Effect of Initial Concentration on Stability of Panipenem in Aqueous Solution

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The effect of initial panipenem (CAS No. 87726-17-8) concentration on its degradation in an aqueous solution was investigated. The degradation of panipenem followed pseudo-first-order kinetics at all the pH values tested. However, in an acidic solution, the degradation rate increased with the initial panipenem concentration. On the other hand, in an alkaline solution, the degradation rate was not affected by its initial concentration. In an acidic solution, the plots of the first-order rate constants versus initial panipenem concentrations showed a linear relationship. Theoretically, the first-order rate constant is independent of the initial concentration, and therefore, the results suggested unusualness. We investigated the results obtained under acidic conditions in detail to find a very complex reaction mechanism: panipenem and its degradation products are factors causing the unusual increase in the degradation rate. Moreover, it was shown that the dissociation of the carboxyl group played an important role in the degradation of panipenem.

Key words panipenem; solution; stability; kinetics; degradation

Panipenem (PAPM, CAS No. 87726-17-8, Fig. 1), which was discovered and developed in Sankyo Co., Ltd., is a carbapenem antibiotic with a broad antibacterial spectrum and potent bactericidal activity.[1—5)]

In the previous report, the degradation kinetics of panipenem in aqueous solution at a fixed initial panipenem concentration (5 mg/ml≈15 mM) was studied with regards to pH, temperature, ionic strength, and buffer catalysis.[6)] The degradation of panipenem followed first-order kinetics, and the pH dependence of the apparent first-order rate constants was explained using the specific acid-base catalysis theory and considering the dissociation of the carboxyl group.

In the study reported here, the effect of panipenem concentration on the degradation of panipenem was investigated. It had been previously reported that the degradation of carbapenem antibiotics, imipenem and meropenem, followed a pseudo-first-order degradation in an aqueous solution, and the initial concentration was considered to affect the degradation of the drugs.[7—11)] However, the mechanism of how the concentration affected the degradation had not been elucidated.

In this report, we investigated the effect of the initial panipanem concentration on the degradation of panipenem, in particular, in an acidic solution.

Experimental

Materials Panipenem was obtained from lots produced in-house (Sankyo Co., Ltd.) and used without further purification. 2-(*N*-Morpholino) ethanesulfonic acid (MES, pK_a=6.15) and 3-(*N*-Morpholino) propanesulfonic acid (MOPS, pK_a=7.20) were purchased from Dojin Chemicals Co., Ltd. All the other chemicals used were of reagent grade.

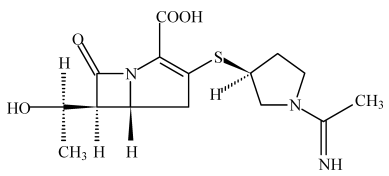

Fig. 1. Chemical Structure of Panipenem (PAPM)
$C_{15}H_{21}N_3O_4S$=339.42.

Kinetic Runs Aqueous buffer solutions were adjusted to the desired pH with concentrated aqueous sodium hydroxide or diluted hydrochloric acid. The buffer species used were, citrate (pH 2.0—4.5), MES (pH 5.0—6.5), MOPS (pH 7.0—8.0), and carbonate (pH 8.5—10.0). Then, panipenem was weighed and added to the buffered solutions. These buffered solutions were placed in test tubes and maintained at the reaction temperatures in a thermostatic water bath (±0.1 °C). The pH values of these buffered solutions were measured with a pH electrode at every sampling time point to confirm to be within desired pH ±0.1. If the pH value deviated, the pH value was re-adjusted by concentrated aqueous sodium hydroxide or diluted hydrochloric acid, and then the kinetic run was continued. The samples in the test tubes were periodically analyzed, and the residual panipenem concentrations were determined by high performance liquid chromatography (HPLC). In citrate and carbonate buffers, the buffer concentration effects on the rate constants were observed, so the rate constants were obtained by extrapolating the buffer concentration to zero. In MES and MOPS buffers, the concentration effects were not observed, so the experiments were conducted at the buffer concentration of 0.2 M or 0.5 M, and these buffers showed the sufficient buffer index during the kinetic runs. As for the ionic strength of the solution, it was shown that the ionic strength had no effect on the rate constant in the previous report, so in the experiments in this report, the ionic strength was not adjusted.[6)]

High Performance Liquid Chromatography Analysis HPLC was performed according to the method in The Japanese Pharmacopoeia Fourteenth Edition. The mobile phase was 0.02 M MOPS (pH 8.0) : CH_3CN=50 : 1 (v/v), and *p*-styrenesulfonic acid sodium salt was used as the internal standard. The column, CAPCELL PAK C18 (SHISEIDO Co., Ltd., Japan) was kept at 40 °C during elution. The elution pattern was recorded with a UV detector, which was set at a wavelength of 280 nm.

Effects of Panipenem Degradation Products on the Degradation of Panipenem To consider the effects of panipenem degradation products on the degradation of panipenem, reaction solutions were ice-cooled at specific times to stop the kinetic reaction and obtain solution with panipenem degradation products (first kinetic run). The initial concentrations of these samples were the same (approximately 14 mM) and only their kinetic run time was different. The kinetic runs were carried out at 40 °C, pH 5, for 1, 2, 3, 4, 5, and 6 h, to prepare samples with different residual panipenem concentrations. Then, panipenem powder of equal amount was added to each sample test tube, which contained different amounts of panipenem as well as degradation products, to conduct a second kinetic run, and the effects of the panipenem degradation products on the degradation of panipenem were examined.

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Results and Discussion

Apparent Pseudo-First-Order Plots Kinetic runs were performed, and the residual panipenem concentration was measured by HPLC. Semi-log plots were obtained by plotting the residual percent of panipenem as a function of time (Fig. 2). In Fig. 2, a linear relationship between time and log residual percent was observed, indicating that the degradation of panipenem followed pseudo-first-order kinetics. The apparent first-order rate constants were obtained from the slopes of the semi-log plots.

Effects of Initial Panipenem Concentration on the Degradation Rates The effects of initial panipenem concentrations on the degradation of panipenem were investigated. In an acidic solution (pH 5.0) at 40 °C, panipenem degradation followed a pseudo-first-order reaction. However, the greater the initial panipenem concentration, the higher was the degradation rate (Fig. 3). These results were unusual. If the degradation follows a usual first-order reaction, the degradation rate is not affected by the initial concentration. On the other hand, if the degradation follows a usual second-order reaction, the degradation rate increases with the initial concentration, but the semi-log plot (log residual percent *versus* time) does not show a linear relationship as seen in Fig. 3.

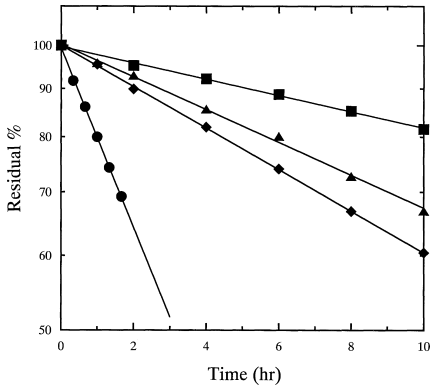

Fig. 2. The Semi-Log Plots of the Observed Pseudo-First-Order Degradation of Panipenem at Various pH Values at 40 °C

The pH values of the solutions were 5.0 (●, in 0.2 M MES), 6.0 (▲, in 0.2 M MES), 7.0 (■, in 0.2 M MOPS), and 8.0 (◆, in 0.2 M MOPS). The apparent first-order rate constants were obtained from the slopes of the semi-log plots.

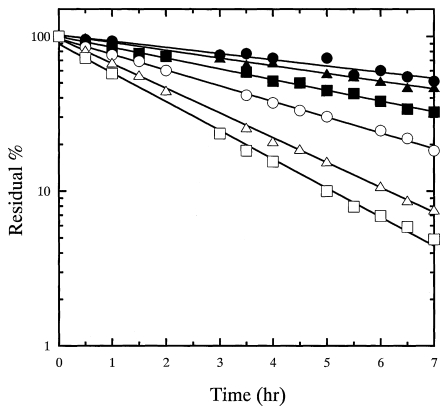

Fig. 3. The Effects of the Initial Concentration on the Degradation Rate (40 °C, pH 5.0)

●: 1 mg/ml, ▲: 2 mg/ml, ■: 5 mg/ml, ○: 10 mg/ml, △: 20 mg/ml, □: 25 mg/ml.

Furthermore, when the apparent pseudo-first-order rate constants, which were obtained from the slopes in Fig. 3, were plotted against the initial panipenem concentrations (Fig. 4), a good linear relationship was observed. The correlation coefficient (r^2) of the linear regression line was 0.9984.

Mathematical Explanation (1) In Fig. 3, as a linear relationship was observed in the semi-log plots, indicating pseudo-first-order kinetics, Eq. 1 was used to express the pseudo-first-order reaction.

$$\frac{d}{dt}\left[\mathrm{PAPM}\right] = -k_{\mathrm{obs}} \cdot \left[\mathrm{PAPM}\right] \tag{1}$$

where k_{obs} is the apparent first-order rate constant, and [PAPM] is the panipenem concentration in the solution.

In Fig. 4, the plots of apparent first-order constants *versus* initial panipenem concentrations showed a linear relationship. Therefore, Eq. 2 was used to express the linear function.

$$k_{\mathrm{obs}} = k_0 + k_1 \cdot [\mathrm{PAPM}]_0 \tag{2}$$

where k_0 is the intercept, k_1 is the slope, and $[\mathrm{PAPM}]_0$ is the initial concentration of panipenem.

Kinetic runs were performed at 40 °C, varying the pH and initial panipenem concentration, to obtain k_0 and k_1 at each tested pH value. The obtained k_0 and k_1 values were plotted against pH in Fig. 5 (k_0 *versus* pH) and Fig. 6 (k_1 *versus* pH). The influence of pH on k_0 and k_1 (regression lines in these Figures) will be discussed later in this report.

Effects of Panipenem Degradation Products on the Degradation Rates Based on the experimental results

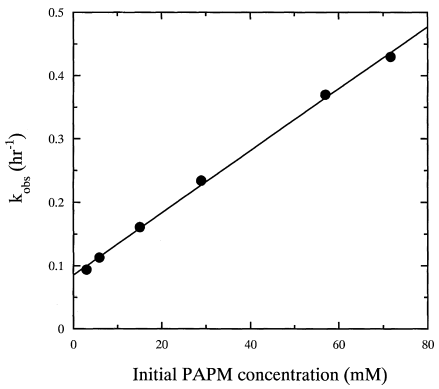

Fig. 4. The Plots of Pseudo-First-Order Rate Constants *versus* the Initial Panipenem Concentration (1—25 mg/ml, 40 °C, pH 5.0)

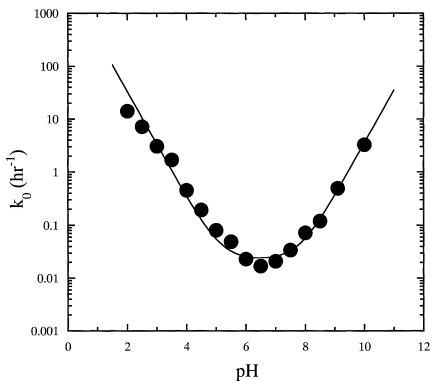

Fig. 5. The Plot of k_0 *versus* the pH Value (40 °C)

(Figs. 3—6), it was thought that the reaction in the solution followed a complex mechanism in the acidic condition. To examine this in detail, the effects of panipenem degradation products on the degradation of panipenem were investigated, by adding panipenem powder to the test tube which contained the reacted solutions (second kinetic run). The buffer used were 0.5 M MES (pH 5.0), at 40 °C. Both in the first and second kinetic run, the pH value of the solution during kinetic run was checked and maintained within 5.0±0.1. In the second kinetic run, the degradation also followed the first-order reaction. The results are shown in Table 1.

In Table 1, the rate constants of samples B, C, D, E, F, G, and H were similar within the experimental error, but different from that of sample A. Surprisingly, these results showed that the rate constants depended only on the total added concentration of panipenem ("[Initial]+[Added]" in Table 1).

In Figs. 3 and 4, the degradation rate increased with initial panipenem concentration. If the degradation products have no effect on the degradation rate and the initial panipenem concentration alone ("[Remaining]+[Added]" in Table 1) is the determining factor, the rate constants of samples B—G, after adding additional panipenem, should be different each other resulting from the difference of the total panipenem concentration. However, the rate constants of samples B—G, which have different concentration of "[Remaining]+[Added]" (Table 1), are equal, and the values of samples B—G are similar to that of sample H. Thus, in samples B—G, the determining factor is total panipenem concentration ("[Initial]+[Added]" in Table 1).

In the non-degraded solutions, the initial panipenem concentration was the determining factor in Figs. 3 and 4, and this was also confirmed from the results in Table 1 (sample A and sample H). However, considering the effect of the degradation product in Table 1 (samples B—H), the total panipenem concentration ("[Initial]+[Added]" in Table 1) was the determining factor. These results suggested that the degradation products affected the degradation of panipenem to the same extent as panipenem itself.

Catalytic Effects of Acetate Buffer As mentioned in the previous report, panipenem degradation followed pseudo-first-order kinetics in acetate buffer, and acetate buffer had a catalytic effect.[6] At pH 4 and pH 5, the acetate buffer concentrations and the first-order rate constants showed a linear relationship (Fig. 7).

Since the pK_a value of acetic acid at 25 °C is 4.76, the calculated ratio of undissociated acetic acid is 0.852 and 0.365, in pH 4.0 and pH 5.0 solutions, respectively.[12] To consider the effect of the undissociated portion of acetic acid in detail, the graph of the first-order rate constants *versus* concentration of the undissociated acetic acid was drawn (Fig. 8). In Fig. 8, the slopes of the linear plots are identical at pH 4.0 and 5.0. Thus, it is concluded that the undissociated acetic acid has a catalytic effect on the degradation of panipenem, while the dissociated acetic acid does not. From these results, it was shown that the dissociated acetic acid had the general acid catalytic effect.[13]

Mathematical Explanation (2) In Fig. 4, a linear relationship between the initial panipenem concentration and rate constant could be observed, and k_0 and k_1 of Eq. 2 were obtained by linear regression. The same plot as Fig. 4 was drawn for each pH condition, and k_0 and k_1 at each pH value

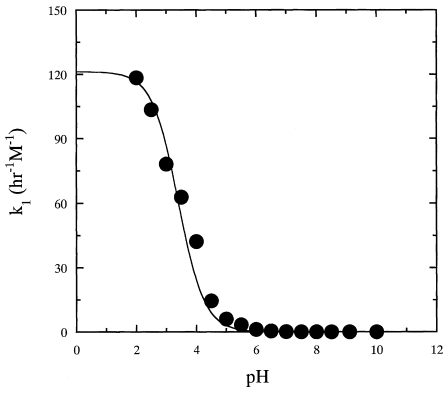

Fig. 6. The Plot of k_1 *versus* the pH Value (40 °C)

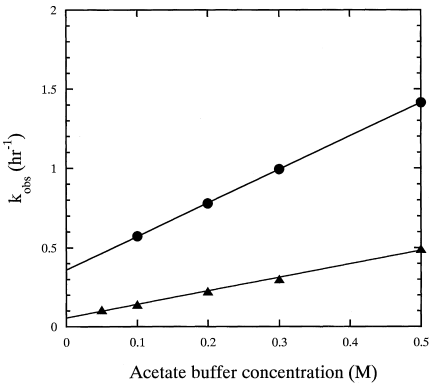

Fig. 7. The Catalytic Effects of Acetate Buffer on the Observed Rate Constants (k_{obs}) at 25 °C, pH 4.0 (●) and pH 5.0 (▲)

Table 1. The Effects of the Degradation Products on the Degradation of Panipenem (40 °C, pH 5.0)

Sample[a]	[I][b] (mM)	[R][c] (mM)	[A][d] (mM)	[I]+[A][e] (mM)	[R]+[A][f] (mM)	k_{obs} (h^{-1})
A	13.8	—	0	13.8	—	0.193
B	13.9	11.6	13.9	27.8	25.6	0.279
C	13.9	9.7	13.9	27.8	23.6	0.280
D	14.0	8.1	14.0	28.0	22.1	0.278
E	14.0	6.7	14.0	28.0	20.6	0.279
F	13.9	5.5	13.9	27.8	19.5	0.273
G	13.9	4.4	13.9	27.8	18.3	0.267
H	27.7	—	0	27.7	—	0.274

a) The initial panipenem concentrations were 13.8 mM and 27.7 mM, in Sample A and Sample H, respectively, and in Sample A and Sample H, no additional panipenem was added. Samples B—G are the solutions which contained degradation products. The first kinetic run time of each sample (Samples B—G) was 1, 2, 3, 4, 5, and 6 h, respectively. The first kinetic runs were stopped by ice-cooling the test solution. *b*) [I] (mM): initial panipenem amount before the first kinetic runs. *c*) [R] (mM): remaining panipenem concentration after the first kinetic runs. *d*) [A] (mM): amount of newly added panipenem. *e*) [I]+[A] (mM): sum of initial and added panipenem amount. *f*) [R]+[A] (mM): sum of remaining and added panipenem amount.

were obtained. The effect of pH on k_0 and k_1 is represented in Figs. 5 and 6, respectively.

As the data plot in Fig. 5 seemed to display specific acid-base catalysis kinetics, Eq. 3 was used to represent the model kinetic equation for the effect of pH on k_0.

$$k_0 = k_H \cdot [H^+] + k_{OH} \cdot \frac{K_W}{[H^+]} + k_{H_2O} \quad (3)$$

where k_H and k_{OH} are the second-order rate constants for the hydrogen-ion-catalyzed degradation and the hydroxide-ion-catalyzed degradation reaction, respectively. k_{H_2O} is the first-order rate constant for the spontaneous water-catalyzed degradation reaction, and K_W is the dissociation constant of water ($K_W = 10^{-13.53}$ at 40 °C).[14]

Non-linear regression was performed on the data plot in Fig. 5, and the unknown parameters in Eq. 3 were calculated to be $k_H = 3.33 \times 10^3$ ($h^{-1} \cdot M^{-1}$), $k_{OH} = 3.50 \times 10^4$ ($h^{-1} \cdot M^{-1}$), and $k_{H_2O} = 2.21 \times 10^{-2}$ (h^{-1}). The regression curve fitted the observed data points well (Fig. 5).

As for the effect of pH on k_1, by looking at the data plot in Fig. 6, an inverse relationship between k_1 and pH is observed until about pH 6.0. As shown above, these results reflected the observation that the rate constant was affected by the initial panipenem concentration in an acidic solution, but not in an alkaline solution.

Based on the results shown in Fig. 6 and the general acid catalytic effect of the undissociated acetic acid shown in Fig. 8, it was hypothesized that the undissociated form of panipenem had the general acid catalytic effect. Then, the portion of the undissociated form of panipenem was calculated, Eq. 4 was used to represent a line passing through the data points of Fig. 6.

$$k_1 = k_1' \cdot f_1 \quad (4)$$

where k_1' is a constant, and f_1 is the proportion of the undissociated form of panipenem. Considering the dissociation constant of the carboxyl group of panipenem, f_1 can be expressed by Eq. 5.

$$f_1 = \frac{[H^+]}{[H^+] + K_a} \quad (5)$$

where K_a is the dissociation constant of the carboxyl group of panipenem. The apparent pK_a value of panipenem was directly measured by a photometric method, and the obtained apparent pK_a value of the carboxyl group was 3.4. Using Eqs. 4 and 5, non-linear regression was performed on the data points in Fig. 6. As a result, it was found that $k_1' = 1.21 \times 10^2$ ($h^{-1} \cdot M^{-1}$) could lead the best fit of the regression curve to the observed data points (Fig. 6).

Thus, it was shown that the rate constants of the degradation of panipenem depended on both the pH value of the solution and initial panipenem concentration. Equation 6 was obtained from Eqs. 2—5.

$$k_{obs} = \left\{ k_H \cdot [H^+] + k_{OH} \cdot \frac{K_W}{[H^+]} + k_{H_2O} \right\} + \left\{ k_1' \cdot \frac{[H^+]}{[H^+] + K_a} \right\} \cdot [PAPM]_0 \quad (6)$$

where k_H, k_{OH}, K_W, k_{H_2O}, k_1', and K_a are constants, and $[H^+]$ and $[PAPM]_0$ are variables.

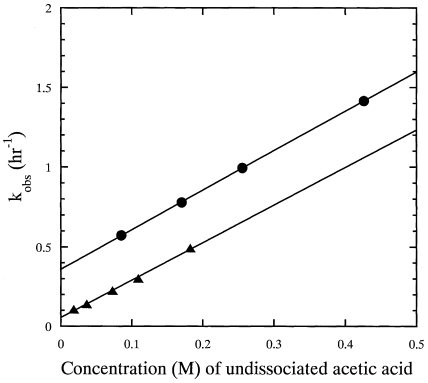

Fig. 8. The Catalytic Effects of Undissociated Acetate Buffer on the Observed Rate Constants (k_{obs}) at 25 °C, pH 4.0 (●) and pH 5.0 (▲)

From Eq. 6, the rate constant can be expressed as a function of pH and the initial panipenem concentration, by considering the specific acid-base catalysis theory and the general acid catalysis theory about the carboxyl group of panipenem.

Based on Eq. 6, different initial panipenem concentrations would produce different k_{obs} values, which was a result observed in our study (samples A and H in Table 1, and Fig. 4). This demonstrates the effect of panipenem itself on the degradation rate. However looking at samples B—G in Table 1, despite the different amounts of panipenem concentrations ("[Remaining]+[Added]" in Table 1), the rate constants were the same. This indicates that panipenem degradation products also affect the degradation rate. Moreover, the rate constants of samples B—G were equal to the rate constant of sample H, which contained no degradation products at the beginning of the kinetic run. Thus, it can be considered that panipenem and its degradation products affect the degradation of panipenem to the same extent.

Conclusions

The degradation of panipenem in an aqueous solution was investigated, and it was shown that the degradation mechanism of panipenem was very unusual. The degradation rate followed a pseudo-first-order reaction. However, in an acidic solution, the rate constants were affected by the initial panipenem concentration. This unusual phenomenon could be explained by taking into account the catalytic effect of the degradation products and the amount of the undissociated form of the carboxylic group of panipenem.

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