

A Fluorescence Spectroscopic Study of the Interaction Between Norfloxacin and DNA

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Résumé

Nous avons étudié l'interaction entre la norfloxacine (NFA) et l'ADN par les méthodes spectrales de fluorescence et UV. Les résultats de l'expérience indiquent que l'ADN peut fortement diminuer l'intensité de fluorescence de la norfloxacine par inhibition statique. Quand les concentrations de NFA se situent dans la gamme entre 5.86×10^{-7} et 1.95×10^{-6} molL⁻¹, il existe une relation linéaire entre l'intensité de fluorescence du système NFA+ADN et la concentration d'ADN. Lorsque la concentration de NFA est à 5.86×10^{-6} molL⁻¹, on observe une bonne linéarité dans la gamme de concentration d'ADN entre 2.0×10^{-5} et 2.22×10^{-4} mol L⁻¹. Nous avons aussi examiné les effets des substances interférentes et l'optimisation de la procédure. Trois échantillons synthétiques ont été mesurés de façon satisfaisante.

Abstract

The interaction between norfloxacin (NFA) and DNA was studied by fluorescence and UV spectra methods. The results of the experiment indicate that DNA can strongly decrease the fluorescence intensity of norfloxacin by static quenching. When the NFA is in the concentration range from 5.86×10^{-7} to 1.95×10^{-6} molL⁻¹, there is a linear relationship between the fluorescence intensity of the system of NFA+DNA and the concentration of DNA. Also, when the concentration of the NFA is 5.86×10^{-6} molL⁻¹, there is good linearity in the range of DNA concentration

from 2.0×10^{-5} - 2.22×10^{-4} molL⁻¹. The effects of interference substances and optimization of procedure were investigated. Three synthetic samples were determined satisfactorily.

Keywords: Norfloxacin, fluorescence, static quenching, DNA.

Introduction

The design of small molecules that target a 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).

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 with quinolone antibacterial agent. In the preliminary investigation, Palu (10,11) used fluorometric and radiometric measurements to study the mechanism of quinolone-DNA interaction. Tuma *et al.* (12) have studied the effect of covalently appended quinolones on termini of DNA duplexes. Tabarrini *et al.* (13) have synthesized 6-hydroxy derivative as a new desfluoroquinolone and also studied its DNA binding properties. Son (14) and his coworkers have studied the binding mode of norfloxacin to calf thymus DNA.

Quinolones are gyrase inhibitors that are widely used as antibiotics in the clinic. Norfloxacin (NFA) [1-ethyl-6fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) quinoline -3-

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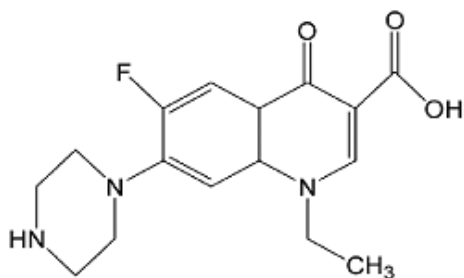


Figure 1. Structure of NFA

carboxylic acid is a synthetic broadspectrum fluoroquinolone antibacterial agent for oral administration that acts *in vitro* against Gram-positive and Gram-negative aerobic bacteria. It has been used for a number of years effectively in humans and animals in the treatments of several bacterial infections, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Shigella*.

The structure of NFA is shown in figure 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 grouping in the form of $-\text{COOH}$ and $-\text{C}=\text{O}$; these are capable of chelate formation with metal ions. Norfloxacin also inhibits DNA synthesis and is bactericidal (15,16).

The interaction between norfloxacin and DNA is studied here by fluorescence and UV spectra methods. There is a linear relationship between the fluorescence intensity of the NFA-DNA system and the concentration of DNA. When the concentration of the NFA is $5.86 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$, there is a good linearity in the concentration of DNA ranging from 2.0×10^{-5} – $2.22 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$. It is a good method due to its high sensitivity and selectivity.

Experimental

Apparatus

The fluorescence spectra were measured with a Shimadzu RF-540 spectrofluorometer with a $1 \times 1 \text{ cm}$ 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 was 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 for synthesis are of analytical reagent grade unless otherwise stated. Double distilled water is used throughout the work.

The working concentration of NFA (made by the Wu Han Wu Ging Medicine Company) solution was $1.22 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$. 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 to dissolve DNA with occasional gentle shaking. The concentrations of stock solutions of nucleic acids were determined by the absorbance at 260.0 nm . The working concentration of the nucleic acid solution was $6.3 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$; this solution was prepared by diluting the stock solution with deionized water. Tris (hydroxymethyl) aminomethane–HCl solution was used to control the acidity, and $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ NaCl}$ was used to adjust the ionic strength of the aqueous solutions.

Methods

To a dry 25 mL volumetric flask were added 0.6 mL of the NFA solution, 1.0 mL of Tris-HCl solution, and appropriate volumes of nucleic acid. The mixture was then diluted to 12.5 mL with doubly distilled water and vortexed. Five minutes later, the fluorescence spectra were measured with the following settings of the spectrofluorometer: excitation wavelength (λ_{ex}), 285 nm ; emission wavelength (λ_{em}), 445 nm . Both the excitation and emission slits are 10 nm . All the absorption and fluorescence spectra measurements were obtained against a blank treated in the same way but without nucleic acid.

Results and Discussion

Spectral characteristics of NFA-DNA system

As shown in figure 2, the typical emission spectra of NFA with and without DNA were studied. The excitation and emission wavelengths of NFA are 285 nm and 445 nm , respectively. When DNA is added, the intensity of fluorescence of NFA at 445 nm is quenched by 44% . The integration of a small molecule with DNA can have three binding mechanisms (17). One of these is the intercalative model. Another one is the groove-binding model, and the last one is the long-range assembly of the organic dyes on the molecular surface of nucleic acids. The quenching of fluorescence indicates that NFA can bind with DNA

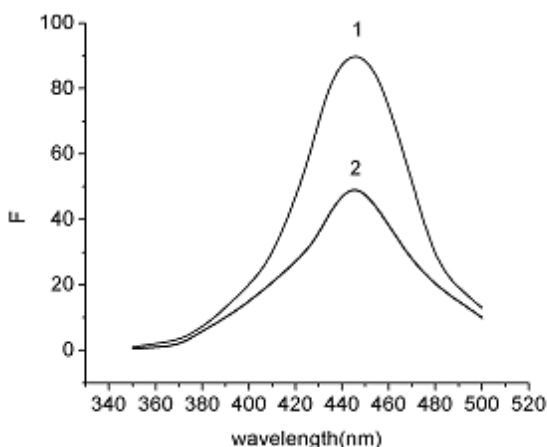


Figure 2. Fluorescence spectra of NFA-DNA system. The concentrations of NFA and DNA are respectively $1.95 \times 10^{-6} \text{ molL}^{-1}$ and $4.0 \times 10^{-5} \text{ molL}^{-1}$. 1. NFA; 2. NFA+DNA

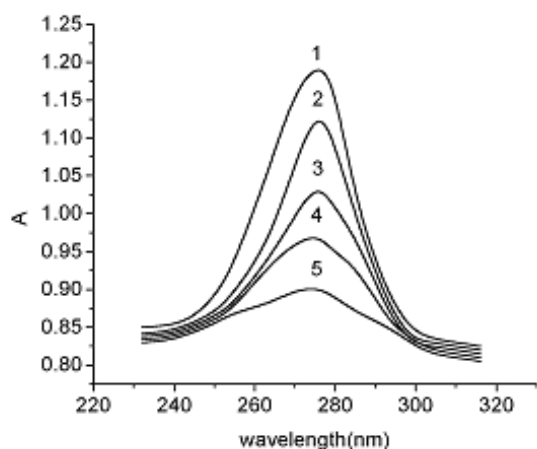


Figure 3. UV spectra of NFA-DNA system. The concentration of NFA is $1.95 \times 10^{-5} \text{ molL}^{-1}$. 1. pure NFA, The concentration of DNA: 2. $2.016 \times 10^{-5} \text{ molL}^{-1}$ 3. $6.048 \times 10^{-5} \text{ molL}^{-1}$ 4. $1.008 \times 10^{-4} \text{ molL}^{-1}$ 5. $1.411 \times 10^{-4} \text{ molL}^{-1}$

strongly, and perhaps NFA interacts with DNA by the intercalative model (18). According to this, the method can be used to determine DNA.

The absorption spectra of NFA obtained at a constant NFA concentration and differing DNA concentrations are presented in Figure 3. In the determination, pure DNA with the same concentration of DNA as the sample is made as a reference solution. This is done to avoid the effects of the absorbance of DNA at 260 nm. NFA has a strong absorption peak at 275 nm. Significant spectral changes in the absorption spectra quenched by DNA indicate that NFA has bonded with DNA. With an increase of the DNA concentration, the absorption of

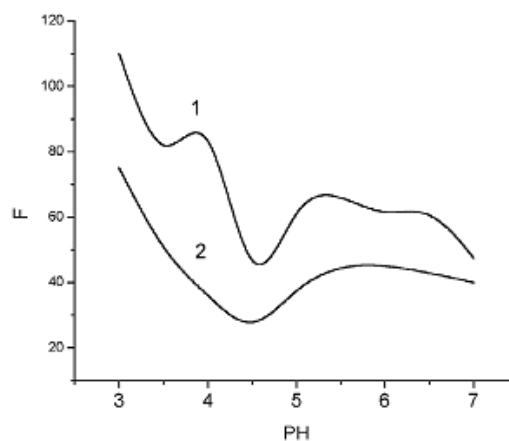


Figure 4. The effect of pH on the NFA-DNA system. The concentrations of NFA and DNA are respectively $1.95 \times 10^{-6} \text{ molL}^{-1}$ and $8.064 \times 10^{-5} \text{ molL}^{-1}$. 1. NFA, 2. NFA+DNA

the NFA-DNA system at 272 nm decreases strongly. As the inference of DNA is eliminated by the reference solution, the decrease of the peak is not simple addition.

Effect of pH

The effect of pH on the NFA-DNA system in aqueous solution was investigated (figure 4). As shown in Figure 1, the structure of the NFA molecule contains carbonyl, amino and carboxyl groups. The protonation of the nitrogen atom and oxygen atom in different pH solutions will influence the electronic cloud distribution of system directly, which will then affect the chelate formation ability of NFA. When the pH is in the range of 2-4, the form of quinline occupies a maximal proportion (19), so there is the most strong intensity of fluorescence. When the pH is below 3, some of the double-strand DNA turns to single-strand DNA. As a result, the optimum pH for the NFA-DNA system is 4. From controlling the concentration of NFA and DNA, it can be concluded that 1 mL Tris-HCl is the most suitable amount.

Effect of ionic strength

The effect of controlling the ionic strength using 0.1 molL^{-1} NaCl solutions is shown in Figure 5. With increasing concentration of NaCl, the fluorescence intensity of the NFA-DNA system increases first and then decreases. There is the same rule for NFA. As the NFA can chelate metal, the ionic strength has a great effect on the system.

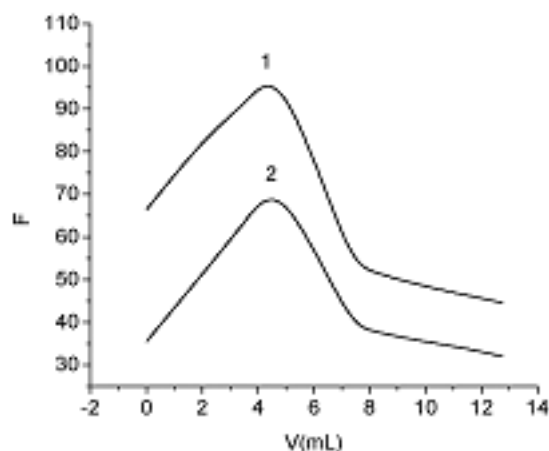


Figure 5. The effect of ionic strength on the NFA-DNA system. The concentrations of NFA and DNA are respectively $1.95 \times 10^{-6} \text{ molL}^{-1}$ and $8.064 \times 10^{-5} \text{ molL}^{-1}$. 1. NFA + NaCl, 2. NFA+DNA+ NaCl

Table 1. Interferences of co-existing substances

Co-existing Substances	Concentration (mgL^{-1})	Change of ΔI (%)
Zn ²⁺	2.62×10^4	-2.5
Cu ²⁺	8.19×10^4	2.75
K ⁺	160	-0.35
Ba ²⁺	1.024	1.05
Ni ²⁺	3.28×10^4	0.22
Mn ²⁺	25.6	3.25
As ⁵⁺	80	-3.86
Cr ³⁺	5.24×10^2	3.12
Ag ⁺	----	----
Pb ²⁺	80	2.59
Sn ⁴⁺	1.024×10^2	-3.74
Hg ²⁺	6.4	1.66
Br ⁻	800	-2.1
L-cysteine	0.384	-2.66
BSA	0.016	0.01
Glucose	0.016	0.45

Tolerance of co-existing substance

The influences of co-existing substances, such as metal ions, proteins, and amino acids have been tested. The substance is added to the sample of NFA+DNA. Then the sample is tested by the spectrofluorometer, and the fluorescence intensity of the sample with and without

the co-existing substance is compared. The solution of the co-existing substance is diluted until the substance has little interference on the system. The results are shown in Table 1.

$$\Delta I = (F_0 - F) / F_0 \quad (1)$$

F_0 is the fluorescence intensity of the sample without the co-existing substance, and F is the fluorescence intensity of the sample with the co-existing substance presence when the substance has little interference on the system. When ΔI is smaller than 5%, the concentration of the co-existing substance is allowed.

Among the tested metal ions, K⁺, Pb²⁺, As⁵⁺ and Mn²⁺ can be allowed with relatively high concentration levels. It has been proved that they tend to bind exclusively to the phosphate groups of DNA molecule, stabilizing the Watson-Crick double stranded helix (18). In contrast, those ions, such as Zn²⁺ and Cu²⁺, which appear to bind exclusively with the base moiety of DNA to form internal chelates, interstrand complexes, or cross-links (20), can be allowed with relatively low concentration. Ag⁺ has a large interference effect on the system.

The effect of temperature

The effect of temperature of the system was studied as follows.

The efficiency of quenching of a fluorophore species by a quencher species follows the Stern-Volmer relationship.

$$F_0/F = 1 + K_{sv}[Q] \quad (2)$$

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 described by the Stern-Volmer equation. Static quenching implies either the existence of a sphere of effective quenching or the formation of a ground-state non-fluorescent complex. When dynamic quenching happens, the ground-state non-fluorescent complex cannot be formed. $[Q]$ 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 a straight line with a slope of K_{sv} and y-axis intercept. For dynamic quenching, K_{sv} increases with increasing temperature. On the other hand, for static quenching, K_{sv} decreases with increasing solvent temperature.

Figure 6 is the Stern-Volmer plot of the NFA-DNA system. As shown in Figure 6, the term F_0/F linearly increases with increasing concentration of quencher. The

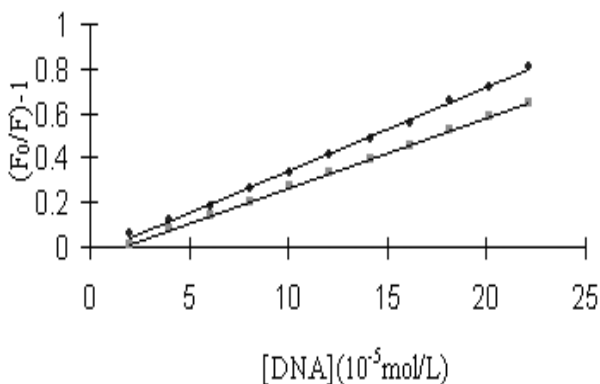


Figure 6. Fluorescence quenching stern-volmer plots of NFA with increasing concentration of DNA. The concentration of NFA is $1.95 \times 10^{-6} \text{ mol/L}$. circle 30°C square 40°C

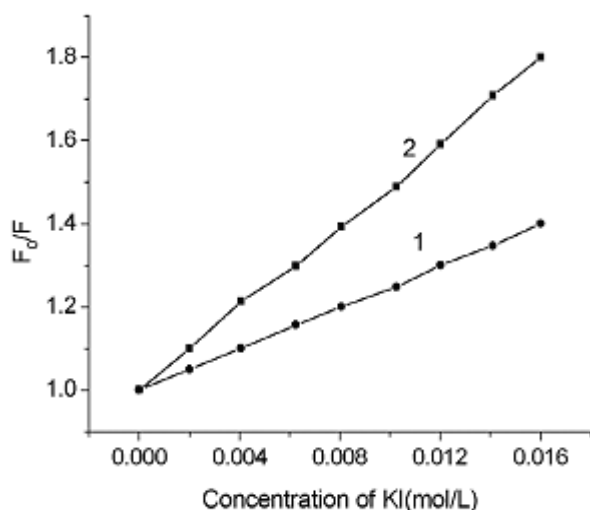


Figure 7. The effect of KI. The concentrations of NFA and DNA are respectively $1.95 \times 10^{-6} \text{ mol/L}$ and $8.064 \times 10^{-5} \text{ mol/L}$. 1. NFA + KI, 2. NFA + DNA + KI

coefficients are 0.9974 (30°C) and 0.9998 (40°C), respectively. At the same time, the quenching efficient of NFA fluorescence by DNA undergoes an intense decrease with increasing temperature: $K_{30^\circ\text{C}} = 37600 \text{ Lmol}^{-1}$ and $K_{40^\circ\text{C}} = 31500 \text{ Lmol}^{-1}$, respectively. It is suggested that the static quenching is attributable to the formation of a non-fluorescent ground state complex between NFA and DNA (21).

Effect of KI

KI is a kind of anion quencher. Small molecules integrate with nucleic acids using three binding mechanisms (22). If NFA interacts with DNA by the intercalative binding model, the base pairs and negative

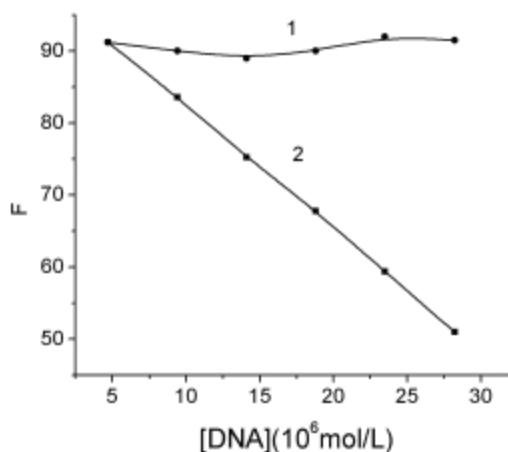


Figure 8. The effect of denatured DNA and natural DNA on the NFA-DNA system. $C_{\text{NFA}} = 1.95 \times 10^{-6} \text{ mol/L}$. 1. NFA + denatured DNA 2. NFA + DNA + natural DNA

electric charge of the phosphate bond will prevent the anion quencher from approaching the small molecule, and so the small molecule is protected. So if the small molecule interacts with DNA by the intercalative binding model, the quenching efficiency of the fluorescence intensity would be higher when DNA is added; however, if the small molecule interacts with DNA by the groove-binding model, the quenching efficiency of the fluorescence intensity would be higher without DNA. In the experiment (figure 7), KI can quench NFA with or without DNA. Without DNA the quenching efficiency is higher, so it is concluded that NFA interacts with DNA by the intercalative binding model.

Effect of denatured DNA and natural DNA

As shown in Figure 8, the interactions of native DNA and denatured DNA with NFA are compared. Double-strand DNA was converted into single-strand DNA by the unwinding of its double helix by being incubated at 100°C for 30 minutes and then immediately cooled in ice-water for 10 minutes. The experimental results showed that native DNA can linearly quench the fluorescence intensity of the system of NFA in a certain concentration range. However, denatured DNA cannot quench the fluorescence intensity of NFA as well as native DNA. The denatured DNA is split into two string-like soft polynucleotide chains from the original rigid double-helix structure, and this could cause the difference in the fluorescence quenching. The main reason might be associated with the intercalation of NFA into native DNA base pairs.

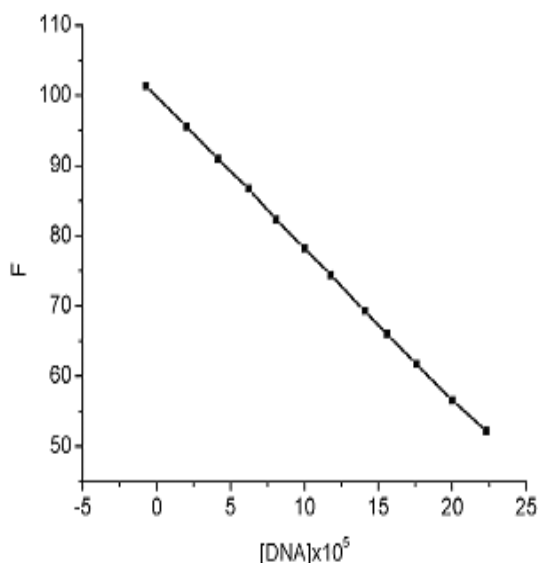


Figure 9. Calibration curve. The concentration of NFA is $5.86 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$

Table 2. Determination results of synthetic samples

Sample	Concentration of DNA ($\times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$)	Foreign Substances	Found ($\times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$)	Recovery (%)
CtDNA	4.21	Pb ²⁺ , Sn ⁴⁺ , Mn ²⁺	4.35	100.3
CtDNA	8.7	Cr ³⁺ , L-cysteine	8.51	98.24
CtDNA	12.96	Zn ²⁺ , K ⁺ , Ca ²⁺	13.1	100.08

Concentration: $C_{\text{Pb}^{2+}}=0.02 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{Sn}^{4+}}=0.02 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{Mn}^{2+}}=2.4 \times 10^{-6} \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{Cr}^{3+}}=0.01 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{L-cysteine}}=0.41 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{Zn}^{2+}}=0.04 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{K}^{+}}=0.01 \text{ mg}\cdot\text{L}^{-1}$; $C_{\text{Ca}^{2+}}=0.01 \text{ mg}\cdot\text{L}^{-1}$.

Calibration curve

The calibration curve is obtained according to the above standard procedure. According to the experiment, it is concluded that there is a linear relationship between the fluorescence intensity of the NFA-DNA and the concentration of DNA when the concentration of NFA is in the range of 5.86×10^{-7} - $1.95 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$. When the concentration of NFA is $5.86 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$ and the concentration of nucleic acid is in the range from 2.0×10^{-5} - $2.22 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$, there is the best linear relationship.

As shown in Figure 9, the linear regression equation is $I = -2.1916(\text{DNA}) + 100.68$ $r^2 = 0.9986$. The detection limit (3σ) is $2.6 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$.

Analysis

The present method was applied to the determination of nucleic acid in synthetic samples constructed based on the tolerance of co-existing species. Table 2

summarizes the results. The determination of nucleic acids by this method is reliable, precise, and simple.

Conclusions

In summary, the fluoroquinolone antibiotic NFA can interact with DNA by the intercalative binding model. This behavior is of great importance with regard to the 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, *J. Biochem.*, **31**, 10809 (1992).
2. M. Gellert, K. Mizuuchi, M.H. O'Dea and H.A. Nash, *J. Proc. Natl. Acad. Sci. USA*, **73**, 3872 (1976).
3. M. Gellert, K. Mizuuchi, M.H. O'Dea, T. Itoh and J. Tomizawa, *J. Proc. Natl. Acad. Sci. USA*, **74**, 4772 (1977).
4. A. Sugino, C.L. Peebles, K.N. Kreuzer and N.R. Cozzarelli, *J. Proc. Natl. Acad. Sci. USA*, **74**, 4767 (1977).
5. D.C. Hooper, J.S. Wolfson, K.S. Souza, C. Tung, G.L. McHugh and M.N. Swartz, *J. Antimicrob. Agents Chemother.*, **29**, 639 (1986).
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, *J. Biochem.*, **28**, 3886 (1989).
7. L.L. Shen and A.G. Pernet, *J. Proc. Natl. Acad. Sci. USA*, **82**, 307 (1985).
8. L.L. Shen, S.K. Tanaka and D.T.W. Chu, *Current Pharma. Design*, **3**, 169 (1997).
9. A.Y.C. Saiki, L.L. Shen, C.M. Chen, J. Baranowski and C.G. Lerner, *J. Antimicrob. Agents Chemother.*, **43**, 1574 (1999).
10. G. Palu, S. Valisena, G. Ciarrocchi, B. Gatto and M. Palumbp, *J. Proc. Natl. Acad. Sci. USA*, **89**, 9671 (1992).
11. G. Palu, S. Valisena and M. Peracchi, *J. Biochem. Pharmacol.*, **37**, 1887 (1988).
12. J. Tuma, W.H. Connors, D.H. Stitelman and C.

- Richert, *J. Am. Chem. Soc.*, **124**, 4236 (2002).
13. O. Tabarrini, C. Sissi, A. Fravolini and M. Palumbo, *J. Nucleosid Nucleotides*, **19**, 1327 (2000).
 14. G.S. Son, J.A. Yeo, M.S. Kim, S.K. Kim and A. Holmen, *J. Am. Chem. Soc.*, **120**, 6451 (1998).
 15. M.D. Bethesda. *Drug Information 88*. Authority of the Board of Directors of the American Society of Hospital Pharmacists Press (1988).
 16. J.B. Lippincott. *Drug Facts and Comparison*. Philadelphia and Toronto (1988).
 17. E.C. Long and J.K. Barton, *Acc. Chem. Res.*, **23**, (273) 1990.
 18. S.Q. Wu, W.J. Zhang and S.G. Chen, *Spectrochim. Acta Part A*, **57**, 1317 (2001).
 19. L.M. Song, W.J. Cao and C. Dong, *J. Spectrosc. Spectral Analysis*, **21**, 518 (2001).
 20. K.B. Jacobson and J.E. Turner, *Toxicology*, **16**, 1 (1980).
 21. H. Zhang, Y.C. Hong and C. Yu, *Chinese J. Pharm. Anal.*, **16**, 9 (1996).
 22. G. Pasternack, J. Bermadou and B. Meunier, *Angew Chem. Int. Ed. Engl.*, **34**, 746 (1995).