostatic effect to form complex compound. Thus, the solubility of CTAB increases.

The hydrotrope-solubilization action of cephanone

Figure 3a–g shows the partial phase diagrams of CTAB/*n*-$C_5H_{11}OH$/cephanone (aq). It is obviously seen from Fig. 3 that there are four distinguishing points with the increasing cephanone content:

1. The solubility of CTAB in water increases, which shows the hydrotrope action of cephanone.
2. Both the solubility of pentanol in water and that of water in pentanol increase with the addition of cephanone. When the cephanone content in the solvent is greater than 25%, pentanol and water can be miscible at any weight ratio of pentanol/water. Figure 4 further shows the effect of cephanone on the solubility of pentanol in water, or water in pentanol. It can be seen from Fig. 4 that the solubility of pentanol in water and that of water in pentanol increase with the addition of cephanone. When cephanone content is between 25% and 50%, pentanol and water can be miscible at any weight ratio of pentanol/water. The probable reason for this phenomenon is that the complex compound of cephanone···*n*-$C_5H_{11}OH$···H_2O forms in the CTAB/*n*-$C_5H_{11}OH$/cephanone(aq) system.
3. The solubilization amount of pentanol in the O/W microemulsion (L_1) and that of water in the W/O microemulsion (L_2) increase, which makes the O/W and W/O microemulsion regions larger and the multiphase region (M_1) decrease. When 15% cephanone solution is used as solvent, the O/W and W/O microemulsions coalesce with each other. When the content of cephanone is 35% in water, the multiphase region (M_1) on the left of the phase diagram disappears completely and becomes an isotropic region. This result probably shows that the location of cephanone in the palisade of the O/W and the W/O microemulsion droplets causes the order degree of the surfactant in the O/W and W/O microemulsion droplets to be increased. So the film intensity of O/W and W/O microemulsion droplets enhances and then the stability of O/W and W/O microemulsions increases and thus the solubilization amount of pentanol in O/W microemulsion and that of water in W/O microemulsion increase.
4. The lamellar liquid crystal region gradually shrinks (LLC). The result indicates that the presence of cephanone makes the stability of lamellar liquid crystal decrease, which can be verified by the effect of cephanone on the order degree of surfactant molecules in the amphiphilic bilayer in the lamellar liquid crystal through IR absorption spectra.

With the polarized angle being 90°, the IR polarizing absorption spectra are measured with the incident angle being 0° and 45°, respectively. The oriented angle of hydrocarbon chain in CTAB molecule of the lamellar liquid crystal can be calculated from the following equations [14]:

$$A_{i,45}/A_{i,0} = n_s\ (1+n_s)\ \Big[2\sin^2 i/\Big(\tan^2\varphi\Big)n_f^4 + \cos i\cdot\cos r/n_s\Big]/(\cos r\ +\ n_s\cos i) \tag{1}$$

$$\cos^2\alpha + \cos^2\beta + \cos^2\gamma = 1 \tag{2}$$

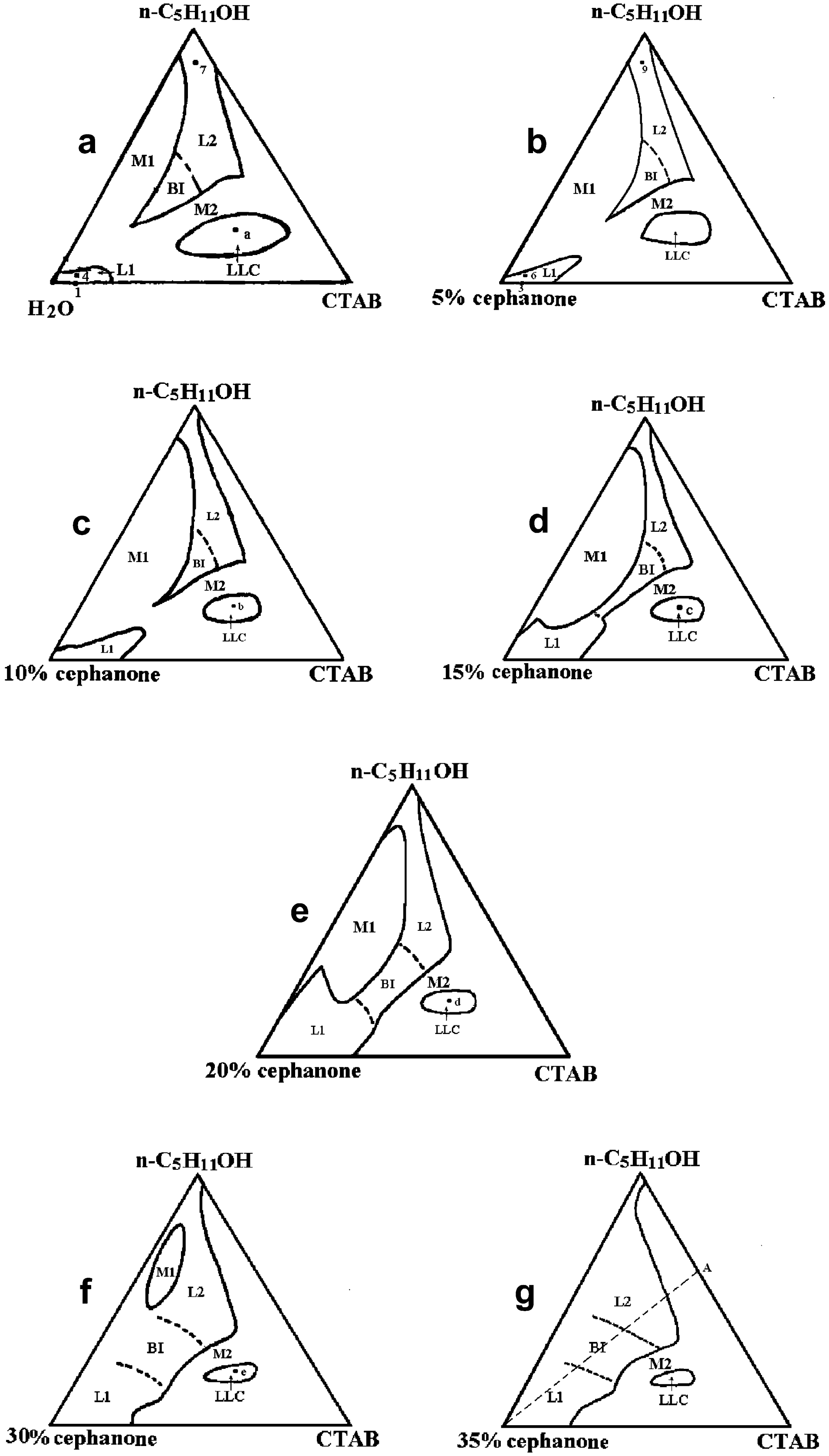
In Eq. (1) A is the absorbance of IR spectra, n_s is the refrangibility of the base substance CaF_2 (1.415), n_f is the refrangibility of the lamellar liquid crystal,i is the incident angle, r is the refraction angle, which can be calculated by $\sin r = \sin i/n_s$, ϕ is the angle between the transition moment of symmetric or asymmetric flexing vibration and the normal of amphiphilic bilayer in lamellar liquid crystal. In Eq. (2) α, β are the angles between the transition moment of asymmetric and symmetric flexing vibration of CH_2group and the normal of amphiphilic bilayer in lamellar liquid crystal, γ is the angle between the hydrocarbon chain and the normal of the amphiphilic bilayer in the lamellar liquid crystal. The value of γ shows the order degree of lamellar liquid crystal. The lower the γ value is, the higher the order degree of lamellar liquid crystal will be.

Table 1 shows that with the weight ratio of CTAB/*n*-$C_5H_{11}OH$/cephanone (aq) = 50/20/30, the oriented angle of hydrocarbon chain in CTAB molecule of the lamellar liquid crystal increases with cephanone content. This result shows that with the increase of cephanone content, the order degree of lamellar liquid crystal decreases and so does the stability of lamellar liquid crystal.

The effect of cephanone on the stability of microemulsion and lamellar liquid crystal

The structure of the microemulsion can be determined by the measurement of the electric conductivity κ in the CTAB/*n*-$C_5H_{11}OH$/cephanone (aq) system. According to the percolation theory, in W/O microemulsion region, the electric conductivity κ can be expressed as follows[1, 12, 15, 16, 17]:

Fig. 3a–g The partial phase diagrams of cephanone (aq)/CTAB/n-$C_5H_{11}OH$ system: L_1—O/W microemulsion region; L_2—W/O microemulsion region; BI—the bicontinuous region; LLC—the lamellar liquid crystal region; M_1, M_2—the multiphase region



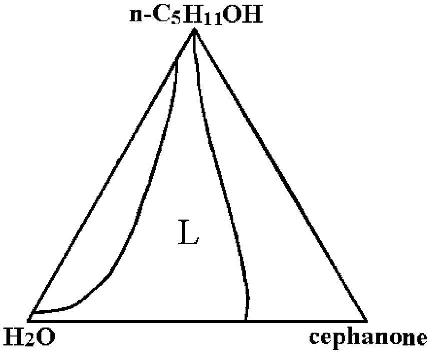


Fig. 4 The partial phase diagram of cephanone/n-$C_5H_{11}OH/H_2O$ system: L—the isotropic region

$$\kappa = A(\varphi_e - \varphi)^a \quad \varphi_e > \varphi \tag{3}$$

$$\kappa = A(\varphi - \varphi_e)^a \quad \varphi > \varphi_e \tag{4}$$

where ϕ is the water content, ϕ_e is the percolation coefficient, A and a are constants.

A typical curve of electric conductivity κ vs cephanone aqueous content ϕ is shown in Fig. 5. According to the percolation theory, the curve can be divided into four parts, each corresponding to a certain structure [1, 12]. In the first part, where $\phi < \phi_e$, (a < 1), the system shows small values of κ increasing slowly with ϕ because the electric conductivity chain has not been established. In the second part, where $\phi_e < \phi < \phi_b$, (a = 1), the electric conductivity chain has formed, and κ increases linearly with ϕ. The system corresponds to W/O structure. In the third part, where $\phi > \phi_m$, with the increase of ϕ the concentration of conducting droplets which can conduct electricity reduces and κ decreases. The system corresponds to O/W structure. And in the last part, where $\phi_b < \phi < \phi_m$, the electric conductivity κ increases slowly, and the system corresponds to the bicontinuous structure [1, 12, 18, 19, 20].

According to the results above, the isotropic region in Fig. 3 can be divided into three parts: O/W, W/O, and bicontinuous (BI). Figure 3 shows that the lamellar liquid crystal phase region deduces with the increase of cephanone content, which corresponds to the increase of the bicontinuous region. So the hydrotrope-solubilization action of cephanone on the CTAB/n-$C_5H_{11}OH$/H_2O system is related to the destabilization of the lamellar liquid crystal.

Microemulsion droplet of the CTAB/n-$C_5H_{11}OH$/cephanone(aq) system with different structures is also confirmed by the freeze-fractured images shown in Fig. 6. From the images of the freeze-fracture, O/W and W/O microemulsion droplets can be seen clearly, but the bicontinuous system shows a network structure.

The location of cephanone in the microemulsion

The hydrotrope-solubilization action of cephanone in CTAB/n-$C_5H_{11}OH/H_2O$ system is probably related to the location of cephanone in the palisade of O/W and W/O microemulsion droplets, which makes the film intensity and the stability of O/W and W/O microemulsions increase.

The location of cephanone in the palisade of O/W and W/O microemulsion droplets can be confirmed by the micropolarity of microemulsion droplets, which can be determined by the I_1/I_3value of the microen-

Table 1 The effects of cephanone on the oriented angle of hydrocarbon chain of CTAB molecule in the lamellar liquid crystal (CTAB/n-$C_5H_{11}OH$/cephanone (aq) = 50/20/30)

Samples[a]	Cephanone (wt%)	H_2O	n_f[b]	Wavenumber (cm^{-1})	Vibration mode	α[c]	β[d]	γ[e]
a	0	30.0	1.432	2924	$v_{as}(CH_2)$	61.8°		41.5°
				2854	$v_s(CH_2)$		62.3°	
b	3.0	27.0	1.437	2924	$v_{as}(CH_2)$	60.5°		45.3°
				2854	$v_s(CH_2)$		59.2°	
c	4.5	25.5	1.440	2924	$v_{as}(CH_2)$	57.2°		48.3°
				2854	$v_s(CH_2)$		59.1°	
d	6.0	24.0	1.444	2924	$v_{as}(CH_2)$	55.5°		52.2°
				2854	$v_s(CH_2)$		56.6°	
e	9.0	21.0	1.450	2924	$v_{as}(CH_2)$	54.3°		54.7°
				2854	$v_s(CH_2)$		55.2°	

[a] Samples of a, b, c, d, e correspond to the points in Fig. 3a, Fig. 3c, Fig. 3d, Fig3e, and Fig. 3f, respectively.

[b] n_f is the refrangibility of the lamellar liquid crystal

[c] α is the angle between the transition moment of asymmetric flexing vibration and the normal of amphiphilic bilayer in lamellar liquid crystal

[d] β is the angle between the transition moment of symmetric flexing vibration and the normal of amphiphilic bilayer in lamellar liquid crystal

[e] γ is the oriented angle between the hydrocarbon chain and the normal of the amphiphilic bilayer in the lamellar liquid crystal

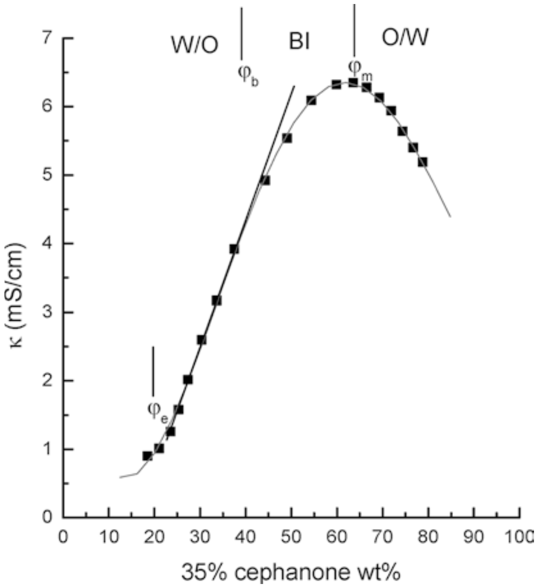


Fig. 5 The curve of electrical conductivity κ vs the content of cephanone aqueous. The weight ratio of CTAB:n-$C_5H_{11}OH$ = 45:55. The route of conductivity measurement is the line from A to the solvent corner in Fig. 3g

vironment where pyrene exists [13] (Table 2). Pyrene is a strongly hydrophobic compound and its solubility in water is very low (2–3 μmol/L). In the presence of micelles and microemulsions, pyrene is preferentially solubilized in the palisade of these aggregates. When pyrene transfers to the inside of the micelles or O/W microemulsion droplets, the polarity of the micelles or microemulsion droplets where pyrene exists decreases, and then the I_1/I_3 value decreases. This is in contrast in the W/O microemulsions. In CTAB/H_2O micelle system, pyrene exists in the palisade of CTAB micelle, where the polar group and hydrocarbon chain link, and the I_1/I_3 is 1.336 (Sample 1). When cephanone is added, the I_1/I_3 value decreases from 1.336 (Sample 1) to 1.306 (1.0%, Sample 2) and 1.253 (5.0%, Sample 3), respectively. It shows that cephanone exists among the polar groups of CTAB micelle, which makes pyrene transfer to the inside of micelle, and the I_1/I_3 value deceases. When cephanone is added to the O/W microemulsion of CTAB/n-$C_5H_{11}OH$/H_2O system, the value of I_1/I_3further decreases from 1.189 (Sample 4) to 1.152 (1.0%, Sample 5) and 1.119 (5.0%, Sample 6), respectively. The above result shows that the addition of cephanone can make the pyrene further transfer to the inside of the palisade of O/W microemulsion droplets. It is well known that cosurfactant pentanol always exists among the polar groups of surfactant molecules in the O/W microemulsion droplets, so the decease of I_1/I_3value indicates that cephanone is located between n-pentanol and pyrene in the palisade of O/W microemulsion droplets. In the CTAB/n-$C_5H_{11}OH$/H_2O W/O microemulsion system, the I_1/I_3 value decreases from 1.079 (Sample 7) to 1.068 (1.0%, Sample 8) and 1.046 (5.0%, Sample 9) when cephanone is added. It reveals that the addition of cephanone can make pyrene transfer slightly to the

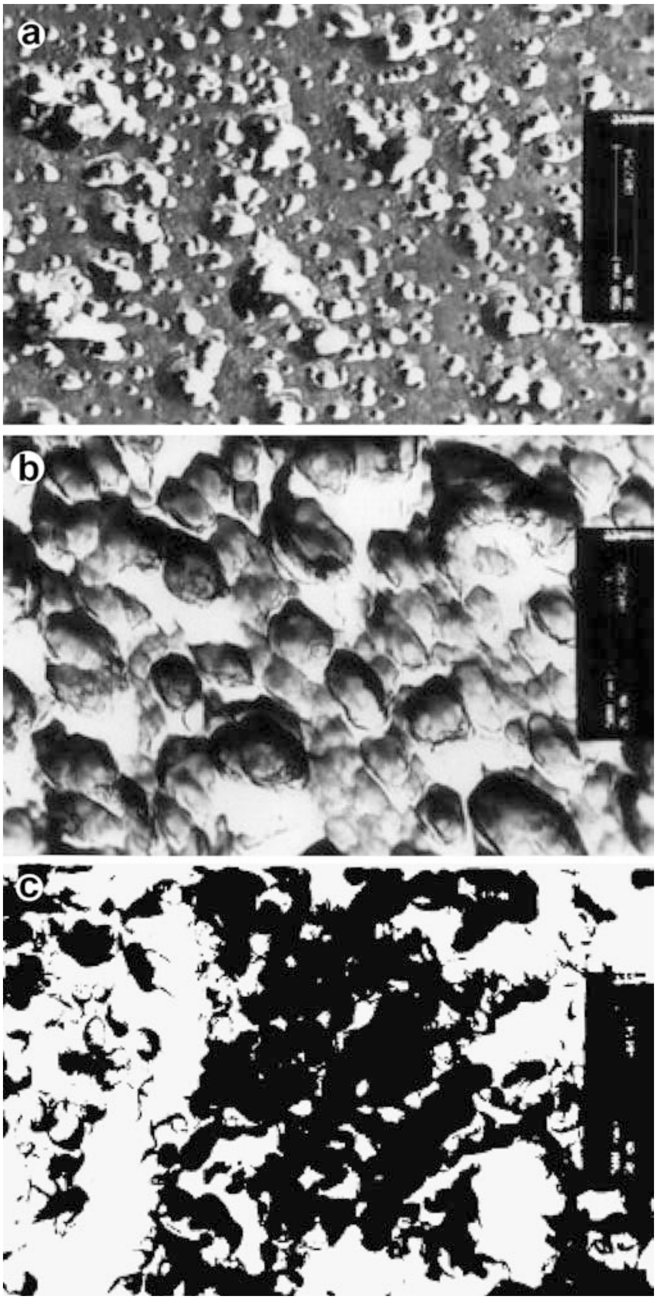


Fig. 6a–c The freeze-fractured TEM images of CTAB/n-$C_5H_{11}OH$/20% cephanone(aq) system: **a** –O/W (CTAB:n-$C_5H_{11}OH$:20% cephanone(aq) = 2:8:90); **b** –BI (CTAB:n-$C_5H_{11}OH$:20% cephanone(aq) = 25:50:25); **c** –W/O (CTAB:n-$C_5H_{11}OH$:20% cephanone(aq) = 2:90:8)

Table 2 The effects of cephanone on the micropolarity (I_1/I_3) of CTAB/n-$C_5H_{11}OH/H_2O$ system

Samples[a]	Content, wt%				Media	I_1/I_3
	CTAB	n-$C_5H_{11}OH$	H_2O	Cephanone		
1	5.0		95.0		Micelle	1.336
2	5.0		94.0	1.0	Micelle	1.306
3	5.0		90.0	5.0	Micelle	1.253
4	5.0	3.0	92.0		O/W	1.189
5	5.0	3.0	91.0	1.0	O/W	1.152
6	5.0	3.0	87.0	5.0	O/W	1.119
7	5.0	87.0	8.0		W/O	1.079
8	5.0	86.0	8.0	1.0	W/O	1.068
9	5.0	82.0	8.0	5.0	W/O	1.046

[a] Samples of 1, 3, 4, 6, 7, 9 correspond to the points in Fig. 3a, Fig. 3b, respectively

outside of the palisade of W/O microemulsion droplets.

The effects of cephanone on the distribution coefficient of n-$C_5H_{11}OH$ in microemulsion

The distribution coefficient K of n-$C_5H_{11}OH$ between CTAB/n-$C_5H_{11}OH/H_2O$ O/W microemulsion and water continuous phase can be formulated as [21]

$$K = X_A^{mic}/X_A^{aq} \tag{5}$$

where $X_A{}^{mic}$, $X_A{}^{aq}$ is the molar fractions of n-$C_5H_{11}OH$ in the microemulsion and water continuous phase, respectively. By introducing mole concentration, Eq. (5) can be expressed as

$$K = 55.5\left(C_A - C_A^{aq}\right)/C_A^{aq}\left[\left(C_A - C_A^{aq}\right) + (C_S - \mathrm{cmc})\right] \tag{6}$$

where C_A is the total concentration of n-$C_5H_{11}OH$, $C_A{}^{aq}$ is the concentration of n-$C_5H_{11}OH$ in the water phase, C_S is the total concentration of surfactant (mol L^{-1}). The minimum concentration of C_S is 0.02 mol·L^{-1}, and the cmc of CTAB is 8.9×10^{-4} mol·L^{-1}. So cmc can be ignored for its smaller value, and Eq. (6) can be simplified as

$$K = 55.5\left(C_A - C_A^{aq}\right)/C_A^{aq}\left[\left(C_A - C_A^{aq}\right) + C_S\right] = 55.5K' \tag{7}$$

$$K' = \left(C_A - C_A^{aq}\right)/C_A^{aq}\left(C_S + C_A - C_A^{aq}\right) \tag{8}$$

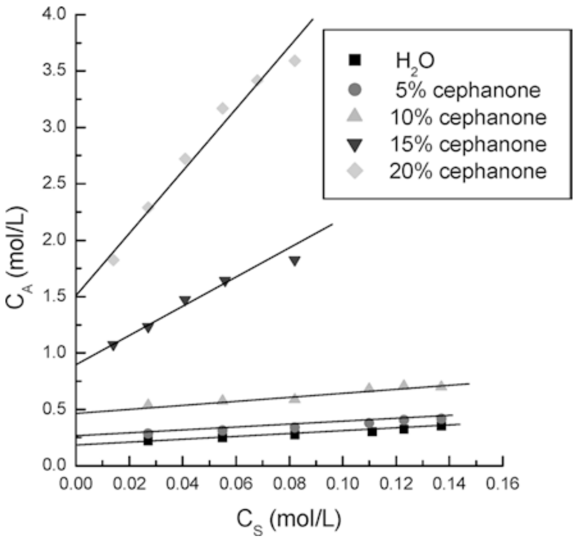


Fig. 7 The solubility of pentanol as a function of CTAB with different cephanone concentration

Table 3 The distribution coefficient K of n-$C_5H_{11}OH$ between CTAB/n-$C_5H_{11}OH/H_2O$ microemulsion droplets and water continuous phase

Cephanone (wt%)	K
0	165.6
5.0	120.1
10.0	72.9
15.0	52.5
20.0	34.2

From Eq. (7) we obtain

$$C_A = \left[K'C_A^{aq}/\left(1 - K'C_A^{aq}\right)\right] \times C_S + C_A^{aq} \tag{9}$$

Equation (9) shows that C_A is linear function with C_S, if the change of $C_A{}^{aq}$ with C_S can be ignored. Solubility of n-$C_5H_{11}OH$ in CTAB/n-$C_5H_{11}OH/H_2O$ O/W microemulsion in the presence of cephanone is shown as a function of C_S (Fig. 7). As can be seen from Fig. 7, it is apparent to be a linear relation. Ignoring the change of C_A with C_S, Eq. (9) can be applied to the experimental result in Fig. 7. Accordingly, the line relation allows to evaluating partition coefficient K of n-$C_5H_{11}OH$ at its solubility in CTAB/n-$C_5H_{11}OH/H_2O$ O/W microemulsion. In Fig. 7 K′ can be calculated from the slope k and the intercept $C_A{}^{aq}$, and then the distribution coefficient K can be calculated from K = 55.5 K′. The values of K in the presence of cephanone are listed in Table 3.

Table 3 shows that the distribution coefficient K of n-$C_5H_{11}OH$ between CTAB/n-$C_5H_{11}OH/H_2O$ O/W microemulsion and water phase decreases with the increasing content of cephanone. The probable reason is that with the formation of a complex compound of cephanone···n-$C_5H_{11}OH$···H_2O, the solubility of pentanol in water increase, and then the molar fraction increase.

The mechanism of the hydrotrope-solubilization action of cephanone

According to the theory of cohesive energy for surfactant aggregation, if the amphiphilic bilayer is marked by C, the water layer by W and the oil layer by O, the ratio R is expressed as [22]

$$R = A_{CO}/A_{CW} \tag{10}$$

where A_{co} is the interaction energy between the C-phase and the O-phase, and A_{cw} is the interaction energy between the C-phase and the W-phase. $R < 1$ shows the interaction energy between the C-phase and the O-phase is smaller than that between the C-phase and the W-phase. Therefore the convex tendency of the C-phase is from the C-phase to the W-phase and the O/W structure is formed. In contrast, $R > 1$ indicates that the convex tendency of the C-phase is from the C-phase to the O-phase and the W/O structure is formed. When $R = 1$, the convex tendency from the C-phase to the W-phase is equal to that from the C-phase to the O-phase, and the lamellar structure is formed. If one part of the aggregation exists with convex tendency from the C-phase to the O-phase ($R > 1$) and the other part from the C-phase to the W-phase ($R < 1$), the average value of R is almost equal to 1, and the bicontinuous structure (BI) is formed.

In the amphiphilic bilayer of the lamellar liquid crystal of CTAB/*n*-$C_5H_{11}OH/H_2O$ system, on the one hand, the existence of cephanone in the amphiphilic bilayer makes the interaction energy between the C-phase and the O-phase decrease ($R < 1$), so the convex tendency of the C-phase is from the C-phase to the W-phase and the O/W structure forms; on the other hand, the cosurfactant pentanol always exists among the polar groups of surfactant molecules in the amphiphilic bilayer of the lamellar liquid crystal, which makes the interaction energy between the C-phase and the W-phase decrease ($R > 1$), so the convex tendency of the C-phase is from the C-phase to the O-phase, and the W/O structure forms. If the convex tendency from the C-phase to the O-phase ($R > 1$) and the tendency from the C-phase to the W-phase ($R < 1$) are almost equal, $R \approx 1$, the bicontinuous structure (BI) is formed. It is obvious that with the addition of cephanone the lamellar liquid crystal is destabilized and transforms to bicontinuous structure because the effect of cephanone and that of *n*-pentanol are equal.

Conclusions

1. The location of cephanone between *n*-pentanol and pyrene in the palisade of the O/W and W/O microemulsion droplets of CTAB/*n*-$C_5H_{11}OH/H_2O$ system strengthens the film intensity and the stability of O/W and W/O microemulsion droplets. Thus the solubilization amount of water in W/O microemulsion and that of *n*-$C_5H_{11}OH$ in O/W microemulsion increase, and then W/O and O/W microemulsion regions are enlarged, and even linked together.
2. The location of cephanone in lamellar liquid crystal reduces the stability of the lamellar liquid crystal, and then the structure transfers from lamellar liquid crystal phase to the bicontinuous structure, which makes lamellar liquid crystal region shrink with the addition of cephanone.

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