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Studies on Interaction of Norfloxacin, Cu^{2+}, and DNA by Spectral Methods

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The interactions of norfloxacin (NFA), DNA, and Cu^{2+} are studied by fluorescence and UV-spectra method. According to the experimental results, it can be concluded that NFA can form a steady binary complex with Cu^{2+}. There is a linear relationship between the Fluorescence intensity of the norfloxacin–Cu^{2+}–DNA system and the concentration of DNA. And when the concentration of the NFA is 1.95×10^{-5} mol L^{-1}, they possess a good linearity in the concentration of DNA ranged from 4.7×10^{-6} to 2.8×10^{-5} mol L^{-1}.It is a good method due to the high sensitivity and selectivity.

KEY WORDS: Norfloxacin; DNA, Cu^{2+}; fluorescence; static-quenching style.

INTRODUCTION

The design of small molecules that target specific site along a DNA helix has become a subject of considerable interest. Small molecules serve as an analogue in the study of protein-nucleic acid recognition, provide site-specific reagents for molecular biology, and yield rationales for new drug design. Many small molecules have already been proven to be useful as sensitive probes of local nucleic-acid structure [1]. Also the determination of nucleic acids is very important for many biological studies since it is often used as a reference for measurement of other components.

Although a large amount of biological data has indicated that DNA gyrase was the target for quinolone compound [2–5], Shen and his coworkers [6–9] have carried out an extensive experiment which concluded that DNA could bind by quinolone antibacterial agent. In the preliminary investigation, Palu [10] used fluorometric and radiometric measurements to study mechanism of quinolone–DNA interaction. Tuma *et al*. [11] have studied the effect of covalently appended quinolones on termini of DNA duplexes. Tabarrini *et al*. [12] have synthesis 6-hydroxy derivative as new desfluoroquinolone and also studied its DNA-binding properties. Son and coworkers [13] have studied the binding mode of norfloxacin to Calf Thymus DNA.

The interest of this field is diverse, since metal ions were found playing an important role in the structures of DNA and RNA. They realized that the major difference in the experimental conditions between their work and that of Shen and coworkers was Mg^{2+} concentration. Later, gel electrophoresis experiments by Tornaletti and Pedrini [14] showed that ciprofloxacin was able to unwind the DNA double helix in the presence of Mg^{2+}. Sophie [15] used NMR spectra and the results showed that in the presence of magnesium, pefloxacin bound strongly to DNA. Wu [16] studied the fluorescence spectra of fluoroquinolone antibiotics including ofloxacin, norfloxacin, and ciprofloxacin complexed with Co^{2+}, the fluorescence spectral data appeared that the fluoroquinolone antibiotic cannot directly complex with ATP but indirectly complex with Co^{2+}, which was playing an intermediary role. Yuan [17] used fluorescence method studied the interaction of between ciprofloxacin and DNA mediated by Cu^{2+}, Claudia *et al*. [18] studied the interaction of a number of novel 6-substituted quinolone derivatives with DNA

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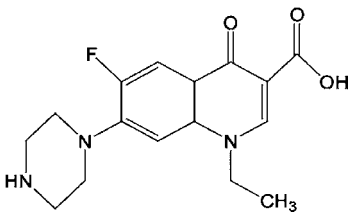

Fig. 1. Structure of NFA.

in the presence or absence of magnesium ions by fluorometric techniques. Skauge [19] studied the interaction between ciprofloxacin and DNA mediated by Mg^{2+}.

Quinolones are gyrase inhibitors that be widely used as antibiotics in the clinic. Norfloxacin (NFA) [1-ethyl-6fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl) quinoline-3-carboxylic acid] is a synthetic broad-spectrum fluoroquinolone antibacterial agent for oral administration, which is greatly effective against Gram-positive and Gram-negative aerobic bacteria *in vitro* that are resistant to other antibacterial. It has been used for a number of years effectively in human and animals' treatment in several bacterial infections, such as Escherichia coli, Staphylococcus aurues, Pseudomonas, aeruginosa, and Shigella.

The structure of NFA is shown in Fig. 1. NFA is related to other quinolones including cinoxacin, ciprofloxacin, enoxacin, ofloxacin, and nalidixic acid. It is the derivative of 4-quinolone that has an active group in the form of the −COOH and −C=O, which are capable of chelate formation with metal ions [20]. The fluorine atom provides increased potency against Gram-negative organisms and its activity. Norfloxacin also inhibits DNA synthesis and is bactericidal [21,22].

The interaction between norfloxacin and DNA mediated by Cu^{2+} is studied by fluorescence and UV spectra method. The fluorescence intensity of NFA can be quenched by DNA and Cu^{2+} respectively, both form a ground-state complex. The above results, further suggest that NFA has a weak interaction with DNA but indirectly complex with Cu^{2+}, which is playing an intermediary role during the interaction. There is a linear relationship between the fluorescence intensity of the norfloxacin- Cu^{2+}-DNA system and the concentration of DNA. The method is good for its high sensitivity and selectivity.

EXPERIMENTAL

Apparatus

The fluorescence spectra were measured with a Shimadzu RF-540 spectrofluorometer with a 1×1 cm cross-section quartz cell (Kyoto, Japan). The absorption measurements were performed on a Perkin-Elmer lambda 17 UV/vis spectrophotometer (P-E Co., America). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution, and a pHB-4 pH meter is used to measure the pH of the solution. A super thermostat circulating water bath was used for maintaining the different temperatures of the systems for the fluorescence quenching experiments.

Reagents

All reagents used were of analytical reagent grade. Double distilled water is used throughout the work unless otherwise stated.

The working concentration of NFA (made in Wu Han Wu Ging Medicine Company) solution was 1.22×10^{-4} mol L^{-1}. The stock solution of DNA was prepared by dissolving commercially purchased calf thymus DNA (Baitai Biochemical Co., Chinese Academy of Sciences) in doubly distilled water at 0∼4°C. Twenty-four hours or more were needed for dissolving DNA completely with occasional gentle shaking. The concentrations of stock solutions of nucleic acid were determined by the absorbance at 260.0 nm. The working concentration of the nucleic acid solution was 6.1×10^{-4} mol L^{-1}, which was prepared by diluting the stock solution with deionized water. The stock solution of $CuCl_2$ was prepared by dissolving $CuCl_2 \cdot 6H_2O$ with deionized water. The working concentration of Cu^{2+} was 1.0×10^{-34} mol L^{-1}.Tris (hydroxymethyl) aminomethane-HCl solution was used to control the acidity. 0.1 mol L^{-1} NaCl was used to adjust the ionic strength of the aqueous solutions.

Procedures

In a dry 25 mL volumetric flask were added 0.2 mL of the NFA solution, 0.4 mL of the $CuCl_2$ solution, 1.6 mL of Tris-HCl solution, and appropriate volumes of nucleic acid. The mixture was diluted to 12.5 mL with doubly distilled water and vortexes. Five minutes later, the fluorescence spectra were measured with the following settings of spectrofluorometer: excitation wavelength (λ_{ex}), 285 nm; emission wavelength (λ_{em}), 445 nm; Both the emission and excitation slit were 10 nm. And then all the absorption and fluorescence spectra measurements were obtained against the blank treated in the same way without nucleic acid.

RESULTS AND DISCUSSION

Spectral Characteristics of NFA–Cu^{2+}–DNA System

The fluorescence spectra of NFA and its decreased fluorescence intensity by Cu^{2+} and DNA are shown in

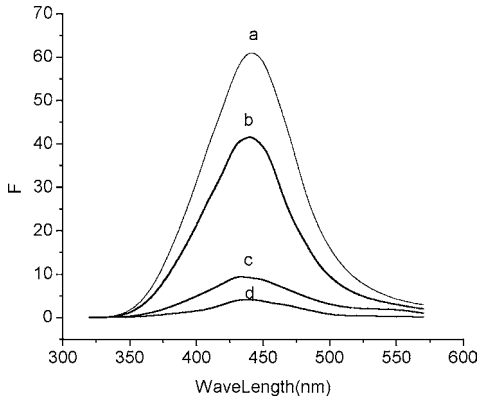

Fig. 2. Fluorescence spectra of NFA–Cu^{2+}–DNA system. $C_{NFA} = 1.95 \times 10^{-64}$ mol L^{-1}, $C_{DNA} = 1.63 \times 10^{-5}$ mol L^{-1}, $C_{Cu}^{2+} = 8 \times 10^{-64}$ mol L^{-1} a. NFA b. NFA + DNA c. NFA + Cu^{2+} d. NFA + DNA + Cu^{2+}.

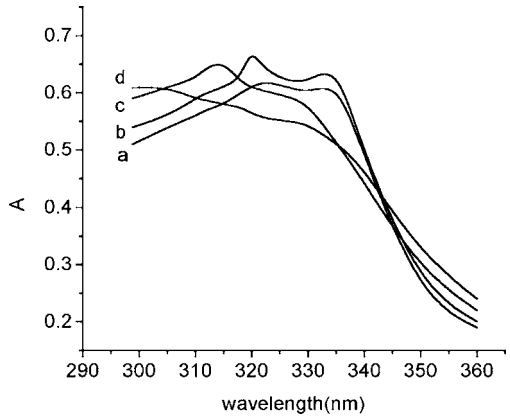

Fig. 3. UV spectra of NFA-DNA system. $C_{NFA} = 3.9 \times 10^{-64}$ mol L^{-1}, $C_{DNA} = 2.9 \times 10^{-3}$ mol L^{-1} $C_{Cu}^{2+} = 3.2 \times 10^{-54}$ mol L^{-1} a. NFA, b. NFA + DNA c. NFA + Cu^{2+} d. NFA + DNA + Cu^{2+}.

Fig. 2. In the system, when 1.0 mL of DNA was added, the fluorescence intensity of NFA was quenched by 31% at 445 nm (Fig. 2, curve *a,b*). In the present of Cu^{2+}, the intensity of fluorescence of NFA can be quenched apparently by 85% with a little blue shift (Fig. 2, curve *a,c*). When DNA and Cu^{2+} were added at the same time, the fluorescence intensity of NFA was obviously decreased lowest by 93% (Fig. 2, curve *a,d*). The results indicate the form of a nonfluorescence ground state ternary complex of NFA–Cu^{2+}–DNA.

The UV spectra of the system are also studied in Fig. 3. It can prove the form of ternary complex of NFA–Cu^{2+}–DNA further. In the study, NFA displays three characteristic absorption peaks located at 270, 322, and 335 nm. In particular, special attention has been paid to the peaks at 322 and 335 nm. In the present of Cu^{2+}, the peaks at 322 and 335 nm disappear and a new peak at 315 nm forms at the same time (Fig. 3, curve *a,c*). It suggests that an interaction takes place between NFA and Cu^{2+}. When Cu^{2+} coexists with DNA in NFA system, the spectrum of absorbs has a strong hypochromic effect with a little blue shift. (Fig. 3, curve *a,d*).

Effect of pH

As Fig. 1 showed the NFA molecule possesses carbonyl, amino and carboxyl groups. Protonated nitrogen atom and oxygen atom in different pH solution influence electronic cloud distribution of system directly, which affect the chelate formation ability of NFA. When the pH was in the range of 2∼4 controlled by Tris-HCl, the keto-form of quinoline pharmaceutical molecule occupy maximum proportion [16], so there was most strong intensity of fluorescence, as shown in Fig. 4. Part of Cu^{2+} hydrolysis in alkaline solution, which makes Cu^{2+} to coordinate with NFA difficultly. So control proper acidity of system is very important. The maximum ΔF of NFA–Cu^{2+}–DNA system was obtained at pH 4. Thus pH 4 was selected.

Effect of Ionic Strength

In Fig. 5, the effect of ionic strength controlling by 0.1 mol L^{-1} $MgCl_2$ and NaCl solutions are shown. There was no obvious change with fluorescence intensity of the system when Na^+ was added. But when Mg^{2+} was added, the fluorescence intensity of the NFA–Cu^{2+}–DNA system increased obviously with the increasing amount of the Mg^{2+}. The main reason is that the anion of phosphate on the backbone of DNA is shield by the cation of Mg^{2+}, which competes with NFA–Cu^{2+}, thus leading to an increase of free NFA–Cu^{2+}.The ability of masking of

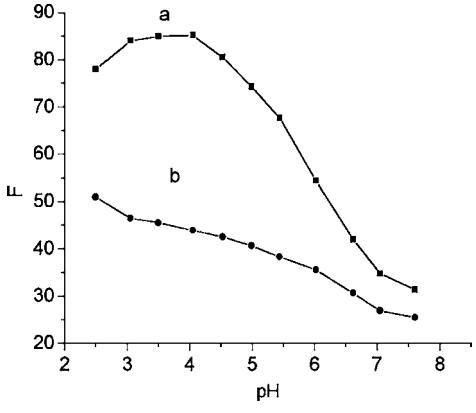

Fig. 4. Effect of pH. $C_{NFA} = 1.95 \times 10^{-6}$ mol L^{-1}, $C_{DNA} = 2.9 \times 10^{-5}$ mol L^{-1}, $C_{Cu}^{2+} = 8 \times 10^{-74}$ mol L^{-1} a. NFA, b. NFA + Cu^{2+} +DNA.

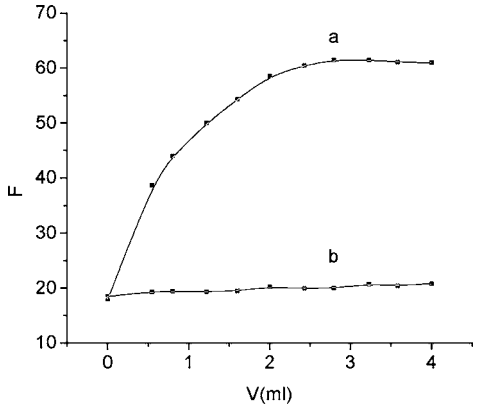

Fig. 5. The effect of ionic strength on the NFA–Cu^{2+}–DNA system. $C_{NFA} = 1.95 \times 10^{-6}$ mol L^{-1}, $C_{DNA} = 2.9 \times 10^{-5}$ mol L^{-1}, $C_{Cu}^{2+} = 8 \times 10^{-74}$ mol L^{-1} a. NFA + Cu^{2+} + DNA + Mg^{2+} b. NFA + Cu^{2+} + DNA + Na^{+}.

a divalent cation Mg^{2+} is much stronger than that of a monovalent Na^{+}, so just Mg^{2+} can lead to the increase of fluorescence intensity of the system.

Effect of Temperatures

The effect of temperatures of the system was studied as follows. The efficiency of quenching of a fluorophore species by a quencher species follows the Stern–Volmer relationship.

$$F_0/F = 1 + K_{sv}[Q] \quad (1)$$

Where, F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively. Either the collisional quenching of fluorescence or the static quenching of fluorescence can be depicted by the Stern–Volmer equation. [Q] is the concentration of the quencher. K_{sv} is the Stern–Volmer quenching constant. If a system obeys the Stern–Volmer equation, a plot of F_0/F versus [Q] will give straight line with a slope of K_{sv} and y-axis intercept. For dynamic quenching, K_{sv} increases with increasing temperature. And for static quenching, K_{sv} decreases with increasing solvent's temperature.

Figure 6 shows the Stern–Volmer plot of the NFA–Cu^{2+}–DNA system. As shown in Fig. 6, the term, F_0/F, linearly increases with the increasing concentration of quencher. The coefficients are 0.9772 (30°C), 0.9968 (40°C), respectively. At the same time, the quenching efficient of NFA + Cu^{2+} fluorescence by DNA undergoes an intense decrease with increasing temperature, $K_{30°C} = 40{,}100$ L mol^{-1}, $K_{40°C} = 26{,}000$ L mol^{-1}, respectively. It indicates that the static quenching is attributable to the formation of a nonfluorescence ground state complex between NFA + Cu^{2+} and DNA.

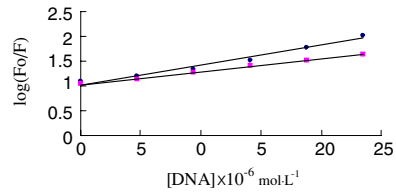

Fig. 6. Fluorescence quenching stern-volmer plots of NFA + Cu^{2+} with increasing concentration of DNA. $C_{NFA} = 1.95 \times 10^{-6}$ mol L^{-1}, $C_{Cu}^{2+} = 3.2 \times 10^{-64}$ mol L^{-1} (circle) 30°C (square) 40°C.

Effect of Denatured DNA and Natural DNA

As shown in Fig. 7, the interactions of native DNA and denatured DNA with NFA–Cu^{2+} were compared. Double-strand DNA was converted into single-strand DNA with the unwinding of its double helix by being incubated at 100°C for 30 min and immediately cooled in ice-water for 10 min. The experimental result shows native DNA can linearly quench the fluorescence intensity of the system of NFA–Cu^{2+} in certain concentration range. But denatured DNA could not quench the fluorescence intensity of NFA–Cu^{2+} system at all. The denatured DNA split into two string-like soft polynucleotide chains from the original rigid double-helix structure, which cause the difference in the fluorescence quenching. The main reason might be associated with the intercalation of NFA–Cu^{2+} into native DNA base pairs.

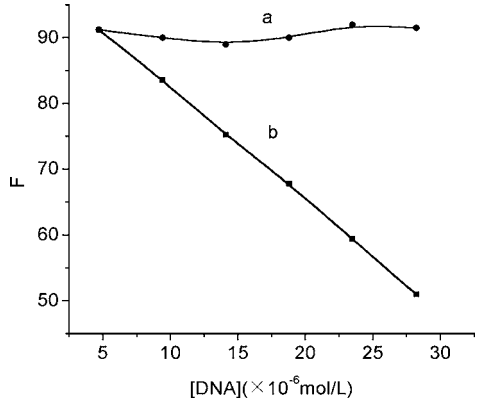

Fig. 7. The effect of denatured DNA and natural DNA. on the NFA- Cu^{2+}-DNA system. $C_{NFA} = 1.95 \times 10^{-6}$ mol L^{-1}, $C_{Cu}^{2+} = 8 \times 10^{-74}$ mol L^{-1} a. NFA + Cu^{2+} + denatured DNA b. NFA + Cu^{2+} +DNA + natural DNA.

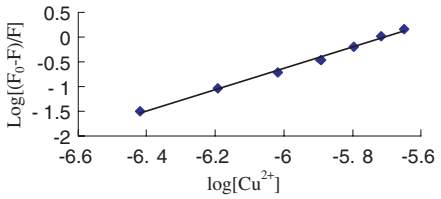

Fig. 8. Estimation of composition of the NFA–Cu^{2+} complex. C_{NFA} 1.95×10^{-6} mol L^{-1}.

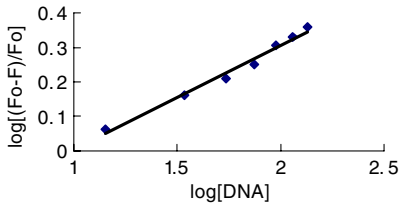

Fig. 9. Estimation of composition of the NFA–DNA complex. C_{NFA} 1.95×10^{-6} mol L^{-1}.

Calibration Curve

The calibration curve is obtained according to the above standard procedure. There exists a linear relationship between the fluorescence intensity of the complex NFA–Cu^{2+}–DNA and nucleic acid when the concentration of nucleic acid is in the range of $4.7 \times 10^{-6} \sim 2.8 \times 10^{-54}$ mol L^{-1}.

The linear regression equation is $I = 99.467 - 1.7082$ C(DNA); $r = 0.9996$.

The Composition of the Binary Complex

The composition of the binary complex can be deduced from the following formula [24].

$$M + nL = MLn \tag{2}$$

$$\log[(F_0 - F)/F] = \log Ka + n \log[M] \tag{3}$$

where, M is the quencher, L is the pharmaceutical molecule with a fluorophore, MLn is the binary complex whose resultant constant is Ka, F_0 is the fluorescence of the overall amount of pharmaceutical molecule (bound and unbound), F is the fluorescence of unbound pharmaceutical molecule. A plot of log $[(F_0 - F)/F]$ versus log $[M]$ will give straight line with a slope of n and y-axis intercept log Ka.

Figure 8 is obtained by keeping the NFA concentration (1.95×10^{-6} mol L^{-1}) constant and changing the concentration of Cu^{2+}. The data are well fitted to Eq. (3) and the slope is 2.2. The coefficient is 0.9940. The result indicates that the fluoroquinolone antibiotics can form a stable 1:2.2 complexes with Cu^{2+}.

The composition of the complex NFA–DNA was also determined by the above method. A 1:0.3 mole ratio of NFA to DNA is conformed by the experimental results. Figure 9 shows the result. The coefficient is 0.9840. The mole ratio of NFA to DNA is far smaller than that of NFA–Cu^{2+} system. It is suggested that the force between NFA and Cu^{2+} is much stronger than that between NFA and DNA.

The Reaction Mechanism

In the experiment, deduced from the fluorescence and UV absorption spectra, a ground-state binary complex is formed between NFA and Cu^{2+}, and a ground-state ternary complex also can be formed among NFA, Cu^{2+}, and DNA. Small molecular integrate with nucleic acids have three binding mechanisms [25]. One is intercalative binding model. Another one is the groove-binding model. The last one is the long-range assembly of the organic dyes on the molecular surface of nucleic acids. If the NFA–Cu^{2+} complex interact with DNA according to intercalation model, the chromophore would bury itself in the stack of DNA bases, leading to spectral changes caused by the interactions of the complex with the electronic states of the DNA bases. Aromatic π–π^* states of NFA–Cu^{2+} seem to interact strongly with the electronic states of the DNA bases.

Earlier studies have pointed out that norfloxacin binds to DNA bases via hydrogen binding. The hydrogen bonding donors thus become available for pairing with the acceptors on the drug molecules (carbonyl or fluoro groups). Both C_4 carbonyl group on the quinolone ring and C_3 carboxylic acid group are potential hydrogen-bond acceptors. But it is discovered that the interaction between NFA and DNA is weak without Cu^{2+} in the experiment. The result of experiment indicates a possible interaction mechanism for NFA binding to double-stranded DNA. That is, firstly, NFA forms a binary complex with Cu^{2+}, then forms a ternary complex with DNA by intercalation binding form, and finally, releases Cu^{2+} to form hydrogen bonds between NFA and DNA. Cu^{2+} plays an intermediary role in the interaction between the NFA and DNA.

CONCLUSIONS

In summary, the fluoroquinolone antibiotic, NFA, can complex with Cu^{2+} and DNA, which gives information concerning the antibiotic–nucleotide interaction. From the experimental results, we can conclude that NFA can form a steady binary complex with NFA–

Cu^{2+} can interact with DNA by intercalative binding model, and Cu^{2+} plays an intermediary role. This behavior is of great importance with regard to relevant biological role of fluoroquinolone antibiotics in the human body.

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REFERENCES

1. Y. Jenkins, A. E. Friedman, N. J. Turro, and J. K. Barton (1992). Characterization of dipyridophenazine complexes of ruthenium(II): The light switch effect as a function of nucleic acid sequence and conformation. *Biochem*. **31**, 10809–10816.
2. M. Gellert, K. Mizuuchi, M. H. O'Dea, and H. A. Nash (1976). DNA Gyrase: An enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. U.S.A*. **73**, 3872–3876.
3. M. Gellert, K. Mizuuchi, M. H. O'Dea, T. Itoh, and J. Tomizawa (1977). Nalidixic acid resistance: A second genetic character involved in DNA gyrase activity. *Proc. Natl. Acad. Sci. U.S.A*. **74**, 4772–4776.
4. A. Sugino, C. L. Peebles, K. N. Kreuzer, and N. R. Cozzarelli (1977). Mechanism of action of nalidixic acid: Purification of escherichia coli nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. *Proc. Natl. Acad. Sci. U.S.A*. **74**, 4767–4771.
5. M. G. Cormican, S. A. Marshall, R. N. Jones (1995). Cross-resistance analysis for DU-6859a, a new fluoroquinolone, compared to six structurally similar compounds (ciprofloxacin, clinafloxacin, fleroxacin, levofloxacin, ofloxacin, and sparfloxacin). **22**, 51.
6. L. L. Shen, L. A. Mitscher, P. N. Sharm, T. J. O'Donnell, W. T. D. Chu, C. S. Cooper, T. Rosen, and A. G. Pernet (1989). Mechanism of inhibition of DNA gyrase by quinolone antibacterials: A cooperative drug-DNA binding model. *Biochemicals* **28**, 3886.
7. L. L. Shen and A. G. Pernat (1985). Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: The target of the drugs is DNA. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 307–311.
8. L. L. Shen, J. Baranowski, J. Fostel, D. A. Montgometry, and P. A. Lartey (1992). DNA topoisomerases from pathogenic fungi: Targets for the discovery of antifungal drugs. *Antimicrob. Agents Chemother.* **36**, 2778–2784.
9. A. Y. C. Saiki, L. L. Shen, C. M. Chen, J. Baranowski, and C. G. Lerner (1999). DNA cleavage activities of *Staphylococcus aureus* gyrase and topoisomerase IV stimulated by quinolones and 2-pyridones. *Antimicrob. Agents Chemother.* **43**, 1574–1577.
10. G. Palu, S. Valisena, G. Ciarrocchi, B. Gatto, and M. Palumbp (1992). Quinolone binding to DNA is mediated by magnesium ions. *Proc. Natl. Acad. Sci. U.S.A* **89**, 9671–9675.
11. J. Tuma, W. H. Connors, D. H. Stitelman, and C. Richert (2002). On the effect of covalently appended quinolones on termini of DNA duplexes. *Am. Chem. Soc*. **124**, 4236–4246.
12. V. Cecchetti, O. Tabarrini, et al. (1999). Studies on 6-aminoquinolones: Synthesis and antibacterial evaluation of 6-amino-8-ethyl- and 6-amino-8-methoxyquinolones. *Bioorg. and Med. Chem*. **7**, 2465–2471.
13. G. S. Son, J. A. Yeo, M. S. Kim, S. K. Kim, and A. Holmen (1998). Binding mode of norfloxacin to calf thymus DNA. *Am. Chem. Soc*. **120**, 6451–6457.
14. S. Tornaletti and A. M. Pedrini (1988). Studies on the interaction of 4-quinolones with DNA by DNA unwinding experiments. *Biochim. Biophys. Acta*. **949**, 279.
15. L. Sophie, J. M. Nicole, and T. C. Marie (1998). NMR investigation of pefloxacin-cation-DNA interactions: The essential role of Mg^{2+}. *Int. J. Pharmaceut*. **164**, 57–65.
16. S. Q. Wu, W. J. Zhang, X. G. Chen, Z. D. Hu, M. Hooper, B. Hooper, Z. F. Zhao (2001). Fluorescence characteristic study of the ternary complex of fluoroquinolone antibiotics and cobalt (II) with ATP. *Spectrochim. Acta Part A: Mol. Biomol. Specrtosc*. **57**, 1317–1323.
17. X. Y. Yuan, L. Chen, J. L. Qiao (1999). Study on the ternary complex of CPFX, Copper(II) and DNA by the method of fluorescence. *Spectroscopy and Spectral Anal*. **19**, 572–574.
18. S. Claudia, A. Magda, C. Ecchetti, et al. (1998). Mg^{2+}-mediated binding of 6-substituted quinolones to DNA: Relevance to biological activity. *Bioorg. Med. Chem*. **6**, 1555–1561.
19. T. Skauge, I. Turel, and E. Sletten (2002). Interaction between ciprofloxacin and DNA mediated by Mg^{2+}-ions. *Inorg. Chim. Acta* **339**, 239–247.
20. S. K. Bhowal and T. K. Das (1991). Spectrophotometric determination of some recently introduced antibacterial drugs using ferric chloridd. *Anal. Lett*. **24**, 25.
21. M. D. Bethesda (1988). Drug Information 88, Authority of the Board of Directors of the American Society of Hospital Pharmacists Press, America.
22. J. B. Lippincott (1988). Drug facts and comparison, published by facts and comparision Div., Spain.
23. Y. Xu, H. X. Shen, and H. G. Huan. (1997). Fluorescence characteristics of iron ()-norfloxacin-guanylic acid ternary complex. *Anal. Chem*. **25**, 419.
24. C. W. Kumar, and H. E. Asuncion (1993). DNA binding studies and site selective fluorescence sensitization of an anthryl probe. *Am. Chem. Soc*. **115**, 8547.