The Effect of Temperature and pH on the Solubility of Quinolone Compounds: Estimation of Heat of Fusion

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Received May 28, 1993; accepted November 18, 1993

Although many reports involving fluoroquinolone agents have been published in the past decades, only a few address preformulation studies. In this paper, we describe the effect of temperature and pH on the aqueous solubility of two typically used quinolones, ciprofloxacin and norfloxacin. We measured the aqueous solubilities over the pH range of 5.5 to 9.5 at temperature of 6, 25, 30, and 40°C. The intrinsic solubilities and the thermodynamic dissociation constants were determined from solubility data and the temperature dependence of the intrinsic solubility was evaluated using van't Hoff and Hildebrand plots. The heat of fusion was determined from these two plots. When the heat of fusion was compared to that measured from differential scanning calorimetry (DSC) studies, we found that the Hildebrand method overestimated, and the van't Hoff equation underestimated, the heat of fusion. From the absolute values of the relative errors, the Hildebrand plot produced the better results. DSC results show that the heat of fusion is 15.41 kcal/mol for ciprofloxacin and 7.88 kcal/mol for norfloxacin.

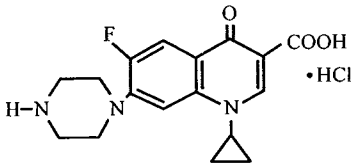
KEY WORDS: ciprofloxacin; norfloxacin; solubility; dissociation constants; heat of fusion.

INTRODUCTION

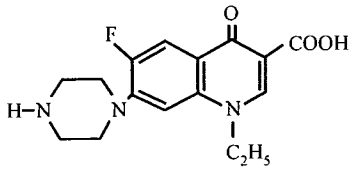
Quinolone antibacterials have aroused much interest because of their potency and efficacy (1–3). The first analogue of this class of synthetic agents used clinically was nalidixic acid for treatment of urinary tract infections. In the past decade, many fluoroquinolone agents have been developed or are under development into appropriate dosage forms. However, only a few address preformulation studies (4–8). Ross and Riley (4) reported the effect of temperature on solubilities of quinolones, but they did not build a detailed correlation between temperature and solubility. To date, there is no information in the literature on important physicochemical parameters such as heat of fusion.

Ciprofloxacin and norfloxacin are two clinically used quinolones. The structures of ciprofloxacin and norfloxacin are shown below.

We studied the effect of temperature and pH on their solubilities. We measured the solubilities of ciprofloxacin and norfloxacin over the pH range from 5.5 to 9.5 and at temperatures of 6, 25, 30, and 40°C. The intrinsic solubility and the thermodynamic macroscopic dissociation constants



Ciprofloxacin



Norfloxacin

were determined from the pH–solubility profiles. The temperature dependence of the intrinsic solubility of ciprofloxacin and norfloxacin was evaluated using van't Hoff and Hildebrand plots, and the heat of fusion was determined from both plots. We found that the Hildebrand plot gave a better prediction of the heat of fusion.

MATERIALS AND METHODS

Materials

Ciprofloxacin (Lot 41H0008) and norfloxacin (Lot 18F0428) were purchased from Sigma Chemical Co. (St. Louis, MO). Phosphoric acid (85%; Lot 2796) was purchased from Mallinckrodt, Inc. (Paris, KY). Tetrabutylammonium hydroxide solution in water (40%; Lot 01007AZ) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Potassium hydroxide (45%; Lot A44141) was purchased from J. T. Baker Chemicals Co. (Phillipsburg, NJ). Acetonitrile and methanol were UV grade and purchased from Burdick and Jackson (Muskegon, MI).

Methods

Solubility Studies

Solubility studies were performed in a shaking air bath (Lab-Line Instruments Inc., Melrose Park, IL) or with the help of a rotating sample holder (Berkeley, CA) at 6, 25, 30, and 40°C. Solubility was determined over the pH range of 5.5 to 9.5 in increments of about 0.5 pH unit. Excess drug was added to the different buffer solutions, which were maintained at 0.3 ionic strength with NaCl. The solutions were placed in amber vial for light protection and agitated for 3 days. At the end of this time period, the samples were withdrawn and filtered with Sterile Acrodisc 13 0.2-μm syringe filters (Ann Arbor, MI). Precaution was taken to avoid supersaturation or undersaturation. The pH's of filtered solutions were measured and concentrations assayed by HPLC. The solid phase at pH 6 and 9 was then dried and the melting point determined by differential scanning calorimetry (DSC). All solubility measurements were performed in triplicate.

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0724-8741/94/0400-0522$07.00/0

HPLC Assay

HPLC assays were performed using a Microsorb C18 reverse-phase column (3 μm, 4.6 × 50 mm) and UV detector at a wavelength of 278 nm for both ciprofloxacin and norfloxacin. The mobile phase used was 77% 100 m*M* H_3PO_4, 19 m*M* tetrabutylammonium hydroxide, potassium hydroxide (45%) to pH 2.5, 23% methanol/acetonitrile (90/10). The flow rate was 1 mL/min. Injection volume was 10 μL using an ICI LC 1600 autosampler.

DSC Studies

A Perkin–Elmer Model DSC-4 differential scanning calorimeter was used for all measurements. Transition temperature and transition energy were obtained simultaneously. Prior to the measurements, the DSC-4 was calibrated by measuring the melting profile of high-purity indium. For each experiment, approximately 5 mg of sample was sealed in a hermetic aluminum pan, with an empty encapsulated pan as a reference. The temperature program was a linear ramp of 10°C/min from 50 to 300°C. The cell was purged with nitrogen throughout the duration of the DSC run. Norfloxacin was used in DSC studies as received and ciprofloxacin was collected from precipitation after titrating to pH 7. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

DSC Studies

Typical DSC curves for ciprofloxacin and norfloxacin are shown in Figs. 1 and 2. The onset temperature T_m and the heat of fusion ΔH_m are shown in Table I.

Determination of Intrinsic Solubility and Dissociation Constants

It is well-known that the solubility of a zwitterionic compound depends on its ionization constants and intrinsic solubility. Several authors have discussed the determination of apparent macroscopic dissociation constants from pH–solubility profiles (4,9,10). Peck and Benet (11) developed a general method for determining thermodynamic dissociation constants from pH dependent solubility data. For a zwitterionic compound with two ionizable groups, the macroscopic ionization is

$$H_2A^+ \rightleftarrows HA \rightleftarrows A^- \quad (1)$$

The total solubility at any pH is the sum of the concentration of each different species present:

$$S_t = [H_2A^+] + [HA] + [A^-] \quad (2)$$

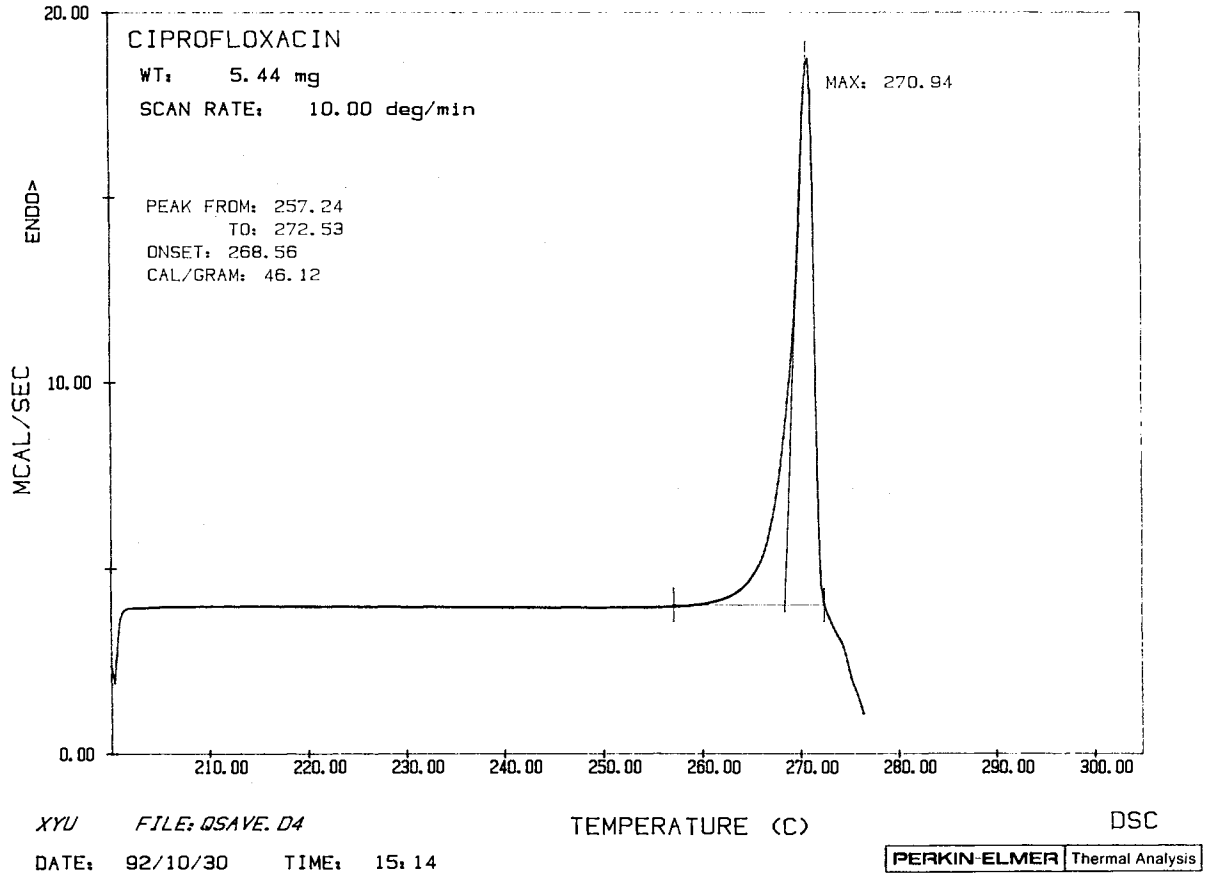

Fig. 1. Typical DSC curve for ciprofloxacin. The mean onset temperature T_m and heat of fusion ΔH_m are 268.3°C and 15.41 kcal/mol.

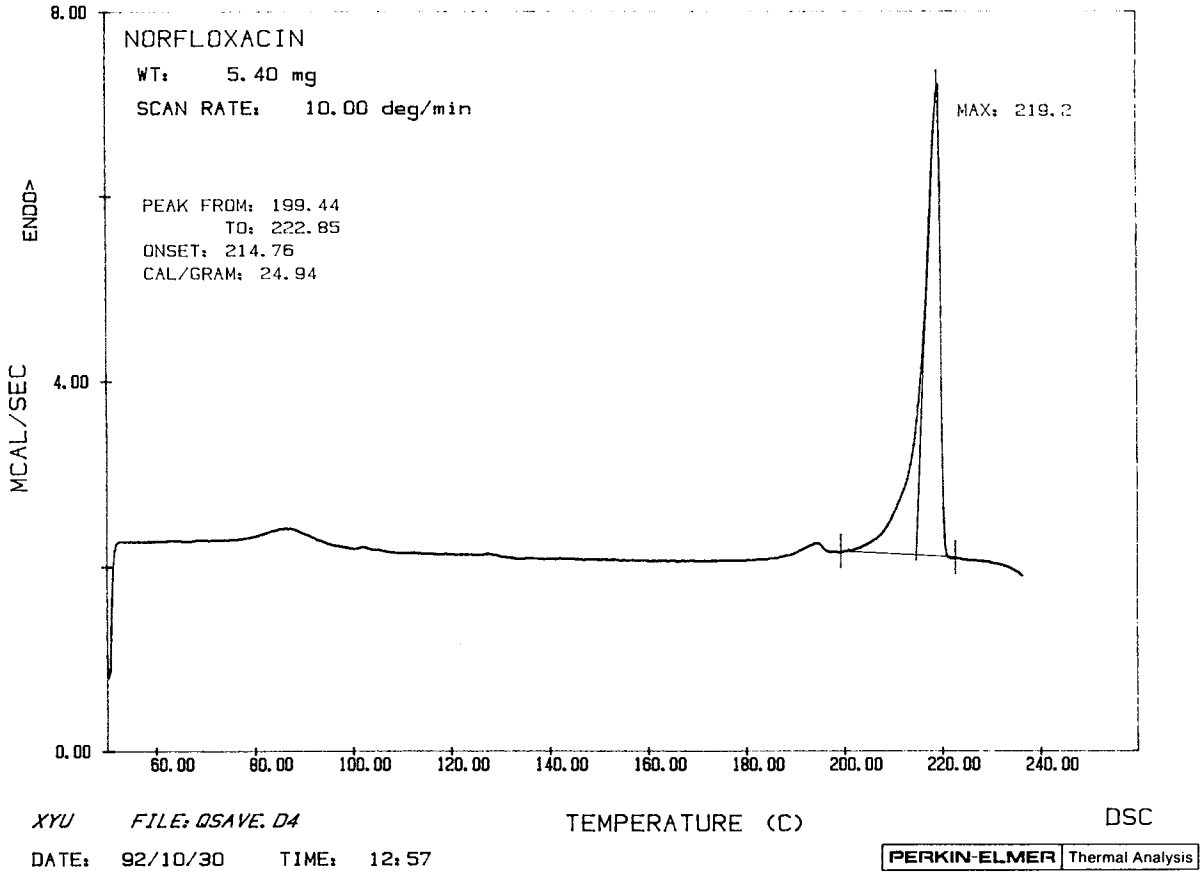

Fig. 2. Typical DSC curve for norfloxacin. The mean onset temperature T_m and heat of fusion ΔH_m are 215.1°C and 7.88 kcal/mol.

Therefore, we can express the solubility of a zwitterionic compound with two ionizable groups by

$$S_t = S_0\left(1 + \frac{a_{H^+}}{K_{a_1}\gamma_{H_2A^+}} + \frac{K_{a_2}}{a_{H^+}\gamma_{A^-}}\right) \quad (3)$$

or

$$S_t = S_0\,(1 + 10^{pK_{a_1} - pH - \log\gamma_{H_2A^+}} + 10^{pH - pK_{a_2} - \log\gamma_{A^-}}) \quad (4)$$

where S_t is the total solubility, S_o is the intrinsic solubility of the species HA, K_{a_1} and K_{a_2} represent the thermodynamic macroscopic dissociation constants, and $\gamma_{H_2A^+}$ and γ_{A^-} are the activity coefficients. The activity coefficients can be estimated using the Davis equation (12):

$$-\log\gamma = Az^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \quad (5)$$

where A is a constant for a given temperature and solvent system, z is the charge on the ion, and I is the total ionic strength. Therefore, S_o, K_{a_1}, and K_{a_2} can be determined by fitting Eq. (4) to the solubility data.

Table I. Onset Temperature and Heat of Fusion for Ciprofloxacin and Norfloxacin from DSC Study

Compound	ΔH_m (kcal/mol)	T_m (°C)
Ciprofloxacin	15.41 (0.15)[a]	268.3 (0.3)
Norfloxacin	7.88 (0.09)	215.1 (0.5)

[a] Standard deviation in parentheses.

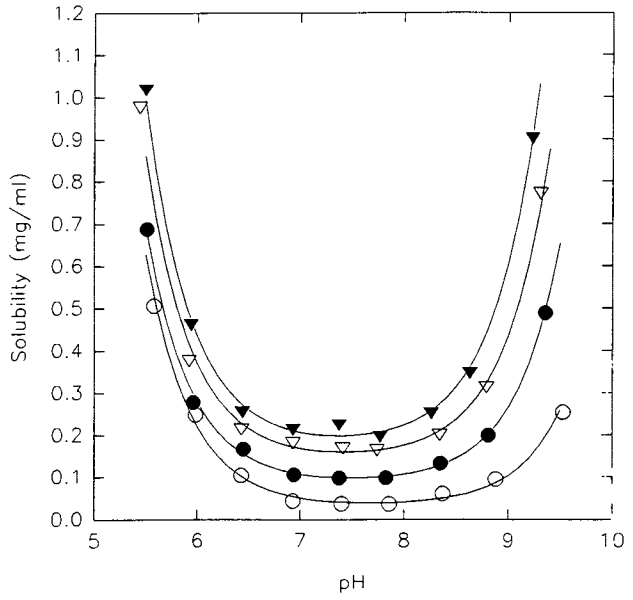

Fig. 3. Solubility of ciprofloxacin as a function of pH at temperatures of 6°C (○), 25°C (●), 30°C (▽), and 40°C (▼). The solid lines represent predictions from Eq. (4).

Table II. Intrinsic Solubility and pK_a of Ciprofloxacin

Temperature (°C)	Estimated parameter		
	S_o (mg/mL)	pK_{a_1}	pK_{a_2}
6	0.037 (0.003)[a]	6.15 (0.07)	8.66 (0.07)
25	0.086 (0.003)		
30	0.14 (0.004)		
40	0.17 (0.01)		

[a] Standard deviation in parentheses.

Table III. Intrinsic Solubility and pK_a of Norfloxacin

Temperature (°C)	Estimated parameter		
	S_o (mg/mL)	pK_{a_1}	pK_{a_2}
6	0.27 (0.01)[a]	6.54 (0.04)	8.50 (0.03)
25	0.37 (0.02)		
40	0.85 (0.04)		

[a] Standard deviation in parentheses.

Figure 3 shows the solubilities of ciprofloxacin as a function of pH at 6, 25, 30, and 40°C. Fitting the data to Eq. (3) using nonlinear regression gives the intrinsic solubility. Table II shows the estimated values. Figure 4 shows the solubility of norfloxacin as a function of pH at 4, 25, and 40°C. We fitted the solubility data of norfloxacin to Eq. (3) and obtained the intrinsic solubility. The results are shown in Table III.

Temperature Dependence of Intrinsic Solubility

The temperature dependence of solubility has been extensively investigated. The ideal solubility of a solid solute at any absolute temperature T can be given by (13,14)

$$\ln X = -\frac{\Delta H_m(T_m - T)}{RTT_m} + \frac{\Delta C_p}{R}\left[\frac{(T_m - T)}{T} + \ln\frac{T}{T_m}\right] \quad (6)$$

where X is the ideal mole fraction solubility of the solute, ΔH_m corresponds to the heat of fusion, and ΔC_p is the difference in heat capacity between the liquid and the solid forms of the compound. By assuming the same heat capacity for both liquid and solid forms, Eq. (6) becomes the van't Hoff equation:

$$\ln X = -\frac{\Delta H_m(T_m - T)}{RTT_m} \quad (7)$$

or

$$\ln X = -\frac{\Delta H_m}{R} * \frac{1}{T} + \text{constant} \quad (8)$$

Therefore, the heat of fusion can be determined from the slope $-\Delta H_m/R$ of the plot of $\ln X$ against $1/T$.

Hildebrand and others observed that there are advantages to plotting $\ln X$ against $\ln T$ (15–17). As a result, an alternative assumption that the ΔC_p can be estimated by the entropy of fusion has been offered. Employing this alternate estimation of C_p, Eq. (6) is simplified into

$$\ln X = \frac{\Delta H_m}{RT_m}\ln\frac{T}{T_m} \quad (9)$$

or

$$\ln X = \frac{\Delta H_m}{RT_m} * \ln T + \text{constant} \quad (10)$$

and the heat of fusion can be determined from the slope $\Delta H_m/R_mT_m$ of the plot of $\ln X$ versus $\ln T$. Equation (8) has found many applications in the pharmaceutical area (18–21).

We used both Eq. (8) and Eq. (10) to analyze quinolone intrinsic solubility temperature dependence data. The plot of

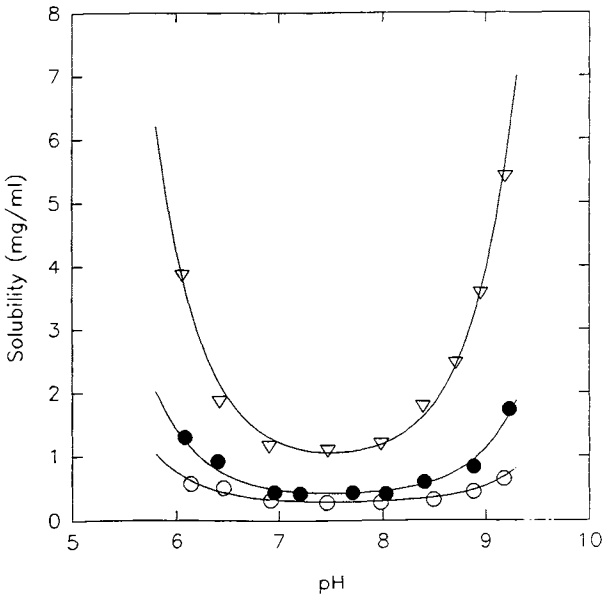

Fig. 4. Solubility of norfloxacin as a function of pH at temperatures of 6°C (○), 25°C (●), and 40°C (▽). The solid lines represent predictions from Eq. (4).

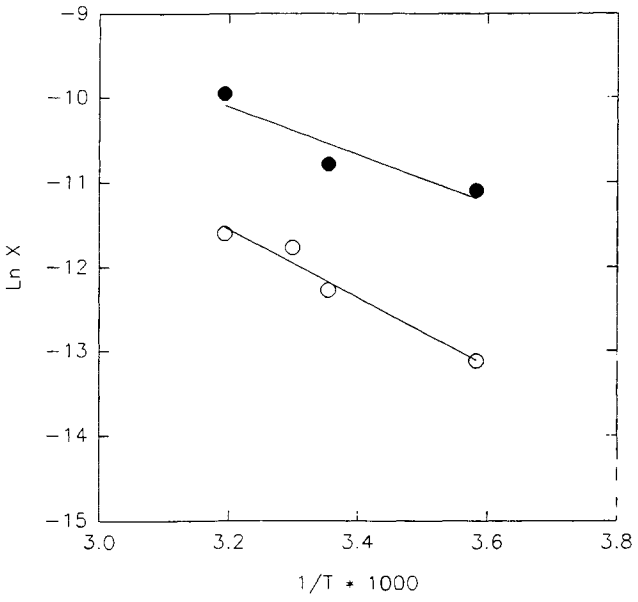

Fig. 5. Plot of $\ln X$ versus $1/T$ for ciprofloxacin (○) and norfloxacin (●), where X is the mole fraction intrinsic solubility. The solid lines represent predictions from Eq. (8).

Table IV. Heat of Fusion for Ciprofloxacin and Norfloxacin from Solubility Study

Compound	ΔH_m (kcal/mol) van't Hoff Eq. (8)	ΔH_m (kcal/mol) Hildebrand Eq. (10)
Ciprofloxacin	8.13 (−47.2%)[a]	14.95 (3.0%)
Norfloxacin	5.65 (−28.3%)	9.41 (19.4%)

[a] Relative error, compared to DSC results in Table I, in parentheses.

$\ln X$ versus $1/T$ is shown in Fig. 5 for ciprofloxacin and norfloxacin. By fitting experimental data to Eq. (8), we obtained

$$\ln X = \frac{-4.09 * 10^3}{T} + 1.55, \quad r = 0.98 \qquad (11)$$

for ciprofloxacin and

$$\ln X = \frac{-2.84 * 10^3}{T} - 1.01, \quad r = 0.94 \qquad (12)$$

for norfloxacin. From Eqs. (11) and (12), we determined the heat of fusion, and the results are shown in Table IV.

The plot of $\ln X$ versus $\ln T$ is shown in Fig. 6 for ciprofloxacin and norfloxacin. By fitting experimental data to Eq. (10), we obtained

$$\ln X = 13.89 \ln T - 91.32, \quad r = 0.98 \qquad (13)$$

for ciprofloxacin and

$$\ln X = 9.70 \ln T - 65.81, \quad r = 0.94 \qquad (14)$$

for norfloxacin. Again, from Eqs. (13) and (14), we are able to estimate the heat of fusion.

Table IV shows the heat of fusion for ciprofloxacin and norfloxacin obtained from the solubility studies. Compared to the DSC results in Table I, the Hildebrand method gave

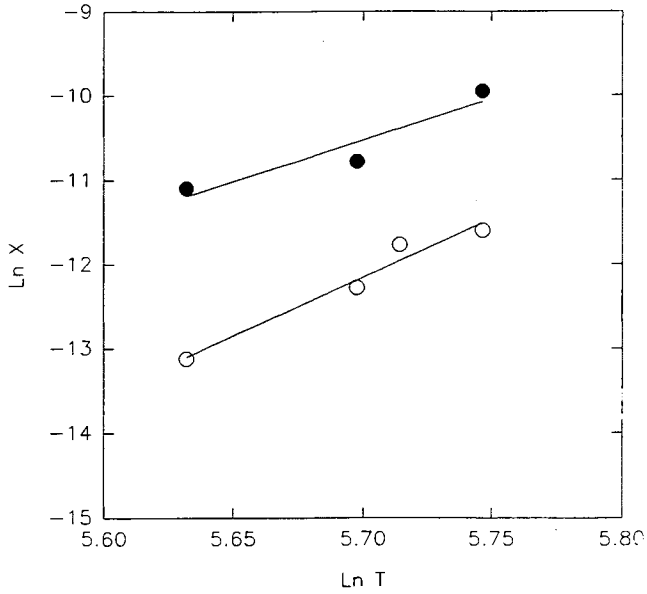

Fig. 6. Plot of $\ln X$ versus $\ln T$ for ciprofloxacin (○) and norfloxacin (●), where X is the mole fraction intrinsic solubility. The solid lines represent predictions from Eq. (10).

Table V. Comparison of Experimental pK_a Values with Literature Values

Compound	Experimental pK_{a_1}	Experimental pK_{a_2}	Literature pK_{a_1}	Literature pK_{a_2}	Ref. no.
Ciprofloxacin	6.15	8.66	6.09	8.74	5
			6.00	8.80	8
Norfloxacin	6.54	8.50	6.30	8.38	5
			6.40	8.70	8
			6.22	8.51	9

overestimates, and the van't Hoff equation gave underestimates. From the absolute values of the relative errors, the Hildebrand plot produced the better results. Better estimates might be obtained using the nonlinear solubility–temperature relationships proposed by Grant *et al.* (22), Prankerd and McKeown (23), and Prankerd (24).

pK_a Temperature Dependence

We also evaluated the thermodynamic dissociation constants at each temperature by nonlinear regression from solubility–pH profiles. We found that pK_{a_1} and pK_{a_2} for both compounds do not markedly change with temperature. There is good agreement between our dissociation values and those reported previously in the literature, as shown in Table V.

ACKNOWLEDGMENTS

We are indebted to Gregory E. Amidon, Shri C. Valvani, and Walter Morozowich for their help and comments.

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