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Interactions of Aluminum, Magnesium, and Calcium Ions with Nalidixic Acid[1]

Masahiro Nakano, Masakazu Yamamoto, and Takaichi Arita

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

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Interaction of nalidixic acid with aluminum ion was revealed by spectrophotometric study. Stoichiometry of the nalidixic acid-aluminum ion complex was estimated to be 3 to 1 by the method of continuous variations. The interaction was further supported by an increase in solubility of the drug with increasing concentration of aluminum ion, by a decrease in (carbon tetrachloride/acetate buffer, pH 3.3) partition coefficient with increasing concentration of aluminum ion in the aqueous layer, and by a decrease in the permeation rate of the drug through a cellulose membrane in the presence of aluminum ion in the donor compartment.

Interaction of nalidixate anion with magnesium and calcium ions was also shown spectrophotometrically.

Keywords——nalidixic acid; spectral change; aluminum ion; alkaline earth ions; method of continuous variations; stoichiometry; solubility; partition coefficient; permeation rate; cellulose membrane

Nalidixic acid has been used for the treatment of urinary tract infections caused by gram-negative bacteria. Its mechanism of action appears to be inhibition of deoxyribonucleic acid (DNA) synthesis.[3] Its congeners, piromidic acid has been employed clinically in Japan[4] and oxolinic acid in the United States and Great Britain.[5]

Its unique molecular structure (see Chart 1) among antibacterial agents prompted us to study its interaction with a variety of substances such as metal ions, some organic molecules, and adsorbents.[6]

Although metal (sodium, calcium, and copper) salts of nalidixic acid have been prepared and documented in a patent,[7] studies dealing with interaction of the drug with metal ions are limited to that with ferric ions.[8,9] The stoichiometry of the ferric complex, however, is somewhat controversial, *i.e.* the ratio of nalidixic acid to ferric ion has been reported to be 3 to 1 (pH is not cited) according to Dick and Murgu,[8] whereas it has been proposed to be 2 to 1 at pH 1.0 according to Ruzicka *et al.*[9] Differences in pH of the solution may have pronounced effects on the stoichiometry of the metal complexes.

In the present study, interactions of nalidixic acid with metal ions which are often contained in antacid formulations were examined. Since alumium hydroxide and other aluminum compounds are frequently found in antacid formulations, coadministration of nalidixic acid and aluminum-containing antacid may have some consequence in the bioavilability of the drug.[10] In addition, possible interactions of magnesium and calcium ions with the drug have also been examined.

1) Pharmaceutical Studies on Urinary Tract Antiseptics, Part 1.
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan.*
3) W.A. Goss, W.H. Deitz, and T.M. Cook, *J. Bacteriol.*, **88**, 1112 (1964).
4) M. Shimizu, Y. Sekine, H. Higuchi, H. Suzuki, and S. Nakamura, *Antimicrob. Ag. Chemother.*, 123 (1970).
5) P.T. Mannisto, *Clin. Pharmacol. Therap.*, **19**, 37 (1976).
6) M. Yamamoto, M. Nakano, Y. Tanaka, and T. Arita, to be published.
7) G.Y. Lesher and M.D. Gruett, Belg. Pat. 612258, July 3, 1962.
8) I. Dick and N. Murgu, *Rev. Chim.* (Bucharest), **15**, 757 (1964), through *Chem. Abstr.*, **62**, 15600*h* (1965).
9) E. Ruzicka, J. Lasovsky, and P. Brazdil, *Chem. Zvesti*, **29**, 517 (1975).
10) E.W. Martin "Hazards of Medication," J.B. Lippincott Co., Philadelphia, 1971, p. 695.

Changes in physico-chemical properties of the drug in the presence of these cations, which are suggestive of interaction of the drug with these ions, are presented in this paper. No effort has been made to isolate the complexes and analyze the solid complexes by chemical and physical means, but instead changes in physico-chemical properties of the drug in solution upon complexation have been examined.

Because solubility, partition, and permeation profiles of the drug in the presence of the metal ions give some basic information on the effect of these ions on the absorption rate of the drug, changes in these properties in solution were studied in addition to some spectroscopic measurements.

Experimental

Materials——Nalidixic acid was used as supplied from Daiichi Seiyaku Co., Tokyo. Aluminum sulfate octadecahydrate, magnesium chloride hexahydrate, calcium chloride dihydrate, hydrochloric acid, acetic acid, sodium acetate (anhydrous), tromethamine, and carbon tetrachloride purchased from Wako Pure Chemical Industries, Osaka, were of reagent grade and used without further purification. Ethanol (Kanto Chemical Co., Tokyo) was distilled prior to use. Seamless cellulose tubing, type 20/32 manufactured by Visking Co., Chicago was employed in permeation experiments.

Spectrophotometric Studies——The stock solution of 10^{-3} M nalidixic acid in ethanol was used for preparations of its dilute solutions for spectrophotometric studies. The pH of solution was maintained at 3.3 by 0.185 M acetate buffer and at 8.8 by 0.524 M tromethamine–hydrochloric acid buffer.[11]

Spectra of nalidixic acid in buffer solutions were obtained with a model 323 recording spectrophotometer (Hitachi, Manufacturing Co., Tokyo) or Acta M-VI spectrophotometer (Beckman Instruments, Palo Alto, California).

Examination of stoichiometry in nalidixic acid-aluminum ion complex was made by measuring the absorbance at 330 nm of solutions prepared according to the method of continuous variations.[12] Nalidixic acid (10^{-4} M) in 0.185 M acetate buffer, pH 3.3 and 10^{-4} M aluminum sulfate solution in the same buffer were mixed in different volume ratios.

Measurements of absorbance according to the method of continuous variations and those for determination of the drug in solubility, partition, and permeation studies were made with a model 200-20 digital reading spectrophotometer (Hitachi Manufacturing Co.). Absorbance in the presence of aluminum ion was usually read at the wave length corresponding to the isosbestic point (313 nm, see Fig. 2) in the spectra of nalidixic acid at pH 3.3.

Solubility Measurements——Glass-stoppered test tubes containing an excess amount of nalidixic acid powder in 0.185 M acetate buffer, pH 3.3 were immersed in a constant temperature water bath (Type K1, Ikemoto Rika Industry, Tokyo) maintained at 30.0° and the suspension was agitated by means of Teflon magnetic bars, 10 mm long, placed in the test tubes. Seven days equilibration time was allowed for prior to filtration through a sintered glass disk and dilution with the buffer for spectrophotometric measurements.

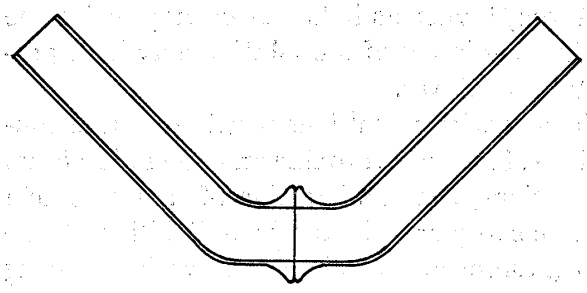

Fig. 1. The Glass Permeation Cell Employed in the Present Study

Partition Studies——Glass stoppered test tubes containing 4 ml of 10^{-4} M nalidixic acid in 0.185 M acetate buffer, pH 3.3 and 4 ml of carbon tetrachloride were immersed in the constant temperature water bath at 30.0° and liquid layers were agitated by means of Teflon magnetic bars, 10 mm long, placed in the test tubes. Thirty min equilibration time was allowed for and the concentration of the drug remaining in the aqueous phase was determined spectrophotometrically after appropriate dilution with the buffer.

Permeation Studies——A glass dialysis cell depicted in Fig. 1 was employed. The cell was constructed from greaseless joints (Kokura Glass Industries, Kitakyushu) 30 mm in internal diameter. A piece of cellulose membrane which had been washed thoroughly was clamped between the two half cells with Teflon

11) T. Ando, H. Terayama, K. Nishizawa, and T. Yamakawa, eds., "Seikagaku Kenkyuho II," Asakura Shoten, Tokyo, 1967, p. 811.
12) F.J.C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill Book Co., New York, N.Y., 1961, pp. 47—51.

O-ring by a stainless steel circular holder with tightening screws and the assembled cell was placed on a platform in the constant temperature water bath maintained at 30.0°. A drug solution (50 ml) in 0.185 M acetate buffer, pH 3.3 was pipetted into the donor compartment whereas the buffer solution (50 ml) into the receptor compartment. Both solutions had been warmed to 30.0° before pipetted into each compartment. The cell was shaken horizontally at a rate of about 75 cpm. Every 2 hr, a small volume of sample was removed from the receptor compartment, for immediate absorbance reading prior to its return to the receptor solution.

Results and Discussion

Absorption Spectra

Nalidixic acid dissociates over a neutral to alkaline pH region to form nalidixate anion with a pK_{a_2} of 5.99 whereas it is protonated in strong acid solution to form naphthyridinium cation with a pK_{a_1} of -0.86.[13] The drug is in its undissociated form over a pH range of 1.6—3.6 as shown in Chart 1.

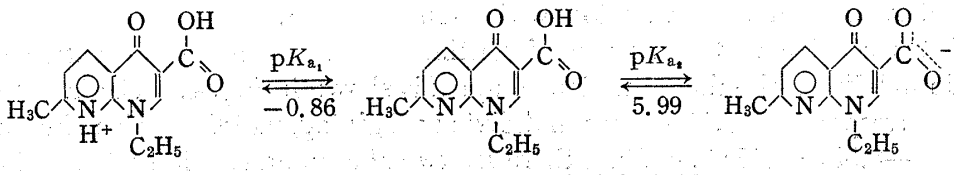

Chart 1. Ionization of Nalidixic Acid

Nalidixic acid in its undissociated form has an absorption maximum at 316 nm as shown in Fig. 2, no symbol. The dissociated form of the drug, on the other hand, has an absorption maximum at around 334 nm[9,14] (see Fig. 3).

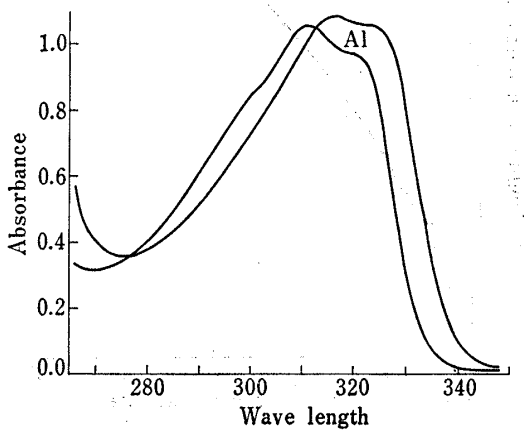

Fig. 2. Absorption Spectra of 10^{-4} M Nalidixic Acid in 0.185 M Acetate Buffer, pH 3.3 in the Presence (Al) and Absence (no symbol) of Aluminum Sulfate (5×10^{-3} M)

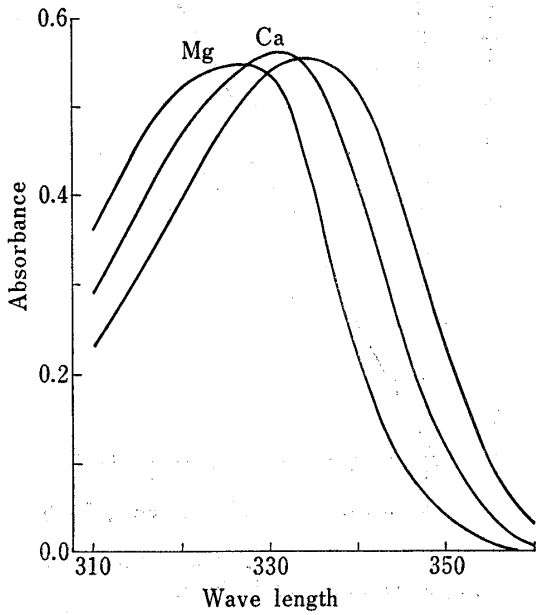

Fig. 3. Absorption Spectra of 5×10^{-5} M Nalidixate in 0.524 M Tromethamine Buffer, pH 8.8 in the Presence and Absence (no symbol) of 10^{-2} M Magnesium Chloride (Mg) or Calcium Chloride (Ca)

13) J. Sulkowsha and R. Staroscik, *Pharmazie*, **30**, 405 (1975).
14) E.F. Salim and I.S. Shupe, *J. Pharm. Sci.*, **55**, 1289 (1966).

Changes in absorption spectrum of nalidixic acid may be used as a simple test of possible interactions of the drug with metal ions. It has been reported that upon complexation with ferric ion, nalidixic acid exhibited a new absorption maximum at 425 nm at pH 10.[9]

Upon addition of aluminum ion to the solution containing the undissociated form of nalidixic acid, the absorption maximum shifted from 316 nm to 311 nm as shown in Fig. 2, curve Al. Any new absorption maximum in visible wave length region as observed with ferric ion[9] was not found. Little change in absorption spectrum of nalidixic acid was observed even in the presence of 10^{-2} M magnesium chloride or calcium chloride (not shown in the figure), indicating negligible degree of interaction of nalidixic acid with alkaline earth metal ions in weakly acidic solutions.

The absorption maximum of nalidixate ion shifted from 334 nm to 327 or 331 nm by the addition of 10^{-2} M magnesium chloride or calcium chloride, respectively as shown in Fig. 3. Although high concentration of aluminum sulfate could not be employed because of formation of poorly soluble aluminum hydroxide at pH 8.8, spectral change observed with the addition of a small amount of aluminum sulfate (not shown in the figure) does suggest some degree of interaction of nalidixate ion with aluminum ion.

The plot according to the method of continuous variations for estimation of the stoichiometry in nalidixic acid-aluminum ion complex is shown in Fig. 4. The intersecting point of two lines corresponds to 8.55×10^{-5} M nalidixic acid giving, nalidixic acid: aluminum sulfate $=8.55:(10.00-8.55)=8.55:1.45$ Since there are two aluminum ions in each aluminum sulfate (Al_2SO_4), the above ratio corresponds to nalidixic acid to aluminum ion ratio of, $8.55:2\times1.45=8.55:2.90=2.95:1.00\simeq3:1$.

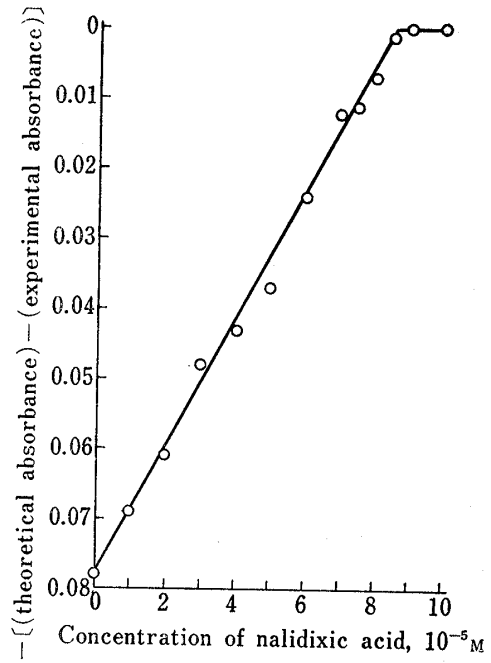

Fig. 4. A Plot according to the Method of Continuous Variations.

The sum of concentration of nalidixic acid and that of aluminum sulfate was 10^{-4}M.

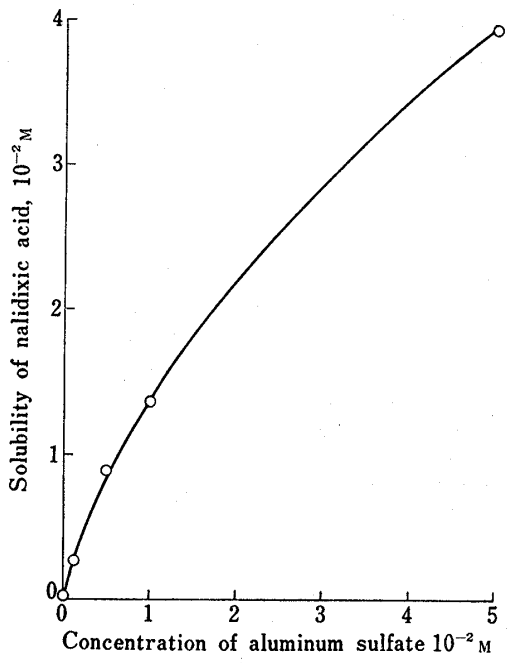

Fig. 5. Solubility Profile of Nalidixic Acid in 0.185 M Acetate Buffer, pH 3.3 with Varying Concentration of Aluminum Sulfate at 30.0°

Solubility

Changes other than spectral shift in physical properties of nalidixic acid in solution were examined to confirm interaction of nalidixic acid with aluminum ion. A solubility profile of the drug with increasing concentrations of aluminum sulfate is shown in Fig. 5. Aqueous solubility was increased from 1.55×10^{-4} M in the absence of aluminum ion to about 3.92×10^{-2}M

in 5×10^{-2} M aluminum sulfate solution, some 250-fold increase in solubility being observed. Since pH was kept constant, any increase in solubility is attributed to appearance of a new species, *i. e.*, observed solubility=(solubility of nalidixic acid)+(concentration of the drug in nalidixic acid-aluminum ion complex).

Compared with the aluminum salt, an increase in solubility of the drug in the presence of magnesium and calcium ions was much less. Solubility of nalidixic acid at magnesium concentration of 10^{-2} M was only 1.72×10^{-5} M (not shown in the figure).

Partition Coefficient

Nalidixic acid partitions between carbon tetrachloride and 0.185 M acetate buffer, pH 3.3 with a partition coefficient of 2.35. With an increasing concentration of aluminum ion, the partition coefficient decreased as shown in Fig. 6. At 10^{-3} M aluminum sulfate, it decreased to 0.105. The result may be rationalized by interaction of nalidixic acid with aluminum ion in the aqueous layer and poor solubility of the complex in carbon tetrachloride.

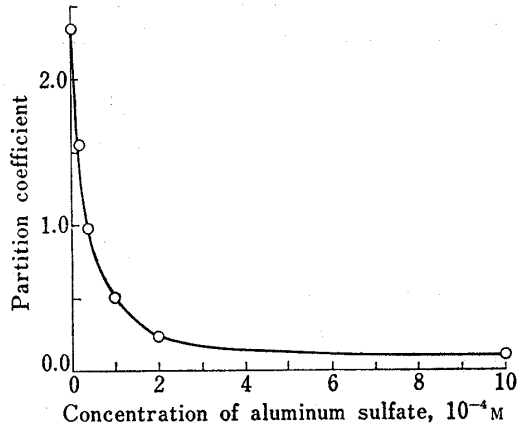

Fig. 6. Changes in (Carbon Tetrachloride/0.185 M Acetate Buffer, pH 3.3) Partition Coefficient of Nalidixic Acid (Initial Concn., 10^{-4} M) with Varying Concentrations of Aluminum Sulfate at 30.0°

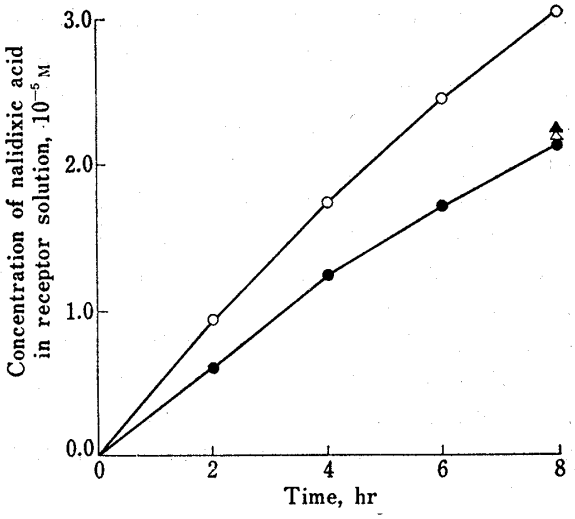

Fig. 7. Permeation Profiles of Nalidixic Acid (Initial Concn., 10^{-4} M) through Cellulose Membrane (Area=7.1 cm^2, Labeled Pore Size=240 nm, Labeled Thickness=20.3 μm) from 50 ml of Donor Solution either in 0.185 M Acetate Buffer, pH 3.3 (○) or in 0.254 M Tromethamine Buffer, pH 8.8 (△) in the Presence (Closed Symbol) and Absence (Open Symbol) of 3×10^{-5} M Aluminum Sulfate at 30.0

Each data point represents the mean of 2 experiments. Data points for experiments at pH 8.8 for the first 6 hr almost overlapped those for experiments at pH 3.3 in the presence of aluminum ion and are excluded from the figure for clarity.

Permeability

Permeation profiles of nalidixic acid at two pH values in the presence and absence of aluminum sulfate are shown in Fig. 7. An undissociated form of nalidixic acid (pH 3.3) permeated faster than the dissociated form of the drug (pH 8.8). Such an effect of charge state of permeants on dialysis rate has been reported.[15]

Permeation of nalidixic acid at pH 3.3 through the cellulose membrane was clearly inhibited in the presence of aluminum ion, whereas little effect of the presence of aluminum ion was noted at pH 8.8. An increase in the size of permeating species due to interaction of the drug with aluminum ion, resulting in some 3-fold increase in the size, may be a cause of reduced permeation rate.

15) R. Withington and J.H. Collett, *J. Pharm. Pharmacol.*, **25**, 273 (1973).

Almost identical permeation profiles of nalidixate ion at pH 8.8 in the presence and absence of aluminum ion may be rationalized by the degree of interaction between nalidixate anion and aluminum ion being less than that between nalidixic acid and aluminum ion.

General Discussions

Solubility, partition, and permeation studies not only supported that the spectral change of nalidixic acid upon addition of metal ions is due to complexation but also revealed some properties of the complex. The complex is shown to be water-soluble, less soluble than nalidixic acid in a nonpolar solvent, and less permeable through the pores of a dialysis membrane than the drug.

The extent of interaction between the drug and aluminum ion seems to be greatest in weakly acidic pH region where the drug is in its undissociated (unionized) form. At alkaline pH, where the drug is in its dissociated (anionic) form, spectroscopic study indicated that the drug interacts with aluminum ion, but the extent of interaction seems to be smaller than that at weakly acidic pH as observed in permeation studies (Fig. 7). Spectral change of the drug at strongly acidic pH, where the drug is in its protonated (cationic) form, in the presence of aluminum ion indicates some degree of interaction of the drug with the metal ion, but because of unphysiological condition (18N H_2SO_4), no quantitative measurement was attempted.

As to a possible structure of the complexes, participation of carbonyl and carboxylic acid groups in interactions with multivalent metal ions may be considered,although further studies are necessary to eliminate alternative possibility, *i. e.* participation of nitrogens in naphthyridin ring system in the interaction. Such drugs as oxolinic acid, which have carbonyl and carboxylic acid groups but lack aromatic nitrogen may be employed for such a study.

The effort of determining formation constants of the nalidixic acid-metal ion complexes has been unsuccessful. Clinical significance of these interactions following coadministration of nalidixic acid and aluminum-containing antacid preparations will be examined in human volunteers.

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