Quenching of Ofloxacin and Flumequine Fluorescence by Divalent Transition Metal Cations

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Received June 13, 2006

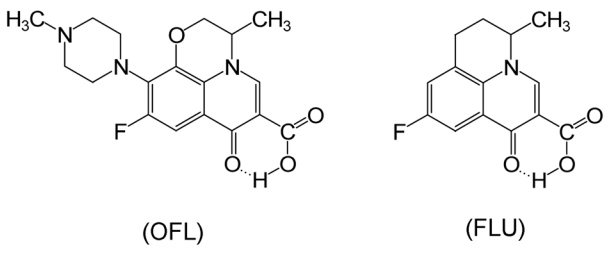
This study examined the quenching of ofloxacin (OFL) and flumequine (FLU) fluorescence by Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} in an aqueous solution. The change in the fluorescence intensity and lifetime was measured at various temperatures as a function of the quencher concentration. According to the Stern-Volmer plots, the fluorescence emission was quenched by both collisions (dynamic quenching) and complex formation (static quenching) with the same quencher but the effect of static quenching was larger than that of dynamic quenching. Large static and dynamic quenching constants for both OFL and FLU support significant ion-dipole and orbital-orbital interactions between fluorophore and quencher. For both molecules, the static and dynamic quenching constants by Cu^{2+} were the largest among all the metal quenchers examined in this study. In addition, both the static and dynamic quenching mechanisms by Cu^{2+} were somewhat different from the quenching caused by other metals. Between Ni^{2+} and FLU, a different form of chemical interaction was observed compared with the interaction by other metals. The change in the absorption spectra as a result of the addition of a quencher provided information on static quenching. With all these metals, the static quenching constant of FLU was larger than those of OFL. The fluorescence of OFL was quite insensitive to both the dynamic and static quenching compared with FLU. This property of OFL can be explained by the twisted intramolecular charge transfer in the excited state.

Key Words : Fluorescence quenching, Fluoroquinolone antibiotics, Transition metal cation, Stern-Volmer plot, Intramolecular charge transfer

Introduction

Quinolone antibiotics such as ofloxacin (OFL), flumequine (FLU) *etc*., have been widely prescribed for the treatment of infectious diseases on account of their safety, good tolerance, and broad antibacterial spectrum with less resistance, as shown in Scheme 1.[1-3] Quinolone antibiotics with different structures and substituents exhibit completely different antibacterial responses and chemical properties as the physicochemical properties of environments are changed.[4-9]

The ability of the quinolone antibiotics to interact with some cellular components is mediated by their complexation with divalent metal cations.[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 a nonmagnesium divalent cation.[11-15] Because the two groups of drugs, OFL, which has the piperazinyl group at the 7-carbon, and FLU, which does not have this group, have quite different photochemical properties, it has been suggested that there is a major difference between these two kinds of drugs in terms of either their mode of action or their mechanism of penetration into a bacterium.[4,7] Therefore, knowledge on the physicochemical properties of these drugs *in vivo* is very important for understanding the mechanism of antibacterial activity and cutaneous photosensitization.[16,17] Several studies have been carried out using systems that mimic biological environments such as aerosol-OT (AOT) reverse micelle or H_2O-CH_3OH and H_2O-CH_3CN solvent mixtures because it is difficult to perform this type of work *in vivo* directly.[5-8]



Scheme 1

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a given substance. Quenching processes involve a chemical interaction of the fluorophore and quencher.[18,19] One such process is static quenching, whereby a nonfluorescent ground-state complex is formed between a ground-state fluorophore and quencher. Another important quenching mechanism is collisional or dynamic quenching, which involves the collision and subsequent formation of a transient complex between an excited state fluorophore and a ground-state quencher. Dynamic quenching is diffusion controlled because the quencher must

diffuse to the fluorophore during the lifetime of the excited-state. The excited-state complex dissociates upon radiative and nonradiative deactivation, leaving both the fluorophore and quencher in the ground state.

The aim of this study was to examine the fluorescence quenching of the OFL and FLU by several divalent cations such as Cu^{2+}, Ni^{2+}, Co^{2+}, and Mn^{2+}. All the metal quenchers belong to the first transition metal series from atomic number 25 to 29 without iron. Iron was not used in this study because it is mainly present as the +3 ion. It was reported that 1 : 1 complexes are formed by an electrostatic ion-dipole interaction between these drugs and cations using the 4-keto oxygen and the ionized 3-carboxylic acid group.[20] Therefore, the ionic radii of cations will be one of the most important factors in determining the formation constant (K_f). There is usually a steady decrease in radius as the number of 3*d* electrons increases from Mn^{2+} to Ni^{2+}. However, the ionic radius begins to increase when the number of 3*d* electrons increases to more than Ni^{2+}. Hence, the ionic radius of Cu^{2+} will be slightly larger than that of Ni^{2+}.[21] Due to the electronic structure of the 3*d* orbital of $Co^{2+}(3d^7)$, $Ni^{2+}(3d^8)$ and $Cu^{2+}(3d^9)$, the additional orbital-orbital interaction between the metal and ligand (antibiotics) will be relatively strong. However, the orbital-orbital interaction with Mn^{2+} will be weak because the 3*d* of Mn^{2+} is half filled. Therefore, the K_f of Mn^{2+} is expected to be small compared with Ni^{2+}, Co^{2+} and Cu^{2+}.[20] The difference in the K_f value among Ni^{2+}, Co^{2+} and Cu^{2+} is not large. For all of these metal ions, FLU has somewhat larger K_f values than OFL on account of its smaller molecular size. Furthermore, for OFL, the positive charge on the zwitterions makes it more difficult for the metal cations to approach the binding site. However, this type of electrostatic repulsion does not occur in FLU. Therefore, a fluorescence quenching study using divalent transition metal cations should provide further insight into the chemical properties of quinolone antibiotics.

Experimental Section

Reagents and materials. OFL and FLU were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The chemicals used as metal quenchers [$CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$] were obtained from Aldrich Chemical Co. (Milwaukee, WI; ACS reagent grade). These chemicals were dried for 48 hr in a vacuum (1×10^{-3} torr) with P_2O_5 at room temperature. Tris(hydroxymethyl)aminomethane (Tris), KCl and HCl were purchased from Aldrich to prepare a buffer solution (pH = 7.5). The sample solutions were prepared using quadruply distilled water, which was obtained by passing doubly distilled water through a Barnstead (U.S.A.) Nanopure II deionization system. Low concentration solutions (below 3×10^{-5} M) were used as the sample in order to avoid any solute-solute interactions and self-absorption. The procedure for preparing the dilute solution and eliminating the dissolved oxygen in the sample was explained elsewhere.[8] A Tris and KCl mixed solution was used to prepare the Tris buffer. The pH was adjusted using the appropriate amount of 0.1 M HCl. The final concentration of this buffer was 0.050 M Tris and 0.10 M KCl.

Instruments. UV/Vis absorption spectra were obtained using a Perkin-Elmer Lambda 900 spectrophotometer with a 1.0 nm spectral bandwidth. The steady-state fluorescence emission spectra were obtained on a Perkin-Elmer LS-50B spectrofluorometer with 5 nm slits. The sample temperature was controlled using a Julabo MP-5A temperature circulator (± 0.5 °C). After adjusting the temperature, the samples were allowed to equilibrate for 25 min. The quantum yields (Φ) were measured using quinine sulfate (Φ = 0.546) and anthracene (Φ = 0.27) as a reference.[22-24] A fluorescence lifetimes were obtained by phase-modulation method. A SLM 48000S (SLM Aminco, Rochester, NY) spectrofluorometer with a 300W Xe lamp was used to do this measurements. For all lifetime measurements, the repetition rate of the Pockel's cell was 5 MHz and the modulation frequencies were obtained for the harmonic content of this pulse train.[19,25-27] Multifrequency phase and modulation data were fitted by a theoretical model using commercially available software. The time resolution of this system was 10 ps.

Results and Discussion

Absorption and fluorescence emission spectra. Figure 1 shows the UV/visible absorption spectra and the steady-state fluorescence emission spectra of the molecules in water. The absorption spectrum of OFL contains two bands, a strong peak at ~285 nm and a small peak at ~330 nm. In FLU, a strong absorption band was observed at ~245 nm region and small peak at ~325 nm. The change in the absorption spectra of OFL and FLU showed different pattern depending on the environments.[5,6] The fluorescence spectra of OFL showed strong, broad structureless bands with large Stokes' shifts. The fluorescence properties of OFL were quite sensitive to the physicochemical properties of the solvent.[5,6] The fluorescence properties of FLU were very different from those of OFL, for example, the position of the fluorescence emission bands, Stokes' shift and fluorescence quantum yields were insensitive to the properties of the solvents.

Because OFL has an electron donor, the piperazinyl group, and an electron acceptor, the keto oxygen, excited-state intramolecular charge transfer (ICT) was observed in the aqueous solution.[5] The formation of the ICT state is accompanied by an isomerization reaction where the donor moiety twists with respect to the acceptor. Therefore, OFL is regarded as a good twisted intramolecular charge transfer (TICT) molecule in the excited-state.[5-7] Since the ICT in the S_1 state is accelerated by a highly polar and protic environment such as water, OFL is present in aqueous solutions mainly in the charge transferred zwitterionic form, [S_1(CT)], as shown in Scheme 2. However, OFL is present in the molecular form, [S_1(M)], in organic solvent such as CH_3OH or CH_3CN. In the S_1(CT) form, the lone pair electrons of the 1-nitrogen atom of the piperazinyl group attached directly to 7-carbon will participate to some extent the delocalization of

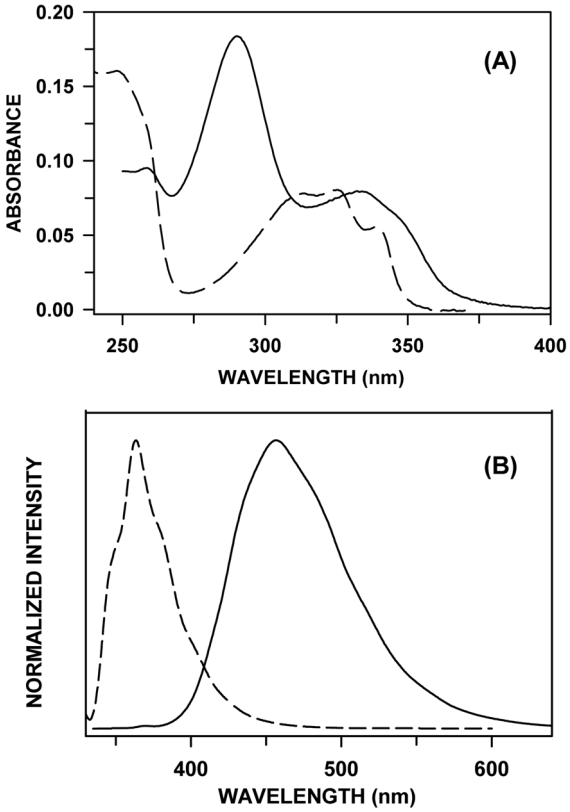


Figure 1. (A) Absorption spectra of 7×10^{-6} M OFL (——) and FLU (– – –) in water. (B) Steady-state fluorescence emission spectra of 7×10^{-6} M OFL (——) and FLU (– – –) in water at room temperature. OFL: λ_{ex} = 330 nm, FLU: λ_{ex} = 325 nm.

the π electrons in the quinoline nucleus. Owing to these reasons, OFL exhibits specific fluorescence properties in various systems that mimic biological environments such as AOT micelle or aqueous-organic solvent mixtures.[5-8]

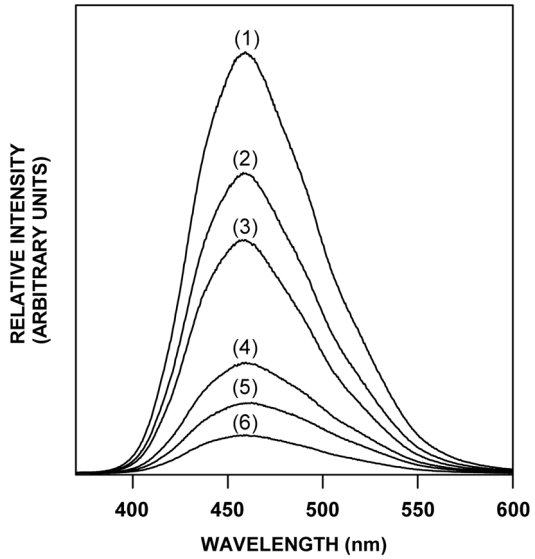
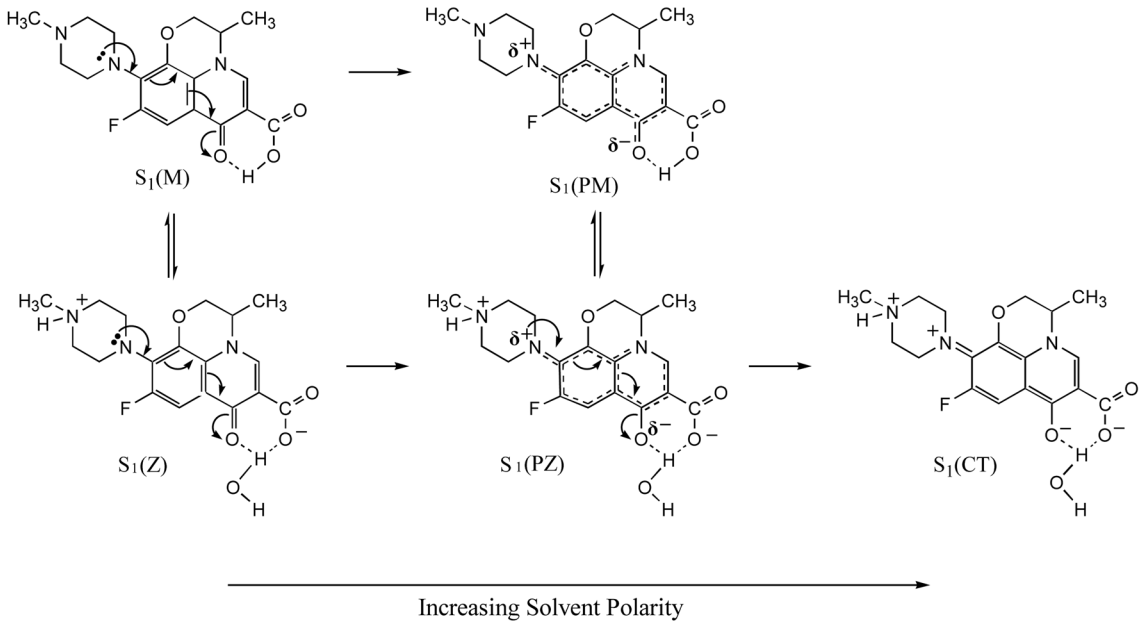


Figure 2. Fluorescence emission spectra of 7×10^{-6} M OFL aqueous solution at 0 °C in the presence of Cu^{2+} (λ_{ex} = 330 nm). Concentration of Cu^{2+} (M): (1) 0; (2) 2.6×10^{-6}; (3) 3.9×10^{-6}; (4) 5.2×10^{-6}; (5) 6.5×10^{-6}; (6) 9.1×10^{-6}.

Fluorescence quenching. When metal quenchers, Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+}, were added to an OFL and FLU solution, appreciable quenching occurred even at very low concentrations (from $< 2 \times 10^{-6}$ M for Cu^{2+}). However, the shape of the fluorescence spectra remained the same, with no change in the position of the maxima until the intensity fell below the detection limit as shown in Figure 2. Figure 3, 4, 5 and 6 show the Stern-Volmer plots of antibiotics by Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} at various temperatures, respectively. Several characteristic features can be observed in these plots.

First, the change in the fluorescence intensity as a function



Scheme 2

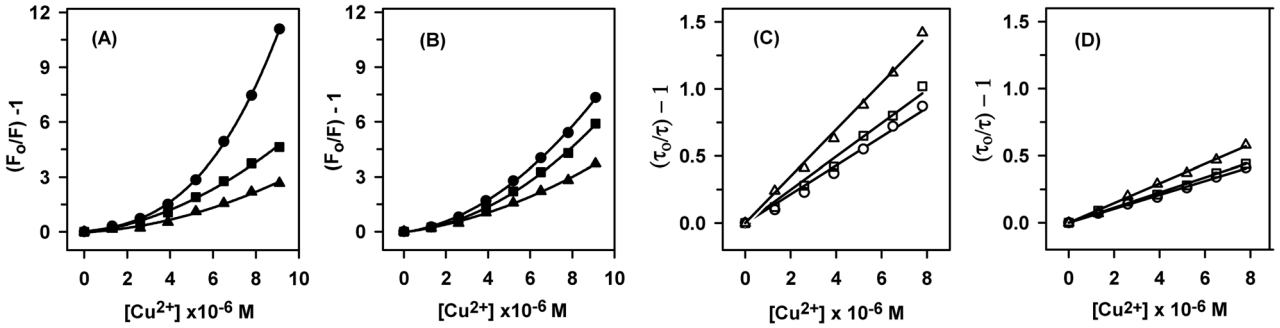


Figure 3. Stern-Volmer plots for the quenching of antibiotics fluorescence by Cu^{2+} at various temperatures: (A) OFL; 10 °C (●), 30 °C (■), and 60 °C (▲), (B) FLU; 10 °C (●), 30 °C (■), and 60 °C (▲), (C) OFL; 10 °C (○), 30 °C (□), and 60 °C (△), (D) FLU; 10 °C (○), 30 °C (□), and 60 °C (△).

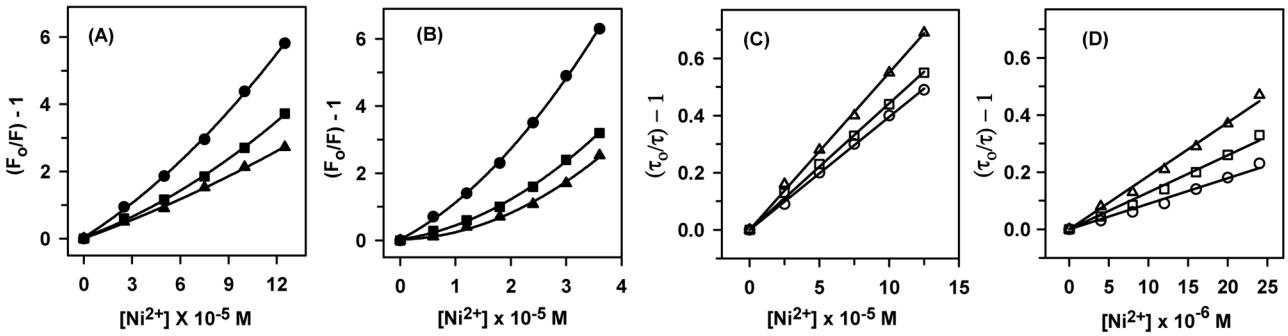


Figure 4. Stern-Volmer plots for the quenching of antibiotics fluorescence by Ni^{2+} at various temperature: (A) OFL; 10 °C (●), 30 °C (■), and 60 °C (▲), (B) FLU; 10 °C (●), 30 °C (■), and 60 °C (▲), (C) OFL; 10 °C (○), 30 °C (□), and 60 °C (△), (D) FLU; 10 °C (○), 30 °C (□), and 60 °C (△).

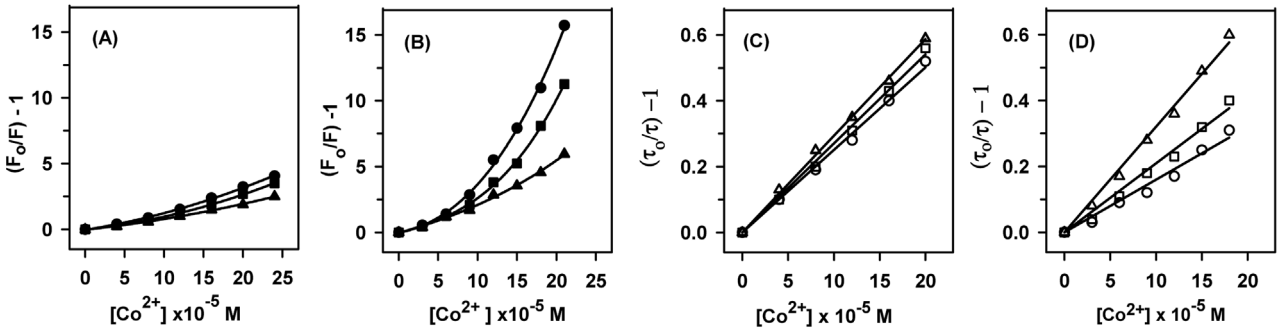


Figure 5. Stern-Volmer plots for the quenching of antibiotics fluorescence by Co^{2+} at various temperature: (A) OFL; 10 °C (●), 30 °C (■), and 60 °C (▲), (B) FLU; 10 °C (●), 30 °C (■), and 60 °C (▲), (C) OFL; 10 °C (○), 30 °C (□), and 60 °C (△), (D) FLU; 10 °C (○), 30 °C (□), and 60 °C (△).

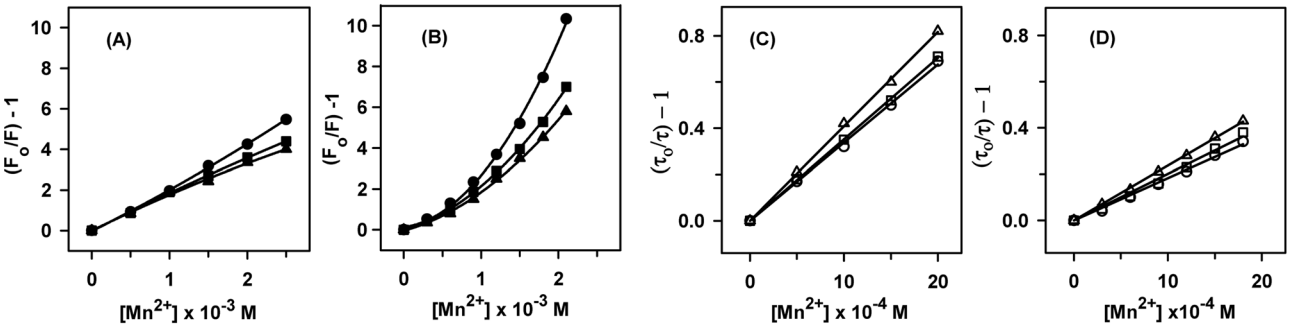


Figure 6. Stern-Volmer plots for the quenching of antibiotics fluorescence by Mn^{2+} at various temperature: (A) OFL; 10 °C (●), 30 °C (■), and 60 °C (▲), (B) FLU; 10 °C (●), 30 °C (■), and 60 °C (▲), (C) OFL; 10 °C (○), 30 °C (□), and 60 °C (△), (D) FLU; 10 °C (○), 30 °C (□), and 60 °C (△).

of quencher concentration, $[(F_o/F) - 1]$ *vs* [Q], increases more rapidly when the temperature is reduced, where F and F_o are the fluorescence intensities in the presence and absence of the quencher, respectively. Q is the quencher. This suggests that static quenching has a large influence on the overall quenching process. Increased temperature is likely to reduce the stability of the complexes, resulting lower static quenching constants. Second, most of the $[(F_o/F) - 1]$ *vs* [Q] plots have a concave curvature towards the y-axis. If the fluorophore is quenched both by collisions and by complex formation with the same quencher, the Stern-Volmer equation can be modified as follows[19]

$$F_o/F = (1 + K_D[Q])(1 + K_S[Q]), \quad (1)$$

where $K_D = k_q\tau_o$ is the Stern-Volmer quenching constant, k_q is the bimolecular quenching constants, τ_o is the fluorescence lifetime in the absence of quencher, and K_S is the static quenching constant. Because this equation is second order with respect to [Q], the upward curvature observed suggests that both static and dynamic quenching occur by the same quencher. Third, the change in the lifetime as a function of the quencher concentration, $[(\tau_o/\tau)-1]$ *vs* [Q] plots, indicates that the slope increases with increasing temperature, where τ is the fluorescence lifetime in the presence of a quencher. In addition, these plots are almost linear straight lines. This change in lifetime by the quencher at various temperatures shows the dynamic portion of the observed quenching.[19] Dynamic quenching can be expressed by the following Stern-Volmer equation :

$$\tau_o/\tau = 1 + k_q\tau_o[Q] = 1 + K_D[Q] \quad (2)$$

Because dynamic quenching depends upon diffusion, k_q is expected to be proportional to T/η (T = temperature, η = solvent viscosity). The viscosity is expected to decrease with increasing temperature. Therefore, k_q will increase with the increasing temperature. On the other hand, for static quenching, the complexed fluorophores are nonfluorescent and the only observed fluorescence is from the uncomplexed fluorophores. Because the uncomplexed fraction is unperturbed, the lifetime remains τ_o. Therefore, $\tau_o = \tau$ for static quenching. In contract, for dynamic quenching, $\tau_o/\tau = F_o/F$.

Table 1 shows the fluorescence quenching constants of OFL and FLU by divalent metal cations at different temperatures. Another specific feature is exhibited in this table. The most of the k_q by all of the metal cations are considerably larger than those possible for a diffusion-controlled quenching in solution (about 10 $M^{-1}.ns^{-1}$), particularly for quenching by Cu^{2+}. Usually, large k_q beyond the diffusion-controlled limit indicates that some types of binding interaction exist between fluorophore and quencher.[19,28] Therefore, this fact supports that the ion-dipole and orbital-orbital interaction between antibiotics and metal quencher are relatively strong. As expected, the k_q of OFL and FLU by Mn^{2+} approach to the diffusion-controlled rate. In our laboratory, further studies are underway to understand this quenching mechanism in detail.

Almost all the static and dynamic quenching constants by Cu^{2+} were larger than those by other metal quenchers with the exception of the K_S of FLU fluorescence by Ni^{2+}, as shown in Table 1. Figure 3 shows that the curvature of $[(F_o/F) - 1]$ *vs* $[Cu^{2+}]$ plots of OFL increases more rapidly with decreasing temperature than those of FLU. Because the K_D of OFL are larger than that of FLU, the $[(\tau_o/\tau) - 1]$ values for OFL will also increase more rapidly with increasing Cu^{2+} concentration at all temperatures. In addition, the lifetime of both OFL and FLU decreases gradually with increasing temperature but the temperature dependence of the OFL lifetimes is larger than that of FLU, as shown in Table 2. Because the lifetimes of OFL are much longer than those of FLU, the k_q of OFL are smaller than the half of the k_q of FLU. With increasing temperature, the k_q of OFL by Cu^{2+} increases more rapidly than that of FLU. The K_S of OFL and FLU with Cu^{2+} are similar at 10 °C but the K_S of OFL decreases more rapidly with increasing temperature than that

Table 1. Fluorescence Quenching Constants, $K_S(M^{-1})$, $K_D(M^{-1})$, $k_q(M^{-1}\,ns^{-1})$, for Quinolone Antibiotics with various Divalent Cations. The uncertainty is ≤ 7%

(1) OFL

Cations	10 °C			30 °C			60 °C		
	K_s	K_D	k_q	K_s	K_D	k_q	K_s	K_D	k_q
Cu^{2+}	1.35×10^6	1.08×10^5	2.00×10^4	4.12×10^5	1.24×10^5	2.69×10^4	1.89×10^5	1.74×10^5	4.06×10^4
Co^{2+}	15100	2540	460	13100	2740	550	7040	3010	730
Ni^{2+}	33300	3900	690	19900	4420	840	8970	5540	1290
Mn^{2+}	25	350	67	–	370	79	–	410	99

(2) FLU

Cations	10 °C			30 °C			60 °C		
	K_s	K_D	k_q	K_s	K_D	k_q	K_s	K_D	k_q
Cu^{2+}	1.48×10^6	5.17×10^4	4.97×10^4	1.09×10^6	5.62×10^4	5.92×10^4	4.40×10^5	7.32×10^4	9.12×10^4
Co^{2+}	2.09×10^5	1520	1660	10.7×10^4	1950	2180	2.34×10^4	3090	3660
Ni^{2+}	2.96×10^6	8550	9750	1.37×10^6	1.24×10^4	1.43×10^4	8.46×10^5	1.81×10^4	2.20×10^4
Mn^{2+}	9340	183	190	5170	200	230	3920	240	290

Table 2. Fluorescence lifetimes of OFL and FLU at various temperatures in water. The uncertainty is ≤ 5%

	10 °C	30 °C	60 °C
OFL	5.48 ns	4.81 ns	4.12 ns
FLU	932 ps	879 ps	818 ps

of FLU. Therefore, both the dynamic and static quenching of FLU fluorescence by Cu^{2+} is more effective than that of OFL. However, the change in K_S and k_q of OFL as a function of temperature is greater than that of FLU. Cu^{2+} is well known as a strong quencher because it is a good electron scavenger on account of its electronic structure (d^9). Quenching by this type of substance most likely involves the donation of an electron from the fluorophore to the quencher. The ion-dipole interaction between Cu^{2+} and the molecule will also be strong due to the large nuclear charge and the relatively small ionic radius compared with other metals. Furthermore, Cu^{2+} usually introduces easily accessible low energy levels, which can give rise to energy- and electron-transfer processes, and is capable of quenching the fluorescent excited states of the molecules.[29-31]

When Ni^{2+}, Co^{2+} and Mn^{2+} are used as quenchers, the Stern-Volmer plots, $[(F_o/F) - 1]$ *vs* [Q] and $[(\tau_o/\tau) - 1]$ *vs* [Q], show a different pattern compared with those of Cu^{2+}. In contrast to Cu^{2+}, the curvature of the $[(F_o/F) - 1]$ *vs* [Q] plots of OFL by Ni^{2+}, Co^{2+} and Mn^{2+} is small, and these plots are almost linear compared with those of FLU. In the dynamic portion of the observed quenching by Ni^{2+}, Co^{2+} and Mn^{2+}, the temperature dependence of the $[(\tau_o/\tau) - 1]$ *vs* [Q] plot of OFL is smaller than that of FLU. In particular, for quenching by Ni^{2+}, the K_S and k_q of FLU are very large compared with those of OFL (approximately 100 times in K_S and more than 10 times in k_q). This very large K_S values between FLU and Ni^{2+} suggests some kinds of specific chemical interaction in this fluorophore-metal couple. The relatively large K_S and k_q of OFL and FLU by Ni^{2+} quenching can be explained partially by the smaller ionic radius and larger nuclear charge of Ni^{2+} than Co^{2+} and Mn^{2+}. When Co^{2+} was used as a quencher, the static and dynamic quenching constant for both OFL and FLU was much smaller than those of Cu^{2+} and Ni^{2+}. The slope of the $[(F_o/F) - 1]$ *vs* $[Co^{2+}]$ plots for OFL was small and almost linear at all temperatures but the inclination of these plots for FLU was quite large and strongly dependent on the change in temperature. Therefore, for the OFL - Co^{2+} couple, there is a relatively small decrease in the K_S value with temperature. However, for the FLU - Co^{2+} couple, K_S is large and this value decreases quite rapidly with increasing temperature. Although the k_q of both OFL and FLU fluorescence by Co^{2+} was small, the k_q of FLU was larger than that of OFL. In addition, the temperature dependence of k_q for the FLU - Co^{2+} couple was larger than that of OFL. In the case of Mn^{2+}, the K_S and k_q of both OFL and FLU were quite small. In particular, the K_s of the OFL - Mn^{2+} couple at 30 °C and 60 °C were too small to obtain with certainty. The $[(F_o/F) - 1]$ *vs* $[Mn^{2+}]$ plots of OFL have a small slope and these plots at 30 °C and 60 °C have a slight downward curvature; very small negative deviation from linearity. However, for FLU, the $[(F_o/F) - 1]$ *vs* $[Mn^{2+}]$ plots at various temperatures have a large slope and a significant concave curve towards the y-axis. Both K_S and k_q of FLU by Mn^{2+} were larger than that of OFL - Mn^{2+}. In addition, the change in the quenching constants of OFL and FLU by Mn^{2+} as a function of temperature was similar to the case of Co^{2+}. It is clear that there is very weak orbital-orbital interaction between Mn^{2+} and the antibiotics. The ion-dipole interaction between Mn^{2+} and the molecules will also be weak because of the relatively large ionic radius and small nuclear charge.

The $[(F_o/F) - 1]$ *vs* $[Cu^{2+}]$ plots of OFL have a large upward curvature compared with those the plots of FLU, particularly at low temperatures. However, the $[(F_o/F) - 1]$ *vs* [Q] plots of OFL, where Q is Ni^{2+}, Co^{2+} or Mn^{2+}, were almost linear but these plots of FLU were concave towards the y-axis, with a relatively large slope, particularly for Co^{2+} and Mn^{2+}. The $[(\tau_o/\tau) - 1]$ *vs* $[Cu^{2+}]$ plots of OFL have a stronger temperature dependence relative to that plots of FLU. However, for Ni^{2+}, Co^{2+} and Mn^{2+}, the temperature dependence of the $[(\tau_o/\tau) - 1]$ *vs* [Q] plots for FLU was stronger than those for OFL. This suggests that the dynamic and static quenching mechanism by Ni^{2+}, Co^{2+} and Mn^{2+} are similar but basically somewhat different from those of Cu^{2+}.

Interaction between antibiotics and metal quenchers. The change in the absorption spectra as a result of the addition of a metal quencher can provide more information on static quenching because the formation of the ground state complex will frequently result in a change in the absorption spectrum of the fluorophore. Therefore, the changes in the absorption spectra of OFL and FLU by metal cations were examined in a pH 7.5 buffer solution. In this physiological pH, OFL exists mainly as zwitterions, but FLU exists as an anion.[20] In neat water, Cu^{2+} can precipitate as $Cu(OH)_2$, but in this buffer, there was no evidence of the formation of a precipitate until the Cu^{2+} concentration reached 1×10^{-3} M. The complexes between these molecules and metal cations will mainly be formed by the ion-dipole interaction suggested previously[20] because no strong absorption bands due to orbital-orbital interactions between metal *d* orbital and ligand were observed, as shown in Figure 7. When Cu^{2+} was added to the OFL solution, the strong absorption band around 288 nm moved to a longer wavelength (~6 nm) with a gradual increase in absorbance. The weak absorption band around 333 nm shifted to a shorter wavelength (~9 nm) with a small increase in absorbance. In addition, a small absorption peak appeared around 370 nm after the addition of Cu^{2+} [see Figure 7(A)]. In this case, there will be some orbital-orbital interactions in addition to the ion-dipole interaction between OFL and Cu^{2+} because the change in the absorption bands and the appearance of a new band are relatively significant. These complexes will involve both the 4-keto oxygen and the ionized 3-carboxylic acid groups because these ketone and carboxyl group correspond to a different chromophore and both these two absorption bands change as a result of the formation of a complex.[20] This

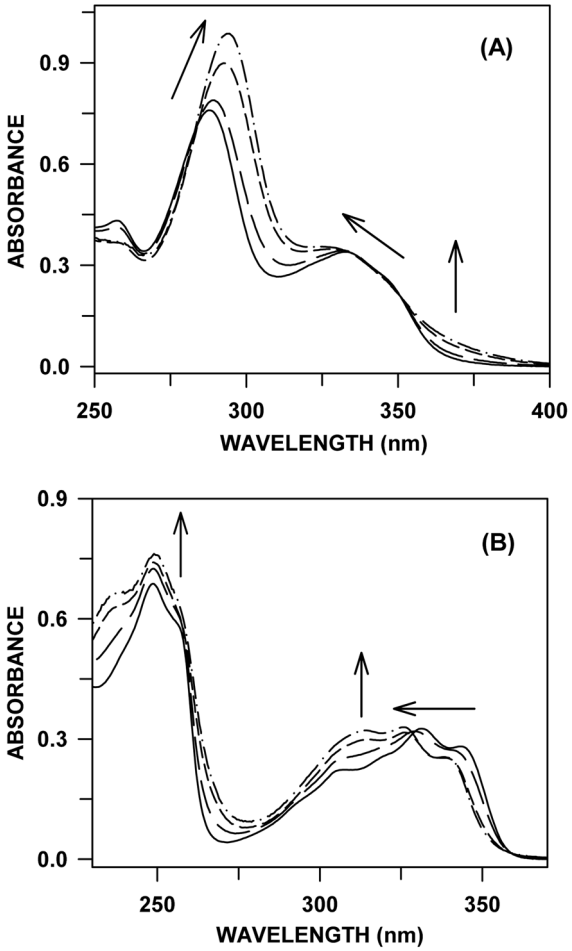


Figure 7. Change in the absorption spectra of 3×10^{-5} M OFL, (A), and FLU, (B), by the addition of Cu^{2+}. The concentration of Cu^{2+} in both (A) and (B) are as follows: (——) 0.0 M, (– – –) 1.0×10^{-4} M, (-----) 5.0×10^{-4} M, (–·–·–) 1×10^{-3} M. Arrows indicate the change of spectra with the increase of Cu^{2+} concentration.

relatively strong orbital-orbital interaction can explain the large static quenching constant between OFL and Cu^{2+}. The crystal structure of the ciprofloxacin (CIP) - Cu^{2+} complex was recently reported.[32] In that study, it was shown that metal cations are usually bonded to the oxygen atoms of the carbonyl and carboxylic groups of the CIP and exhibit an octahedral, or square pyramidal geometry, with additional water molecules or counter ions in the remaining coordination sites. CIP and OFL belong to the same class of quinolone antibiotics and both have the same molecular structure except for a minor difference in the side group. Therefore, it can be assumed that OFL - Cu^{2+} complex will have a similar structure to CIP - Cu^{2+} complex. When Ni^{2+}, Co^{2+}, and Mn^{2+} were added, the change in the spectra showed approximately the same pattern but the spectral change was small compared with the case of Cu^{2+}. The extent of the spectral change as a result of the addition of quenchers is consistent with the size of K_S between OFL and metals. The change in the absorption spectrum of OFL as a result of the addition of Mn^{2+} was smallest among all of the metal quenchers.

When Cu^{2+} was added to the FLU solution, the band around 330 nm moved to a shorter wavelength (~5 nm), as shown in Figure 7(B). Moreover, the absorbance of the side band around 310 nm and the strong band around 250 nm increases without any change in the band position. Therefore, in addition to an ion-dipole interaction, there is some type of orbital-orbital interactions between Cu^{2+} *d*-orbital and FLU. However, the change in the weak absorption band around 285-350 nm was more significant. Therefore, the chemical interaction between Cu^{2+} and the oxygen of the carboxylic group of FLU is greater than that between Cu^{2+} and the oxygen of the carbonyl group because FLU should have two chromophores : one assigned from the nitrogen atom at position 1 to the carboxyl group, which corresponds to the weak absorption band around 325 nm, and the other from 7 and 8-carbon atoms to the carbonyl group, which corresponds to the strong absorption band around 245 nm.[20] When Co^{2+} and Mn^{2+} were added, a similar change in the spectra was observed but this change was relatively small compared with the case of Cu^{2+}. Similar to the OFL, the extent of the spectral change due to the addition of metal quenchers is consistent with the size of the K_S values. Between FLU and Ni^{2+}, some different chemical interactions compared with other metals exist because a different form of spectral change was observed due to the addition of Ni^{2+} (Data are not shown). The change in the weak absorption band around 330 nm was similar to the case of Cu^{2+} but the strong absorption band around 250 nm shifts slightly to a longer wavelength with the small decrease of absorbance and a new band at 235 nm increases rapidly. When Cu^{2+} is added, this new band is small, which is not observed significantly when Co^{2+} and Mn^{2+} are used. Because the spectral shape of both absorption bands at ~245 nm and ~325 nm changes significantly as a result of the addition of Ni^{2+}, it can be suggested that both the carboxylic and carbonyl oxygen are involved in the chemical interaction between Ni^{2+} and FLU to a similar extent. This observation can explain why the static and bimolecular quenching constants between FLU and Ni^{2+}, especially the K_S value, are much larger than those between OFL and Ni^{2+}.

Specific fluorescence properties of OFL. For all the metal quenchers, the static quenching constants of FLU were much larger than those of OFL, as shown in Table 1. The difference in the K_S values between OFL and FLU with all the metal quenchers was large compared with the difference in the formation constants (K_f). The k_q of FLU by all the cations were also larger than those of OFL but the difference in k_q values between OFL and FLU by the same cations was relatively small compared with the difference in the K_S values. The difference in the k_q or K_S values between OFL and FLU was small when the strongest quencher, Cu^{2+}, was used. However, the k_q or K_S values of FLU were much larger than those of OFL when relatively weak quenchers, Ni^{2+}, Co^{2+}, and Mn^{2+}, were used. For the fluorescence quenching of both OFL and FLU by metal quenchers, static quenching overwhelmed dynamic quenching. In addition, the static quenching was more sensitive to changes in temperature

than dynamic quenching. When Cu^{2+} was used, the K_S of OFL decreased more rapidly compared with the K_S of FLU with increasing temperature. However, with Co^{2+} quenching, the K_S of FLU decreased much more rapidly than the K_S of OFL at the same temperature change. When Ni^{2+} was added, the change in K_S with temperature for OFL and FLU were similar. In the dynamic quenching by Cu^{2+}, the increase in the k_q of OFL with increasing temperature was larger than that of FLU. However, in the dynamic quenching by Co^{2+} and Ni^{2+}, the k_q of FLU change more rapidly with increasing temperature than those of OFL. When Mn^{2+} was added, the increase of k_q with temperature for OFL and FLU was similar.

Although most of the OFL is present in S_1(CT) form in aqueous solution as shown in Scheme 2, small amount of S_1(Z) and S_1(PZ) species also exists due to the resonance. The additional resonance forms in the excited-state usually lead to a stronger fluorescence emission. In addition, during excitation and deactivation between the S_o and S_1 state in water, the geometry and dipole moment of OFL change greatly.[5-8] The rate of internal conversion usually decreases if the geometry change between different electronic states increases. Therefore, the OFL in water has a very high quantum yield and a short lifetime compared with the OFL in organic solvents. Due to these reasons, the OFL is quite insensitive to fluorescence quenching by divalent metal cations in aqueous phase, particularly static quenching, compared with FLU. All these phenomena are one of the characteristic chemical properties of the OFL molecule.

Conclusions

The fluorescence emission of OFL and FLU was quenched by both collisions and complex formation with the same quencher when Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} were added. In this quenching, both K_S and k_q are very large. This fact supports the existence of relatively strong ion-dipole and orbital-orbital interaction between antibiotics and metal cations. Furthermore, static quenching has a more important effect on the fluorescence emission of both OFL and FLU. In both dynamic and static quenching, the quenching mechanism by Cu^{2+}, which is the strongest quencher of all the metal cations examined, is somewhat different from the mechanism by Ni^{2+}, Co^{2+}, and Mn^{2+}. This might be because Cu^{2+} is an excellent electron scavenger and introduces easily accessible low energy levels, which can quench the molecules. For the quenching of OFL fluorescence by all the metal cations, both carboxylic and carbonyl oxygen atoms will be involved in the chemical interaction between these metals and OFL. Also, in the chemical interaction between Ni^{2+} and FLU, both the carboxylic and carbonyl oxygen of FLU will be involved. However, when Cu^{2+}, Co^{2+} and Mn^{2+} are used as a quencher in the FLU solution, only the carboxylic oxygen will significantly be involved in the chemical interaction between the cation and FLU. Therefore, the K_S and k_q, particularly the K_S, of the FLU-Ni^{2+} couple are much larger than those of the other quenchers. The complex formation constants of FLU are only 1.5-2 times larger than those of OFL for all of these metal quenchers. However, FLU has much larger static quenching constants. Furthermore, the bimolecular quenching constants of FLU by all the metal quenchers were also much larger than those of OFL.

Because OFL is a TICT molecule in the excited state, this molecule shows many characteristic fluorescence properties in various biological mimic systems. This study finds that OFL is quite insensitive to dynamic and static quenching compared with FLU even though the decrease in the lifetime of OFL with increasing temperature is relatively large in water. In addition, this phenomenon was significant when Ni^{2+}, Co^{2+}, and Mn^{2+} were used as quencher. OFL will exhibit this fluorescence properties because this molecule has additional resonance forms in the S_1 state, and geometry and dipole moment greatly change during electronic transition between the S_o and S_1 state in water. This result is another peculiar fluorescence property that provides further evidence that TICT occurs at the S_1 state of OFL in water. These chemical properties of OFL may be related to the significant improvement in the antibacterial activity due to the introduction of a piperazinyl group at the 7-carbon compared with the FLU. FLU is not a TICT molecule because it does not have a piperazinyl group. Hence, FLU does not have these types of spectral properties. Therefore, a study of the photophysical and photochemical properties of OFL and FLU will provide important information on the mechanism of the antibacterial activity and cutaneous photosensitization of these antibiotics.

Acknowledgements. This study was partially supported by GSNU Chemistry Research Fund in 2005.

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