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ORIGINAL CONTRIBUTION

Junhong Qian
Rong Guo

Hydrolysis of cephanone in the micelles with different charges

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J. Qian · R. Guo (✉)
School of Chemistry and Chemical Engineering, Yangzhou University, 225002 Yangzhou, China
E-mail: guorong@yzu.edu.cn

Abstract The hydrolysis of cephanone in water and micelles with different charges was studied by UV-vis absorption spectroscopy. The change of pH with the hydrolysis of cephanone was determined. The mechanism of the hydrolysis and the effect of the acidity of the media on the hydrolysis were studied. The results show that the hydrolysis rate of cephanone increases with the acidity. Compared with water, SDS micelles accelerate this hydrolysis, whereas CTAB and Triton X-100 micelles suppress it. The effects of the micelles with different charges on the hydrolysis are explained by the proton concentration of the microenvironment where cephanone exists and by the charge density of the polar group of the cephanone molecules.

Keywords Cephanone · Hydrolysis · SDS · Triton X-100 · CTAB · Micelle

Introduction

Cephanone, which inhibits the synthesis of mucopeptide of cell walls of gram-positive bacterial, is a β-lactam with high medicinal value [1, 2, 3]. Unfortunately, it hydrolyses easily in acidic or basic media and loses its drug efficacy. It is an intriguing task to improve stability and inhibit the hydrolysis. Micellar solutions can affect many chemical reactions [4, 5] and in recent years there have been a lot of interest in reports about micelles as microreactors [6, 7, 8, 9, 10, 11]. In view of the hitherto unreported effect of micelles on the stability of cephanone, the hydrolysis of cephanone in micellar solutions of different surfactants was studied by UV-vis spectroscopy.

Experimental section

Materials

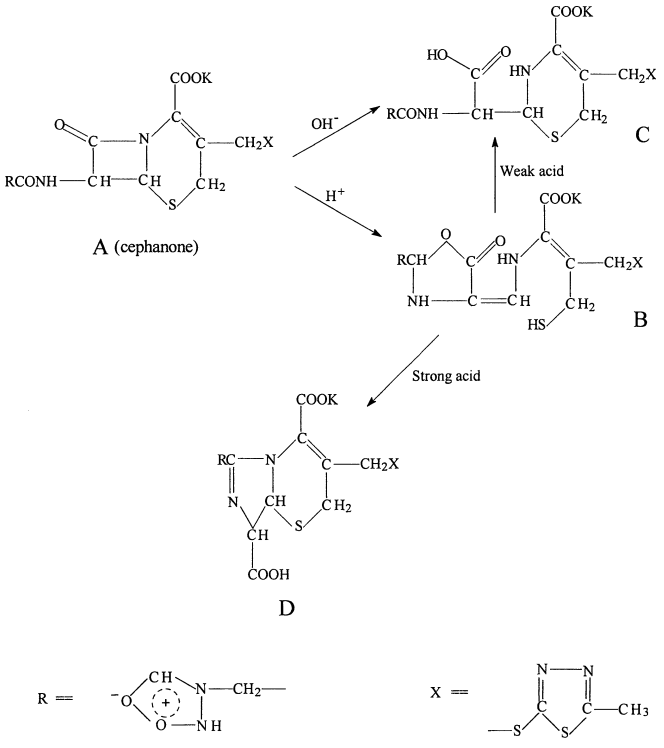
Sodium dodecylsulfate (SDS, Sigma, 98%) was recrystallised twice in ethanol. The surface tension of SDS solution had no minimum around the critical micelle concentration (cmc). Cetyl trimethylammonium bromide (CTAB, Sigma, 99%), Triton X-100 (Aldrich, 99%), cephanone (The Pharmaceutial Factory of Chongqing, 1,000,000 units) and pyrene (Aldrich, 99%) were used as received. Water was twice-distilled.

Measurement of hydrolysis

On account of the similarity between the structure of cephanone and of penicillin G (potassium salt), the mechanism of the hydrolysis of cephanone was studied according to that of penicillin G salt [12].

Cephanone contains negatively charged groups such as carbonyl and carboxyl groups (Scheme 1). These groups can be attacked by electrophilic reagents, which leads to the hydrolysis of cephanone. Protons attack the carbonyl groups at position 2 to form a carbocation that attracts the carbonyl group at position 1 to form an internal ester (product B). The hydroxide ion attacks the carbonyl at position 2 to form product C in basic media. The intermediate product B of the hydrolysis can be further hydrolysed into the final product D in strong acidic media, or into the final product C in weak acidic or basic media.

Cephanone and product C do not show absorption above 280 nm. Product D has an absorption peak around 282 nm, and the intermediate product B around 320 nm (Fig. 1). Triton X-100 and SDS both absorb around 282 nm, while the other reagents show no absorption above 280 nm. Thus, the absorbance values (A) around 320 nm and 282 nm can be used to quantitatively indicate the relative contents of products B (in water, SDS, Triton



Scheme 1 Mechanism of the hydrolysis of cephanone

X-100 and CTAB micelles) and D (in water and CTAB micelle), respectively.

The hydrolysis curves at 50 ± 0.1 °C were obtained by plotting the absorbance A versus time *t*. The concentration of cephanone was 0.1% (w). The UV-vis absorption spectra were recorded on a UV-240 ultraviolet spectrometer (Shimadzu).

Measurement of the critical micelle concentration (cmc)

The cmc of SDS and CTAB were determined by the electroconductivity method, that of Triton X-100 by surface tension measurement. The critical micelle concentration of CTAB, SDS and Triton X-100 at 50 ± 0.1 °C were 9.35×10^{-3} mol L^{-1}, 9.46×10^{-3} mol L^{-1} and 2.92×10^{-4} mol L^{-1}. The concentrations of surfactants studied in this paper were above the cmc.

Determination of pH value

The pH value containing 0.1% cephanone was determined by using a pHS-25 acidimeter (Shanghai Rex Instrument Factory) at 50 ± 0.1 °C.

Determination of distribution coefficient

The distribution coefficient K_D of cephanone between the micelles and water was determined by UV-vis spectroscopy at a cephanone concentration of 3×10^{-3} mol L^{-1}. The effect of the surfactant on the distribution coefficient was considered. K_D is obtained from [13]:

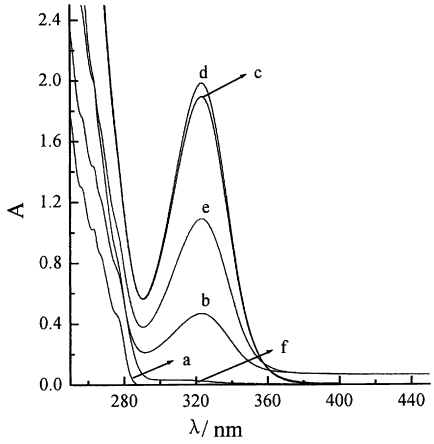


Fig. 1 The change of UV-vis spectra of 0.1%wt aqueous cephanone with time: *a* 4 min, *b* 318 min, *c* 432 min, *d* 723 min, *e* 821 min, *f* ∞

$$\frac{1}{E_\psi - E'_w} = \frac{1}{K_D(E_m - E_w)} \times \frac{1}{C_D} + \frac{1}{E_m - E_w} \quad (1)$$

where C_D is the concentration of surfactant, E_Ψ is the apparent absorbance coefficient of cephanone and E_W and E_m are the absorbance coefficients of cephanone in water and micelle, respectively.

Determination of micropolarity

The micropolarity in micelles was determined by the steady-state fluorimetry with pyrene (1.4×10^{-7} mol L^{-1}) as the probe molecule in a RF-5310PC fluorescence spectrophotometer (Shimadzu). Pyrene has five emission peaks when it is excited at 338 nm. The intensity ratio (I_1/I_3) at 373 nm to that at 384 nm indicates the polarity of the microenvironment around the probe molecule [14, 15], from which the location of cephanone in micelles is derived [16].

All solutions were deoxygenated by bubbling pure nitrogen for 15 min before measurements. The steady-state fluorescence spectra of pyrene in the micelles were determined at 50 ± 0.1 °C.

Results

Curve a in Fig. 2 shows the absorbance A of the intermediate product B in water with time *t*. The concentration of the intermediate product B remains almost constant during the initial stage (t < 150 min), then rises abruptly and reaches a maximum at about 710 min, and decreases at *t* > 710 min. PH increases slightly in the initial stage, then decreases (Fig. 2b) and reaches a minimum at about 700 min. At *t* > 700 min, it remains almost constant. Obviously, the time corresponding to the minimum pH value corresponds to the maximum concentration of the intermediate product B in water.

In acid medium (pH = 2.5) the time needed for the intermediate product B to reach the maximum concentration ($A_{max} = 0.70$) is about 11 min (Fig. 3). The

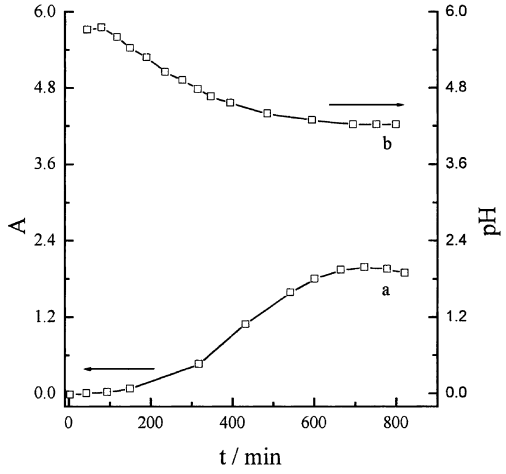


Fig. 2 Absorbance of the intermediate product B (*curve a*) and pH (*curve b*) with time in water (cephanone content 0.1%)

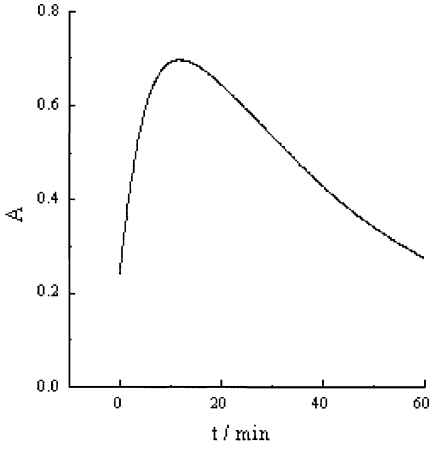


Fig. 3 Absorbance of the intermediate product B as a function of time in water at pH 2.5 (cephanone content 0.1%)

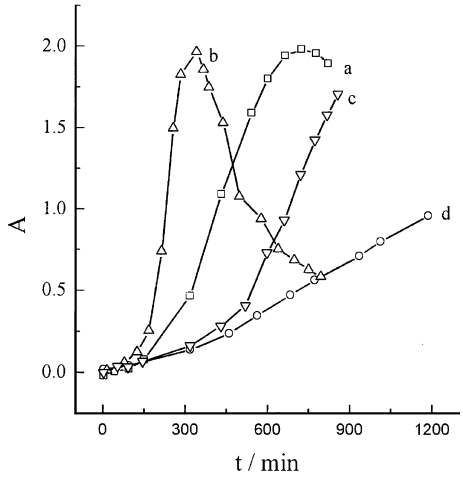


Fig. 4 Absorbance of B as a function of time in water (*a*) and micellar systems (*b–d*). Weight ratio of surfactant:H_2O = 1:99, cephanone content 0.1%. Surfactants: *b* SDS, *c* Triton X-100, d CTAB

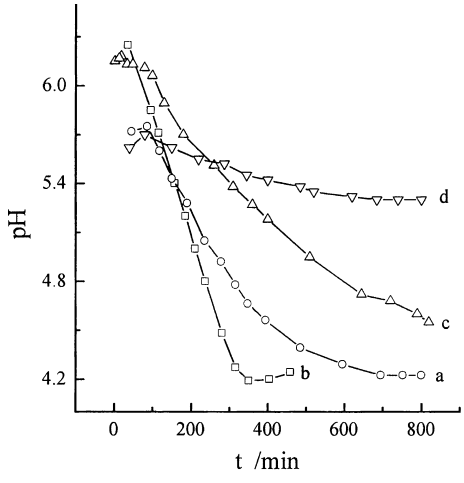


Fig. 5 pH of different systems as a function of time. Weight ratio of surfactant:H_2O = 1:99, cephanone content 0.1%. *a* Water, *b* SDS solution, *c* Triton X-100 solution, *d* CTAB solution

concentration decreases faster than that in water (Fig. 2a). Obviously, the time for the intermediate product B to reach its maximum decreases with the increasing H^+ concentration, indicating that acidic surroundings can catalyse the hydrolysis of cephanone into B and from B to D.

The above results show that:

1. The hydrolysis of cephanone is acid-catalysed
2. The hydrolysis of cephanone in water is a self-catalysed reaction, where formation of B reduces the pH of the system, which in turn affects the formation rate of B

The concentration of B changes little within the first 100 min then it increases to a sharp maximum for water and SDS solutions (Fig. 4). The formation rates of B within the first 100 min are similar in water, CTAB and Triton X-100 solutions, but it is somewhat higher in the presence of SDS micelles. In Triton X-100 and CTAB micelle systems, the concentration of B increases steadily within the experiment time. The formation rates in Triton X-100 and CTAB micellar solutions are slower than those in water and SDS solutions, and it is slowest in CTAB micellar solutions. Thus, SDS micelle can promote while CTAB and Triton X-100 micelles can inhibit the hydrolysis of cephanone.

The pH value first increases and then decreases rapidly to the minimum at about 700 min in water (Fig. 5a). The decrease is steepest in SDS solutions, and the pH value reaches the minimum at about 350 min (curve b). The time for pH to reach the minimum again corresponds to the maximum concentration of B in water and SDS solutions (Fig. 4a, b). In Triton X-100 micellar

systems, pH changes little within the first 100 min, then decreases at a lower rate than in water (Fig. 5c). In CTAB micelle system, the pH value first increases to its maximum and then decreases slowly (Fig. 5d). Obviously, the pH do not show minima in CTAB and Triton X-100 micellar systems, corresponding to the observation that the concentration of B did not reach a maximum within the experiment time. Figure 5 also shows that the H^+ concentration in each system is fairly low within 100 min, so that the hydrolysis rate of cephanone did not change within 100 min.

The concentration of B in water is distinctly less than that in CTAB micellar system at the same pH (Fig. 6), but it can be seen from Fig. 7 that the formation rate of the final product D in water at pH 2.5 is higher. These results reveal that B may be located in the CTAB micelles and the interaction between B and the continuous water phase is reduced, so that the concentration of D is smaller in CTAB solutions than in water. The results further indicate that in strong acidic media, CTAB inhibits the hydrolysis of cephanone, because the stability of B is enhanced, and the formation rate from B to D is reduced. In Triton X-100 and SDS micellar systems, the concentration of D could not be determined because both Triton X-100 and SDS absorb around 282 nm.

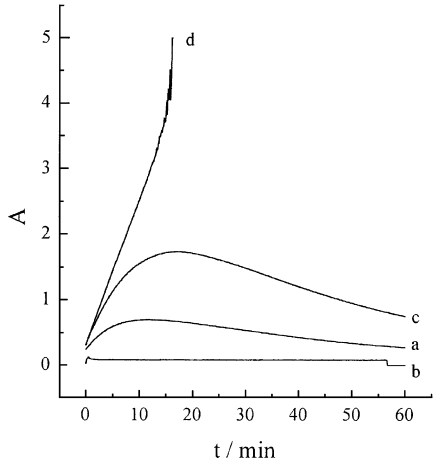


Fig. 6 Absorbance of B as a function of time. Weight ratio of surfactant:H_2O = 1:99, cephanone content 0.1%, pH 2.5. *a* Water, *b* SDS solution, *c* Triton X-100 solution, *d* CTAB solution

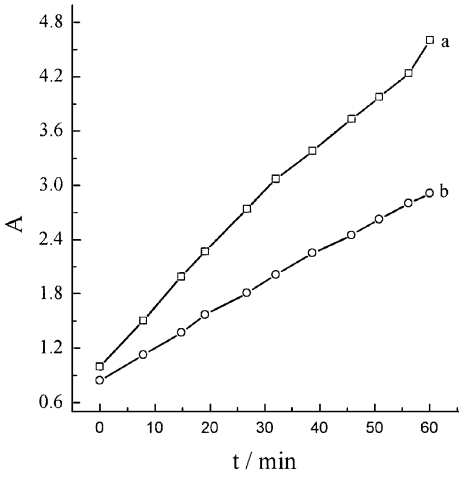


Fig. 7 Absorbance of the final product D as a function of time (cephanone content 0.1%, pH 2.5). *a* Water, *b* CTAB micellar solution (CTAB content 1%)

Discussion

PH-dependent hydrolysis of cephanone

Cephanone is hydrolysed in water by protons. Thus, in the initial stage of hydrolysis of cephanone, pH increases (Fig. 2). With the increasing concentration of the intermediate product B, pH decreases because of the dissociation of the -SH group of B. After the pH decrease to the minimum ($t > 700$ min), B begins to hydrolyse into final product C (Scheme 1), which decreases pH. On the other hand, the decreasing concentration of B increases pH. The almost constant pH value at $t > 700$ min in water indicates that the formation of B and the transformation into C are in equilibrium (Fig. 2).

Effects of micelles on the hydrolysis of cephanone

The hydrolysis of cephanone in micellar systems may be represented as [11, 12]:

$$\begin{array}{ccc} \mathrm{M} + \mathrm{S} & \overset{k}{\rightleftharpoons} & \mathrm{MS} \\ k_0 \searrow & & \swarrow k_\mathrm{m} \\ & \mathrm{B} & \end{array} \qquad (2)$$

where M is the micelle, S cephanone, MS the micelle-cephanone complex and B the intermediate product. k_0 and k_m are the rate constants for the hydrolysis of uncomplexed and complexed cephanone; k is the equilibrium constant for the formation of the micelle-cephanone complex. Assuming k_0 is constant, the apparent reaction constant k_a is only related to k_m.

As shown above, micelles with different charges influence the hydrolysis of cephanone in different ways. Cephanone is ionised into cephanone$^-$ and K^+ in aqueous solution and its polar groups (carboxyl, carbonyl and amino groups) are attacked by protons, which leads to the rupture of β-lactam ring (Scheme 1). Because pH in these studies was < 7 and the variation patterns of the pH value were similar, the mechanisms of the hydrolysis of cephanone should be the same. It follows that the different hydrolysis reactions in different systems are related to the charges of the

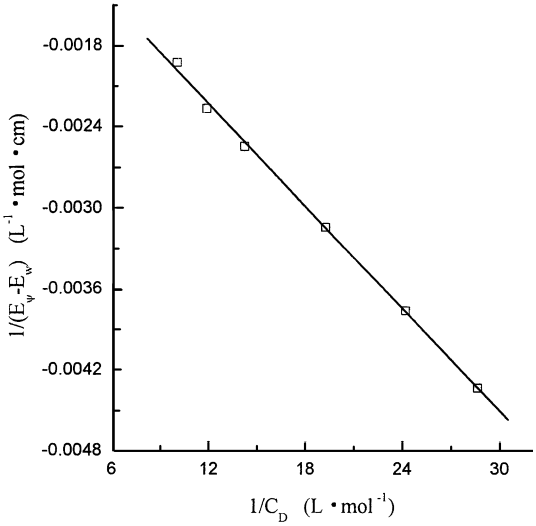


Fig. 8 Plot of $(E_{\psi}-E_{w})^{-1}$ versus $c_{SDS}{}^{-1}$

surfactants and the interaction between cephanone and surfactants.

In the SDS micelles, the -O-SO_3^- groups repel the cephanone anions, which leads to the higher negative charge density of cephanone anions and enhances the hydrolysis of cephanone. The distribution coefficient of cephanone between SDS micellar solutions and water (Eq. 1, Fig. 8) is 7.1. Table 1 shows that cephanone can be adsorbed at the surface of SDS micelles, which increase the contact of cephanone molecules with H^+ adsorbed at the surface of SDS micelles. Thus, the hydrolysis of cephanone is enhanced in the SDS micellar solutions.

There are three factors affecting the microenvironment of cephanone in Triton X-100 micelles. Firstly, the hydrogen bond between the amino group in cephanone and the oxygen of oxyethylene chain in Triton X-100 enables cephanone to be solubilised in the interphase of the micelles, which decreases the contact of cephanone with water and enhances the stability of cephanone. Secondly, the surface of the micelles can adsorb protons by hydrogen bonds, which decreases the hydrolysis rate of cephanone. Thirdly, the higher proton concentration at the surface of the micelles increases the hydrolysis rate of cephanone adsorbed at the surface of the micelles. The latter two effects on the hydrolysis of cephanone are opposite and almost cancel each other out. Therefore, the hydrolysis of cephanone is inhibited due to the location of cephanone in the interphase of the micelles. The distribution coefficient of cephanone between Triton X-100 micelles and water is 23.6 (Table 1), which shows that the uptake of cephanone molecules in Triton X-100 micelles is stronger than in SDS micelles. Therefore, the hydrolysis rate of cephanone in Triton X-100 solutions is lower than that in SDS solutions (Fig. 4).

In CTAB solutions, the electrostatic interaction causes cephanone to be located in the interior of the CTAB micelles, and the hydrolysis rate of cephanone decreases. The distribution coefficient of cephanone between CTAB micelles and the aqueous phase is 187 (Table 1) and much higher than in the other systems and in agreement with the lower hydrolysis rate compared to the other systems (Fig. 4).

The location of cephanone in the micelles can be verified by the determination of the micropolarity of the micelles with pyrene as the probe molecule [16] (Table 2). When 1% cephanone or 2% cephanone are added to the CTAB solutions, the I_1/I_3 value decreases from 1.349 to 1.323 and 1.305, respectively. In Triton X-100 solutions, the addition of cephanone decreases I_1/I_3 1.487 to 1.474 (cephanone 1%) and 1.462 (cephanone 2%). In SDS micellar solutions the intensity ratio remains almost unchanged. This also reveals that cephanone is not solubilised in SDS micelles due to the electrostatic repulsion between cephanone molecules and SDS.

Effects of micelles on the pH value

Figure 5 also shows similar trends of pH but the absolute pH values in water, SDS, Triton X-100 and CTAB micellar systems are different (5.7, 6.2, 6.1 and 5.6). It is obvious that the surface of the micelles of SDS adsorbs protons. The surface of the micelles of the non-ionic surfactant Triton X-100 can also adsorb protons by hydrogen bonds, which increases the solution pH. However, the effect of hydrogen bonds is weaker than

Table 1 Distribution coefficient and Gibbs free energy of cephanone in different surfactant micelles (50 ± 0.1 °C)

Micelle	K_D	ΔG (kJ mol^{-1})
SDS	7.1	−5.27
Triton X-100	23.6	−7.18
CTAB	187.5	−13.47

Table 2 I_1/I_3 value of pyrene in different system (at 50 ± 0.1 °C)

Content (wt%)				I_1/I_3
Surfactant		H_2O	Cephanone	
SDS	1.0	99.0		1.107
	1.0	98.0	1.0	1.103
	1.0	97.0	2.0	1.098
Triton X-100	1.0	99.0		1.487
	1.0	98.0	1.0	1.474
	1.0	97.0	2.0	1.462
CTAB	1.0	99.0		1.349
	1.0	98.0	1.0	1.323
	1.0	97.0	2.0	1.305

the electrostatic interaction so that the pH in Triton X-100 solutions is slightly lower than in SDS solutions. The surface of the micelles of the cationic surfactant CTAB adsorbs OH^- ions, which decreases the pH.

Conclusions

The location of cephanone in the interior of CTAB and Triton X-100 micelles enhances the stability of cephanone and inhibits the hydrolysis of cephanone. However, the hydrolysis of cephanone is increased in SDS micellar solutions due to the electrostatic repulsion between cephanone anions and SDS and the increased proton concentration at the surface of the SDS micelles.

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