# Catalytic effect of cationic surfactants on degradation of cephalexin in aqueous solution

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Penicillins and cephalosporins have been known to undergo remarkably facile cleavage of their β-lactam bonds. Recently, Yamana & Tsuji (1976) reported the kinetics of the hydrolysis of six therapeutically useful cephalosporins. They showed the pH-rate profile of the degradation of cephalexin. Cephalexin is fairly acid stable and much more susceptible to hydroxide-ion catalysed degradation. At neutral pH, its degradation involves intramolecular-nucleophilic attack of the side-chain α-amino group on the β-lactam carbonyls. On the other hand, vigorous interest has been shown in the kinetics and mechanisms of organic reactions which occur in the presence of surface-active agents (Fendler & Fendler, 1970). The interactions between the substrate and the specifically oriented hydrophobic and hydrophilic parts of the micelle are chiefly responsible for the spectacular rate enhancements or inhibitions exhibited by micelles on organic reactions.

We found that the degradation of cephalexin was subject to marked catalysis by hexadecyltrimethylammonium bromide (CTAB). We have investigated some of the characteristics of the degradation of cephalexin in the presence of CTAB.

Hydrolysis of cephalexin was at 37° and ionic strength 0·25 by using appropriate buffer systems. The rate of hydrolysis was determined by iodometric titration (Finholt, Jürgensen & Kristiansen, 1965) and/or by measuring the loss of the characteristic ultraviolet absorbance at 261 nm due to the β-lactam bond. Good first order plots were obtained and the pseudo-first-order rate constants were determined.

The pseudo-first-order rate constant for the degradation of cephalexin in the absence of surfactant, $k_0$, was 0·0450 $h^{-1}$ at pH 6·5. Its range of concentrations was 0·05–10 mM and in this range the observed rate constants did not vary, within the limits of experimental error, as a function of substrate concentration. The observed rate constants, $k_\psi$, for the degradation of cephalexin at pH 6·5 in the presence of various additives are shown in Table 1. In the presence of cationic surfactants such as CTAB and benzalkonium chloride above their critical micelle concentrations (cmc), the degradation of cephalexin increased by a factor of about 9 to 14. On the contrary, in the presence of anionic surfactant, sodium lauryl sulphate (SLS), the degradation of cephalexin was slightly protected. Positively charged tetraethylammonium chloride did not alter the degradation of cephalexin. Consequently, it appears that the enhancement of the degradation of cephalexin is a specific effect of cationic micelles.

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Table 1. *Effect of various additives on the stability of cephalexin at pH* 6·5, 37° *and* $\mu$ = 0·25.

| Additives | $k_\psi$ ($h^{-1}$) |
|---|---|
| None | 0·0450 |
| Hexadecyltrimethylammonium bromide (CTAB) (20 mM) | 0·6482 |
| Benzalkonium chloride (1%) | 0·4188 |
| Sodium lauryl sulphate (20 mM) | 0·0317 |
| Tetraethylammonium chloride (20 mM) | 0·0464 |

Spectrophotometrically determined. The concentration of cephalexin was 0·1 mM except for benzalkonium chloride (5 mM).

The catalytic effects of buffers on the degradation of penicillins and cephalosporins have been reported (Finholt & others, 1965; Yamana & Tsuji, 1976). The degradation of cephalexin was measured at constant pH, constant ionic strength, varying only the buffer concentration. Plots of the observed rate constants *vs* the total buffer concentration gave a reasonably straight line at constant pH. Extrapolation of such plots to zero buffer concentration provides, as intercepts, $k_{pH}$, corresponding to the non buffer-catalysed degradation of cephalexin. The $k_{pH}$ values at pH 4·0, 6·5 and 10·0 were 0·0033, 0·0051 and 0·0721 $h^{-1}$ in the absence of CTAB, and 0·0051, 0·0234 and 0·3584 $h^{-1}$ in the presence of 20 mM CTAB, respectively. Consequently, micellar catalysis of the degradation of cephalexin could occur in the absence of buffers. In addition, the presence of CTAB micelles enhances the catalytic effect of a buffer on the degradation of cephalexin. The micellar effects increased at higher pH values. Cephalexin is an amphoteric cephalosporin and has three ionic species. The percent of total concentration of each ionic species has been calculated as a function of pH by Purich, Colaizzi & Poust (1973). At pH 6·5, cephalexin exists essentially as a zwitterion, whereas, at lower or higher pH values, it exists mainly as a cation or an anion respectively. The increase of the micellar effect at higher pH values can be rationalized considering the interactions between the drug and positively charged CTAB micelles.

The apparent heats of activation for cephalexin degradation were determined following the Arrhenius-type plots at temperature ranging from 30 to 50°. The calculated heats of activation were 18·9 kcal $mol^{-1}$ (79 kJ $mol^{-1}$) in the absence of CTAB and 17·9 kcal $mol^{-1}$ (75 kJ $mol^{-1}$) in the presence CTAB. A little decrease in the activation energy by the addition of CTAB was observed.

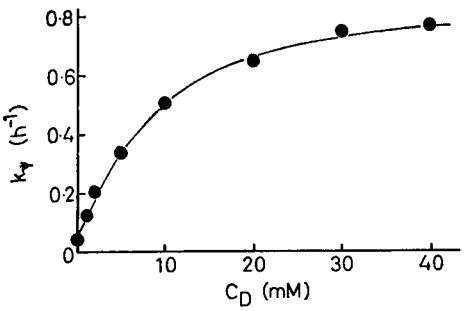


FIG. 1. Plot of the observed pseudo-first-order rate constant, $k_\psi$ ($h^{-1}$), against the CTAB concentration, $C_D$ (mM) for the degradation of cephalexin at pH 6·5, 37° and $\mu = 0{\cdot}25$. The initial concentration of cephalexin was 0·1 mM. Spectrophotometrically determined.

In Fig. 1, the observed rate constant, $k_\psi$, for the degradation of cephalexin is plotted against the concentration of CTAB. The rate constant first increased rapidly and then gradually approached to a constant value as a function of CTAB concentration. The cmc of CTAB, 0·087 mM, was obtained by measuring the surface tensions of CTAB solutions at pH 6·5, 25° and $\mu = 0{\cdot}25$. Therefore, the CTAB concentrations shown in Fig. 1 are greater than the cmc.

By making a number of assumptions and simplifications, the kinetic form of micellar catalysis has been successfully treated in terms of micelle (M)—substrate (S) complex formation and reaction in the aqueous and micelle phase (eqn 1),

$$\begin{array}{ccc} & \overset{K}{} & \\ M + S & \rightleftarrows & MS \\ & \downarrow k_o \quad \downarrow k_m & \\ & P \quad P & \end{array} \qquad \ldots \quad \ldots \quad (1)$$

where $k_o$ and $k_m$ are the rate constants for product formation in the bulk solvent and in the micellar phase, respectively, and K is the micelle-substrate binding constant (Fendler & Fendler, 1970). The observed rate constant, $k_\psi$, for product formation is given by equation 2.

$$k_\psi = \frac{k_o + k_m K[M]}{1 + K[M]} \qquad \ldots \quad \ldots \quad (2)$$

which can be rearranged to give equation (3)

$$\frac{1}{k_o - k_\psi} = \frac{1}{k_o - k_m} + \left(\frac{1}{k_o - k_m}\right)\left(\frac{1}{C_D - cmc}\right)\frac{N}{K} \qquad (3)$$

using the relation of equation (4)

$$[M] = \frac{C_D - cmc}{N} \qquad \ldots \quad \ldots \quad (4)$$

where $C_D$ is the total concentration of the surfactant and N is the aggregation number. A plot of the left-hand side of equation 3 *vs* $1/(C_D - cmc)$ should result in a straight line, from which $k_m$ and K/N can be calculated. As expected, a good straight line was obtained by plotting the data shown in Fig. 1 according to equation 3 ($r = 0{\cdot}9993$). The fit of experimental data to this equation indicates that this micellar catalysis obeys Michaelis-Menten (saturation) kinetics. From the intercept, $k_m$ is calculated to be 0·9706 $h^{-1}$. This value means that a rate augumentation of more than 20-fold occurs in the micellar phase. On the other hand, K/N is calculated to be $1{\cdot}00 \times 10^2$ $M^{-1}$ from the slope of the straight line.

The rate of micellar catalysed reaction is influenced by the ionic strength of the medium (Fendler & Fendler, 1970). The rate constants for the degradation of cephalexin were evaluated as a function of ionic strength ($\mu = 0{\cdot}25-2{\cdot}0$) by addition of sodium chloride at pH 6·5. In the absence of CTAB, the reaction rates were not influenced by the ionic strength change. But in the presence of 20 mM CTAB, the reaction rates were markedly suppressed with increasing salt concentration. At $\mu = 2{\cdot}0$, $k_\psi$ was 0·0768 $h^{-1}$, which was near to the non-micellar value (0·0450 $h^{-1}$). The salt effect in the micellar system primarily arises from competitive occupation of the counterion site. The inhibition of micellar catalysis has been treated similarly in terms of micelle-substrate complex formation and reaction in the aqueous and micellar phase by making the additional assumptions that the interaction of the substrate, S, with the micelles does not affect their formation or their interaction with the inhibitor, I, and that inhibition is competitive (eqn 5).

$$\begin{array}{ccc} & \overset{K}{} & \\ M + S & \rightleftarrows & MS \\ I \downarrow\uparrow K_I & \downarrow k_o & \downarrow k_m \\ MI & P & P \end{array} \qquad \ldots \quad \ldots \quad \ldots \quad (5)$$

The observed rate constant, $k_\psi$, in the presence of inhibitor is given by equation 6.

$$k_\psi = \frac{k_o + k_o K_I[I] + k_m K[M]}{1 + K[M] + K_I[I]} \qquad \ldots \quad \ldots \quad (6)$$

Combination of equations 4 and 6 gives equation 7 (Fendler & Fendler, 1970).

$$\frac{k_m - k_o}{k_\psi - k_o} = 1 + \frac{N}{K(C_D - cmc)} + \frac{K_I[I]N}{K(C_D - cmc)} \qquad (7)$$

A plot of the left-hand side of equation 7 *vs* inhibitor concentration should result in a straight line. When the concentration of added sodium chloride was given as [I], a straight line was obtained ($r = 0{\cdot}9985$) and the inhibitor constant, $K_I$, was calculated to be 31·5 $M^{-1}$. The linearity of the plot justifies the assumption that the inhibition is competitive and that incorporation of an inhibitor molecule in a micelle prevents incorporation of the substrate.

Considering the competitive occupation of the

counterion site in the micellar systems, the substrate, itself, could compete at the binding site. To examine this possibility, the effect of initial concentration of cephalexin was investigated in the presence of 20 mM CTAB at pH 6·5. The observed rate constant, $k_\psi$, was found to be decreased as the initial concentration of cephalexin increased. At its initial concentrations of 0·1, 1·0, 2·0, 5·0 and 10·0 mM, $k_\psi$ were 0·6482, 0·6254, 0·5620, 0·4951 and 0·3905 $h^{-1}$, respectively. Using equation 7, giving the initial concentration of cephalexin as [I], a good straight line was obtained ($r = 0·9973$). This indicates that the substrate molecules are competitively occupying the binding site of CTAB micelles.

These observations demonstrate that the degradation of cephalexin is specifically accelerated by cationic micelles and the catalysis is explicable in terms of the interactions between cephalexin and the micelles. Many factors undoubtedly contribute to the catalysis. The dependence of the extent of micellar catalysis on pH reflects the importance of electrostatic interactions.

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# The polymorphism of acetohexamide

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Analytical studies made in recent years on the oral hypoglycaemic compound acetohexamide do not report any incidence of polymorphism, although this phenomenon is known to occur with other sulphonylureas, and both tolbutamide (Kuhnert-Brandstätter & Wunsch, 1969; Kuhnert-Brandstätter & Bachleitner-Hofmann, 1971; Simmons, Ranz & others, 1972; Burger, 1975b) and chlorpropamide (Burger, 1975a) have been shown to exist in different polymorphic forms. No significant differences have been reported in the biological activities of these forms, but the *in vitro* availability of chlorpropamide from tablets has been shown to be affected by the type of polymorph used in the tablet preparation (Burger, 1976a, b). This paper presents evidence to show that acetohexamide can also exist in at least two polymorphic forms that can easily be detected by infrared spectroscopy.

Acetohexamide was kindly supplied by Eli Lilly and Company Ltd. A sample of acetohexamide U.S.P. reference standard was also obtained. Infrared spectra were recorded from potassium bromide discs using a Perkin-Elmer Model 357 grating spectrometer. Nmr spectra were recorded with a Perkin-Elmer R32 (90 MHz) spectrometer. Differential scanning calorimetry was carried out with a Perkin-Elmer DSC-1B apparatus using pans with crimped aluminium covers and a heating rate of 4° $min^{-1}$. For the determination of melting points, samples were inserted into an Electrothermal melting point apparatus at a temperature of 170°.

*Preparation of polymorph A.* Acetohexamide (1 g polymorph A or B) was dissolved in a minimum volume of glacial acetic acid (about 30 ml) by heating on a boiling water bath, and the solution allowed to crystallize at room temperature. The crystals were washed well with cold water, and dried at 105°, m.p. 180–183° dec.

*Preparation of polymorph B.* Acetohexamide (1 g polymorph A or B) was dissolved in chloroform (30 ml) by heating on a boiling water bath, and the solution left to crystallize at room temperature. The crystals obtained were dried at 105°, m.p. 183–185° dec.

The nmr spectra of the two polymorphic forms of acetohexamide were determined in trifluoroacetic acid and found to be identical showing therefore that the two species were chemically the same.

Infrared spectroscopy revealed a number of differences by which the polymorphs could easily be identified. The spectra recorded were unchanged when the re-crystallized forms A or B were dried *in vacuo* at 60° instead of at 105° at atmospheric pressure. The main distinctions between the forms were as follows:

1. The infrared spectrum of polymorph A (Fig. 1) shows a strong well-resolved doublet with maxima at 3310 and 3240 $cm^{-1}$, and a second medium intensity doublet at 2940 (with a shoulder at 2920) and 2850 $cm^{-1}$.
2. In the infrared spectrum of polymorph B (Fig. 2), the first doublet is replaced by a single strong peak at 3360 $cm^{-1}$, and the second appears as a medium intensity band at 2940 $cm^{-1}$ with shoulders at 2890 and 2850 $cm^{-1}$.
3. Between these two bands, polymorph B shows a broad medium intensity band at 3095 $cm^{-1}$ with a shoulder at 3150 $cm^{-1}$. This band is almost absent from the spectrum of polymorph A.
4. Form A produces a single strong band at 1685 $cm^{-1}$;

* Correspondence.