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Effect of Self-Association on Rate of Penicillin G Degradation in Concentrated Aqueous Solutions

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Abstract □ The apparent rate of degradation of penicillin G potassium micellar solutions of 500,000 units/ml, a concentration commonly encountered in vials reconstituted for storage in the refrigerator, was investigated and compared to that of nonmicellar solutions of 8000 units/ml at 25°, ionic strength of 1.1 *M*, and pH range from 5.0 to 9.5. In the micellar solutions the apparent rate of the H^+-catalyzed degradation was increased twofold but that of water- and OH^--catalyzed hydrolysis was decreased two- to threefold. Consequently, the pH–rate profile of the micellar solutions was shifted to higher pH values and the pH of minimum degradation was found to be at 7.0 compared to 6.5 for the nonmicellar solution of the same ionic strength. Compared at their respective pH–rate profile minima, micellar penicillin G is 2.5 times as stable as the nonmicellar solution under the conditions of constant pH and ionic strength.

Keyphrases □ Penicillin G potassium degradation—concentrated aqueous micellar solutions compared to nonmicellar solutions, pH–rate profiles □ Degradation of penicillin G potassium—concentrated micellar aqueous solutions compared to nonmicellar solutions, pH–rate profiles □ Micellar solutions of penicillin G—degradation compared to nonmicellar solutions, pH–rate profiles

The kinetics of penicillin G degradation in aqueous solution have been studied extensively. The effects of pH, temperature, buffers, ionic strength, metal ions, and model catalysts that simulate penicillinase and other enzymes have been investigated (1–6). The effect of surfactants below and above their critical micelle concentration (CMC) was also studied, with the finding that at pH 6.5 all surfactants studied enhanced the rate of penicillin G degradation (7).

The present study was undertaken to assess the kinetics of penicillin G degradation at concentrations above the CMC, particularly at 500,000 units/ml as encountered in hospital usage, and to obtain a comparison of the pH of minimum degradation for solutions above and below the CMC. Instead of buffer, the pH-stat technique was utilized to maintain a constant pH in these studies. The dilution obtained in all studies was negligible due to the very high concentration of titrant (15 *M* KOH) used.

BACKGROUND

In the earlier studies (1–7), relatively low concentrations of penicillin G were used (8000 units/ml or 0.5% w/v). Literature pertaining to the kinetics of penicillin G degradation at high concentration is limited to observations of the percent degraded after a certain time under specific conditions. Although several results tend to indicate that penicillin G degrades faster at higher concentration, no definitive comparison between the degradation rates at the high and low concentrations can be made because the solutions studied were either unbuffered (8, 9) or inadequately and not proportionally buffered (10, 11). Inadequate buffering of concentrated solutions of penicillin G could give an inordinately large apparent rate of degradation due to a marked pH decrease from the production of large quantities of penicilloic acid.

It has been common practice, particularly in hospitals, to batch-reconstitute buffered penicillin G solutions at a concentration of 500,000 units/ml (30% w/v) and store them frozen until needed. Periodically, the frozen solutions are moved into the refrigerator and kept in a liquid state ready to be diluted with intravenous infusion fluid and administered to patients. According to the manufacturer, sterile solutions in the refrigerator may be kept for 1 week without significant loss of potency. Knowledge of the pH of minimum degradation at this high concentration becomes essential.

The formation of micelles of penicillin G in aqueous solution was first observed by Hauser *et al.* (12). Later studies (13, 14) found the CMC to be relatively high, *i.e.*, 0.25 molal, 130,000 units/ml, or 8.26% (w/v). This value was further confirmed using the data of the NMR (100 MHz) chemical shift of the aromatic protons (15). It was indicated that the number of penicillin G ions that come together to form the aggregates is probably not very large.

In recent years, reactions in micellar systems have been increasingly investigated. Extensive reviews of the kinetic and mechanistic implications of micellar catalysis have been presented (16, 17). From the drug product stability point of view, micellization is important because it can either increase or decrease the stability of a drug, depending on the nature of the micelle and the reaction involved. Thus, catalytic effects of micelles have been observed when both reactants are enriched within the micelles for a bimolecular process. A study (18) of the accelerated fading of cationic triphenylmethane dyes in alkaline solutions of the quaternary surfactant cetyltrimethylammonium bromide has become classic. The micelle itself can serve as a substrate for the reaction, *e.g.*, micellization enhances the rate of H^+-catalyzed hydrolysis of sodium lauryl sulfate by 30-fold over that in a single ion state (19). Conversely,

when one reactant is enriched and the other reactant is excluded from the micelle, a rate decrease in the micellar phase is observed. Thus, sodium lauryl sulfate has been shown to inhibit the OH^--catalyzed hydrolysis of benzocaine (20).

EXPERIMENTAL

Materials—Penicillin G potassium, 1595 units/mg[1], was used. All reagents were of analytical grade. Imidazole[2] was recrystallized twice from benzene and washed with anhydrous ether. Double-distilled water[3] was used in all studies.

Analytical Method—The intact penicillin G concentration was determined according to a spectrophotometric[4] method (21). Molar absorptivity at 325 nm, determined from the standard curve, was 26.3×10^3 which is in excellent agreement with that previously reported (21).

Kinetic Studies—The pH of the penicillin G potassium solution was maintained constant at the desired value with the aid of pH-stat[5]. A 50-ml reaction vessel (V520) was immersed in the thermostated jacket (V525), through which water of 25° was circulated from a constant-temperature bath and circulator[6] (sensitivity ±0.1°). Inserted into the reaction vessel was a glass electrode (G 202C), a saturated calomel reference electrode (K 401), a stirrer, and a delivery tube for titrant (15 *M* KOH). The proportional band of the titrator was set to provide small incremental additions of titrant.

Penicillin G potassium solutions above the CMC were prepared at a concentration of 30% (w/v) in solutions of 0.3 *M* potassium chloride so that the final solutions had an ionic strength of 1.1 *M*. At selected time intervals, 40 μl of sample was withdrawn with a microsyringe[7] and diluted with water to 100 ml in a volumetric flask. Two 1-ml samples of the diluted solution were then pipetted into two separate 10-ml test tubes, and each was assayed separately according to the method described by Bundgaard and Ilver (21).

Solutions of penicillin G potassium below the CMC were prepared at a concentration of 0.5% (w/v) in solutions of 1.1 *M* potassium chloride. The same procedure was followed, except that 1 ml of sample was withdrawn and diluted with water to 50 ml in a volumetric flask.

In both micellar and nonmicellar solutions, the initial rate was determined by following 5–10% of the entire course of the reactions. The observed pseudo-first-order rate constants were then calculated by linear regression analysis of a plot of the logarithm of penicillin G concentration against time.

RESULTS AND DISCUSSION

The concentration of micellar penicillin G used was very high (500,000 units/ml, 0.8 *M*, or 30% w/v). It would not be possible to keep the pH constant using buffer systems, because a very high buffer capacity would be required. Many buffer systems are known to have marked catalytic effects so that a high buffer concentration is not desirable. Although citrate buffer has little catalytic effect at neutral pH (3), the buffer capacity is quite low under these conditions. Large citrate concentrations increase the ionic strength of the solution, which, in turn, enhances the rate of the OH^--catalyzed reaction (3) as well as that at the pH of minimum degradation. Therefore, the concentration of citrate buffer found in commercial products is limited to 1.5% (w/v). The present study was conducted at a constant ionic strength of 1.1 *M* to take into account the contributions of 30% penicillin G potassium and 1.5% citrate buffer. The change in ionic strength due to counterion binding to the penicillin G micelle was assumed to be negligible for these apparently small aggregates.

The results of kinetic studies in nonmicellar and micellar solutions of penicillin G are recorded in Table I. The rates at pH 5 and 5.5 were increased and those at pH 8.5 and 9.0 were decreased twofold in micellar systems relative to nonmicellar solutions.

Table I—Effect of Concentration on the Magnitude of the Observed Pseudo-First-Order Rate Constants and the Corresponding t_{90} at 25° and Ionic Strength of 1.1 *M*

	Nonmicellar (0.5% w/v)		Micellar (30% w/v)	
pH	$10^3 k_{obs}$, hr^{-1}	t_{90}, hr	$10^3 k_{obs}$, hr^{-1}	t_{90}, hr
5.0	10.2	10.3	18.8	5.6
	9.02	11.7	18.2	5.8
5.5	2.88	36.6	6.22	17.0
	—	—	7.57	13.9
6.0	1.49	70.8	2.25	46.9
6.5	1.16	90.8	0.760	138.8
7.0	1.41	74.8	0.466	226.4
	—	—	0.443	238.1
7.5	—	—	0.669	157.7
8.0	2.52	41.9	1.03	102.4
8.5	3.87	27.3	1.77	59.6
	4.49	23.5	2.04	51.7
9.0	11.4	9.2	5.46	19.3
9.5	—	—	16.4	6.4
	—	—	18.7	5.6

Penicillin G is an acid with a pKa of 2.80. The degradation is known to be first order with respect to penicillin G and is catalyzed by acid and base. The pH–rate profile is linear in the pH region of 4–6 and above 8 and has a minimum at pH 6.50–6.75. Above pH 5, penicillin G exists mostly in the anionic form. In the pH region investigated, the principal reactions in both nonmicellar and micellar states are attacks of H^+, water, and OH^- on penicillin G anion. It is also conceivable that in the micelle the molecules could be aligned to facilitate intramolecular or intermolecular attack on the β-lactam carbonyl carbon by the amide carbonyl oxygen (22). The acid-catalyzed reaction of undissociated penicillin G was not significant under the study conditions.

According to Thakkar and Wilham (15), penicillin G ions aggregate primarily by hydrophobic interactions involving the benzyl side chains. The ionized carboxylate groups on the thiazolidine ring form the periphery of the micelle in intimate contact with the surrounding water. Therefore, the micelles formed are of the anionic type. The greater negative charge density of the micelles would exert stronger electrostatic attraction toward hydrogen and sodium ions to be bound as counterions. This would facilitate isomerization to penicillenic acid, since it is known that the formation of penicillenic acid is a result of the reaction of penicillin G ion with hydrogen ions or the kinetically equivalent spontaneous rearrangement of undissociated penicillin (3, 23).

The association between penicillin G ion and hydrogen ions to form the reactive species, undissociated penicillin G, might also be favored by a larger positive entropy change for the micellar reaction. This can be explained in terms of hydration and electrostatic entropy changes. In terms of hydration of ions, the explanation of the entropy factor is that the two oppositely charged ions coming together to form a neutral molecule become partially dehydrated and cause breakdown of the "iceberg" structure of coordinated water molecules around the ions, leading to a decreased orientation of solvent molecules or an increase of entropy (24). The larger charge density of the micelle causes the formation of a more ordered structure, consisting of water molecules and counterions about its outer, polar surface. Thus, there would be a greater overall change in randomness in micellar systems, since the number of electrostatically bound water molecules released in the formation of neutral undissociated penicillin G would be greater for the micelle state than for the single ion state.

In terms of electrostatic theory, the entropy change resulting solely from bringing two ions from infinite separation to the equilibrium distance r is given by (25):

$$\Delta S_{el} = \frac{Z_A Z_B e^2}{Dr}\left(\frac{\partial \ln D}{\partial T}\right)_p \qquad \text{(Eq. 1)}$$

where Z_A and Z_B are the charges of the two reacting ions, and D is the dielectric constant of the medium. The quantity $(\partial \ln D/\partial T)$ is always negative, because thermal motion overcomes the orienta-

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Eastman Kodak Co., Rochester, N.Y.
[3] Corning AG-3 distillator, Corning Glass Works, Parkersburg, W.Va.
[4] Gilford 240 spectrophotometer, Gilford Instrument Labs., Monrovia, Calif.
[5] Radiometer A/S, Copenhagen, Denmark, comprised of a TTT2 automatic titrator, a TTA3 titration assembly, an SBR2c titrigraph, and an ABU11 autoburet (0.25 ml).
[6] Haake F-Junior, Haake Instruments, Rochelle Park, N.J.
[7] Drummond Scientific Co., Broomall, Pa.

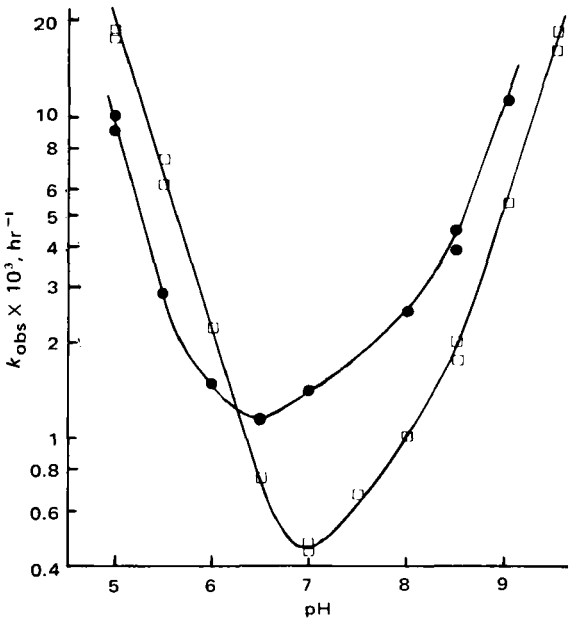

Figure 1—*The pH–rate profiles of penicillin G in 0.5% (w/v) nonmicellar (●) and 30% (w/v) micellar (□) concentrations.*

tion of dipoles in an electric field (26). Thus, for a reaction of oppositely charged penicillin and hydrogen ions, ΔS_{el} has a positive value and is inversely proportional to the dielectric constant of the medium. The surface of micelles appears to be less polar than water itself (27). In the present micellar system, the carboxylate group of the penicillin G ion might be regarded as being attached to a region of lower dielectric constant than water in the bulk of the solution; therefore, ΔS_{el} is larger and more positive than in nonmicellar solution.

At pH around and above neutrality, the main degradation is the water- and OH^--catalyzed hydrolysis of the β-lactam ring to form penicilloic acid. The micellar state somewhat protects the β-lactam ring from nucleophilic attack, because it is located farther inside the micelle surface. Furthermore, the OH^- is excluded from the anionic micelle by electrostatic repulsion. In contrast with the H^+-catalyzed degradation, the OH^- attack on the negatively charged penicillin G ion is not favored by entropy change. The transition state is a more highly charged ion which would be expected to be strongly hydrated, leading to a decrease of entropy. The electrostatic entropy change becomes more negative in the micellar phase than in the bulk of the solution. These unfavorable factors lead to a decrease of rate in the micellar system at pH around and above neutrality.

Figure 1 shows the pH–rate profiles of penicillin G in nonmicellar and micellar concentrations of 0.5 and 30% (w/v), respectively. The profile of micellar penicillin G was shifted to higher pH values. The pH of minimum degradation was obtained at 7.0 for micellar and 6.5 for nonmicellar penicillin G. The present results clearly indicate that micellar solutions of penicillin G degrade slower than nonmicellar solutions above pH 6.25, provided the pH of the solution is held constant. Under the conditions of the previous studies where the penicillin G solutions were either unbuffered (8, 9) or inadequately and not proportionally buffered (10, 11), the faster degradation rate observed at higher concentration can be attributed to a faster decrease of the pH of the solution since penicillin G itself has no buffering activity. The decrease in pH renders the H^+-catalyzed reaction more significant, leading to a faster degradation rate.

Based on the present results, micellar solutions of penicillin G are most stable if they are buffered at pH 7.0, although solutions of concentration less than the CMC (130,000 units/ml or 8.26% w/v) are most stable at pH 6.5. Micellar solutions buffered at pH 7.0 are approximately 2.5-fold more stable than nonmicellar solutions buffered at the pH of the minimum hydrolysis rate.

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