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Thermodegradation Kinetics of the New Antibacterial Fluoroquinolone Derivative, Sparfloxacin, in Aqueous Solution

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The degradation kinetics of sparfloxacin (5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) were examined as functions of pH (1.0—10.5), temperature (100—120 °C) and buffer concentration (0.01—0.05 M).

The degradation kinetics for sparfloxacin in aqueous solution followed a pseudo-first-order reaction under all experimental conditions. No appreciable effect of buffer species on the degradation of sparfloxacin was observed within the experimental conditions. The log *k*–pH profiles showed specific-acid catalysis at a pH below 4.0, and there were inflection points near pH 6 and 9 corresponding to the pK_{a1} and pK_{a2} values. Arrhenius data in this study showed that the degradation of sparfloxacin was negligible at room temperature between pH 1.0—10.5.

Key words degradation kinetics; fluoroquinolone derivative; sparfloxacin; stability

Sparfloxacin (SPFX), 5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, is a synthetic antibacterial agent and also a fluoroquinolone derivative. The degradation kinetics of a fluoroquinolone derivative, orbifloxacin, in aqueous solution has been reported.[1]

The objective of this investigation was to study the degradation kinetics of SPFX in aqueous solution under various storage conditions such as pH, temperature and buffer concentration, and to obtain the information for the stability.

Experimental

Materials SPFX was prepared in our laboratories. Other chemicals used in this experiment were reagent grade commercial products.

Kinetic Study The thermodegradation kinetic study of SPFX was performed in 0.1 N HCl and 0.02 M buffer solutions of various pHs (2.5—10.5) at an ionic strength of 0.1. The buffers used as solvents were citrate buffer (pH 2.5—3.5), acetate buffer (pH 4.0—5.6), phosphate buffer (pH 6.0—8.6), borate buffer (pH 9.0—9.5) and carbonate buffer (pH 10.5). And five buffer solutions (pH 3.0 citrate buffer, pH 5.0 acetate buffer, pH 7.2 phosphate buffer, pH 9.0 borate buffer and pH 10.5 carbonate buffer) of 0.01 and 0.05 M were used for investigating the catalytic effect of the buffer species. The ionic strength of each buffer was adjusted to 0.1 with sodium chloride. The sample solutions were prepared by dissolving an amount of SPFX (0.05 mg/ml) in each of the buffers described above. Two ml of these sample solutions were sealed in 10-ml glass ampoules and stored in constant-temperature ovens at selected temperatures (100, 110 and 120 °C). At designated storage intervals, these samples were taken out from the ovens and stored in a −20 °C freezer. At the time of analysis, these samples were thawed by warming at 80 °C for 5 min and then assayed by HPLC. Separately, the pH values of these sample solutions for the degradation kinetic study were measured at 25, 40, 50 and 70 °C, and these pH values were plotted against the reciprocal of the absolute temperature. Then, the pH values of these sample solutions at 100, 110 and 120 °C were obtained by extrapolation.

Assay of Intact SPFX The rates of thermodegradation were monitored by reversed-phase HPLC. The HPLC consisted of a Shimadzu model LC-6A pump, an SPD-2A UV-detector, a Chromatopack C-R5A integrator and an SIL-6A injector with a 100-μl loop. A Develosil ODS-5 column (4 mm i.d. × 150 mm, particle size 5 μm, Nomura Chemical Co., Ltd.) was used in this study. Column temperature was maintained at 40 °C by a column oven. A mobile phase system of 0.1 M citrate buffer (pH 3.5)–methanol–dioxane (84 : 12 : 5) was used for the determination of SPFX. The flow rate was 1.2 ml/min and the column eluent was monitored at 290 nm.

Results and Discussion

Reaction Order and Rate Constants The time course of the decrease in concentration of SPFX at 100 °C is shown in Fig. 1. The relationship between the logarithm of residual percentage and the storage time was linear, indicating a pseudo-first-order reaction for the degradation of SPFX in aqueous solution. The regression lines were linear and the correlation coefficients were above 0.98 under the all experimental conditions. The pseudo-first-order rate constants (*k*) were determined from the slopes of the graph by a statistical regression analysis method.

Effect of Buffer Species The effect of general acid/base catalysis caused by buffer species on the degradation kinetics of SPFX was studied by varying the total buffer concentration from 0.01 to 0.05 M, while maintaining constant pH, temperature (100 °C) and ionic strength. The degradation rates did not vary under the various concentrations of buffer at each specific pH range. This fact suggests that no appreciable effects on the degradation of SPFX were related to any buffer species used in

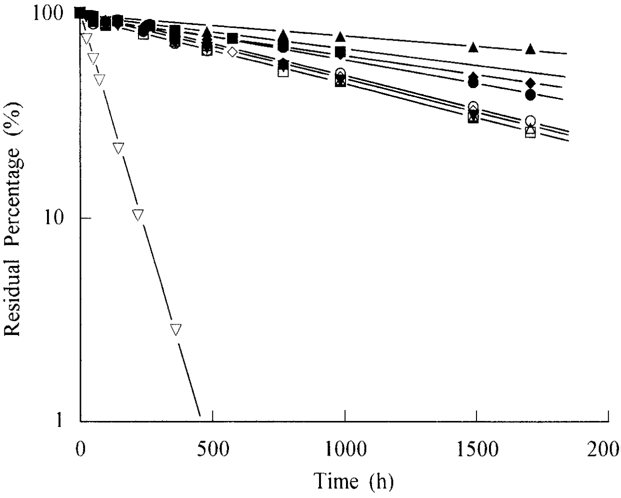

Fig. 1. Apparent First-Order Plots for the Degradation of SPFX at Various pH Values, 100 °C and Ionic Strength of 0.1

▽, pH 1.04; ■, pH 2.81; ●, pH 3.45; ▲, pH 4.77; ◆, pH 6.09; □, pH 6.66; ○, pH 7.62; △, pH 8.37; ◇, pH 8.99; ▼, pH 9.68.

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this study.

log k–pH Profile The log k–pH profiles for the degradation of SPFX (Fig. 2) were constructed from the logarithm of the pseudo-first-order rate constants and pH values at 100, 110 and 120 °C. The log k–pH profiles show that SPFX is the most stable at pH 4—5 and most unstable in strongly acidic solutions. In the acidic region below pH 4, the log k–pH profiles indicated specific hydrogen-ion catalysis of cationic species. The degradation rates increased gradually with an increase in pH from 5 to 7 and were almost constant from pH 7 to 8. These results can be explained by an increase in the neutral species (or zwitterion of SPFX) attacked by water molecules or by kinetically equivalent specific hydroxide-ion catalysis of cationic species. Then, the log k–pH profiles between pH 8 and 9 indicate the water-catalysis of anionic species or the kinetically equivalent hydroxide-ion catalysis of the neutral species (or zwitterion). Two inflection points in the log k–pH plots near pH 6 and 8 indicate that the dissociation of a carboxyl group and dimethylpiperazinyl group in SPFX influence the degradation rate.

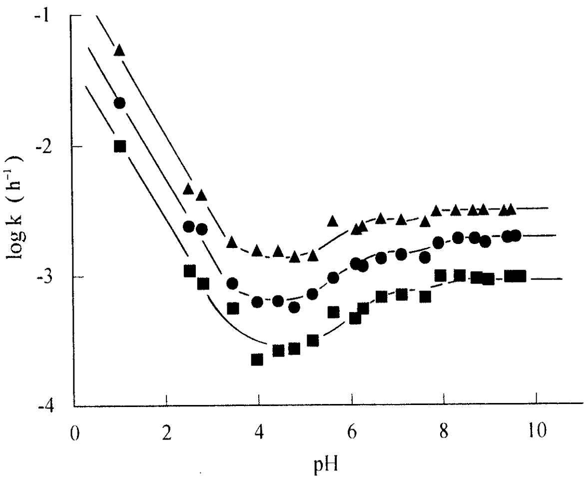

Fig. 2. log k-pH Profiles for the Degradation of SPFX at Various Temperatures

■, 100 °C; ●, 110 °C; △, 120 °C.

SPFX have been known to degrade to decarboxylated SPFX (Chart 1) in aqueous solution.[2)] In the previous report[1)] it has been described that orbifloxacin decomposes in alkaline solution. Orbifloxacin has a fluorine atom at the 5-position and a hydroxide ion appears to attack the C–F bond at the 5-position. However, the degradation mechanism of SPFX in an alkaline condition seems to be different from that of orbifloxacin. The specific hydroxide-ion catalysis of an anionic species was not observed in the log k–pH profiles for the degradation of SPFX, and the hydroxylated compound at the 5-position was not detected in the alkaline solution of SPFX under severe conditions. These results suggest that hydrolysis of the C–NH_2 bond at the 5-position does not occur during the degradation of SPFX.

Thus, the log k–pH profiles could be fitted to the following relationship:

$$k=(k'_{\mathrm{H}}\cdot a_{\mathrm{H}}+k'_{\mathrm{H_2O}})\cdot\frac{a_{\mathrm{H}}^2}{a_{\mathrm{H}}^2+K_{\mathrm{a1}}\cdot a_{\mathrm{H}}+K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}}+k_{\mathrm{H_2O}}\cdot\frac{K_{\mathrm{a1}}\cdot a_{\mathrm{H}}}{a_{\mathrm{H}}^2+K_{\mathrm{a1}}\cdot a_{\mathrm{H}}+K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}}+k''_{\mathrm{H_2O}}\cdot\frac{K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}}{a_{\mathrm{H}}^2+K_{\mathrm{a1}}\cdot a_{\mathrm{H}}+K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}} \tag{1}$$

where k'_{H} is the second-order rate constant for the hydrogen-ion-catalyzed degradation of cationic species; $k'_{\mathrm{H_2O}}$, $k_{\mathrm{H_2O}}$ and $k''_{\mathrm{H_2O}}$ are the first-order rate constants for the water-catalyzed (or spontaneous) degradation of cationic, neutral and anionic species, respectively; a_{H} is the activity of the hydrogen ion; and K_{a1} and K_{a2} are the dissociation constants of the carboxyl group and dimethylpiperazinyl group of SPFX, respectively.

As shown in Fig. 2, the line represents the theoretical curve calculated from Eq. 1 and the points indicate ex-

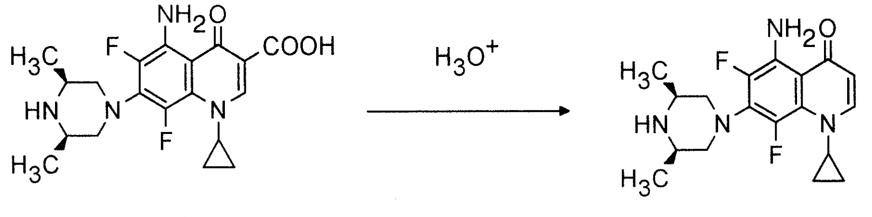

Chart 1. Degradation Pathway of SPFX

Table 1. Catalytic Rate Constants and Arrhenius Parameters for the Degradation of SPFX

	k'_{H} ($\mathrm{M}^{-1}\cdot\mathrm{h}^{-1}$)	$k'_{\mathrm{H_2O}}$ (h^{-1})	$k_{\mathrm{H_2O}}$ (h^{-1})	$k''_{\mathrm{H_2O}}$ (h^{-1})	pK_{a1}	pK_{a2}
Temperature (°C)						
100	1.67×10^{-1}[a)]	3.07×10^{-4}[a)]	6.81×10^{-4}[a)]	9.74×10^{-4}[a)]	6.06[a)]	8.16[a)]
110	3.57×10^{-1}[a)]	6.71×10^{-4}[a)]	1.42×10^{-3}[a)]	1.91×10^{-3}[a)]	6.00[a)]	8.06[a)]
120	8.22×10^{-1}[a)]	1.45×10^{-3}[a)]	2.51×10^{-3}[a)]	3.26×10^{-3}[a)]	5.93[a)]	7.99[a)]
25	6.11×10^{-5}[b)]	1.42×10^{-7}[b)]	1.22×10^{-6}[b)]	2.51×10^{-6}[b)]	6.27[c)], 6.71[b)]	8.80[c)], 9.02[b)]
E_{a} (kcal/mol)	23.24	22.59	18.94	17.61		
ln A	29.54	22.39	18.29	16.85		

a) Based on the kinetics for the degradation of SPFX (Eq. 1). *b*) Estimated from the data of the kinetic study on the degradation of SPFX. *c*) Determined by the titration method.

perimental results. The catalytic rate constants obtained from the best fits to the observed $\log k$–pH profiles are given in Table 1. The reasonable agreements indicate that Eq. 1 adequately describes the degradation kinetics.

Dependence of Degradation Rate on Temperature The catalytic rate constants and Arrhenius parameters are listed in Table 1. The activation energies (E_a) for k'_H, k'_{H_2O}, k_{H_2O} and k''_{H_2O} were found to be 23.24, 22.59, 18.94 and 17.61 kcal/mol, respectively. The values of the dissociation constant, pK_{a1} and pK_{a2}, at 100, 110 and 120 °C, were determined by the best fits to the $\log k$–pH profiles (Table 1), and the dissociation constants at 25 °C are estimated from the van't Hoff equation. The pK_{a1} and pK_{a2} at 25 °C derived kinetically ($pK_{a1}=6.71$, $pK_{a2}=9.02$) agreed comparatively well with the values of the dissociation constants ($pK_{a1}=6.27$, $pK_{a2}=8.80$)[2] determined by the titration method.

The stability of SPFX in an aqueous solution at a specific temperature can be predicted from the Arrhenius parameters listed in Table 1. The degradation rate constants predicted over the pH range of 1.0 to 10.5 at room temperature (25 °C) are estimated to be 1.49×10^{-7}—$6.25 \times 10^{-6}\,h^{-1}$.

Consequently, SPFX appears to be very stable in an aqueous solution at room temperature.

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References

1) Morimura T., Ohno T., Matsukura H., Nobuhara Y., *Chem. Pharm. Bull.*, **43**, 1052—1054 (1995).
2) Segawa Y., Fujita K., Nobuhara Y., Nagai Y., *Kagakuryoho No Ryoiki* (*Antibiol. Chemother.*), **10**, 700—707 (1994).