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Stability of Aqueous Bacampicillin Suspension¹⁾

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In this study, the stability of bacampicillin hydrochloride (BAPC) suspension was measured to clarify the kinetics in a concentrated solution. Then, the time courses of BAPC in suspension were examined in the neutral pH region and at 35 °C. In this suspension, not only total BAPC, but also the dissolved BAPC were decreased with time. This result indicates that particles of BAPC precipitate from the solution with time, and the infrared (IR) spectrum of the precipitates suggested that they consisted of BAPC base. Furthermore, the degradation of BAPC in suspension was very much faster than that in solution. This acceleration of the degradation was due to the simultaneous degradation of the precipitated and the dissolved BAPC. In addition, the apparent first-order degradation rate constant of the precipitated particles was larger than that of the dissolved BAPC. Thus, the degradation of the solid seems to be accelerated by hydroxyl ion.

Keywords—bacampicillin; degradation; solubility; stability; suspension; parallel-consecutive reaction; bacampicillin precipitate; sedimentation; bacampicillin base; X-ray diffraction

Bacampicillin hydrochloride (BAPC) is a prodrug of ampicillin (ABPC) and has a superior oral absorbability.²⁾ The stability of BAPC in aqueous solution had been studied in our laboratory, and the degradation reaction was shown to involve specific acid base catalysis similar to those of the other penicillins.³⁾ The concentration used (*ca.* 5.0×10^{-4} M), however, were lower than those (0.01–0.05 M) calculated to be present in the stomach after the usual oral dose of 250–500 mg, based on a stomach juice volume of 50 ml. The stability of penicillins in solution has been extensively examined; for example, the degradation of benzylpenicillin and cyclacillin does not change with increasing concentration, while that of ABPC is accelerated owing to the formation of polymer.⁴⁾ BAPC may also be degraded more quickly as the concentration is increased similarly to ABPC. The stability of BAPC concentrated solution or suspension needs to be studied for this reason. Further, BAPC has a characteristic bitter taste which disappears in the BAPC base form.

In this study, the kinetic behavior of BAPC aqueous suspension was examined in the neutral pH region with the aim of obtaining data for the design of tasteless dosage forms.

Experimental

Materials—BAPC (659 µg/mg, Yoshitomi Pharmaceutical Ind., Ltd.) was used as received.

Reagents—Tween 80 (Wako Pure Chemical Ind., Ltd.) was the normal commercial preparation. Tween 80 solution (0.1%) was prepared in water and adjusted to ionic strength 0.5 with KCl. Toluene and all other reagents were of the highest commercial grade and were used without further purification.

Buffer Solution—Borate buffer (0.1 M, pH 9.0) was made by dissolving 3.09 g of boric acid in water to make 500 ml followed by pH adjustment to 9.0 with NaOH solution. The pH of the buffer was measured at room temperature with a Toa pH meter, model HM-18 ET.

Kinetic Procedures—a) Time Course of BAPC Degradation in Suspension: An exactly weighed amount of BAPC in 1 ml of 0.1% Tween 80 solution was transferred to a flask (30 ml volume), which contained 15 ml of 0.5 M

KCl solution, and the container was rinsed with 0.5 M KCl solution (5 ml), which was added to the flask. The reaction solution had previously been heated to the desired temperature. The flask was stored in a constant temperature bath which was regulated to 35 °C by a thermostat with 0.1 °C precision. The pH of the solution was adjusted to the desired value (0.05 pH unit precision) with 0.2 N NaOH by using a pH stat (Toa, model HMS-10A) to make a suspension, which suspension was stirred at 50 rpm by using a magnetic stirrer. Two samples were taken at suitable intervals. One sample was taken up in 0.0001 N HCl to give a solution of measurable concentration (0.5×10^{-4} — 5.0×10^{-4} M). The other was filtered with a Millipore filter (0.45 μ m) and the resultant filtrate was diluted to the desired concentration as mentioned above. These samples were assayed immediately for intact BAPC and/or ABPC by the simultaneous determination method described previously.⁵⁾

b) Time Course of BAPC Solubility in Suspension: BAPC suspension was prepared in the same way as described above, at pH 6.00 and 0 °C. The starting time of the reaction, however, was taken as one hour after preparing the suspension to allow time for equilibration. Samples were taken out at suitable intervals, filtered (0.45 μ m) and diluted in the same manner as mentioned above.

Analytical Method— I_2 -Colorimetry: The assay method was the same as described previously.⁵⁾

Preparation of BAPC Base—BAPC suspension in 30 ml of toluene was transferred into a separatory funnel together with 15 ml of 0.1 M borate buffer (pH 9.0). The mixture was shaken for 5 min at room temperature, then the toluene layer was taken out, dried over Na_2SO_4 and evaporated under reduced pressure.

Preparation of Amorphous BAPC—An aqueous solution of BAPC was lyophilized to give amorphous BAPC.

Determination of Infrared (IR) and Mass Spectra (MS)—IR spectra were recorded with a JASCO DS-701G grating infrared spectrophotometer using the KBr method. MS were measured with an NEC JMS-DX 300 instrument and analyzed with an NEC JMA-DA 5000 Mass data system.

Determination of Melting Point—The melting point (mp) was measured by using a Yanaco micro melting point apparatus, model MP-S3.

Differential Scanning Calorimetry (DSC)—DSC was carried out with a differential scanning calorimeter (Rigaku TG-DSC) at a scanning speed of 5 °C/min under an N_2 stream.

X-Ray Diffraction Analysis—Powder X-ray diffractometry was carried out under the following condition using an X-ray diffractometer (Rigaku Geigerflex 2025): target, Ni; voltage, 30 kV; divergency, 1 °C; receiving slit, 0.15 mm.

Data Analysis—The amounts of BAPC and/or ABPC were evaluated by a NEC PC-8800 microcomputer using the MULTI program⁶⁾ according to the appropriate reaction scheme.

Results and Discussion

Kinetic Behavior of BAPC in Aqueous Suspension

The time courses of both the precipitated and the dissolved BAPC must be known to define the kinetic behavior in suspension, in addition to the interaction between the

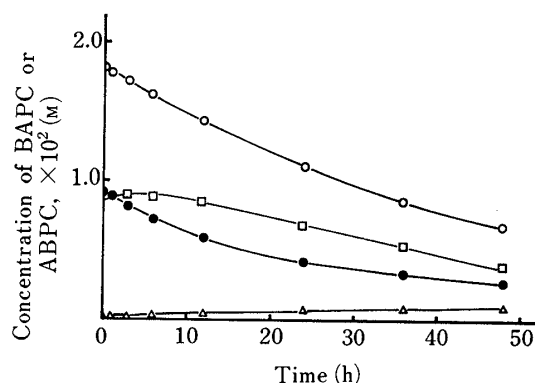


Fig. 1. Time Courses of Bacampicillin and Ampicillin Degradation in Aqueous Suspension at pH 6.00, 35 °C and $\mu=0.5$

○, total bacampicillin; ●, dissolved bacampicillin; □, precipitated bacampicillin; △, ampicillin.

Initial concentration of bacampicillin was 1.8×10^{-2} M. The solid lines of the dissolved and precipitated bacampicillin and the formed ampicillin were calculated by means of Eqs. 5, 6 and 8 from the values summarized in Tables II and III.

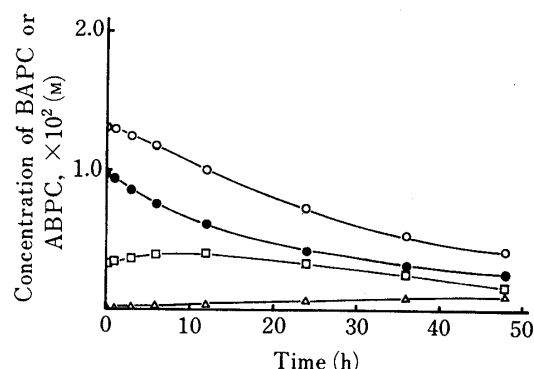


Fig. 2. Time Courses of Bacampicillin and Ampicillin Degradation in Aqueous Suspension at pH 6.00, 35 °C and $\mu=0.5$

○, total bacampicillin; ●, dissolved bacampicillin; □, precipitated bacampicillin; △, ampicillin.

Initial concentration of bacampicillin was 1.3×10^{-2} M. The solid lines were calculated by means of Eqs. 5, 6 and 8 as described in Fig. 1.

precipitates and the solutes. Thus, the kinetic study of BAPC in aqueous suspension was done at pH 6.0 where BAPC degradation is relatively slow. Because the degradation of BAPC is affected by the ionic strength and buffer concentration,³⁾ these time runs were done at $\mu=0.5$ and by using a pH stat. Figures 1 and 2 show the time courses of the concentration of the total and dissolved BAPC. In each case, the total BAPC decreased with time, but the higher the concentration, the faster the rate. Further, the dissolved BAPC also decreased with time. Suspensions of drugs such as aspirin⁷⁾ and procainpenicillin⁸⁾ degrade through a zero-order reaction, because the degradation of these drugs in a solution obeys first-order kinetics and the solubility of the drugs is constant during the reaction. BAPC in solution was already proved to degrade with apparent first-order kinetics.³⁾ Thus, if the suspended particles of BAPC are chemically stable and attain equilibrium rapidly with the dissolved BAPC, the total BAPC in the suspension should obey zero-order kinetics. As shown in Figs. 1 and 2, however, the changes of the total BAPC did not follow zero-order kinetics and the velocity decreased with decreasing BAPC concentration. Furthermore, the solubility of BAPC also decreased regardless of the presence of BAPC particles.

If only the dissolved BAPC degrades and the dissolution rate of the suspended BAPC particles is negligible compared to the degradation rate of the dissolved BAPC, BAPC solubility would decrease with time as indicated above. In this case, the amount of the suspended particles should be constant. However, the difference of the total and the dissolved BAPC decreased with time. On the other hand, in the case where the suspended particles dissolve to some extent, but much more slowly than the degradation rate of the dissolved BAPC, the amount of the suspended particles will decrease.

Thus, the time course of BAPC in the filtrate of the BAPC suspension was followed at pH 6.00 and 35°C (Fig. 3). The total BAPC decreased with time similarly to the results shown in Figs. 1 and 2. The reaction solution gradually changed from clear to opaque. This indicates that a precipitate of BAPC appeared owing to a decrease of the solubility of BAPC. In such a case, assuming that only the dissolved BAPC degrades, the degradation of the total BAPC in the suspension would be much smaller than that in the solution. Figure 3, however, shows that BAPC degradation in the suspension is significantly faster than that in the solution. From these results, it was assumed that the precipitated BAPC particles degraded simultaneously with BAPC in solution, as shown in Chart 1.

Similar degradation behavior was seen in solution at pH 5.00, 6.50 and 7.00. That is to

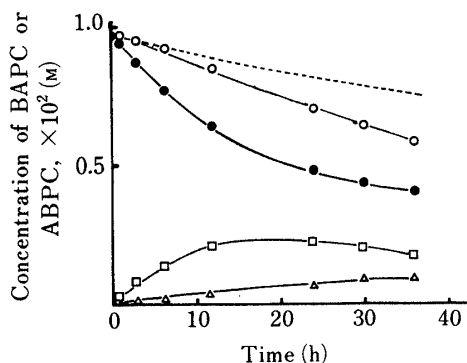


Fig. 3. Time Courses of Bacampicillin and Ampicillin Concentrations in Saturated Solution at pH 6.00, 35°C and $\mu=0.5$

○, total bacampicillin; ●, dissolved bacampicillin; □, precipitated bacampicillin; △, ampicillin.

The dotted line shows the time course of apparent first-order degradation of bacampicillin assuming the initial concentration to be of 1.0×10^{-2} M. The solid lines were calculated by means of Eqs. 5, 6 and 8 as described in Fig. 1.

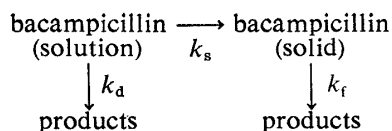


Chart 1

say, both the total BAPC and the dissolved BAPC decreased with time. The total BAPC degradation was accelerated and the amount of dissolved BAPC decreased with increasing pH. Thus, the degradation of the BAPC suspended particles and the dissolved BAPC, and the precipitation of the particles from the solution, all appeared to depend on pH in this suspension. Further, the BAPC solubility in the suspension was also shown to depend on pH. As mentioned above, in BAPC suspension of pH 5.00—7.00, the solubility of BAPC is thought to decrease because of the simultaneous degradation of the suspended particles and the dissolved BAPC, and the precipitation of BAPC.

Dissolution Process of BAPC in Solution

The time courses of BAPC solubility was followed in suspension at pH 6.00 and 0°C at which BAPC degradation was not apparent over 200 h (Fig. 4). The suspension was prepared by adding finely ground BAPC to distilled water at ionic strength 0.5 in a pH stat. Tween 80 was added at 0.1% to the suspension to maintain the suspended state of BAPC. The starting time (0) in Fig. 4 was 1 h after preparation of the suspension.

At each initial concentration, the amount of dissolved BAPC decreased with time. This shows that a high BAPC concentration is produced by the dissolution of BAPC, followed by precipitation of the supersaturated BAPC.

The physical properties of the precipitates were studied. Figure 5 shows the IR spectra of BAPC HCl, freeze-dried BAPC HCl, BAPC base and the precipitates. The IR spectrum of the precipitates was the same as that of BAPC base. Thus, it was assumed that the supersaturated BAPC precipitated as BAPC base, which has very low solubility, after the initial dissolution of BAPC in suspension (Chart 2). As shown in Fig. 4, the change of BAPC solubility seems to correspond to the precipitation process of BAPC, because the determination of the solubility was begun at 1 h after preparation of the suspension and the dissolved BAPC, which was formed by rapid dissolution of all the added BAPC, had changed into BAPC base during the period of the preparation. Thus, the precipitation rate of BAPC base can be calculated from the results shown in Fig. 4. If the precipitation rate is directly proportional to the difference between the dissolved concentration and the saturated concentration of BAPC, the change of the dissolved BAPC at 0°C should follow Eq.1.

$$\frac{d[B]_{\text{sol}}}{dt} = -k_s([B]_{\text{sol}} - [B]_{\text{sat}}) \quad (1)$$

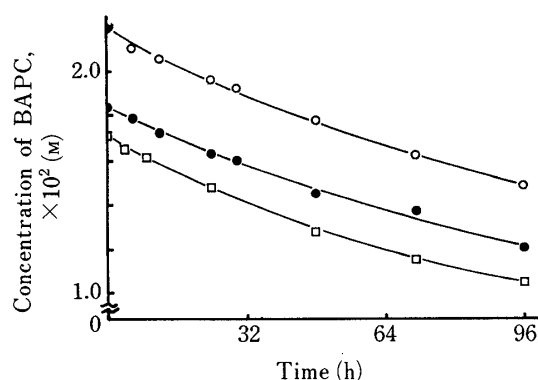


Fig. 4. Time Courses of Bacampicillin Solubility in Aqueous Suspension at pH 6.00, 0°C and $\mu=0.5$

Initial suspended concentration: ○, 0.04 M; ●, 0.03 M; □, 0.02 M. The solid lines are the least-squares best fits to the experimental points, based on the scheme in Chart 2.

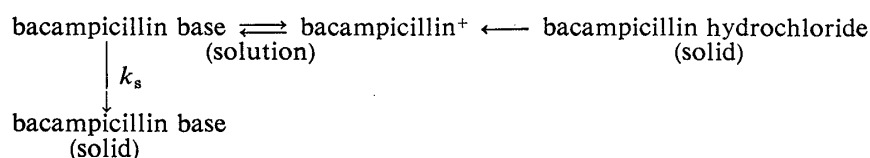


Chart 2

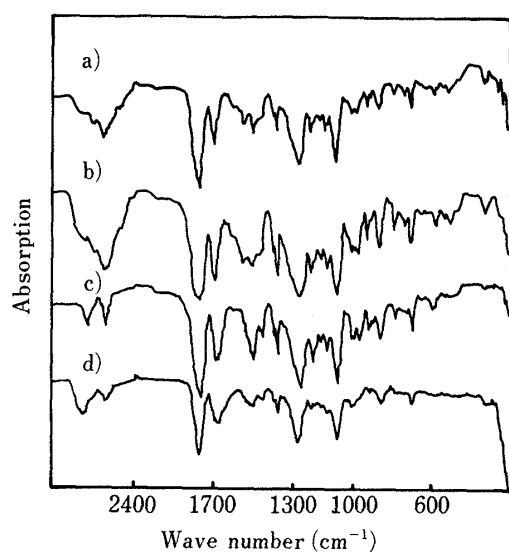


Fig. 5. IR Spectra of Bacampicillin and Precipitates

a) Crystalline bacampicillin hydrochloride; b) freeze-dried bacampicillin hydrochloride; c) bacampicillin base; d) precipitates.

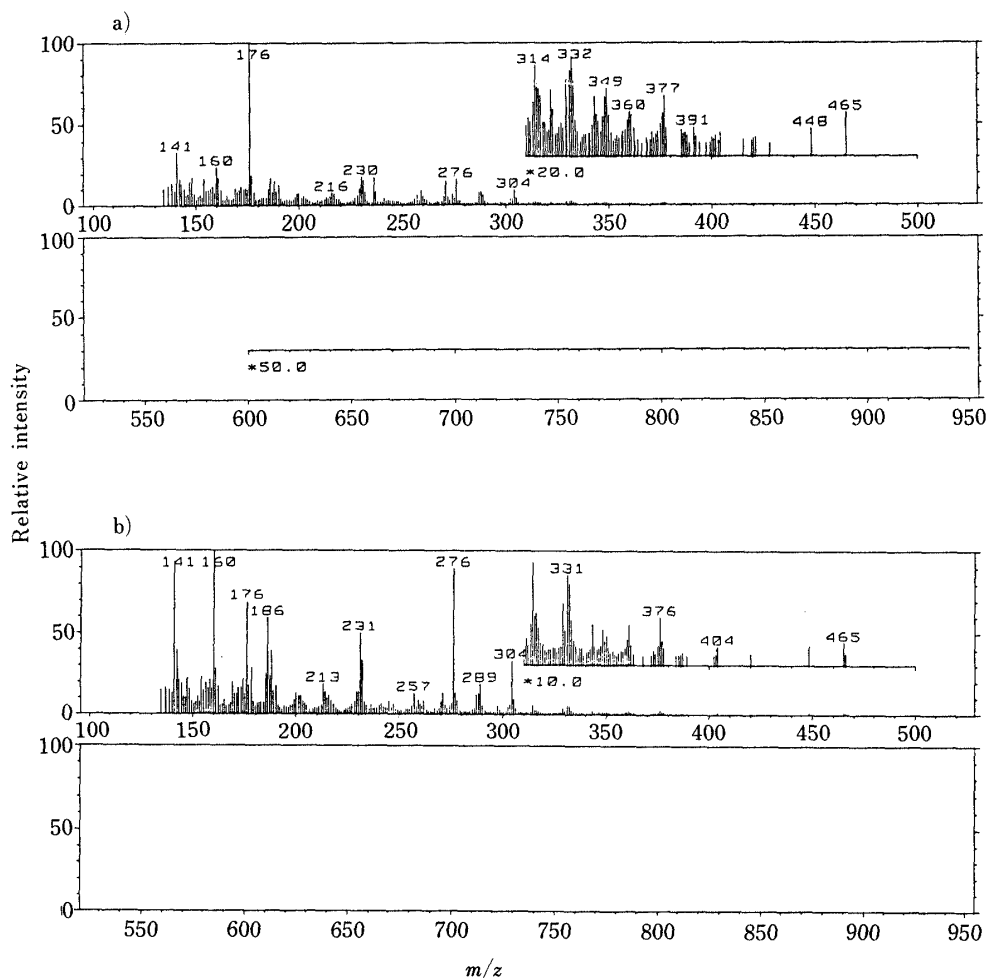


Fig. 6. Mass Spectra of Precipitated Bacampicillin

a) Sample at 0 h; b) sample at 48 h.

where $[B]_{\text{sol}}$ and $[B]_{\text{sat}}$ are the dissolved BAPC and the solubility of BAPC at a given pH, respectively, and k_s is the apparent first-order rate constant of the precipitation. On integrating Eq. 1, Eq. 2 can be obtained.

$$[B]_{\text{sol}} = ([B]_{\text{sol},0} - [B]_{\text{sat}})e^{-k_s t} + [B]_{\text{sat}} \quad (2)$$

where $[B]_{\text{sol},0}$ is the initial concentration of the dissolved BAPC.

The precipitates obtained at 0 and 48 h were analyzed by MS (Fig. 6) and IR spectrometry. The molecular ion peak of BAPC (M_r 465.5) was detected at m/z 465 in each sample. This ion peak was also seen in the MS of authentic BAPC base. No peaks were detected over m/z 465. Thus, the precipitate was concluded to be BAPC itself and not a polymerization product. The IR spectrum of the precipitates was in good agreement with that of BAPC base. The precipitates of BAPC base were obtained even at pH 5.00 regardless of the high solubility of BAPC compared to that at pH 6.00. This result indicates that the solubility of BAPC base is extremely low.

In order to characterize the precipitated BAPC, preliminary examinations by X-ray diffractometry and DSC were performed. The X-ray diffraction pattern shown in Fig. 7 supports the hypothesis that the precipitates, which were originally in crystalline form, are transformed to an amorphous state.

Kinetic parameters, k_s (h^{-1}), $[B]_{\text{sat}}$ were obtained by the least-squares method based on Eq. 2 from the results of Fig. 4 (Table I). The k_s value was almost constant, but the $[B]_{\text{sat}}$ value varied according to the initial BAPC concentration. When the solubility of a solid is constant, mp and the heat of fusion should be constant.⁹⁾ Thus the mp and DSC of BAPC base were determined. The BAPC base started to melt at 87°C and decomposed at 140°C. In the DSC

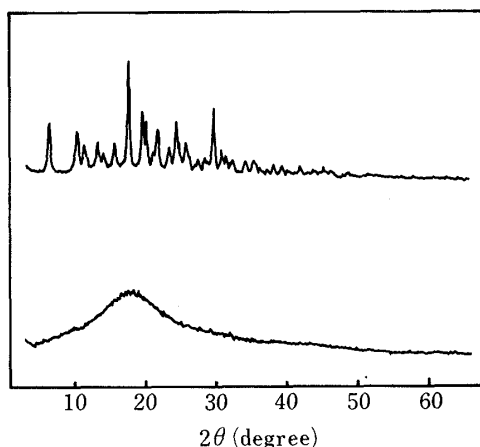


Fig. 7. X-Ray Powder Diffraction Patterns

a) Crystalline bacampicillin hydrochloride; b) bacampicillin base.

TABLE I. Solubility and Precipitation Rate Constant of BAPC in Aqueous Suspension at pH 6.00 and 0°C ($\mu=0.5$)

	BAPC initial concn. (M)		
	0.020	0.030	0.040
$k_s \cdot 10^3$ (h^{-1})	10.0 ± 1.6	8.5 ± 0.9	9.0 ± 1.0
$[B]_{\text{sat}} \cdot 10^2$ (M)	6.3 ± 0.5	7.3 ± 0.7	9.6 ± 0.3

Each value represents the mean $\pm$ S.D.

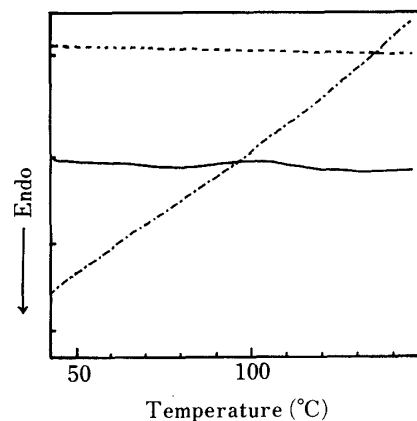


Fig. 8. DSC Thermogram of Bacampicillin Base

—, temperature; ----, sample weight; —, DSC curve.

curve (Fig. 8), no endothermic peak was observed around the mp. Thus, this amorphous BAPC base does not have a constant mp and heat of fusion, resulting in the change of the solubility of BAPC.

BAPC Degradation in Aqueous Suspension

As mentioned in the above section, the precipitation rate of BAPC is given by Eq. 1. It has been proved that the degradation of BAPC in a solution obeys apparent first-order kinetics at constant pH and temperature.³⁾ Then, the change of dissolved BAPC in suspension can be expressed as follows by assuming the degradation rate constant, k_d .

$$\frac{d[B]_{\text{sol}}}{dt} = -k_s([B]_{\text{sol}} - [B]_{\text{sat}}) - k_d[B]_{\text{sol}} \quad (3)$$

On the other hand, the concentration of the suspended particles can be calculated from the difference between the concentrations of total and dissolved BAPC. The time courses of the concentration of the particles are shown in Figs. 1, 2 and 3. The concentration of the precipitated particles increased initially then decreased with time, as shown in these figures.

This result is thought to be due not to the dissolution of BAPC or the precipitation of BAPC base, but to the degradation of BAPC base. If the degradation of the suspended BAPC base proceeds with first-order kinetics in proportion to the concentration of the suspended particles, the first-order degradation of the suspended solids and the precipitation of the solids counterbalance each other. In this case, the change of the suspended particles can be described by Eq. 4.

$$\frac{d[B]_{\text{dep}}}{dt} = k_s([B]_{\text{sol}} - [B]_{\text{sat}}) - k_f[B]_{\text{dep}} \quad (4)$$

where $[B]_{\text{dep}}$ is the concentration of the solid BAPC, k_f is the apparent first-order degradation rate constant of the solid. Equations 3 and 4 can be integrated to give Eqs. 5 and 6, respectively.

$$[B]_{\text{sol}} = \left([B]_{\text{sol},0} - \frac{k_s}{k_s + k_d} [B]_{\text{sat}} \right) e^{-(k_s + k_d)t} + \frac{k_s}{k_s + k_d} [B]_{\text{sat}} \quad (5)$$

$$\begin{aligned} [B]_{\text{dep}} = & \left([B]_{\text{dep},0} + \frac{k_s}{k_f} [B]_{\text{sat}} \right) e^{-k_f t} - \frac{k_s}{k_f} [B]_{\text{sat}} \\ & + \frac{k_s [B]_{\text{sol},0}}{k_s + k_d - k_f} (e^{-k_f t} - e^{-(k_s + k_d)t}) \\ & + \frac{k_s^2 [B]_{\text{sat}}}{k_f (k_s + k_d)(k_s + k_d - k_f)} (k_f e^{-(k_s + k_d)t} - (k_s + k_d)e^{-k_f t} + k_s + k_d - k_f) \end{aligned} \quad (6)$$

where $[B]_{\text{sol},0}$ and $[B]_{\text{dep},0}$ are the initial concentration of the dissolved BAPC and the initial concentration of the solid BAPC, respectively. The solubility and the rate constants which were obtained from the results in Figs. 1, 2 and 3 by the least-squares method⁶⁾ based on Eqs. 5 and 6 are listed in Table II. The resulting degradation rate constant (k_d) at each pH was in good agreement with that obtained from the degradation of BAPC in solution. Thus, the time courses of the dissolved BAPC concentration in suspension support the validity of Eqs. 5 and 6. Further, as shown at pH 6.00, the precipitation rate constant (k_s) and the degradation rate constant of the particles (k_f) were almost constant regardless of the initial BAPC concentration. The results shown in Table II indicate that the suspended particles are unstable at every pH ($k_f > k_d$). This instability of the suspended particles seems to be due to the fact that they are amorphous, consisting of only BAPC base, which is more labile than the ionic species of BAPC in aqueous solution.

TABLE II. Solubility and Rate Constants of BAPC in Aqueous Suspension at Various pH, 35°C and $\mu=0.5$

	pH					
	5.0	6.0			6.5	7.0
$[B]_{\text{initial}}$ (M)	0.029	0.018	0.013	0.010	0.0085	0.0057
$k_s \cdot 10^2$ (h ⁻¹)	2.9 ± 0.2	4.6 ± 0.5	4.7 ± 0.3	5.6 ± 0.9	7.5 ± 0.4	38.0 ± 3.1
$k_f \cdot 10^2$ (h ⁻¹)	2.0 ± 0.1	3.0 ± 0.4	4.9 ± 1.1	3.7 ± 0.5	22.1 ± 3.3	37.2 ± 6.0
$k_d^a) \cdot 10^3$ (h ⁻¹)	3.0 ± 0.2	7.2 ± 0.6	6.9 ± 0.3	7.0 ± 0.3	14.4 ± 2.2	50.7 ± 4.8
$[B]_{\text{sat}}$ 10^3 (M)	25.0 ± 3.1	2.6 ± 0.2	2.4 ± 0.4	3.9 ± 0.8	0.81 ± 0.04	0.69 ± 0.07

a) The values of this run were in good agreement with data obtained in a previous study of bacampicillin.³⁾ Each rate constant or solubility value is the mean ± S.D.

On the other hand, the solubility, $[B]_{\text{sat}}$ varied at pH 6.00, because the suspended particles are amorphous as described previously. The solubility at 0°C was larger than that at 35°C at the same pH, as shown in Tables I and II, but the reason for this was not clarified in the present study. Kinetic parameters obtained from the results at pH 5.00, 6.50 and 7.00 are also listed in Table II. At every pH, the degradation rate constant, k_d was essentially the same as that obtained from the degradation in solution under the same conditions. Thus, the changes of BAPC in suspension at each pH were well described by Eqs. 5 and 6.

All the kinetic degradation parameters, k_d , k_s , k_f , summarized in Table II, were apparently dependent on pH. The saturated solubility of BAPC, $[B]_{\text{sat}}$ decreased with increasing pH. This decrease of the solubility may be attributed to the decrease of water-soluble ionized BAPC according to Henderson-Hasselbalch equation. The apparent first-order degradation rate constant of the particles, k_f , became larger with increase of pH above 6.50, that is, the degradation of the particles was shown to be accelerated by hydroxide anion in a similar manner to that of BAPC in solution. BAPC degradation in solution was proved previously to proceed according to the scheme shown in Chart 3.³⁾ The degradation of dissolved BAPC in suspension is expected to follow the scheme shown in Chart 4.

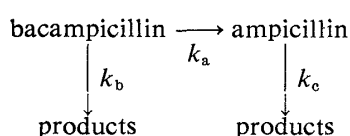


Chart 3

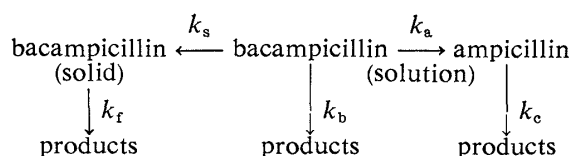


Chart 4

The time course of ABPC in the suspension should then be expressed by Eq. 7.

$$\frac{d[A]}{dt} = k_a[B]_{\text{sol}} - k_c[A] \quad (7)$$

where $[A]$ is the concentration of ABPC, k_a is the apparent first-order degradation rate constant of the ester moiety of BAPC, and k_c is the apparent first-order degradation rate constant of β -lactam cleavage of ABPC. The two differential Eqs. 3 and 7 can be solved to give Eq. 8.

$$\begin{aligned}
 [A] = & \frac{k_a[B]_{\text{sol},0}}{k_s + k_d - k_c} (e^{-k_c t} - e^{-(k_s + k_d)t}) \\
 & + \frac{k_a k_s [B]_{\text{sat}}}{k_c(k_s + k_d)(k_s + k_d - k_c)} (k_c e^{-(k_s + k_d)t} - (k_s + k_d)e^{-k_c t} + k_s + k_d - k_c)
 \end{aligned} \quad (8)$$

TABLE III. Rate Constants of BAPC Degradation in Aqueous Solution
at 35 °C and $\mu=0.5$

	pH			
	5.0	6.0 ^{a)}	6.5 ^{a)}	7.0 ^{a)}
k_d (h ⁻¹)	0.003	0.007	0.014	0.0594
k_a (h ⁻¹)	0.00038	0.0045	0.012	0.0496
k_c (h ⁻¹)	0.0007	0.0008	0.0008	0.0009

a) Taken from ref. 3.

where the rate constant, k_d is the sum of k_a and k_b , which is apparent first-order degradation rate constant of β -lactam cleavage of BAPC. These values of these kinetic parameters are summarized in Table III. The calculated values of ABPC obtained by the use of Eq. 8 with the parameters listed in Tables II and III became lower than the experimental values at pH 5.00—7.00. Thus, ABPC formation in suspension may occur not only from the dissolved BAPC, but also from the suspended BAPC particles. The obtained rate constant, k_f , is considered to include the ABPC formation process.

Consequently, aqueous BAPC suspension was found to be unstable compared to BAPC solution, because BAPC degradation proceeded in the suspended particles and in solution simultaneously.

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References and Notes

- 1) A part of this work was presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985.
- 2) B. A. Ekstrom and B. O. H. Sjöberg, Ger. Patent 2144 (1972) [*Chem. Abstr.*, **77**, 491 (1972)].
- 3) H. Fujiwara and S. Kawashima, *Chem. Pharm. Bull.*, **33**, 1202 (1985).
- 4) A. Tsuji and T. Yamana, *Chem. Pharm. Bull.*, **22**, 2434 (1972).
- 5) H. Fujiwara, S. Kawashima, and M. Ohhashi, *Chem. Pharm. Bull.*, **30**, 1430 (1982); *idem, ibid.*, **30**, 2181 (1982); H. Fujiwara, S. Kawashima, Y. Yamada, and K. Yabu, *ibid.*, **30**, 3310 (1982).
- 6) K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno, *J. Pharmacobio-Dyn.*, **4**, 809 (1981).
- 7) S. M. Blaug and J. W. Wesolowski, *J. Am. Pharm. Assoc.*, **48**, 691 (1959).
- 8) M. A. Schwartz and F. H. Buckwalter, *J. Pharm. Sci.*, **51**, 1119 (1962).
- 9) Y. Nakai, *PHARM TECH JAPAN*, **1**, 69 (1985).