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# Aqueous solubilities of some variously substituted quinolone antimicrobials

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## Summary

The fluorinated 4-quinolones are a group of orally active antimicrobials, which are active against a wide range of gram-negative organisms and gram-positive cocci. These compounds are essentially zwitterions at physiological pH and, therefore, in their lowest state of solubility. The objective of this study was to evaluate the solubility of a series of the fluoroquinolones as a function of pH, temperature, and salt concentration. The aqueous solubilities ($\mu = 0.15$ with NaCl) at 25 and 37 °C, the apparent macroscopic dissociation constants ($\mu = 0.15$ with NaCl), and the melting points of a series of fluoroquinolone antimicrobials were determined. The intrinsic solubility ($\mu = 0.15$ with NaCl) at 25 °C for this group of compounds was found to range from $1.28 \times 10^{-4}$ M (0.0297 mg $ml^{-1}$) to $7.64 \times 10^{-3}$ M (2.75 mg $ml^{-1}$). At pH values between 5 and 7, if the solubility of quinolone was greater than 5 mg $ml^{-1}$, the observed solubility was greater than that predicted by theory. At pH values below 5, there was evidence of the salt forms of the quinolones limiting the solubilities. The effect of NaCl on the solubility of lomefloxacin mesylate was studied and showed that increasing ionic strength may suppress the solubility more than could be explained by the common-ion effect alone. The apparent macroscopic dissociation constants ($\mu = 0.15$ with NaCl) were found to range from 5.46 to 6.30 for the carboxylic acid function. The range of apparent macroscopic dissociation constants for the piperazinyl function was found to be $9.0 \pm 0.3$ for all compounds except those with a methyl substituent on the piperazinyl nitrogen, in which case, the range was $7.8 \pm 0.4$.

## Introduction

The first member of the quinolone carboxylic acid family of antimicrobials introduced into clinical practice, nalidixic acid, was used primarily in the treatment of urinary tract infections. Although it was active against most Enterobacteriaceae, *Pseudomonas* and gram-positive bacteria were resistant and problems with development of bacterial resistance or superinfection with inherently resistant species severely limited its use.

In recent years, synthesis of the newer 4-quinolones has renewed the interest in this family of compounds. The addition of the 6-fluoro and 7-piperazinyl groups to the molecule resulted in enhanced activity against Enterobacteriaceae, as well as activity against a wide range of gram-negative organisms and gram-positive cocci. The in-

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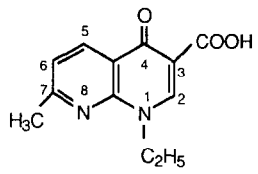
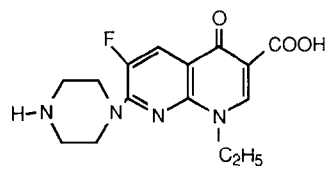
creased potency of the new fluoroquinolones has greatly expanded their potential clinical usefulness (Desplaces et al., 1986; Mitscher et al., 1989).

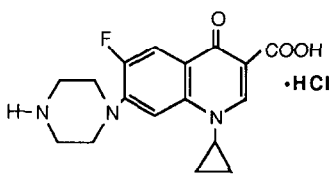
Numerous structurally related compounds have been synthesized. Unfortunately, the activity of these compounds varies in vitro and does not necessarily correspond to activity in vivo. These differences in activity may be due to differences in absorption and tissue penetration which may potentially be predicted by the physicochemical



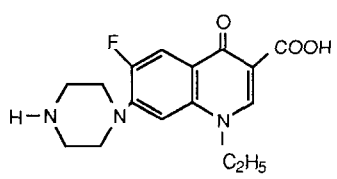

NALIDIXIC ACID (I)



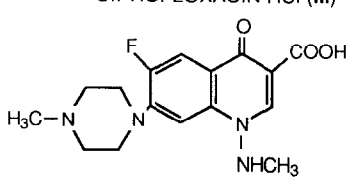

ENOXACIN (II)



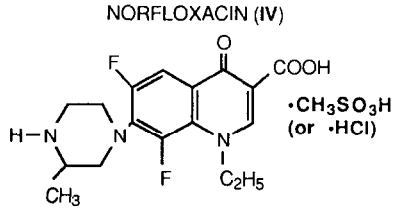

CIPROFLOXACIN HCl (III)



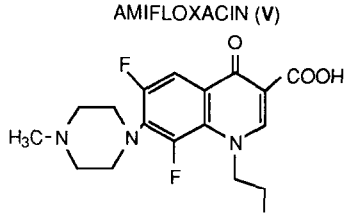

NORFLOXACIN (IV)



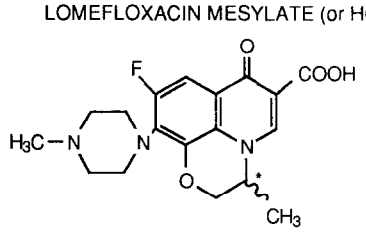

AMIFLOXACIN (V)



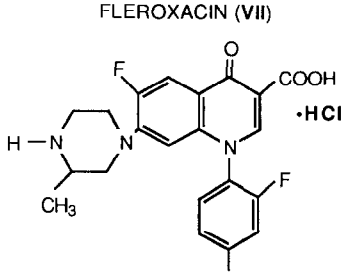

LOMEFLOXACIN MESYLATE (or HCl) (VI)



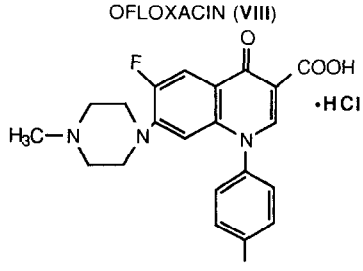

FLEROXACIN (VII)




OFLOXACIN (VIII)




TEMAFLOXACIN HCl (IX)




DIFLOXACIN HCl (X)

Fig. 1. Structures of quinolones studied.

properties (Mitscher et al., 1989). Other concerns are related to the toxicities of these compounds, one of which is precipitation or crystallization in urine. In animal studies, crystalluria was a problem but may have been due to the low aqueous solubilities of the quinolones within the normal pH range of the urine of the animals studied (monkeys and rats) which is more alkaline than the normal human urine. Crystalluria in man has been rare and transient during clinical investigations of the quinolones (Christ et al., 1988; Paton and Reeves, 1988); however, precipitation in the urine is possible in man, especially in those individuals with a more alkaline urine than normal. Therefore, the objective of this study was to evaluate the solubility of a series of model 4-quinolone antimicrobials as a function of pH, temperature, and salt concentration. Structures of the quinolones chosen for study are shown in Fig. 1. Nalidixic acid was included for comparison. Ciprofloxacin HCl and norfloxacin are in clinical use worldwide, whereas the other compounds studied here are in various stages of development.

## Materials and Methods

*Materials*

All the quinolones were supplied by their respective manufacturers: amifloxacin (Sterling-Winthrop, Rensselaer, NY), ciprofloxacin HCl (Miles Labs, West Haven, CT), difloxacin (Abbott Labs, North Chicago, IL), enoxacin (Warner-Lambert, Ann Arbor, MI), fleroxacin (Hoffmann-LaRoche, Nutley, NJ), lomefloxacin mesylate and lomefloxacin HCl (G.D. Searle, Skokie, IL), ofloxacin (Ortho Labs, Raritan, NJ), norfloxacin (Merck, Sharp, and Dohme, West Point, PA), and temafloxacin HCl (Abbott Labs). Nalidixic acid was purchased from Sigma (St. Louis, MO). All solvents were HPLC grade and obtained from commercial sources. All other chemicals were reagent grade obtained from commercial sources. Water was purified in a Milli-Q Water System (Millipore, Bedford, MA) and stored in glass until use.

*Apparatus*

The solubility experiments were performed at constant temperature ($\pm 0.1°$ C) either in a shaking water bath (American Optical, Buffalo, NY or Precision Scientific Group, Chicago, IL), or with a Brinkman Metrohm 614 Impulsomat (Brinkman Instruments, Westbury, NY) equipped with a Metrohm 632 pH meter. All other pH measurements were made using an Orion SA 520 pH meter (Orion Research, Boston, MA) and a Tiny Combination pH electrode (Microelectrodes, Londonderry, NH) or a calomel pH combination glass electrode (Markson, Phoenix, AZ). Samples were assayed by LC using either a Waters chromatographic pump (Waters Associates, Milford MA), or a Beckman Model 110A pump (Beckman Instruments, Fullerton, CA), a Waters Model U6K injector or an Altex 210 injector (Beckman Instruments) fitted with a 20 $\mu$l loop, a Waters Model 440 absorbance detector with a 280 nm filter or a Spectroflow 757 Absorbance Detector (Kratos Analytical, Ramsey, NJ) and an OmniScribe Recorder (Houston Instrument, Austin, TX). All spectrophotometric determinations were conducted using a Hewlett-Packard 8451A Diode Array Spectrophotometer (Hewlett-Packard, San Diego, CA).

*Chromatographic conditions*

All LC assays were conducted using an MOS Hypersil (C8) reversed-phase column (5 $\mu$m, 15 cm $\times$ 4.6 mm, id) and UV detection at 280 nm. The mobile phase used was tetrahydrofuran-acetonitrile-$H_3PO_4$ (10 mM)-triethylamine (10 : 30 : 60 : 0.03, by vol.) with a flow rate of 1.5 ml $min^{-1}$. All injections were made in duplicate.

*Solubility studies*

Solubility determinations were made using the shake-flask method at pH 5, 7, and 9 and constant ionic strength at 25 or 37° C. Excess drug was added to the buffer of interest (pH 5, 0.15 M acetate buffer; pH 7, 0.05 M phosphate buffer; pH 9, 0.15 M borate buffer; $\mu = 0.15$ with NaCl). The pH was adjusted if necessary with the appropriate buffer component. The solutions were protected from light by wrapping the vials in aluminum foil and agitated for 24 h in a shaking

water bath at 25 or 37 °C. The pH of the sample was measured at 24 h and adjusted if necessary. If the pH was adjusted, the sample was agitated for an additional 24 h. This procedure was repeated until the pH was stable (±0.05 pH units). The sample was filtered (5 μm, FS-5005, Burron Medical, Bethlehem, PA), diluted with mobile phase and assayed by LC. All solubility experiments were conducted in triplicate.

The above procedure was unsuccessful when used for amifloxacin because it was not wetted by the solvent. Therefore, approx. 125 mg of amifloxacin was dissolved in 0.15 M NaOH. An aliquot of the solution was diluted with the appropriate buffer solution and the pH adjusted. The aliquot added was in a quantity sufficient to provide drug in excess of solubility at the pH values studied. The solution was allowed to equilibrate and samples were processed as described above.

Temafloxacin HCl solubilities were also determined using a pH-stat. Excess drug, which had been triturated with a mortar and pestle, was added to 40 ml of 0.15 M NaCl. The slurry was placed in a water-jacketed vessel, wrapped in aluminum foil, and maintained at 25 °C with a Haake Water Bath (Haake, Saddle Brook, NJ). The pH was adjusted to greater than 9.5 with 0.15 M KOH. The pH was decreased incrementally with 0.15 M HCl to the desired value and allowed to equilibrate. Samples were removed and centrifuged, diluted with mobile phase, and assayed by LC.

Solubilities of lomefloxacin HCl and lomefloxacin mesylate were also studied as a function of pH and NaCl concentration using the pH-stat method described above. The pH was decreased incrementally with a 0.25 M methanesulfonic acid/NaCl solution to the desired pH value and allowed to equilibrate. A sample was removed, filtered (5 μm, FS-5005, Burron Medical) and diluted with a 0.1 M methanesulfonic acid/NaCl solution. The concentration of each sample was determined spectrophotometrically at 288 nm. The solubility was determined in 0, 0.1, 0.15, 0.2, and 0.3 M NaCl. In this last experiment, no attempt was made to hold ionic strength constant because no salt could be identified that did not lower the solubility of lomefloxacin.

The $pK_a$ values of amifloxacin, ciprofloxacin HCl, and difloxacin HCl were also determined by the solubility method. In these experiments, excess drug was added to a series of buffer solutions from pH 4 to 9 (pH 4–6, 0.15 M acetate buffer; pH 6.5–8, 0.05 M phosphate buffer; pH 8.5–9.0, 0.15 M borate buffer; $\mu = 0.15$ with NaCl). Solutions were protected from light and allowed to equilibrate for at least 48 h in a shaking water bath at 25 °C. The sample was filtered (5 μm, FS-5005, Burron Medical), diluted with mobile phase and assayed by LC. All solubility experiments were conducted in triplicate.

*Spectrophotometric studies*

The $pK_a$ values of all the compounds, except amifloxacin, ciprofloxacin HCl, and difloxacin HCl, were determined spectrophotometrically at ambient temperature (22 ± 1 °C). A wavelength was chosen (for each compound) where the absorbance of the three species (cation, zwitterion, and anion) varied the greatest. The change of absorbance, at the selected wavelength, with pH was monitored. The same total concentration of drug (between $2 \times 10^{-4}$ and $2 \times 10^{-5}$ M depending on the drug) was used for all measurements. Ionic strength was held constant at 0.15 with NaCl. Linearity of absorbance with respect to concentration, up to the concentrations used in these studies, was established for all compounds in their cationic and anionic forms. All spectrophotometric measurements were made in triplicate.

*Melting point determination*

Melting points for all compounds were determined visually, in duplicate, using an Electrothermal[R] Melting Point Apparatus. Those compounds that were provided in their salt form (ciprofloxacin HCl, difloxacin HCl, lomefloxacin mesylate, and temafloxacin HCl) were precipitated in their zwitterionic form before melting point determinations were made. For comparison, melting point determinations were also made on the solids isolated at the end of the solubility experiments.

## Results and Discussion

*Dissociation constants*

In a polar solvent, such as water, the solubility of a solute increases with increasing charge. Therefore, to characterize completely the solubility of any ionizable compound as a function of pH it is necessary to understand its macroscopic dissociation. Nalidixic acid has one ionizable group at pharmaceutically relevant pH values, the carboxylic acid function at position 3. The new fluoroquinolones have an additional ionizable functional group on the piperazinyl ring at position 7. The macroscopic ionization of the newer fluoroquinolones can be illustrated (Scheme 1) using lomefloxacin as an example.

The absorbance of a molecule may be affected by its ionization, resulting in a shift in spectrum with pH. If the spectrum of a compound shifts with pH, the change in absorbance with pH may be used to determine the $pK_a$ value(s). The total absorbance of a molecule at any pH is the sum of the contribution of all the species present. The total absorbance at any pH for a weak acid such as nalidixic acid is described by the relationship:

$$A_T = \frac{A_M[H^+] + A_A K_1}{K_1 + [H^+]} \quad (1)$$

For the fluoroquinolones, which have two ionizable groups, the change in absorbance with pH can be described by:

$$A_T = \frac{A_C[H^+]^2 + A_M K_1[H^+] + A_A K_1 K_2}{[H^+] + K_1[H^+] + K_1 K_2} \quad (2)$$

where $A_T$ is the measured absorbance, $A_C$ is the absorbance of the cation, $A_M$ is the absorbance of the neutral species (or zwitterion), $A_A$ is the absorbance of the anion, and $K_1$ and $K_2$ denote the apparent dissociation constants.

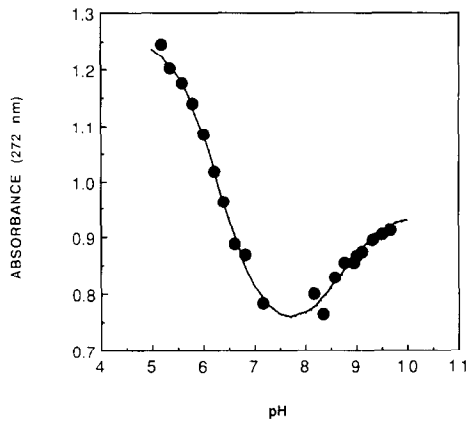
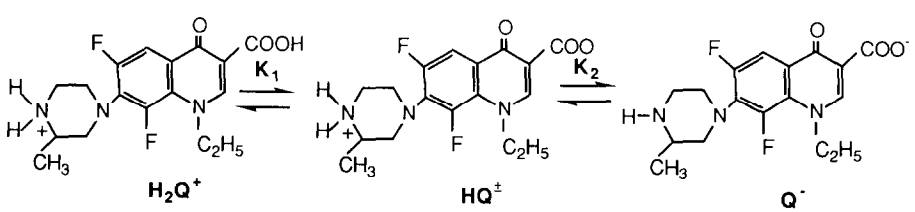



Fig. 2. Enoxacin ($3.40 \times 10^{-5}$ M) absorbance as a function of pH. Calculated line generated by fitting Eqn 2 to experimental points (●) using an RS1 curve-fitting algorithm ($r^2 = 0.9998$).

The apparent $pK_a$ values ($\mu = 0.15$ with NaCl) were determined spectrophotometrically for all the compounds except amifloxacin, ciprofloxacin, and difloxacin which did not have sufficient spectral shifts associated with their second $pK_a$. Spectrophotometrically determined apparent $K_1$ and $K_2$ values for all the fluoroquinolones were calculated by fitting Eqn 2 to the experimental data with an RS1 curve-fitting algorithm. For nalidixic acid, Eqn 1 was used. The value for $r^2$ was greater than 0.999 in all cases. Fig. 2 is a representive plot of absorbance as a function of pH using enoxacin as an example.

Because of their low solubilities and lack of spectral shifts, the dissociation of amifloxacin, ciprofloxacin, and difloxacin was studied by the solubility method. The total solubility at any pH




Scheme 1

is simply the sum of the concentration of each of the different species present.

$$S_t = [H_2Q^+] + [HQ^\pm] + [Q^-] \quad (3)$$

Assuming the component with the smallest charge ($HQ^\pm$) is least soluble in water, the solubility of a compound with two ionizable groups is best expressed by:

$$S_t = S_0 \left( \frac{[H^+]^2 + K_1[H^+] + K_1K_2}{K_1[H^+]} \right) \quad (4)$$

where $S_t$ is the total solubility, $S_0$ the intrinsic solubility of the neutral species (or zwitterion), and $K_1$ and $K_2$ represent apparent dissociation constants.

It should be noted that the intrinsic solubility ($S_0$) is defined as the solubility of the zwitterionic form and should not be confused with the minimum solubility which is the total solubility at pH = p$I$. Intrinsic solubilities, $K_1$, and $K_2$ values for amifloxacin, ciprofloxacin, and difloxacin were determined by fitting Eqn 4 to experimental solubility data with an RS1 curve-fitting algorithm. Results of p$K_a$ determinations are listed in Table 1. The range of apparent p$K_{a_1}$ values for all compounds was 6.0 ± 0.5. The apparent p$K_a$ associated with the piperazinyl function at position 7 was found to be 9.0 ± 0.3 for all compounds except amifloxacin, difloxacin, fleroxacin, and ofloxacin which have a methyl substituent on the piperazinyl nitrogen and correspondingly lower apparent p$K_{a_2}$ values (7.8 ± 0.4). There is good agreement between values given here and those reported previously in the literature (see Table 1).

Although the apparent p$K_{a_1}$ values ranged from 5.46 to 6.31 and the p$K_{a_2}$ values ranged from 7.39 to 9.30 with these compounds, the p$I$ range was much smaller, 6.78–7.56 (see Table 1). Because the isoelectric point occurs near physiological pH in all cases, this physicochemical property may be important in determining biopharmaceutical properties in vivo.

With the exception of amifloxacin and difloxacin, the p$K_a$ values of these compounds were separated by more than 2 pH units and therefore the solubility profiles were in a plateau region at physiological pH. The use of pH adjustment to enhance solubility is not a useful formulation technique with these compounds, perhaps with the exception of amifloxacin and difloxacin, since dramatic changes in solubility with changes in pH

TABLE 1

*Apparent macroscopic ionization constants and isoelectric points of the quinolone antimicrobials*

| Compound | λ (nm) [a] | Experimental | | | Literature values | | |
|---|---|---|---|---|---|---|---|
| | | pI | p$K_{a_1}$ [b] | p$K_{a_2}$ [b] | p$K_{a_1}$ | p$K_{a_2}$ | Reference |
| Amifloxacin | NA | 6.84 | 6.28 [c] | 7.39 [c] | | | |
| Ciprofloxacin | NA | 7.42 | 6.09 [c] | 8.74 [c] | 6.00 | 8.80 | Kitzes-Cohen (1987) |
| Difloxacin | NA | 6.85 | 6.06 [c] | 7.63 [c] | | | |
| Enoxacin | 272 | 7.5 | 6.31 [d] | 8.69 [d] | 6.00 | 8.50 | Dalhoff (1989) |
| Fleroxacin | 272 | 6.78 | 5.46 [d] | 8.10 [d] | 5.70 | 8.00 | Dalhoff (1989) |
| Lomefloxacin | 266 | 7.56 | 5.82 [d] | 9.30 [d] | | | |
| Nalidixic acid | 310 | NA | 5.95 [d] | NA | 6.02 [d] | NA | Staroscik and Sulkowska (1971) |
| | | | | | 6.12 [c] | NA | Staroscik and Sulkowska (1971) |
| Norfloxacin | 274 | 7.34 | 6.30 [d] | 8.38 [d] | 6.40 | 8.70 | Kitzes-Cohen (1987) |
| | | | | | 6.20 | 8.70 | Stein (1987) |
| Ofloxacin | 258 | 7.14 | 6.05 [d] | 8.22 [d] | 5.70 | 7.90 | Kitzes-Cohen (1987) |
| Temafloxacin | 264 | 7.18 | 5.61 [d] | 8.75 [d] | | | |

[a] Wavelength which was monitored for spectrophotometric determinations.
[b] Ionic strength adjusted to 0.15 with NaCl.
[c] Solubility method.
[d] Spectrophotometric.
NA, not applicable.

are only seen at pH values outside the desirable pH range for pharmaceutical formulations.

*Solubility studies*

The solubility of all the compounds were studied at pH 5, 7, and 9 and at 25 and 37 °C. Solubility determinations took a minimum of 48 h because the buffers at an ionic strength of 0.15 were unable to maintain the pH constant, so the pH was readjusted after 24 h. Many of the solutions displayed supersaturation – drug readily dissolved but upon standing for greater than 24 h, precipitation was observed.

The intrinsic solubility of nalidixic acid was calculated using the experimentally determined $pK_a$ value, the solubility at pH 5, and the expression that describes the total solubility of a weak acid:

$$S_t = S_0\left(1 + \frac{K_1}{[H^+]}\right) \quad (5)$$

Intrinsic solubilities for all fluoroquinolones, except amifloxacin, ciprofloxacin, and difloxacin, were calculated using Eqn 4, the experimentally determined spectral $pK_a$ values, and the solubility

TABLE 2

*Intrinsic solubility at 25 °C, melting point, and log D (octanol/water partition coefficient) of the quinolones studied*

| Compound | $S_0$ [a], M (mg ml$^{-1}$) [b] | Melting point (°C) | | log $D$ [e] |
|---|---|---|---|---|
| | | A | B | |
| Amifloxacin | $1.87 \times 10^{-4}$ (0.0621) | 292–298 | 297 | −3.01 [g] |
| Ciprofloxacin | $2.38 \times 10^{-4}$ (0.0792) | 270–275 [c,f] | 265–274 | −1.70 [h] |
| | | 315–320 [d] | | −1.74 [g] |
| Difloxacin | $1.53 \times 10^{-4}$ (0.0609) | 270–276 [c,f] | 292–297 | −0.38 [g] |
| | | 313–318 [d] | | |
| Enoxacin | $1.19 \times 10^{-3}$ (0.383) | 215–222 | 221–222 | −2.15 [h] |
| | | | | −0.29 [g] |
| Fleroxacin | $1.88 \times 10^{-3}$ (0.691) | 272–275 | 273–275 | −1.53 [g] |
| Lomefloxacin | $2.93 \times 10^{-3}$ (1.03) | 235–245 [c] | 237–245 | −1.36 [g] |
| | | 314–319 [d] | | |
| Nalidixic acid | $1.28 \times 10^{-4}$ (0.0297) | 225–228 | 223–226 | 1.87 [i] |
| | | | | 1.24 [g] |
| Norfloxacin | $1.00 \times 10^{-3}$ (0.320) | 217–219 | 214–216 | −2.0 [h,j] |
| Ofloxacin | $7.64 \times 10^{-3}$ (2.75) | 270–273 | 268–269 | −0.48 [h] |
| | | | | −2.4 [g] |
| Temafloxacin | $1.58 \times 10^{-4}$ (0.0659) | 237–245 [c] | 276–280 | −0.41 [g] |
| | | 315–319 [d] | | |

[a] Ionic strength adjusted to 0.15 with NaCl.

[b] mg ml$^{-1}$ of zwitterionic species.

[c] Solid collected immediately after titrating to pH 7 (zwitterionic species).

[d] Salt form as received from manufacturer.

[e] $D$: octanol/water partition coefficient.

[f] Decomposed.

[g] Calculated using the method described by Leo et al. (1971). The values for the $\pi$ substituents were obtained from Leo et al. (1971) or Fujita et al. (1964), whichever was appropriate.

[h] Hirai et al. (1986).

[i] Measured value reported by Hirai et al. (1986) at pH 7.2 was used to calculate $D$ using the relationship:

$$D = D'\frac{([H^+] + K_a)}{[H^+]}$$

[j] Norfloxacin was used as the parent compound for all calculations so the predicted log $D$ value was not calculated.

A: Melting point of the compound prior to the solubility studies; B: melting point of the solid excess collected at the end of the solubility experiments.

at pH 7 (25 °C). Results of the intrinsic solubility calculations are shown in Table 2. Staroscik and Sulkowska (1971) reported the solubility of nalidixic acid at 20 °C to be $8.3 \times 10^{-5}$ M. Their value is slightly lower but comparable with the value of $1.28 \times 10^{-4}$ M that was determined in the present study at 25 °C. The solubility of norfloxacin was reported by Swanson and co-workers (1983) to be 0.45 mg $ml^{-1}$ at pH 7.5 and 25 °C. Using the $K_1$, $K_2$ and $S_0$ values determined in the present study, the solubility of norfloxacin at pH 7.5 can be calculated to be 0.38 mg $ml^{-1}$, a slightly lower value than reported by Swanson et al. (1983).

The intrinsic solubilities of the members of this group of structurally similar compounds varied between $1.28 \times 10^{-4}$ M (0.0297 mg $ml^{-1}$) and $7.64 \times 10^{-3}$ M (2.75 mg $ml^{-1}$) at 25 °C. The six-membered ring of ofloxacin which connects position 1 to position 8 makes it unique among the compounds studied here. The chiral center associated with the ring made it difficult to relate the ofloxacin solubility to the others, since the mixture of *R* and *S*, with which solubility studies were conducted, probably influences the total solubility of ofloxacin. Racemic mixtures may have different intercrystalline forces than their corresponding enantiomer molecules (*R* or *S*) which may result in different solid-state physical properties such as melting behavior and solubility (Repta et al., 1976). Since ofloxacin has the highest intrinsic solubility of all the compounds, it is possible that the ring structure and/or the chiral center may affect the crystal lattice packing and hence the solubility.

Lomefloxacin and fleroxacin both have intrinsic solubilities in excess of 0.5 mg $ml^{-1}$ which was higher than those of the other compounds studied. A particularly interesting observation was that both of these compounds have a fluorine substituent at position 8. Enoxacin differs from nalidixic acid only in the fluorine substituent at position 6 and the piperazinyl group at the 7 position but had an intrinsic solubility that was more than 10 times that of nalidixic acid. The melting point of enoxacin (Table 2) was lower than that of nalidixic acid, indicating that the intermolecular forces in the enoxacin crystal were weaker than those in the nalidixic acid crystal (i.e. enoxacin has a more loosely packed crystal lattice) and this may be the reason for the increase in intrinsic solubility.

Valvani and Yalkowsky (1980) found that the molar solubility of several classes of organic compounds could be predicted from the empirical relationship:

$$\log S = -a(\log D) - b(\mathrm{MP}) - c \qquad (6)$$

where *S* is the molar solubility, *D* the octanol/water partition coefficient, MP is the melting point, and *a*, *b*, and *c* denote constants obtainable by multiple-regression analysis.

Melting points are readily available for most compounds or are easily determined, and partition coefficients can be calculated using the method described by Leo and co-workers (1971) using $\pi$, a substituent constant. When multiple-regression analysis was performed using Eqn 6, the experimentally determined intrinsic solubilities, melting points of the solid excess recovered at the end of the solubility experiments and log *D* values reported in the literature (Hirai et al., 1986) or

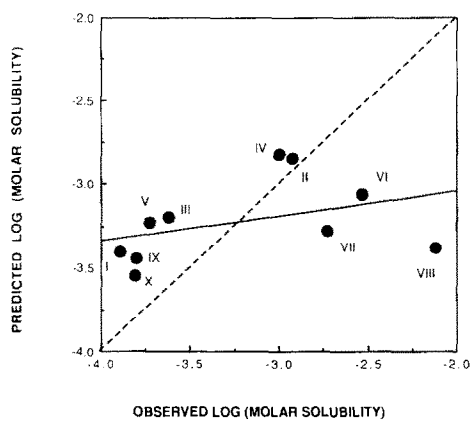



Fig. 3. Results of a multiple regression using $S_0$ values, melting points, and log *D* values in Table 2. Compounds are identified by the numbers corresponding to the structures in Fig. 1. The predicted log *S* was calculated from the regression equation determined to be: $\log S = -0.135(\log D) - 6.51e - 3(\mathrm{MP}) - 1.70$. The regression line is shown by the solid line and fits the equation: $y = -2.7378 + 0.15010x$. The coefficient of correlation (*R*) is 0.387 and the coefficient of determination ($R^2$) is 0.151. The dashed line represents the theoretical line of unit slope and zero intercept.

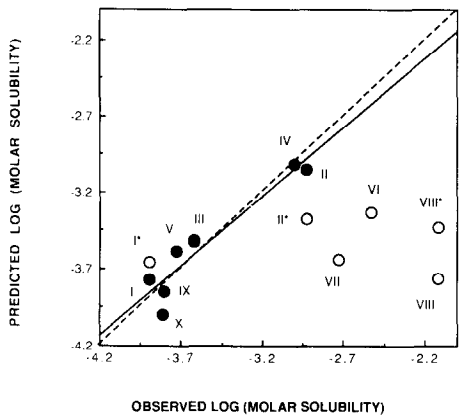



Fig. 4. Results of a multiple regression using $S_0$ values, melting points, and log $D$ values in Table 2 except for ofloxacin, lomefloxacin, and fleroxacin whose values were omitted. Compounds are identified by the numbers corresponding to the structures in Fig. 1. Numbers followed by an asterisk indicate a calculated log $D$ value was used for a compound which had a measured log $D$ value available. The predicted log $S$ was calculated from the regression equation determined to be: $\log S = -0.175(\log D) - 9.03e - 3(\mathrm{MP}) - 1.43$. The regression line is shown by the solid line and fits the equation: $y = -0.35438 + 0.90026x$. The coefficient of correlation ($R$) is 0.948 and the coefficient of determination ($R^2$) is 0.901. The dashed line represents the theoretical line of unit slope and zero intercept. (●) Points included in the regression analysis; (○) values calculated from the regression equation but not included in the analysis.

calculated (Table 2), a coefficient of correlation ($R$) of 0.387 was determined. The results of this regression are shown in Fig. 3. It was noted that most of the points appeared to be well-correlated with the exception of ofloxacin, lomefloxacin, and fleroxacin, therefore, the regression was repeated omitting the three above-mentioned compounds, which resulted in a coefficient of correlation ($R$) of 0.948 (Fig. 4). The log $D$ values were calculated for the compounds with the measured octanol/water partition coefficient (Hirai et al., 1986) and included in Fig. 4 for comparison. It is clear that ofloxacin, lomefloxacin, and fleroxacin have some unique feature that prevents their solubilities from being predicted accurately using Eqn 6. These three compounds have the highest solubilities of the compounds studied and all have electronegative substituents at position 8 which are capable of hydrogen bonding. The failure to correlate with the predicted solubility may simply be due to self-association occurring even at the intrinsic solubility level or may be due to the melting point being a poor predictor of the energy required to remove the molecule from the crystal lattice due to hydrogen bonding or some other specific interaction in the solid state. Further studies are being conducted to clarify these questions.

The solubilities of the model compounds were studied at 25 °C ($S_{25}$) and 37 °C ($S_{37}$). In order to evaluate the change of solubility with temperature, the values of $S_{37}/S_{25}$ were compared. The theoretical basis for this analysis is given in Eqn 7 which shows that the value of $X_2/X_1$ is related to the enthalpy of solution ($\Delta H_{\mathrm{soln}}$):

$$\ln\left(\frac{X_2}{X_1}\right) = \frac{\Delta H_{\mathrm{soln}}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad (7)$$

where $X_1$ and $X_2$ are the mole fraction solubilities at $T_1$ and $T_2$, respectively, and $T_1$ and $T_2$ correspond to the absolute temperatures at which solubility determinations were made.

The value of $S_{37}/S_{25}$ for each drug was not found to be constant at different pH values (Table 3). In most cases, the $S_{37}/S_{25}$ value increased with increasing solubility which is consistent with self-association occurring at higher concentrations. For the fluoroquinolones, the value of $S_{37}/S_{25}$ was greater than unity in all cases except for norfloxacin at pH 5. The reason for this discrepancy is unclear. The $S_{37}/S_{25}$ values at pH 7 varied from 1.10 to 1.88 with a mean of $1.30 \pm 0.25$ ($\pm$S.D.). Since this value was fairly constant between compounds, reasonable predictions of solubilities at 37 °C for the new fluoroquinolones could be made using 25 °C data obtained in vitro.

Fig. 5 shows the solubility of each of the compounds studied as a function of pH. The solubility values determined experimentally at pH 5 exceeded the predicted values for lomefloxacin mesylate, norfloxacin, and ofloxacin. Ciprofloxacin HCl and enoxacin showed this same behavior to a smaller extent. The non-ideal solubility behavior seen with these compounds may be due to self-association.

For all compounds, except those provided in their salt forms, the melting points of the excess solids obtained at the end of the solubility experi-

ments were compared with those of the materials as received from the manufacturers. In all cases, the melting points were the same, consistent with no change in solid state during the solubility experiments. For those compounds provided in their salt form, the melting points of the materials precipitated at pH 7 were compared with those of the excess solid obtained at the end of the solubility experiments, as well as with the salts themselves. Each of the salts melted or decomposed at higher temperatures than the corresponding precipitated solids, consistent with conversion of the salt forms to the zwitterions. For lomefloxacin mesylate, the melting points of the rapidly precipitated solid and the material obtained at the end of the solubility experiments were identical, consistent with no change in the solid state of the zwitterionic material during the solubility experi-

TABLE 3
*Solubility [a] of the model quinolones at pH 5, 7, and 9 at 25 and 37 °C*

| Compound | pH | Solubility (mg ml$^{-1}$)[a] | | | | $S_{37}/S_{25}$ |
|---|---|---|---|---|---|---|
| | | 25 °C | | 37 °C | | |
| | | Mean [b] | S.D. | Mean [b] | S.D. | |
| Amifloxacin | 5 | 1.55 | ±0.45 | 2.05 | ±0.17 | 1.64 |
| | 7 | 0.105 | ±0.007 | 0.12 | ±0.012 | 1.14 |
| | 9 | 2.40 | ±0.14 | 4.95 | ±0.32 | 2.06 |
| Ciprofloxacin HCl | 5 | 3.46 | ±0.20 | 6.19 | ±0.68 | 1.79 |
| | 7 | 0.09 | ±0.01 | 0.15 | ±0.006 | 1.67 |
| | 9 | 0.28 | ±0.02 | 0.68 | ±0.09 | 2.43 |
| Difloxacin HCl | 5 | 0.28 | ±0.05 | 0.58 | ±0.17 | 2.07 |
| | 7 | 0.07 | ±0.002 | 0.08 | ±0.006 | 1.14 |
| | 9 | 1.82 | ±0.035 | 2.65 | ±0.09 | 1.46 |
| Enoxacin | 5 | 16.6 | ±2.7 | 40.7 | ±16.5 | 2.45 |
| | 7 | 0.47 | ±0.03 | 0.60 | ±0.04 | 1.28 |
| | 9 | 0.81 | ±0.085 | 1.76 | ±0.04 | 2.17 |
| Fleroxacin | 5 | 1.02 | ±0.085 | 3.36 | ±0.48 | 3.29 |
| | 7 | 0.77 | ±0.03 | 0.87 | ±0.04 | 1.13 |
| | 9 | 6.03 | ±0.25 | 12.03 | ±0.81 | 2.00 |
| Lomefloxacin mesylate | 5 | 256 | ±7.21 | 296 | ±37.7 | 1.16 |
| | 7 | 1.40 | ±0.03 | 1.64 | ±0.06 | 1.17 |
| | 9 | 2.46 | ±0.18 | 3.75 | ±0.24 | 1.52 |
| Nalidixic acid | 5 | 0.033 | ±0.00 | 0.054 | ±0.005 | 1.64 |
| | 7 | 0.328 | ±0.015 | 0.43 | ±0.056 | 1.31 |
| | 9 | 27.6 | ±1.62 | 26.4 | ±3.20 | 0.96 |
| Norfloxacin | 5 | 161 | ±1.35 | 130 | ±9.6 | 0.807 |
| | 7 | 0.40 | ±0.02 | 0.75 | ±0.01 | 1.88 |
| | 9 | 0.91 | ±0.02 | 2.91 | ±0.095 | 3.20 |
| Ofloxacin | 5 | 95.4 | ±8.3 | 130 | ±10.8 | 1.36 |
| | 7 | 3.23 | ±0.10 | 3.54 | ±0.127 | 1.10 |
| | 9 | 12.1 | ±4.2 | 15.6 | ±1.34 | 1.29 |
| Temafloxacin HCl | 5 | 0.301 | ±0.0104 | 0.69 | ±0.06 | 2.29 |
| | 7 | 0.0965 | ±0.0021 | 0.11 | ±0.004 | 1.17 |
| | 9 | 0.322 | ±0.002 | 0.46 | ±0.03 | 1.43 |

[a] Ionic strength adjusted to 0.15 with NaCl.
[b] Mean ± standard deviation, $n = 3$.

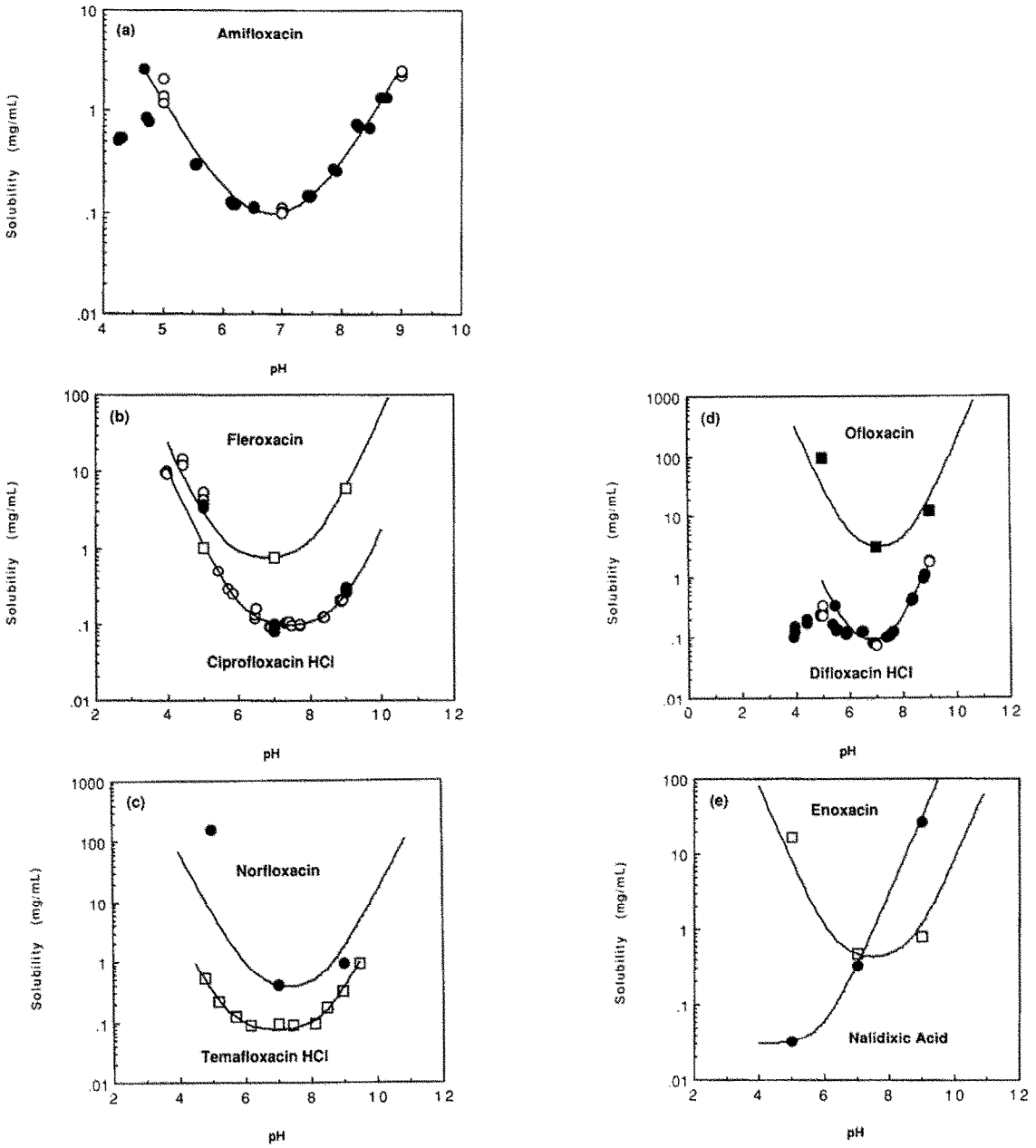



Fig. 5. Quinolone solubility as a function of pH. (a) Amifloxacin solubility experiment (○) 1 and (●) 2; (b) ciprofloxacin HCl solubility experiment (●) 1 and (○) 2; fleroxacin solubility (□); (c) norfloxacin solubility (●); temafloxacin HCl solubility (□); (d) difloxacin HCl solubility experiment (○) 1 and (●) 2; ofloxacin solubility (■); (e) nalidixic acid solubility (●); enoxacin solubility (□). Calculated curves for amifloxacin, ciprofloxacin HCl, temafloxacin HCl, and difloxacin HCl generated by fitting Eqn 4 to experimental points using an RS1 curve-fitting algorithm. Calculated curves for fleroxacin, norfloxacin, ofloxacin, and enoxacin generated using Eqn 4, spectral $pK_a$ values, and $S_0$ values from Table 2. Calculated curve for nalidixic acid generated using Eqn 5, the spectral $pK_a$, and $S_0$ value from Table 2. All data points for each triplicate determination have been included.

ments. For ciprofloxacin HCl, difloxacin HCl, and temafloxacin HCl, the melting points of the rapidly precipitated solids and the materials obtained at the end of the solubility experiments were different, indicating a change in solid state during the solubility experiments. These findings help explain why initial attempts to characterize the solubility of temafloxacin were unsuccessful. When the solubility of temafloxacin HCl was studied at pH 5, 7, and 9, with the sample being allowed to equilibrate for 4 days at 25°C, the between-sample variation was unacceptable. Therefore, the solubility was studied at several different pH values over 10 days, in the same manner as that for the determination of the $pK_a$ values for amifloxacin, ciprofloxacin, and difloxacin. The between-sample variation was acceptable in this case, but the solubility determined at 25°C was much greater than that at 37°C, unlike the other fluoroquinolones studied which had increasing solubilities with increasing temperature. Also, the change of solubility with pH did not follow the behavior expected for a zwitterion. Therefore, the solubility of temafloxacin HCl was studied with a pH-stat. The results shown in Fig. 5c are those obtained from the pH-stat experiment. The solubility varied as a function of pH in an ideal manner when determined with the pH-stat and was one-third that determined over 10 days using the shake-flask method. In addition, the value of $S_{37}/S_{25}$ for temafloxacin at pH 7 following the pH-stat experiment was 1.17, a quite reasonable value when compared to the other compounds studied here.

For a zwitterionic compound at low pH (at least 2 pH units below the $pK_{a_2}$), the change in solubility with pH is similar to that expected for a weak base and can be estimated by:

$$S_t = S_0\left(1 + \frac{[H^+]}{K_1}\right) \tag{8}$$

if the neutral (or zwitterionic) species is limiting the solubility. At low pH, the salt form of a compound may limit the solubility. If this is the case, the total solubility can be estimated from:

$$S_t = S_{(BH_2^+)_0}\left(1 + \frac{K_1}{[H^+]}\right) \tag{9}$$

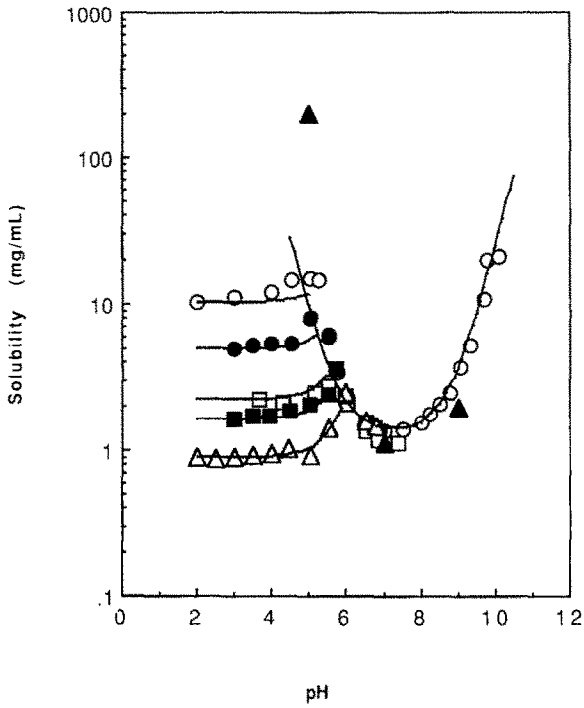



Fig. 6. Lomefloxacin solubility as a function of pH and NaCl concentration. Lomefloxacin solubility as the mesylate salt with no added NaCl (▲), with 0.1 M NaCl (●), with 0.15 M NaCl (□), with 0.2 M NaCl (■), with 0.3 M NaCl (△), and as the HCl salt with no added NaCl (○). Calculated lines below the pH of maximum solubility were generated using Eqn 9, spectral $K_1$, and $S_{(BH_2^+)_0}$ determined at pH 3 for each case. Calculated line above the pH of maximum solubility was generated by fitting Eqn 4 to experimental points for HCl salt using an RS1 curve-fitting algorithm.

The anion associated with the salt will determine the limiting salt value ($S_{(BH_2^+)_0}$). When both the neutral (or zwitterionic) species and the cationic species are limiting the total solubility (i.e. at the intersection of the two lines), the maximum solubility occurs and both Eqns 8 and 9 apply. The pH at which both species are limiting can be calculated from:

$$pH_{max} = pK_1 - \log\left(\frac{S_{(BH_2^+)_0}}{S_0}\right) \tag{10}$$

The pH of maximum solubility is best illustrated in Fig. 6, but for those compounds studied in more detail (amifloxacin, ciprofloxacin HCl, and

difloxacin HCl), salt effects and/or common ion effects can be seen at or below pH 5.

Solubility of a HCl salt in a saturated solution can be expressed according to:

$$K_s = \frac{[BH_2^+]_0[Cl^-]}{[BH_2Cl]_{solid}} \quad (11)$$

and since the concentration of solid is constant:

$$K_s = [BH_2^+]_0[Cl^-] \quad (12)$$

This equation is only appropriately used for sparingly soluble salts when the ionic strength is low or when activities rather than concentrations are used.

Combining Eqn 12 with Eqn 9, the total solubility as a function of added common ion can be studied to estimate the $K_s$

$$S_t = \frac{K_s}{[Cl^-]}\left(1 + \frac{K_1}{[H^+]}\right) \quad (13)$$

A plot of total solubility at a constant pH vs $[Cl^-]^{-1}$ should yield a straight line with a slope of $K_s(1 + K_1/[H^+])$. If $[H^+] \gg K_1$, the slope will have the value of $K_s$.

The effect of NaCl concentration on the solubility of lomefloxacin is shown in Fig. 6. Addition of a common ion ($Cl^-$) resulted in a suppression of solubility of the drug as expected. A plot of solubility at pH 3 vs $[Cl^-]^{-1}$ (Fig. 7) shows a positive deviation from the expected straight line (Eqn 13) with decreasing ionic strength. The positive deviation seen is consistent with an increase in ionic strength resulting in a suppression of total solubility.

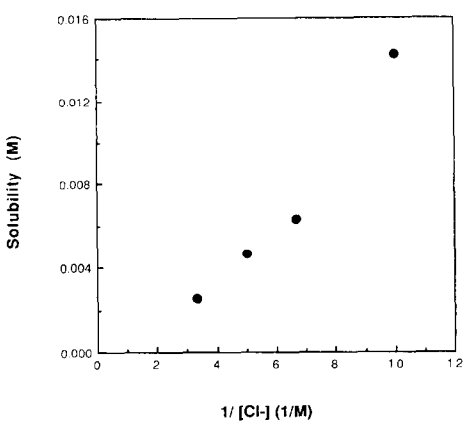



Fig. 7. Lomefloxacin solubility at pH 3 as a function of added $[Cl^-]$.

In conclusion, the solubility and macroscopic ionization constants of a series of quinolone antimicrobials have been determined and insight into the effect of structure on solubility has been provided. Further studies are needed to clarify whether substituents at the 8-position do enhance the solubility without adversely affecting the biological activity and to ascertain if these substituents at the 8-position are the reason for failure to correlate to equation 6.

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