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Structural characterization and DNA-binding properties of Sm(III) complex with ofloxacin using spectroscopic methods

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Abstract. A novel complex Sm(III) complex with ofloxacin was synthesized and characterized on the basis of elemental analyses, molar conductivities, IR spectra, thermal analysis (TG-DSC), ^{1}H-NMR spectra. Then, spectrometric titration, ethdium bromide displacement experiments by UV spectroscopy, ionic influence, viscosity measurements and Circular Dichroism (CD) spectroscopic measurements were conducted to characterize the interaction between the complex and CT-DNA. Results obtained indicate that the complex bound with CT-DNA via an intercalation mechanism. The binding constants and binding sites number of the Sm(III) complex with CT-DNA were 1.80×10^5 l·mol $^{-1}$ and 1, respectively.

Keywords: Sm complex with ofloxacin, structural characterization, DNA-binding properties

1. Introduction

A large amount of biological experiments had elucidated that DNA was the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which caused DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death [1,2]. Of these studies, the interaction of rare earth metals complexes containing multidentate aromatic ligands with DNA had aroused much attention. This was due to their potential application as new therapeutic agents and their photochemical properties, which made them possible probes of DNA structure and conformation [3,4].

The design of metal complexes that bound and target at specific sequences of DNA became significant. A complete understanding of how to directly attach to DNA sites would lead not to novel chemotherapeutics but also to a greatly expand ability for chemists to probe DNA and mechanism of drug, so as to develop highly sensitive diagnostic agents [5].

Ofloxacin((±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxacine-6-carboxilic acid) was a nalidixic acid analog with broad spectrum antibacterial activity. It belonged to the fluorquinolones group, which acted as specific inhibitors of the bacterial DNA-gyrase. The molecule of ofloxacin was of zwitterionic structure, having a favorable solubility in acidic or basic solvents, while its solubility in water, methanol, ethanol and chloroform was poor. However, the metal complexes of ofloxacin could fairly solve in water, DMF, DMSO.

Transitional metals were present in very low concentration *in vivo*, and their ligand environment could be considerably altered when a therapeutically effective dose of drug (e.g., an antibacterial agent) was

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administered. This change in the balance between the metal ion and the ligand might have a profound effect upon the activity of the drug against potentially susceptible bacterial. The formation of complexes might increase the bioavailability of metal ion or the ligand drug, or both [6].

Recently, quite a few scholars had studied the coordination between quinolones and metal cations [7]. Whether the rare earth compounds could cure cancer cells was a heated subject. In some exploitative studies, researchers found, a low dosage of rare earth complexes could have a inhibitory effect on cancer cell [8]. Besides, the formation of complexes increased hydrotropy and liposolubility could enhance the ability of drug molecules in crossing the membrane of a cell, and hence raised the biological utilization ratio and activity of the drug [3].

Up to now, the biological activity and interactions between novel Sm(III) complex with ofloxacin and DNA had not been reported, it aroused our great interest. In order to investigate the mechanism of the drug, we synthesized it. On the basis of studies IR, ^{1}H-NMR spectra, molar conductivity, together with elementary analyses, the reasonable structure of this new compound was proposed.

2. Materials and methods

2.1. Materials

DNA and EB (ethidium bromide) were purchased from Huamei biological company. Ofloxacin was purchased from Jibeier medical company of China. Sm(III) was prepared from its oxide (99.9%) acquired from Guoyao chemical agent company of China. All the experiments were carried out in doubly distilled water buffer containing 5 mM Tris [Tris(hydroxymethyl)–aminomethane] and 50 mM NaCl, and adjusted to pH 7.2 with hydrochloric acid.

2.2. Experimental methods

2.2.1. Synthesis of the complex

Ofloxacin interacted directly with Sm(III) salt in an aqueous medium at natural pH, however, the compound formed in such a way, could not be well defined from a chemical point of view, as the anion of original metal salt was usually incorporated to the compounds formed, giving rising to formation of polymers [9]. To avoid such a polymers formation, the reaction was carried out in an acidic medium as follows: 0.2 g Sm_2O_3 dissolved in proper 1 M HCl at 60°C, and 0.42 g ofloxacin dissolved in 5 ml 1 M HCl in another beaker at 60°C, after a few minutes, mixed two solutions together, stirring thoroughly. After that, the yellow–white precipitate was filtered, washed with acetone for several times, and dried for 8 h at 80°C. Moreover, the complex was monitored by HPLC, showed that it was pure.

2.2.2. Instruments and methods

Carbon, hydrogen, nitrogen were analyzed on an Elemental VarioEL III CHNS analyzer. Sm was analyzed by titration with EDTA. The molar conductance value was determined in DMF on a Shanghai Leici DSS-11A conductivity meter. The thermal behavior was monitored on NETZSCH STA 409 PC thermal analyzer, each operating at a heating rate of 5°C/min and under oxygen as the reaction atmosphere. ^{1}H-NMR spectra were measured on Brucker av 400 spectrometer, using TMS as a reference in DMSO for Sm(III) complex. Infrared spectra (4000–400 cm^{-1}) were determined with KBr disks on a Nicolet 5700 FTIR spectrometer.

The absorption spectra were recorded on a TU-1901 spectra-photometer (Beijing) and the fluorescence spectra were recorded on a Perkin Elmer LS-50B spectrofluorophotometer (USA), using 5 nm/5 nm slit widths.

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature (25.0 ± 0.1°C). The flow time was measured whit a digital stopwatch. Data were presented in a form (η/η_0) versus binding ratio, where η was the viscosity of DNA in presence of compound and η_0 was the viscosity of DNA alone.

The circular dichroism (CD) spectra were recorded on a Jasco J-700 spectropolarimeter using a 1.0 cm path quartz cell and the following acquisition parameters: $\lambda = 190$–400 nm, scan speed 50 nm min^{-1}, resolution step 0.2 nm, sensitivity 50 mdeg, response 1 s, bandwidth 1.0 nm, accumulations 10 times.

3. Results and discussion

3.1. Structural characterization of complex

3.1.1. Structure of Sm(III) complex

Since the crystal structure of the Sm(III) complex had not been obtained yet, we characterized the complex and determined its possible structure by elemental analyses, molar conductivities, IR data, thermal analysis, ^{1}H-NMR spectra.

From the data of elemental analysis and molar conductivity (Table 1), we judged that the type of Electrolyte was nonelectrolyte [10], and estimated that the chemical formula of complex was [Sm(OFLX)$_3$].

Besides, experimental data fitted well with the calculated formula, there was no crystallization water molecules in the complex, as checked by thermal analysis. In addition, the complex did not melt up to 250°C. While the m.p. of the ofloxacin was 228°C.

3.1.2. IR spectra

According to the IR spectra (Fig. 1), we assumed that (CO) part of the ketonic group together with (–OH) of carboxylic group was in the bonding to Sm metal. For if (CO) part of carboxylic group bonded to metal, the band at 1713 cm^{-1} would disappear [11]. At the same time, the antisymmetric and symmetric modes of the carboxylic group would account for the band recorded at 1619–1612 and 1475 cm^{-1} respectively. However, the band at 1713 cm^{-1} did not disappear. As a result, we inferred that (–OH) of carboxylic group participated the bonding, the band at 1713 cm^{-1} shifted to 1702 cm^{-1} properly justified the above assumption, for (CO) chemical environment of (–COOH) had been little changed.

At the same time, the ketonic group participated in the bonding to the metal, because there was an important shift of its stretching bond towards lower wavenumbers, from 1622 cm^{-1} to 1581–1570 cm^{-1} [11,12].

We should conclude that FTIR spectroscopy; by itself did not permit a definitive answer to the way the ligand was bonded to the metal cation ions. However, from the above spectra, the assumption was rather reasonable.

Table 1
Data of elemental analysis and molar conductivity

Complex	Analysis found (Calc.) %				Molar conductance Λ/S·cm^2·mol^{-1} (DMF)
	Sm	C	H	N	
[Sm(OFLX)$_3$]	12.01 (12.18)	52.24 (52.49)	4.89 (4.60)	10.11 (10.20)	59.10

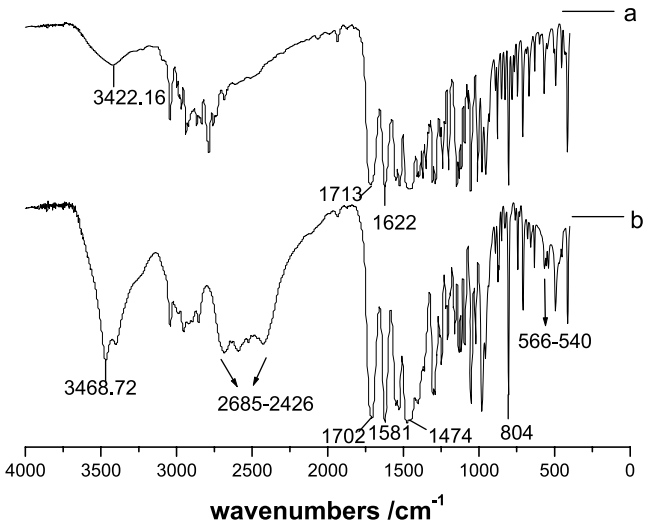

Fig. 1. The IR spectra of OFLX (a) and $Sm(OFLX)_3$ (b).

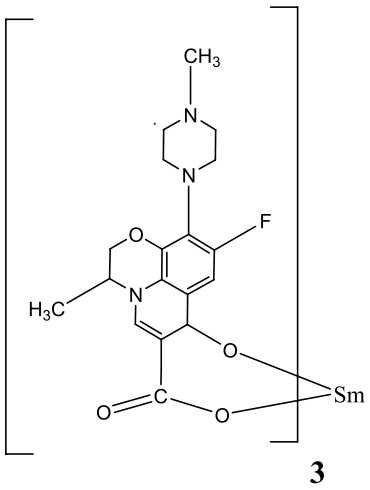

Fig. 2. The likely structure of Sm(III) complex.

3.1.3. NMR spectra

NMR spectrometry was basically another form of absorption spectrometry. With the remarkable developments in NMR magnets, a wealth of information for structure determination had become available. On the basis of related literature [6], for ofloxacin and complex. The vanishing of the peak δ 11.28 (attributed to –COOH group in the ofloxacin), suggested the –COOH bonded to metal. The likely structure of $Sm(OFLX)_3$ could be seen in Fig. 2.

3.2. Fluorescence spectra

Figure 3 showed the emission spectra of Sm(III) complex in the absence and presence of DNA. It could be clearly observed that its fluorescence intensity decreased in the presence of DNA. The results indicated there were strong interactions between the Sm(III) complex and DNA.

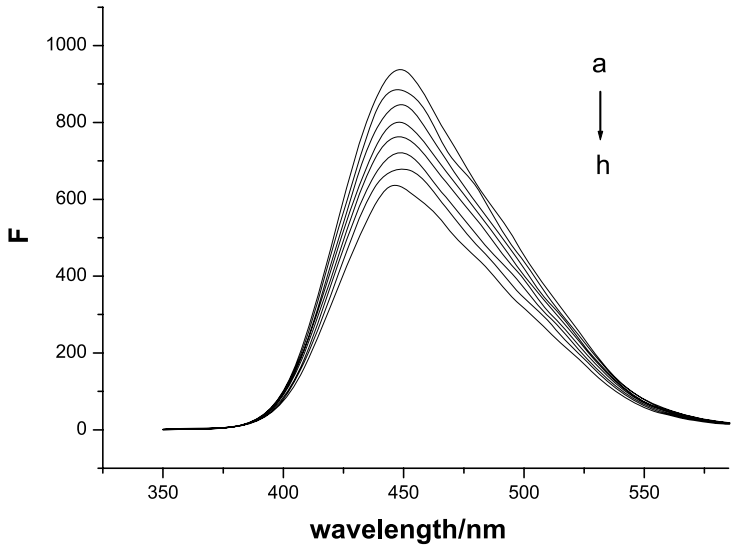

Fig. 3. Fluorescence emission spectra: (a) 5.05×10^{-7} mol·l^{-1} Sm(III) complex; (b)–(h), respectively, 5.05×10^{-7} mol·l^{-1} Sm(III) complex in the presence of 0.86, 1.72, 2.58, 3.44, 4.3, 5.16, 6.02×10^{-6} mol·l^{-1} DNA.

Fluorescence quenching usually divided into dynamic quenching and static quenching. Dynamic quenching was the interaction between quencher and fluorophore at excited state. Their interactions obey the Stern–Volmer equation:

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q], \tag{1}$$

where F_0 and F were the fluorescence intensities in the absence and presence of DNA, respectively. $[Q]$ was the concentration of DNA and K_{SV} was the Stern–Volmer quenching constant. K_q was the quenching rate constant of the biomolecule, τ_0 was the average lifetime of the biomolecule without quencher. When quencher together with fluorophore which was at the ground state to form the non-fluorescence complex, the process of decrease of fluorescence intensity was static quenching. In order to illustrate the mechanism, fluorescence intensity data were analyzed according to the Stern–Volmer equation by plotting F_0/F versus the DNA concentration $[Q]$ at 298 K. From Fig. 4, the relationship between F_0/F and $[Q]$ was a good linearity, R (0.992). Usually, the life time of the fluorescence was 10^{-8} s, and the biggest collision quenching rate constant was 2.0×10^{10} l·mol^{-1}·S^{-1} [13]. With the help of K_{SV} obtained from experiment and Eq. (1), K_q could be calculated, and its value 7.7×10^{12} l·mol^{-1}·S^{-1}, was much bigger than 2.0×10^{10} l·mol^{-1}·S^{-1}. As a result, the quenching mechanism was static quenching.

From the equation $\lg[(F_0 - F)/F] = \lg K + n\lg[Q]$ [14], the plots of $\lg F_0/(F_0 - F)$ vs. $\lg[Q]$ were shown in Fig. 5, binding sites number n and binding constants was 1 and 1.80×10^5 l·mol^{-1}, respectively.

3.3. Competition intercalation tests between Sm(III) complex and EB with DNA

In order to test if the Sm(III) complex could bind to DNA by intercalation, ethidium bromide (EB) was employed, as EB interacted with DNA as a typical indicator of intercalation [15]. Its π^* orbital coupled with the π orbital of base pair, thus decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in bathochromism.

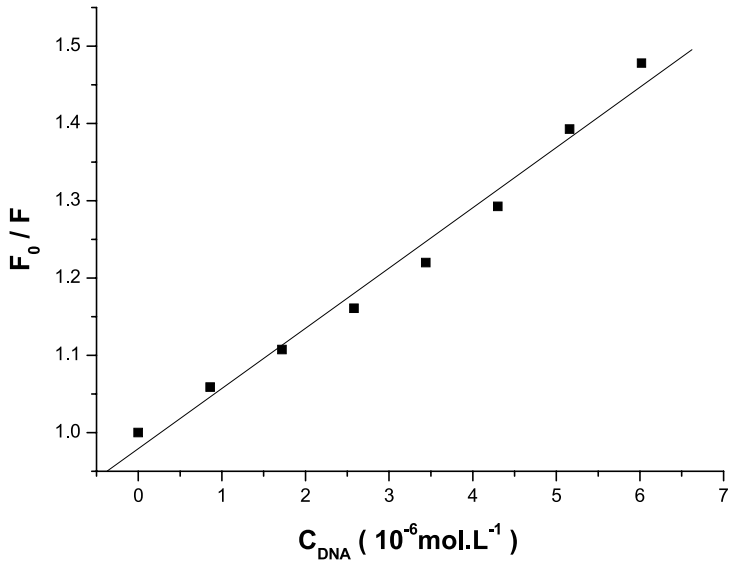

Fig. 4. Stern–Volmer plots of complex–DNA interaction.

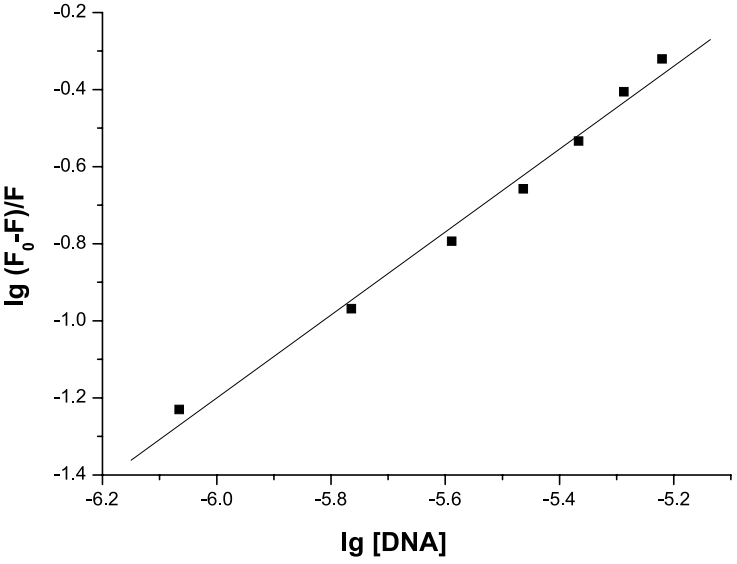

Fig. 5. The number of binding sites n and binding constants between complex and DNA.

As it was shown in Fig. 6, the maximal absorption of EB at 480 nm decreased and shifted to 515 nm, in the presence of DNA, which was the characteristic of intercalation. Figure 6c was the absorption of a mixture solution of EB, Sm(III) complex and DNA. It was found that the absorption at 515 nm increased compared with Fig. 6b. This could result from two reasons: (1) EB bound to the Sm(III) complex strongly, resulting in a decreased amount of EB intercalated into DNA; (2) there exists competitive intercalation between the Sm(III) complex and EB with DNA, thus releasing some free EB from the DNA–EB system. However, the former reason could be precluded since there were no new absorption peaks [5].

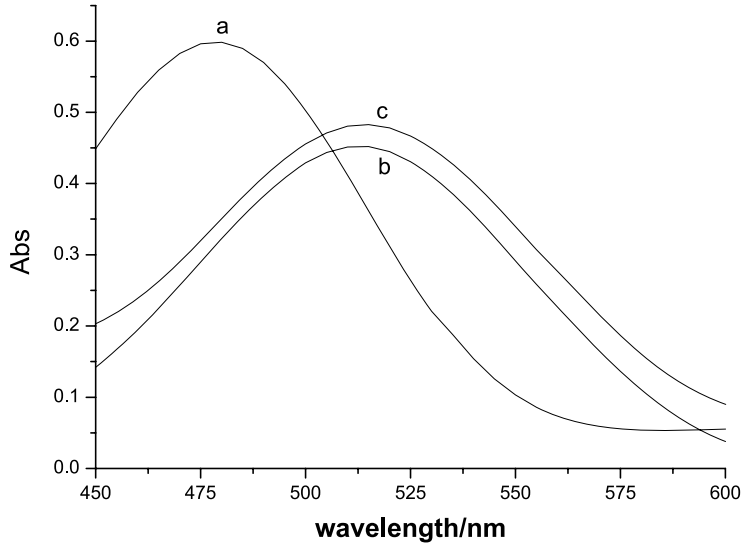

Fig. 6. The visible absorption spectra of (a) 6.6×10^{-5} M EB; (b) (a) + 6.6×10^{-4} M DNA; (c) (b) + 7.5×10^{-4} M Sm(III) complex in Tris-HCl buffer solution.

3.4. Influence of ionic strength on quenching efficiency

The interaction of Sm(III) complex and DNA was investigated under different ionic strength conditions. The NaCl concentration of Tris-HCl buffer (5 mM, pH 7.20) that contained 5.05×10^{-7} mol·l^{-1} Sm(III) complex and 1.65×10^{-6} mol·l^{-1} DNA, the final ionic strength range was 2–12 mM. As shown in Fig. 7, the fluorescence intensity nearly did not change with the increase of ionic strength. Thus, there was no electrostatic interaction between the Sm(III) complex and DNA, and we estimated that the interaction maybe intercalation.

3.5. Viscosity measurements

To further clarify the interaction between the complex and DNA, viscosity measurements were carried out. Photophysical probes generally provided necessary, but insufficient, clues to support an intercalation binding model. Hydrodynamic measurements which were sensitive to length increases (i.e. viscosity, sedimentation etc.) were regarded as the least ambiguous and most critical tests of binding model in solution in the absence of crystallographic structural data [16,17]. A classical intercalation model demanded that the DNA helix lengthen as base pairs are separated to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or link) the DNA helix, reducing its effective length and, concomitantly, its viscosity [18]. Viscosity experimental results clearly showed that Sm(III) complex could intercalate between adjacent DNA base pairs, causing an extension in the helix (Fig. 8).

3.6. Circular dichroism spectroscopy

The CD spectra of the complex with double-stranded DNA could provide us with useful information concerning the complex–nucleotide interaction. The CD spectra of DNA are shown in Fig. 9. They consist of a positive band I at 275 nm and a strong negative one II at 246 nm, characteristic of the

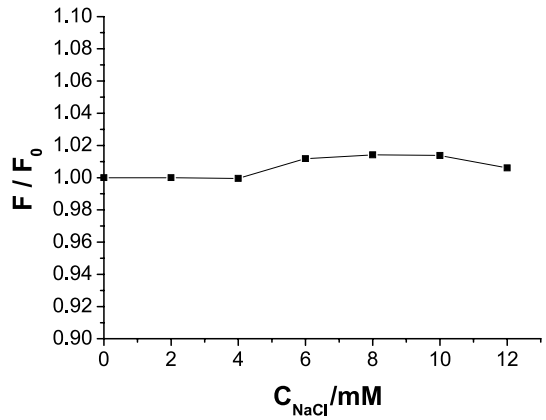

Fig. 7. Influence of ionic strength on quenching efficiency.

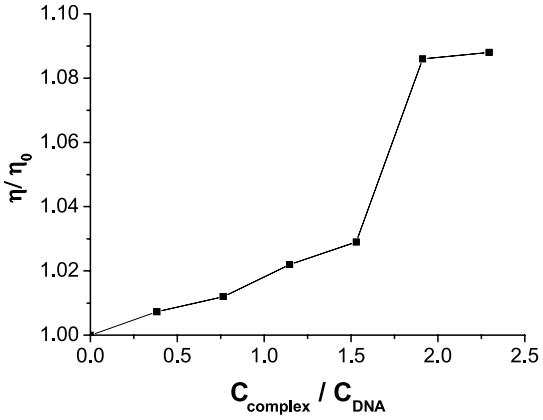

Fig. 8. Effects of complex on the viscosity of DNA solution. Complex was titrated into a DNA solution at 20.2°C. Complex volume: 15, 30, 45, 60, 75, 90 µl ($C_{\text{Complex}} = 2.52 \times 10^{-2}$ mol·l^{-1}, $C_{\text{DNA}} = 6.6 \times 10^{-5}$ mol·l^{-1}, pH 7.20, Tris-HCl).

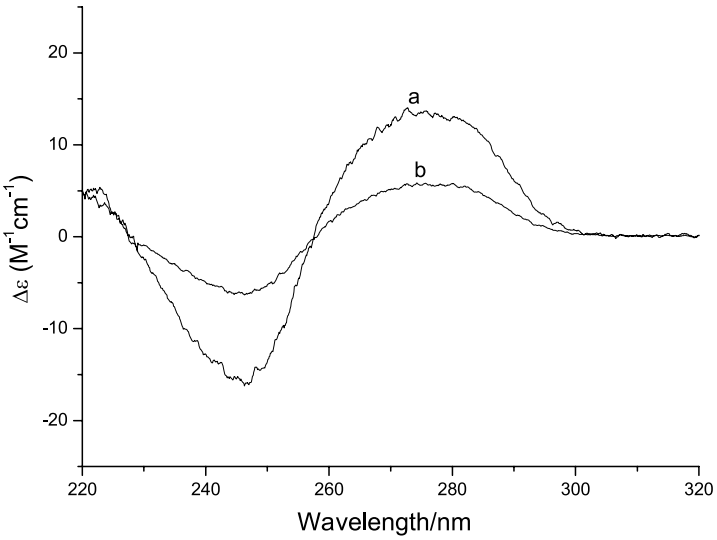

Fig. 9. CD spectra of 5 mM DNA in 5 mM buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) (a) and in the presence of complex (b).

B-form of DNA. When in the presence of complex, there was no shift of $\lambda_{\max}$, suggesting that DNA form has not been transited. However the intensity of the $\lambda_{\max}$ of band I and II decreased, indicating a reduction in DNA helicity which was attributed to complex interstrand crosslinking to the DNA base pairs [19].

4. Conclusions

Taken together, a new complex, $Sm(OFLX)_3$ was synthesized and characterized. The DNA-binding properties of Sm(III) complex were investigated by UV absorption, fluorescence, viscosity measure-

ments, CD spectroscopy. The results indicated that the compounds bound to CT-DNA via intercalation strongly. The binding sites number and binding constant was 1 and 1.80×10^5 l·mol^{-1}, respectively.

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