# Articles

## Ionization and Divalent Cation Complexation of Quinolone Antibiotics in Aqueous Solution

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The protonation and divalent cation complexation equilibria of several quinolone antibiotics such as nalidixic acid (NAL), flumequine (FLU), oxolinic acid (OXO), ofloxacin (OFL), norfloxacin (NOR) and enoxacin (ENO) have been examined by potentiometric titration and spectrophotometric method. The antibacterial activity of these drugs depends upon the pH and the concentration of metal cations such as $Mg^{2+}$, $Ca^{2+}$ in solution. The apparent ionization constants of NAL, FLU, OXO, OFL, NOR and ENO in aqueous solution were found to be 6.33, 6.51, 6.72, 7.18, 7.26, and 7.53, respectively. In aqueous solution, NAL, FLU and OXO were found to be present mainly as two chemical species : molecular and anionic; but OFL, NOR and ENO were present mainly as three chemical species : anionic, neutral zwitterionic and cationic form, in equilibrium. The $pK_{a1}$ and $pK_{a2}$ are found to be 6.10 and 8.28 for OFL; 6.23 and 8.55 for NOR; 6.32 and 8.62 for ENO, respectively. The complex formation constants between OFL, NOR or FLU and some divalent cations are measured at pH 7.5. The 1 : 1 complexes are formed mainly by ion-dipole interaction. FLU has somewhat larger $K_f$ values than OFL and NOR because its molecular size is small and the FLU is present as anionic form at pH 7.5.

### Introduction

Because the quinolone antibacterial agents have a broad antibacterial spectrum, good oral absorption and wide tissue distribution, they have been used extensively in the treatment of infectious diseases.[1] These biologically active molecules are fully or partially ionized at physiological pH, and it has often been shown that the presence of charged groups is necessary for biological activity and solubility. The un-ionized form, however, has a more favorable partition coefficient toward nonaqueous solvents. Therefore, knowledge of the dissociation constants of these drugs may be essential for practical purposes (dissolution rates, rates of gastrointestinal absorption, *etc*.) and for the interpretation of structure-activity relationships.[2,3] The antibacterial activity of quinolones is pH-dependent, because these drugs act by inhibition of bacterial DNA gyrase, a process that depends upon both the pH and concentration of the acid.[4] To this effect, the behaviour of quinolones *in vivo* is influenced significantly by their physicochemical properties, in particular their degree of ionization.[5,6] Furthermore, quinolone antibiotics having different substituents and structures exhibit different antibacterial responses in various environments.[7] For example, with ofloxacin (OFL) and norfloxacin (NOR), which have a piperazinyl group, increases in pH from 5.6 to 8.3 increase their activity in nutrient agar progressively. On the other hand, nalidixic acid (NAL), flumequine (FLU) and oxolinic acid (OXO), which do not have a piperazinyl group, become more active in nutrient agar as the pH falls. Also, because some of these antibiotics contain carboxyl and amine groups, this acid-base behavior may be influnced by the change of the molecular structure in the excited state, leading to intramolecular proton transfer or intramolecular charge transfer.[8] Unfortunately, the ionization of these drugs has not been studied on an active basis. The apparent ionization constants of NAL and OXO have been reported previously.[9] Recently, the ionization equilibria and dissociation constants of several these drugs were studied in aqueous solution and aqueous acetonitrile mixtures.[2,3,10,11] Still, the few reported acid dissociation constants for biologically active substances are often conflicting in the results for the same compound, and an effort to give thermodynamic significance and homogeneity to the collected data is lacking.

It is reported that quinolone antibiotics can form complexes with divalent cations. This chelation of certain metal ions between the carbonyl and carboxyl groups of these molecules plays an important biological role. The ability of the quinolone antibiotics to interact with some cellular components is mediated by their complexation with divalent metal cation.[1] Several studies have shown that a DNA gyrase cannot bind quinolones in the absence of DNA and the amount of quinolone bound to DNA is modulated by the $Mg^{2+}$ con-

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centration.[1,2,7] Some of quinolone antibiotics have been shown to display photocarcinogenic potential and this could be related to their ability to interact with DNA.[1,9] When $Mg^{2+}$ is added to an achievable urinary concentration (5.6 mM), the activity of these antibiotics is reduced for virtually every pH tested, but the antagonistic action of $Mg^{2+}$ on two groups of drugs, with and without the piperazinyl group, does not exhibit a similar pH dependence.[7,10] Furthermore, complex formation with divalent cations of transition metals would be of far greater interest and possible importance, since it has been reported that many enzymes having nucleic acids as substrates or templates contain an nonmagnesium divalent cation.[12-17]

In this work, we determined the ionization constants and complex formation constants with some divalent cations of the quinolone antibacterial agents such as NAL, FLU, OXO, OFL, NOR and enoxacin (ENO) by means of spectrophotometry and potentiometric titration at room temperature. Their molecular structures are presented in Figure 1. As previously stated, OFL, NOR and ENO have a piperazinyl group but NAL, FLU and OXO do not have this substituent. Also, NAL and ENO are naphthyridine derivatives, but FLU, OXO, OFL and NOR are quinoline derivatives. Therefore, from the comprehensive study of the chemical properties of various types of quinolone antibiotics, valuable informations are provided for further insights into the mechanisms of antibacterial activity, phototoxicity of these drugs.

## Experimental Section

NAL, FLU, OXO, OFL, NOR, and ENO were purchased from Sigma and used without further purification. The other chemicals were reagent grade and used as received. Sample solution were prepared using quadruply distilled water, which was obtained by passing distilled water through Barnstead (U.S.A.) Nanopure II deionization system. For the spectrophotometric determination of the ionization constants, the pH of each solution was adjusted by adding $HClO_4$, NaOH or buffer solution. Acetic acid, tris(hydroxymethyl)aminomethane (Tris), ammonium chloride, and borax buffers were used in the pH range 4.2-6.0, 6.0-7.9, 8.1-8.7, and 8.7-9.6, respectively. The concentration of buffers was maintained at lower than 0.01 M. The buffers did not absorb UV light longer than 240 nm. UV/visible absorption spectra were recorded by Uvikon model 943 spectrophotometer at room temperature. The concentration of stock solutions prepared for potentiometric titration was low, owing to the low solubility of drugs in water (NAL, FLU and OXO are $5 \times 10^{-5}$ M; OFL, NOR and ENO are $1 \times 10^{-4}$ M). For back titration, each 200 mL of the stock solution was mixed with 50 mL $1 \times 10^{-3}$ M perchloric acid. Then, the solution was degassed by purging high purity Ar gas for 1 hr. This air-free solution was titrated by $1 \times 10^{-3}$ M sodium hydroxide. DMP400 pH meter (DMS) with glass combination electrode was used for potentiometric titrations. The end point was taken as the point where the first derivative of the titration curve was greatest.

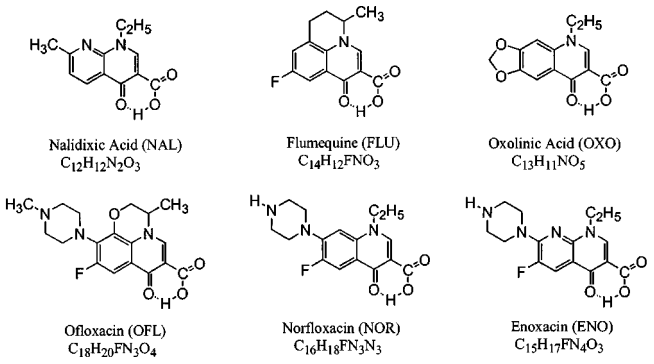



**Figure 1**. Chemical structures of selected quinolone antibiotics. The proposed hydrogen bond is represented by a dotted line.

To find the formation constants with divalent cations, difference spectra were measured at pH 7.5 Tris buffer.[9] The final concentrations of this buffer were 0.05 M Tris and 0.1 M KCl. The pH was adjusted by the appropriate amount of 0.1 M HCl. The concentration of drugs was held constant while the concentration of cation was raised from zero until no further change in the difference spectrum was observed. To find the fraction of total drugs complexed to cation, $\alpha$, at each cation concentration, the height of the difference spectrum maximum was divided by the maximum difference at a saturating concentration of cation. Since only the ionized form of the antibiotics will be involved in complex formation, the formation constant, $K_f$, can be calculated from the expression,

$$K_f = \frac{\alpha}{1-\alpha} \frac{1 + \{[H^+]/K_a'\}}{[M]_T - \alpha[A]_T}$$

where $K_a'$ is the apparent ionization constant and $[M]_T$ and $[A]_T$ are the total cation and total antibiotic concentrations, respectively. The final concentrations of all drugs in this study were $3 \times 10^{-5}$ M.

## Results and Discussion

The UV/visible absorption spectra of quinolone antibiotics in aqueous solution are changed as a function of pH. As shown in Figure 2 (A), the absorption spectra of NAL contain two bands, a strong peak at 258 nm and a weak peak at 332 nm in neutral aqueous solution, respectively. It implies that NAL should have two chromophores : one assigned from the nitrogen atom at position 1 to carboxyl group (Chromophore I) and the other from the methyl group attached to the 7-carbon atom to carbonyl group (Chromophore II). The weak absorption peak of NAL shifts toward the long wavelength side as the pH of solution increases from 5.31 to 7.25. However, any significant shift of the strong absorption peak is not observed at different pH. These results indicate that the weak absorption peak corresponds to the chromophore I, because the carboxyl group will be deprotonated with an increase in pH. On the other hand, the strong absorption peak in the short wavelength region corresponds to the chro-

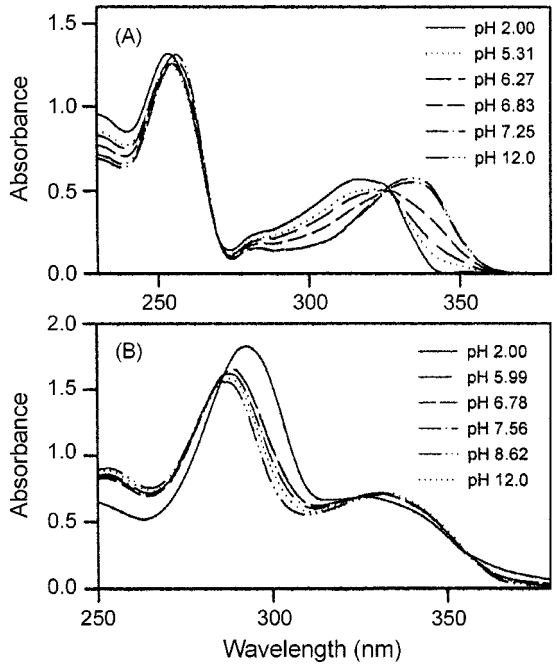



**Figure 2**. Ultraviolet absorption spectra of $5 \times 10^{-5}$ M NAL (A) and OFL (B) in aqueous solution. The actual pH of the solution are shown in the inset of the figures.

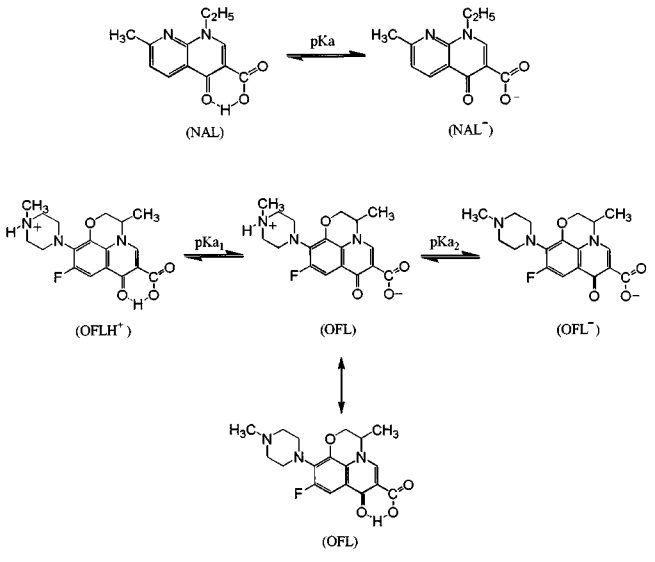
mophore II because this chromophore does not have any functional groups showing significant acid-base properties in pH region 5.31-7.25. FLU and OXO also have two absorption bands, and the shape and band positions of spectra are similar to those of NAL (Data are not shown). Also, the change in the absorption bands of FLU and OXO as a function of pH is about the same as that for NAL, although FLU and OXO do not have a ring nitrogen at position 8, and OXO has electron donating oxygen attached to the 7-carbon atom. Furthermore, NAL, FLU and OXO have one isosbestic point in the UV absorption spectra obtained within the pH ranges of pharmaceutical importance. Therefore, we can suggest that NAL, FLU and OXO have similar types of chromophores.

The absorption spectra of OFL in aqueous solution also have two peaks; strong peak at 287 nm and a weak peak at 332 nm, as shown in Figure 2(B). Contrary to the NAL, the strong absorption peak of OFL shifts toward the short wavelength side much greater compared with the change of the weak absorption peak as the pH of solution increases. This means that the strong peak corresponds to the chromophore involving from the nitrogen atom at position 1 to carboxyl group. The weak absorption peak at the long wavelength side corresponds to the chromophore involving from the nitrogen of the piperazinyl group attached to the 7-carbon to the carbonyl group. The change of absorption spectra of NOR and ENO as a function of pH is similar to that of OFL. Also, the absorption spectra of OFL, NOR and ENO exhibit two isosbestic points as a function of pH. We can suggest that OFL, NOR and ENO contain similar types of chromophores. Therefore, regardless of the naphthyridine or quinoline derivatives, the absorption properties are determined mainly by the presence or absence of piperazine substituent. The UV absorption spectroscopic data for these drugs in various pH solutions are presented in Table 1.

**Table 1**. UV Absorption Maxima ($E_{max}$) and Isosbestic Point ($E_{iso}$) of the Quinolone Antibiotics in Aqueous Solution. $E_{max}$ is Observed in Natural Solution.

| Antibiotics | $E_{max}$ (nm) | | $E_{iso}$ (nm) | |
|---|---|---|---|---|
| NAL | 258 | 332 | 328 | |
| FLU | 248 | 330 | 327 | |
| OXO | 260 | 337 | 286 | |
| OFL | 287 | 332 | 330 | 356 |
| NOR | 272 | 323 | 317 | 345 |
| ENO | 263 | 335 | 295 | 355 |

Quinolone antibiotics have several potentially ionizable functional groups. However, because the p$K_a$ of aniline (4.60) and pyridine (5.23) are very small, the nitrogen atoms at position 1, 8 and $N_1$ of the piperazine ring can hardly exhibit acid – base properties within the pH ranges of pharmaceutical or physiological importance. So, NAL, FLU and OXO have only one relevant ionizable functional group, corresponding to the 3-carboxyl group. One isosbestic point in UV spectra strongly supports the fact that NAL, FLU, and OXO will be present as two chemical species in aqueous solution. In contrast, OFL, NOR and ENO have two relevant ionizable functional groups, the 3-carboxyl group and $N_4$ of the piperazine substituent. Since a carboxyl group is normally a stronger acid than the ammonium group, the first ionization constant, p$K_{a1}$, corresponds to the dissociation of a proton from the carboxyl group and p$K_{a2}$ corresponds to the dissociation of proton from $N_4$ in the piperazinyl group. Also, the two isosbestic points observed for these drugs indicate that OFL, NOR, and ENO will exist mainly as three chemical species in aqueous solution. The ionization equilibria of NAL and OFL are presented in the scheme as below.




To obtain the ionization constants by spectrophotometric

determination, the absorption spectra of the drugs are measured as a function of pH. As shown in Figure 2(A), the absorption spectrum of NAL obtained at pH 2 is considered the spectrum of the molecular species and the spectrum obtained at pH 12 is regarded as that of the ionized species. The largest increase of absorbance as a function of pH is found at 340 nm. Hence, 340 nm is selected as the analytical wavelength for NAL. Using the same method, the analytical wavelength for FLU, OXO, OFL, NOR, and ENO is chosen at 345, 341, 335, 340, and 350 nm, respectively. From the measurement of the absorbance change at this wavelength as a function of pH, we can calculate the concentration ratio of the different chemical species. The apparent ionization constant ($pK_a'$) are calculated from the equation,

$$pK_a' = pH + \log\{(A_I - A)/(A - A_M)\}$$

where $A_I$, $A_M$, and A are the absorbance of the ionized species, the molecular species and the solution at the given pH values, respectively. For OFL, NOR and ENO, $A_I$ is the absorbance of anionic form ($OFL^-$), which is measured at pH 12, and $A_M$ is the absorbance of cationic form ($OFLH^+$), which is measured at pH 2. The apparent ionization constants of the drugs determined by this procedure are summarized in Table 2.

By potentiometric titration, one ionization constant is measured for NAL, FLU, and OXO but two ionization constants are observed for OFL, NOR, and ENO as shown in Table 2. Furthermore, the ionization constants of NAL, FLU and OXO obtained by potentiometric titration and spectrophotometric methods are the same within experimental error. This result is further clear evidence that NAL, FLU and OXO exist mainly as two chemical species, molecular form and anionic form, in equilibrium. Also, the isoelectric points of OFL, NOR and ENO, obtained by averaging the $pK_{a1}$ and $pK_{a2}$ are the same as the apparent ionization constant derived by spectrophotometric method within experimental error. This fact strongly supports that OFL, NOR and ENO exist in three chemical species as a function of pH in equilibrium.

Quinolone antibiotics has a carboxyl group and this part is structurally related with benzoic acid ($pK_a = 4.20$). However, the $pK_{a1}$ of these drugs, indicating the ionization of carboxyl group, are rather high, about 2 pK unit, as shown in Table 2. This result suggests that an intramolecular hydrogen bond exists between the proton of the un-ionized carboxylic acid group and the keto oxygen at position 4. Such an intramolecular hydrogen bond can be found in other cases.[9,18,19] The $pK_{a2}$ of OFL, which corresponds to the ionization of $N_4$ in piperazinyl group, is smaller than the $pK_{a2}$ of NOR and ENO. The protonated form of the secondary amine of NOR and ENO is stabilized by the greater number of water molecules involved in its hydration sphere compared with the corresponding tertiary amine of OFL.[20] Also, the $pK_{a2}$ of OFL will be further decreased by the steric hindrance of $N_4$-methyl substituent in the piperazinyl group. These observations are supported by the early studies for similar secondary and tertiary amines: piperazine, $pK_a = 9.71$ and N-methyl piperazine, $pK_a = 8.98$ in water.[21,22] The acidity of the amine group in piperazine substituent of OFL, NOR, and ENO is still greater than that of piperazine. If the proton can move from the carboxylic group to the $N_4$ in the piperazinyl group *via* hydrogen bonding of water molecules, the ionization of the amine group in the piperazine substituent may arise partly from the combination with the proton produced by ionization of the carboxyl group instead of hydrolysis of water. This proton transfer may contribute to the increase in the acidity of the amine group in this substituent. The $pK_a$ of OXO is slightly higher than that of NAL. Timmer and Sternglanz[9] suggested that this observation might come from the presence of an electron donating oxygen at position 7 and the absence of an electron withdrawing ring nitrogen at position 8. Both of these changes would tend to give the 4-keto oxygen of OXO a greater patial negative charge than that of NAL, strengthening the intramolecular hydrogen bond and thus making OXO the weaker acid. Since FLU does not have an 8-ring nitrogen, the acidity of FLU can be slightly weaker than that of NAL although this difference does not exceed the experimental error. However, although ENO has an 8-ring nitrogen, the acidity of ENO is not stronger than that of NOR. In our laboratory, further study is in progress to understand these properties in detail.

**Table 2**. Ionization Constant of Quinolone Antibiotics in Aqueous Solution using both Spectrophotometric and Potentiometic Titration Methods

| Quinolone Antibiotics | Spectrophotometric Method | Potentiometric titration method | | |
|---|---|---|---|---|
| | $pK_a'$ | $pK_{a1}$ | $pK_{a2}$ | $pK_I$ |
| NAL | 6.33 | 6.41 | | 6.41 |
| FLU | 6.51 | 6.50 | | 6.50 |
| OXO | 6.72 | 6.61 | | 6.61 |
| OFL | 7.18 | 6.10 | 8.28 | 7.19 |
| NOR | 7.26 | 6.23 | 8.55 | 7.39 |
| ENO | 7.53 | 6.32 | 8.62 | 7.47 |

$pK_a'$ and $pK_I$ indicate the apparent ionization constant and isoelectric point, respectively. Uncertainty of all these data is less than 1.5%.

The formation constants with some divalent cations are measured for OFL, NOR and FLU in pH 7.5 buffer solution. In this pH, OFL and NOR exist mainly as zwitterion, but FLU as anion.[23] Table 3 lists the formation constants of each antibiotics with several divalent cations. All drugs have moderate affinities for most of the divalent cations tested. These complexes will mainly be formed by ion-dipole interaction because any significant absorption bands due to orbital-orbital interactions between metals and drugs are not observed. These complexes may involve the 4-keto oxygen and the ionized 3-carboxylic acid groups, since the spectral changes accompanying complex formation are similar to those occurring on protonation of the carboxyl group as shown in Figure 3. This similarity supports our hypothesis of an intramolecular hydrogen bond and its electronic effects in the un-ionized molecules. For cations belonging to the alkaline earth metals, the $K_f$ values decrease going down a group in the periodic table owing to the increase of cationic radii. In transition

metals, the $K_f$ values of $Mn^{2+}$ and $Zn^{2+}$ are relatively small compared with those of $Ni^{2+}$ and $Co^{2+}$. The d orbital of $Mn^{2+}$ is half filled and that of $Zn^{2+}$ is fully filled. So, the orbital-orbital interaction between metal and ligand (antibiotics) will be very weak, and the size of $K_f$ is determined primarily by ion-dipole interaction. If we compare the $K_f$ values of $Ca^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ with the nuclear charge of these metals, $K_f$ values increase gradually as the nuclear charge increases. For $Ni^{2+}(d^8)$ and $Co^{2+}(d^7)$, additional orbital-orbital interactions will exist between metals d orbital and ligands. Therefore, these metals form more stable complexes than $Mn^{2+}$ and $Zn^{2+}$ (see Table 3). For all the ions tested, FLU has somewhat larger $K_f$ values than OFL and NOR because the molecular size of FLU is the smallest among three molecules. Furthermore, for OFL and NOR, the positive charge on the zwitterion makes it harder for the metal cations to aproach the binding site, but for FLU, this kind of electrostatic repulsion does not occur. To determine the composition of the complex clearly, the data were analyzed by Roman Ceba method.[24] The plotted lines obtained in this technique exhibit best straight line only when the composition of the complex is 1 : 1 for all cations (Data are not shown). Also, all of the results in Table 3 are the same as the $K_f$ values obtained by Roman Ceba's within experimental error.

**Table 3**. Apparent Complex Formation Constants of OFL, NOR and FLU with Divalent Cations

| Cation | $\log K_f^*$ | | |
|---|---|---|---|
| | OFL | NOR | FLU |
| $Mg^{2+}$ | 2.9 | 2.9 | 3.2 |
| $Ca^{2+}$ | 2.3 | 2.2 | 3.1 |
| $Sr^{2+}$ | 1.1 | 1.2 | 1.3 |
| $Ba^{2+}$ | 1.1 | 1.1 | 1.1 |
| $Mn^{2+}$ | 3.3 | 3.4 | 3.6 |
| $Zn^{2+}$ | 3.9 | 3.9 | 4.0 |
| $Co^{2+}$ | 4.2 | 4.4 | 4.6 |
| $Ni^{2+}$ | 4.3 | 4.4 | 4.5 |

*Uncertainty is less than 4%.

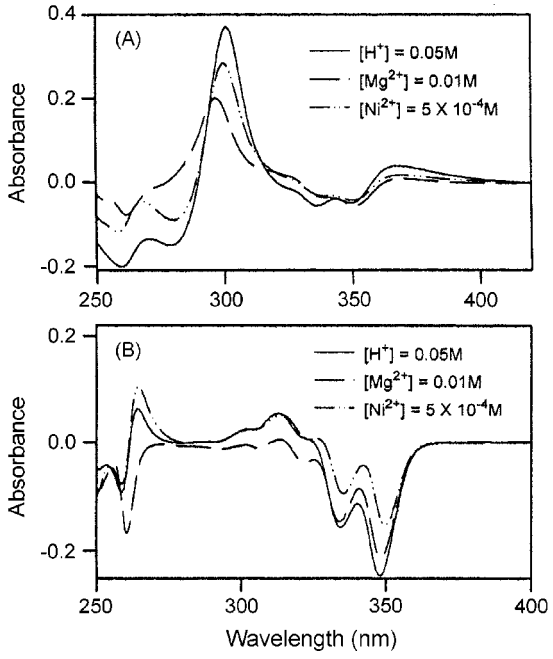



**Figure 3**. Difference spectra of OFL (A) and FLU (B), showing effects of $Mg^{2+}$, $Ni^{2+}$, or $H^+$ versus reference solution of drug at pH 7.5. Final concentrations in reference cuvette: 0.02 M Tris-HCl, 0.1 M KCl, pH 7.5 and $3 \times 10^{-5}$ M OFL or FLU. In sample cuvette, same as reference cuvette plus $Mg^{2+}$ or $Ni^{2+}$. The actual concentrations are shown in the inset of the figure; for the $H^+$ sample, final KCl concentration was reduced to 0.05 M and extra HCl was added to a final concentration of 0.05 M.

The $N_4$ in the piperazinyl group of OFL and NOR may also participate in the complexation with divalent cations. If this interaction is present, the $K_f$ values of NOR are greater than those of OFL because the steric hindrance from the methyl substituent of $N_4$ in the piperazinyl group of OFL results in a weak interaction between the nitrogen atom and metal ion. However, the $K_f$ values of NOR are the same as those of OFL within experimental error. Furthermore, for such an interaction to occur, adjacent molecules would have to interact resulting in 2 : 2 complexing, but this possibility is very low owing to the large excess of the metal ion present in solution.[25] Also, no evidence of 2 : 2 complex formation was observed as described above. Therefore, it is likely that the piperazinyl nitrogen serves to stabilize the complex slightly but can not be considered the primary binding site. It is reported that the formation of 2 : 1 (drug : metal) complexes between NAL and both $Mg^{2+}$ and $Ca^{2+}$ results in higher order solid complexes than observed in our spectroscopic studies as the predominant species in solution.[26] Also, the presence of 2 : 1 (drug : metal) complexes has been indicated for $Mg^{2+}$-fluoroquinolone antibiotics by mass spectroscopy at a drug : metal ratio of 4 : 1 in sample.[27] Therefore, the absence of the evidence of 2 : 1 (drug : metal) complexes in this study may be due partially to the excess of the metal ion present.

For OFL and NOR, the red shift of the strong absorption peak at 285 nm for OFL and 273 nm for NOR, and the blue shift of the small absorption peak at 330 nm are observed as the divalent cations are added (Data are not shown). The lone pair electrons on $N_1$ can conjugate with the 3-carboxylate anion, and this anionic group is destabilized. When divalent cations are complexed between carboxylate anion and 4-keto oxygen, this conjugation will occur more effectively and the energy levels of both the ground and excited states will be stabilized. However, the effect on the excited state may be greater, with the energy differences becoming smaller with complex formation, resulting in significant red shift of strong absorption peak. The intramolecular electron transfer from $N_1$ of the piperazinyl group to 4-keto oxygen will facilitate the complex formation between drugs and metal cations.[8] However, this influence on the metal complex formation will be minor because this intramolecular charge transfer can occur in the excited state. The ground state energy level of this chromophore may be stabilized to a greater extent than

the excited state, owing to the addition of divalent cations, because the stable six-membered chelate ring is formed between the keto and carboxyl oxygen of OFL and NOR. Therefore, small blue shifts of absorption peak at 330 nm are exhibited. For FLU, any shift of the strong absorption peak at 250 nm is not observed, but the blue shift of the small peak around 325 nm is exhibited when cations are added. If the ground state of the chromophore involving $N_1$ to carboxyl group, which corresponds to the absorption peak at 325 nm, is stabilized to a greater extent than the excited state, owing to the formation of six-membered chelate ring, this blue shift of small absorption band can be explained. The effect of $Co^{2+}$ on the absorption spectra of OFL, NOR and FLU is not similar to the effects of the other divalent cations (This effect consists of a broadening of the strong band at short wavelength side). This phenomenon is most significant in FLU. Timmer and Sternglanz[9] also observed similar results for OXO. This fact indicates that these drugs do not form complexes with $Co^{2+}$ of the same type that they form with other divalent cations.

In the present work, ionization constants and cation complex formation constants of some quinolone antibiotics are measured. For these two groups of antibiotics, with and without a piperazinyl group, the relative absorption band positions and intensity are quite different. However, any significant differences of spectroscopic proterties between quinoline (FLU, OXO, OFL and NOR) and naphthyridine derivatives (NAL and ENO) are not observed. The ionization constants obtained by the two different methods, spectrophotometric and potentiometric titration techniques, agree well with each other within experimental error. Between antibiotics and cations, 1 : 1 complexes are formed by ion-dipole interaction, using the 4-keto oxygen and the ionized 3-carboxylic acid group. With $Ni^{2+}$ and $Co^{2+}$, additional orbital-orbital interaction between the metal d orbital and ligand is also observed. The $N_4$ atom at the piperazinyl group of OFL and NOR could not be considered as the primary binding site.

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