# Photodegradation Kinetics of the New Antibacterial Fluoroquinolone Derivative, Orbifloxacin, in Aqueous Solution

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**The photodegradation kinetics of orbifloxacin (1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid) was investigated in aqueous solution at various pH values (1.2—12.5) and at an ionic strength of 0.5. The photodegradation experiments were performed using a fluorescent or a chemical lamp as a light source and the cumulative number of photons during exposure was determined by a ferrioxalate actinometer. It was found that the photodegradation of orbifloxacin followed apparent first-order kinetics under both types of artificial light. The photodegradation rates of orbifloxacin in a neutral medium were higher than those in acidic and alkaline media. Orbifloxacin was most unstable in solution at pH 7.4, and its degradation half-life was 0.9 h. Also, the log *k*–pH profile indicated that the photodegradation rate of orbifloxacin was related to the dissociation of the carboxylic and dimethylpiperazinyl groups and the main photo-labile species was the zwitterionic form. In addition, the photodegradation kinetics of the decarboxylated derivative of orbifloxacin in aqueous solution was investigated to determine the effect of the functional groups on the photodegradation of orbifloxacin.**

**Key words** kinetics; photodegradation; fluoroquinolone derivative; ferrioxalate actinometer; orbifloxacin; log *k*–pH profile

There have been many reports relating to the photodegradation kinetics of drugs in aqueous solution. The effects of the wavelength of light sources, temperature, pH, dissolved oxygen, additives and structural properties have been studied in detail as potential factors affecting the photostability of drugs. In the case of ionizable compounds, such as nifedipine[1] and furosemide,[2] there are large differences between the photostability of the ionic and nonionic forms. Therefore, to obtain information for a formulation study, it is necessary to examine the relationships between dissociation and photodegradation kinetics and to study the photostability of each species involving the dissociated and undissociated functional groups.

In this study, some environmental factors affecting the photostability of orbifloxacin (1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid), a new antibacterial fluoroquinolone derivative, were investigated in aqueous solution. Then the photodegradation kinetics of the decarboxylated derivative of orbifloxacin in aqueous solution was investigated in order to elucidate the effect of the functional groups on the photodegradation kinetics of orbifloxacin and the relationship between structural factors and photodegradation kinetics was examined by comparing the photodegradation kinetics of orbifloxacin with that of decarboxylated orbifloxacin.

## Experimental

**Materials** Orbifloxacin and decarboxylated orbifloxacin (Chart 1) were prepared in our laboratories. All other chemicals used in this experiment were of reagent grade.

**Photodegradation Studies** The photodegradation kinetics of orbifloxacin and decarboxylated orbifloxacin were studied in aqueous solutions at different pH (1.2—12.5) and at total buffer concentration of 0.1 M and ionic strength of 0.5. Citrate buffer (pH 1.2—3.5), acetate buffer (pH 4.4—5.6), phosphate buffer (pH 6.5—8.6), borate buffer (pH 9.0—9.5), carbonate buffer (pH 10.5) and 0.1 N sodium hydroxide solution (pH 12.5) were used as solvents. The ionic strength of each buffer was adjusted to 0.5 with sodium chloride. Orbifloxacin and decarboxylated orbifloxacin were dissolved in these buffers and the resulting solutions (0.1 mg/ml) were used as samples. Twenty-five milliliters each of these sample solutions was put into a test tube of Pyrex glass with a stopper, and the exposure test was performed at room temperature by irradiating these sample solutions with a chemical (FL20S BL, Toshiba Electric Co., Ltd., Tokyo) or a fluorescent lamp (FL15W, Toshiba Electric Co., Ltd., Tokyo) at a distance of approximately 200 mm. At appropriate time intervals, a portion (0.4 ml) of the sample solution was withdrawn from the test tube, mixed with the same volume of internal standard solution (0.1 mg/ml methyl *p*-hydroxybenzoate in methanol) and assayed by HPLC. When the intact orbifloxacin in 0.1 N sodium hydroxide solution was determined, the sample solution irradiated was neutralized with 0.4 ml of 0.1 N hydrochloric acid solution before adding the internal standard solution to avoid hydrolysis of the methyl *p*-hydroxybenzoate used as internal standard.

**Actinometry** For the measurement of light intensity, potassium ferrioxalate was selected as an actinometer.[3] Fe(II) ion formed in the photodegradation solution was determined by spectrophotometry at 550 nm as a complex with 1,10-phenanthroline.

The experimental conditions for actinometry were identical to those for the photodegradation study of orbifloxacin. The light intensities absorbed by the solution under the fluorescent and chemical lamps were found to be $1.86 \times 10^{22}$ photons per hour and $4.33 \times 10^{22}$ photons per hour, respectively.

**Assay of Intact Orbifloxacin and Decarboxylated Orbifloxacin.** The rates of photodegradation were monitored by reversed-phase HPLC. The HPLC equipment consisted of a Shimadzu model LC-6A pump, an SPD-2A UV-detector, a Chromatopack C-R5A integrator and a SIL-6A injector with 100-$\mu$l loop. A Develosil ODS-7 column (4 mm i.d. × 250 mm, particle size 7 $\mu$m, Nomura Chemical Co., Ltd.) was used in this study and the column temperature was maintained at 40 °C in the 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 orbifloxacin and a 0.1 M citrate buffer (pH 3.5)–methanol–dioxane system (84 : 6 : 5) was used for the determination of decarboxylated orbifloxacin. The flow rate was 1.5 ml/min and the column eluent was monitored at 290 nm.

**Determination of Dissociation Constants of Orbifloxacin and Decarboxylated Orbifloxacin** The apparent dissociation constants of orbifloxacin and decarboxylated orbifloxacin were determined spectrophotometrically at room temperature.[14] The apparent dissociation constants of orbifloxacin, $pK_{a1}$ and $pK_{a2}$, which refer to the dissociation of the 3-carboxylic group and the 7-dimethylpiperazinyl group, were 5.60 and 8.90, respectively, whereas the dissociation constant of decarboxylated orbifloxacin, $pK_a$, which refers to the dissociation of the 7-dimethylpiperazinyl group, was 9.06.

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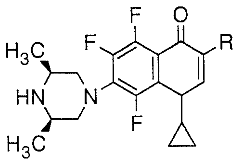

| compound | R |
|---|---|
| orbifloxacin | COOH |
| decarboxylated orbifloxacin | H |

Chart 1

## Results and Discussion

### Reaction Order and Photodegradation Rate Constants

For the precise study of photodegradation kinetics, it is essential that the light intensity per hour is constant during the exposure test. The application of a chemical actinometer to the determination of the light intensity was reported in the study evaluating the photostability of nifedipine.3)

In this report, potassium ferrioxalate was used to measure the number of photons provided by the light sources and the cumulative number absorbed by the solution was used as an index of light intensity. The light intensity of both light sources was constant during the exposure test.

The time course of the photodegradation of orbifloxacin at various pH values under the chemical lamp is shown in Fig. 1. The photodegradation of orbifloxacin was found to follow apparent first-order kinetics at all pH values. The observed first-order rate constants (*k*) were obtained from the slopes of the first-order plots. When kept in the dark, these solutions were completely stable over a period of 30 h.

**Effect of Light Source** The plots of the remaining amount of orbifloxacin against the cumulative number of photons under different light sources at pH 7.5 are shown in Fig. 2. Although the illumination by the fluorescent lamp was more intense than that of the chemical lamp (chemical lamp: 110 lux, fluorescent lamp: 1500 lux), the photodegradation rate under the fluorescent lamp was lower than that with the chemical lamp. The fluorescent lamp irradiates visible light (wavelength: over 420 nm), while the chemical lamp irradiates near ultraviolet rays (wavelength: about 300—400 nm). Therefore, the photodegradation of orbifloxacin is considered to be accelerated by near ultraviolet rays. These results show that the chemical lamp has enough strength to decompose the compound in a short period.

The observed rate constants and half-lives for the degradation of orbifloxacin under both light sources at various pH values are listed in Table 1. The photodegradation rates in neutral solution were higher than those in acidic and alkaline solutions under both light sources, although the spectral irradiation energy of these light sources are different.

**Effect of Initial Concentration of Orbifloxacin** Orbifloxacin solutions of different concentrations (0.05—1.0 mg/ml) in pH 7.5 phosphate buffer were placed under the chemical lamp, and the remaining amount of orbifloxacin was determined at appropriate time intervals. The results are shown in Fig. 3. The photodegradation under all experimental conditions was found to follow apparent first-order kinetics. The photodegradation rate decreased with increasing initial concentration. The report of Connors *et al.*5) suggests that these results can be explained by fact that the quenching of excited orbifloxacin molecules occurred more frequently with increasing initial concentration. Similar results have been reported by Akimoto *et al.*6) and Hanamori *et al.*7)

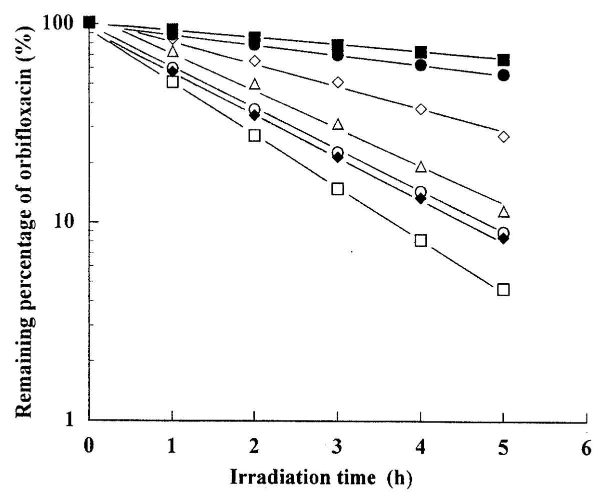

Fig. 1. Apparent First-Order Plots for the Photodegradation of Orbifloxacin under the Chemical Lamp at Room Temperature

■, pH 1.2; ●, pH 2.5; ◇, pH 4.4; ◆, pH 6.2; □, pH 7.3; ○, pH 8.6; △, pH 10.5.

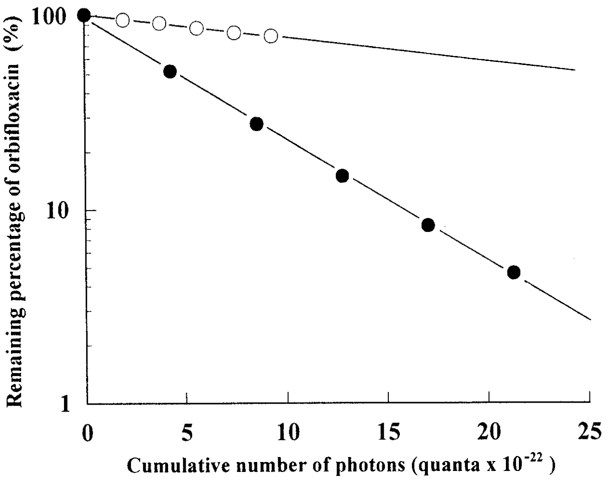

Fig. 2. Effect of the Light Source on the Photodegradation of Orbifloxacin in pH 7.5 Buffer

●, chemical lamp; ○, fluorescent lamp.

Table 1. Kinetic Data for the Photodegradation of Orbifloxacin in Buffer Solutions Exposed to Chemical and Fluorescent Lamps

| pH | Chemical lamp[a] | | Fluorescent lamp[b] | |
|---|---|---|---|---|
| | $k$ ($h^{-1}$) | $t_{1/2}$ (h) | $k$ ($h^{-1}$) | $t_{1/2}$ (h) |
| 2.5 | $8.2 \times 10^{-2}$ | 8.5 | $2.0 \times 10^{-2}$ | 34.7 |
| 4.4 | $11.6 \times 10^{-2}$ | 6.0 | $1.9 \times 10^{-2}$ | 36.5 |
| 6.2 | $49.3 \times 10^{-2}$ | 1.4 | $4.6 \times 10^{-2}$ | 15.1 |
| 7.3 | $61.5 \times 10^{-2}$ | 1.1 | $5.2 \times 10^{-2}$ | 13.3 |
| 8.6 | $48.2 \times 10^{-2}$ | 1.4 | $4.6 \times 10^{-2}$ | 15.1 |
| 10.5 | $26.0 \times 10^{-2}$ | 2.7 | $2.0 \times 10^{-2}$ | 34.7 |

*a*) Light intensity absorbed by the solution was $4.33 \times 10^{22}$ photons/h. *b*) Light intensity absorbed by the solution was $1.86 \times 10^{22}$ photons/h.

**Effects of Buffer Concentration and Ionic Strength** It is known that the buffer concentration affects the photodegradation of piperazine derivatives[6)] and in some cases the photodegradation of drugs is inhibited by halogen, alkaline metal and alkaline earth metal ions.[7)] Therefore, the effect of concentrations of buffers and sodium chloride on the photodegradation of orbifloxacin was investigated under irradiation by the chemical lamp, but it was found that these factors has no significant effect.

**log *k*–pH Profiles for Photodegradation of Orbifloxacin and Decarboxylated Orbifloxacin** The protonation scheme and distribution diagram for cationic, anionic and zwitterionic forms of orbifloxacin are shown in Fig. 4. Orbifloxacin exists mostly in the cationic form at acidic pH, as an anionic form at alkaline pH and as a zwitterionic form (or neutral species) at neutral pH. The fraction of zwitterion seems to be greatest at the isoelectric point (7.25). The log *k*–pH profile for the photodegradation of orbifloxacin with the chemical lamp is shown in Fig. 5. The log *k*–pH profile represents a bell-shaped curve, as shown in Fig. 5, and the photodegradation rate was greatest near pH 7.4, corresponding to the isoelectric point. These results indicate that the photo-reactivity of the zwitterionic form is greater than that of the cationic and anionic forms. There are two inflection points on the log *k*–pH profile near pH 5 and 9. These inflection points suggest that there are large differences between the photodegradation rate of the zwitterionic form and the cationic or anionic forms. Therefore, the photodegradation rates are affected by the dissociation of the carboxylic and dimethylpiperazinyl groups.

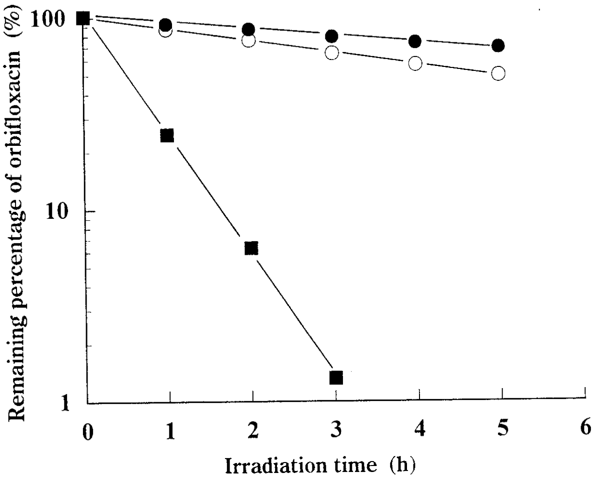

Fig. 3. Effect of the Initial Concentration on the Photodegradation of Orbifloxacin in pH 7.5 Buffer

■, 0.05 mg/ml; ○, 0.1 mg/ml; ●, 1.0 mg/ml.

Thus, the following relationship was fitted to the profile:

$$k=\frac{k_2\cdot a_{\mathrm{H}}^2+k_1\cdot K_{\mathrm{a1}}\cdot a_{\mathrm{H}}+k_3\cdot K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}}{a_{\mathrm{H}}^2+K_{\mathrm{a1}}\cdot a_{\mathrm{H}}+K_{\mathrm{a1}}\cdot K_{\mathrm{a2}}} \qquad (1)$$

where $k_1$, $k_2$ and $k_3$ are the first-order rate constants for the photodegradation of the zwitterion, cation and anion forms of orbifloxacin, respectively, $a_{\mathrm{H}}$ is the hydrogen-ion activity, and $K_{\mathrm{a1}}$ and $K_{\mathrm{a2}}$ are the dissociation constants of the carboxylic and dimethylpiperazinyl groups of orbifloxacin, respectively.

The values of the first-order rate constants, $k_1$, $k_2$, $k_3$ and the dissociation constants, $K_{\mathrm{a1}}$ and $K_{\mathrm{a2}}$, estimated

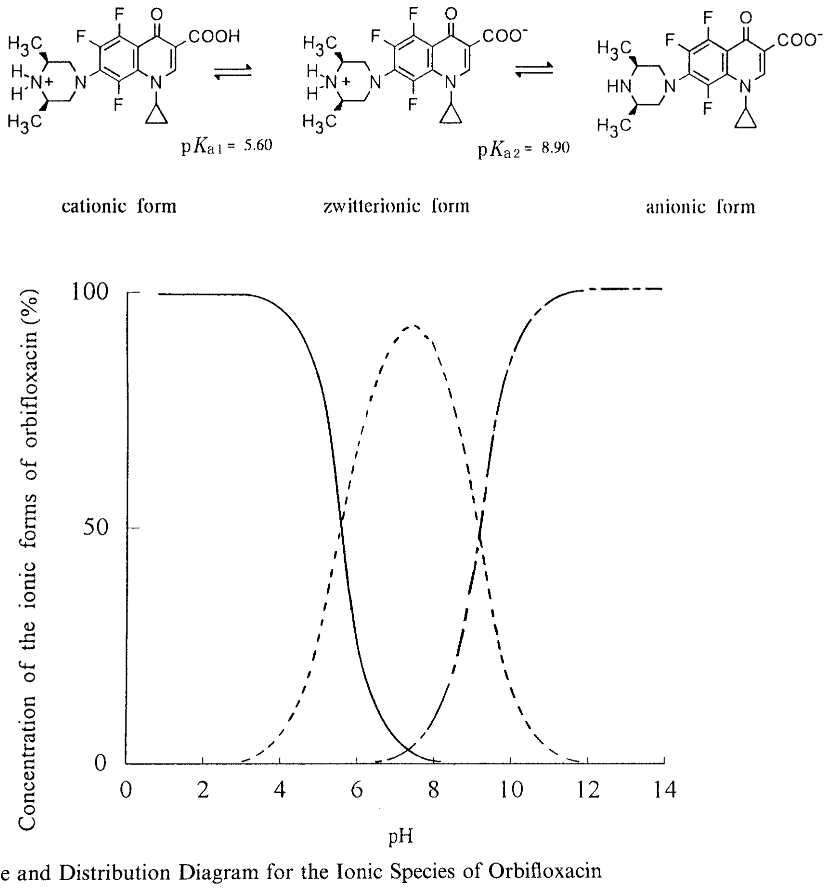

Fig. 4. Protonation Scheme and Distribution Diagram for the Ionic Species of Orbifloxacin

—-—, anionic form; ------, zwitterionic form; ——, cationic form.

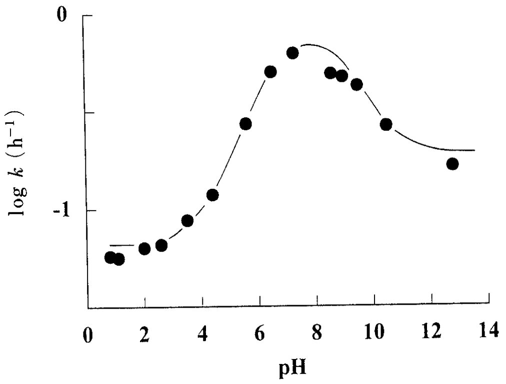

Fig. 5. log *k*–pH Profile for the Photodegradation of Orbifloxacin

from the best fits of the log*k*–pH profile are as follows:

$k_1 = 0.810\,\mathrm{h}^{-1}$
$k_2 = 0.064\,\mathrm{h}^{-1}$
$k_3 = 0.217\,\mathrm{h}^{-1}$
$K_{a1} = 10^{-5.1}$
$K_{a2} = 10^{-9.4}$

In Fig. 4, the line represents the theoretical curve calculated from Eq. 1 and the points show the experimental results. The reasonable agreement indicates that Eq. 1 describes adequately the photodegradation kinetics.

Furthermore, the $\mathrm{p}K_{a1}$ and $\mathrm{p}K_{a2}$ values (5.14 and 9.41) derived kinetically agree comparatively well with the values obtained spectrophotometrically (5.60 and 8.90).

According to Eq. 1, the photostability of orbifloxacin in aqueous solutions can be predicted. Thus, it can be calculated that the photodegradation rate constant of orbifloxacin in a solution of pH 7.4 is $0.76\,\mathrm{h}^{-1}$ ($t_{1/2} = 0.9\,\mathrm{h}$) when the sample solution is exposed to the chemical lamp.

To investigate the influence of structural factors on the degradation rate, the photodegradation kinetics of decarboxylated orbifloxacin was studied at various pH values. The protonation scheme and the distribution diagram for the ionic and nonionic species of decarboxylated orbifloxacin are shown in Fig. 6, and the log*k*–pH profile for the photodegradation of decarboxylated orbifloxacin under the chemical lamp is shown in Fig. 7. The photodegradation of decarboxylated orbifloxacin followed first-order kinetics under various pH conditions. The photodegradation rates were high below a pH of about 8, and decreased with increasing pH above 8. An inflection point near pH 9 shows that there are large differences between the photodegradation rate of the cationic form and the nonionic species. As shown in Figs. 6 and 7, the apparent photodegradation rates increased as the fraction of the cationic species increased. These results suggest that the cationic species of decarboxylated orbifloxacin is photo-labile. The photodegradation rate constants of the cationic and nonionic species were $0.557\,\mathrm{h}^{-1}$ and $0.031\,\mathrm{h}^{-1}$, respectively.

Comparing these log*k*–pH profiles, the effect of structural factors on the degradation rate can be shown clearly. In the case of decarboxylated orbifloxacin, the dissociation of one function, the dimethylpiperazinyl group, seems to promote photodegradation. In the case of orbifloxacin, as the photodegradation rate of the zwitterionic form is greatest, the dissociation of the carboxylic and dimethylpiperazinyl groups seems to promote photodegradation.

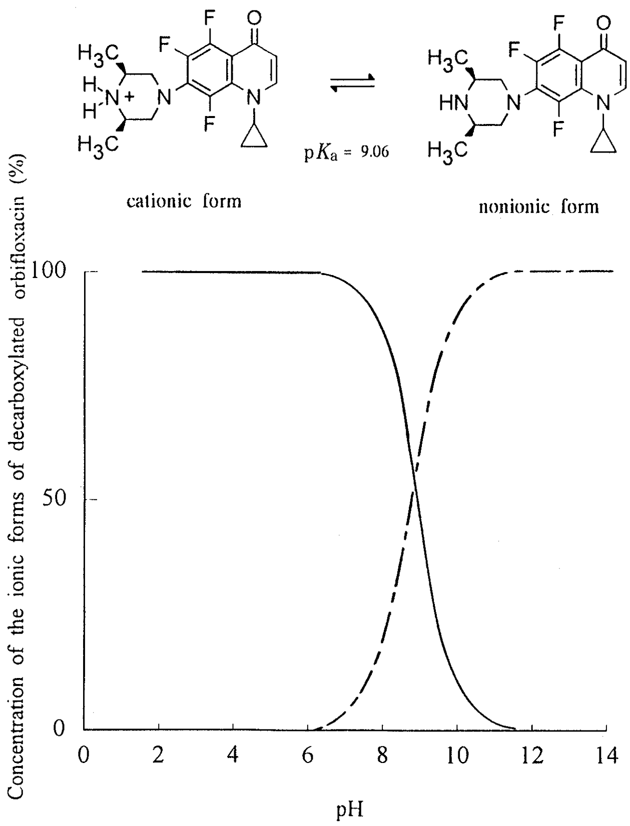

Fig. 6. Protonation Scheme and Distribution Diagram for the Ionic and Nonionic Species of Decarboxylated Orbifloxacin

–·–, nonionic form; ——, cationic form.

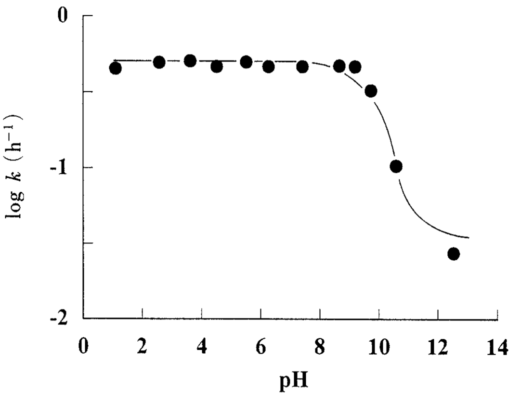

Fig. 7. log *k*–pH Profile for the Photodegradation of Decarboxylated Orbifloxacin

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