Spectroscopic Studies on the Binding of Some Fluoroquinolones with DNA

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ABSTRACT— ***UV-visible spectroscopic methods were used to study the interaction of some fluoroquinolones with calf-thymus DNA. The binding constants of drug-DNA complexes were evaluated and the nature of binding of these drugs with DNA were elucidated. The results suggested that fluoroquinolones bind to DNA through an electrostatic mode of interaction with partial intercalation.***

Keywords— fluoroquinolone, DNA binding, Intercalation, UV-Visible Methods.

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1. INTRODUCTION

Fluoroquinolones are extremely useful for the treatment of a variety of infections, including urinary tract, soft tissue, respiratory, and bone-joint [1,2]. These pharmaceuticals are used as antibiotics [3], especially for the treatment of the Anthrax infections [4]. Conversely, these chemicals may have adverse environmental impact because they are excreted intact and persist in the environment in the wastewater [5]. Because of the extensive usage of these drugs, the presence of fluoroquinolones in aquatic environment has been previously reported by several researchers [6,7,8]. These medications have also been linked to the genotoxicity of waste water effluents and for causing primary DNA damage in bacteria [5].

Several analytical methods have been developed for the determination of fluoroquinolones in their pharmaceutical preparations. These methods include electrochemical [9,10,11], capillary electrophoresis [12,13], chromatographic [14,15], microbiological methods [16], conductometric methods [17]. Within this context, various Spectroscopic methods have been used for the investigation of quinolones concetrations in wastewater [18]. For example, ciprofloxacin was measured in pharmaceutical preparations [19], and in urine samples using absorbing light in the UV region of the spectrum [11,20]. Also their concentration was measured in the visible region through their reaction with iron (III) and measuring the absorbance of the corresponding complex [21], or through ion-pair complex formation [22]. Furthermore, spectrofluorimetric methods based on the charge–transfer reaction were described and ciprofloxacin was determined through transfer reaction with 7,7,8,8-tetracyanoquinodimethane as Π-electron acceptor [23].

Numerous techniques have been applied for studying the interaction between quinolone drugs and DNA [24,25,26]. It was reported that quinolones are active against the DNA-gyrase enzyme, which is a type II topoisomerase. It has been hypothesized that DNA-gyrase introduces negative supercoils in the DNA [27]. Based on this premise, several structural models have been suggested to account for the action of quinolones. The suggested models require a direct interaction between the drug and either single or double-stranded DNA [28,29]. The exact mechanism for the reaction between the drug and the DNA is poorly understood, however, contribution to deeper insight into the mechanism of interaction of this class of antibiotics with DNA is important for greater understanding of their therapeutic efficiency [30].

In this communication, we aim at the elucidation of the interaction of the studied quinolones; ciprofloxacin, norfloxacin, enrofloxacin and nalidixic acid (Figure 1) with DNA in solution using UV-visible spectroscopy, to explore the nature of binding between quinolones and DNA, and to estimate the magnitude of the binding constant (K).

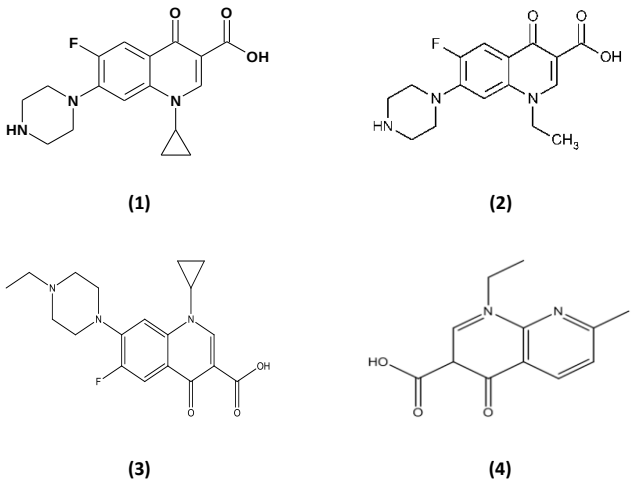

Figure 1: The chemical Structure of: (1) Ciprofloxacin (2) Norfloxacin (3) Enrofloxacin (4) Nalidixic acid

2. EXPERIMENTAL

2.1 Reagents and Solutions

Calf thymus DNA (sodium salt type 1), was purchased from Sigma and was used, as received, without further purification. Solutions of DNA gave ratios of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.8 – 1.9, indicating that DNA was sufficiently free from protein. The concentration of DNA solution expressed in M of nucleotide phosphate (NP), was determined by UV absorbance at 260 nm using molar extinction coefficient (ε) of 6600 $cm^{-1}M^{-1}$.
Ciprofloxacin hydrochloride, norfloxacin, enrofloxacin and nalidixic acid were obtained from Sigma. Stock solutions of $1x10^{-3}$ M were prepared by dissolving the appropriate amount of the drugs with 1 mL of 0.1M glacial acetic acid and then diluting to the mark of 10 mL volumetric flask with distilled water. The solutions were kept at 4.0^{o}C and used within one week.

2.2 Instrumentation

UV-visible spectra were obtained using Shimadzu UV-VIS-NIR Scanning Spectrophotometer, model UV3101PC. The pH measurements were carried out using HANNA pH meter model HI 8424.

2.3 Methodology

The desired concentration of the drugs($1.0x10^{-5}$ M) were prepared by placing 100µL of the stock ($1x10^{-3}$ M) drug solution into 10.0 mL volumetric flask. Different volumes (100, 200, 300, 400 and 500 µL) of 10^{-3} µM stock dsDNA solution were added to achieve various concentrations (10-50 µM) of DNA. The volume was then reconstituted to 10.0 mL with acetate buffer solution. The absorption spectra were recorded in 1 cm optical path length quartz cell.

3. RESULTS AND DISCUSSION

The interaction of ciprofloxacin (CIP), norfloxacin (NOR), enrofloxacin (ENR), and nalidixic acid (NAL) with dsDNA in solution was studied by UV-visible spectroscopy. For example, Figure 2 shows the absorption spectra of the ciprofloxacin drug in the absence and in the presence of various concentrations of dsDNA at pH 5 acetate buffer solution. A continuous decrease in the absorbance maxima of the four drugs was observed with the gradual increase in the concentration of DNA in solution. This hypochromic effect is probably due to the interaction between the electronic states of the intercalating drug chromophores and those of the DNA bases [31]. The strength of this electronic interaction is expected to decrease as the distance of separation between the chromophore and DNA bases increases [32]. The

apparent hypochromism observed suggests a close proximity of the quinolone chromophores to the DNA bases. In addition, a small red shift was observed for the maxima of the drugs with the addition of DNA. This is explained by assuming that the drugs might slide into the base pairs of DNA upon binding, and thus preventing the formation of hydrogen bonding with the solvent water molecules. The hypochromic effect and the red shift in UV-visible spectra upon binding to DNA are considered as indications of an intercalating mode of interaction [33].

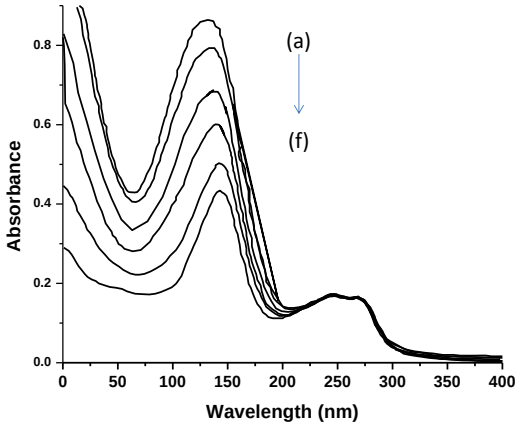

Figure 2: UV/Vis absorption spectra of acetate buffer solution (pH 5) containing 10 µM CIP in the presence of DNA (µM): (a) 0.00; (b) 10.0; (c) 20.0; (d) 30.0; (e) 40.0; (f) 50.0.

Based on variations in absorption spectra of the studied drug upon binding to DNA, the binding constant, K, was calculated from the equation [34]:

$$A_o/(A-A_o) = \varepsilon_G/(\varepsilon_{H-G} - \varepsilon_G) + \varepsilon_G/(\varepsilon_{H-G} - \varepsilon_G) \times 1/K[DNA] \quad (1)$$

Where:

A_o: Absorbance of drug in the absence of DNA.

A: Absorbance of drug in the presence of DNA.

ε_G: Absorption coefficient of drug.

ε_{H-G}: Absorption coefficient of drug-DNA complex.

Using absorbance data extracted from the absorption spectra for the studied quinolones and from the Table 1, the plots of $A_o/(A-A_o)$ versus 1/[DNA] were linear as shown in Figure 3. From the slopes and the intercepts of the straight lines obtained, the values of the binding constants were calculated and tabulated in Table 1. The values of K obtained indicate that these drugs have certain affinities toward DNA.

Table 1: Binding constant (K) values for the interaction of quinolones with DNA

Drug	CIP	NOR	ENR	NAL
$Kx10^{-2}\ M^{-1}$	6.33	7.78	3.62	2.29

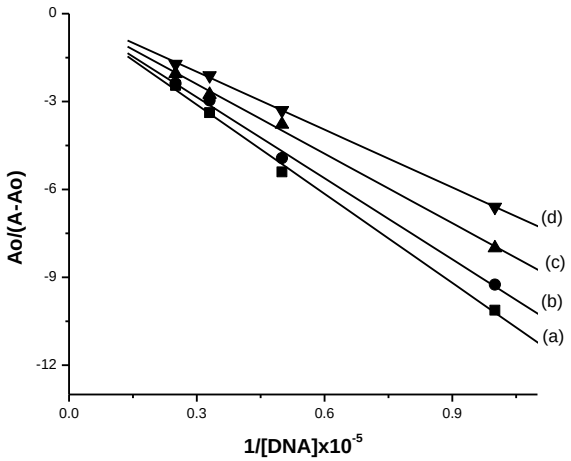

Figure 3: Plot of Ao/(A-Ao) vs. 1/[DNA], [quinolone] = 5×10^{-6}M in acetate buffer solution pH=5, containing [DNA] 10.0-40.0µM

The results in Table 1 reveal that norfloxacin and ciprofloxacin have higher binding constant values than those of enrofloxacin and nalidixic acid. The CIP and NOR quinolones differs only in the substituent (cyclopropyl versus ethyl) at the nitrogen atom of the aromatic heterocyclic ring.

The fact that ciprofloxacin and norfloxacin have comparable binding constant values suggests that the piperazine ring plays an important role in binding to DNA. This explains the small K value of nalidixic acid. The smaller K value of enrofloxacin compared to that of ciprofloxacin and norfloxacin, may be explained by the increased steric hindrance of the ethyl group at the outer nitrogen (N_6) of the piperazine ring in enrofloxacin compared to hydrogen atom in ciprofloxacin and norfloxacin (Figure 4).

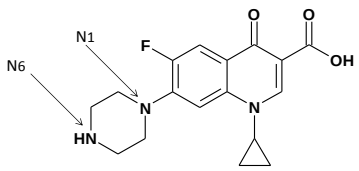

Figure 4: The chemical structure shows the effect of the substituents at nitrogen (N_6) of the piperazine ring.

In addition, the absence of the hydrogen atom of the outer nitrogen (N_6) of the piperazine ring prevents the formation of a hydrogen bonding between enrofloxacin and DNA. Both factors are expected to weaken the binding of enrofloxacin to DNA, and hence, a smaller K value is obtained. However, larger values of K (ca 10^4-10^5 M^{-1}) were obtained for molecules that bind strongly to DNA such as antitumor drugs [35,36]. These results show ciprofloxacin has the properties of an intercalative binder that are agreed with UV–melting curves and fluorescence emission spectra that it has at least two different binding modes; a non-specific binding to DNA molecules, which is electrostatically driven, and a specific non-electrostatically controlled binding [37].

4. CONCLUSIONS

The binding between the studied quinolones and dsDNA does exist. This can be deduced from obtained values of the binding constant (K). Despite the small values of the binding constants for the quinolone-DNA complexes compared to others, typical intercalators shows the interaction is favored in non-intercalative or partial intercalative, that is supported by the hypochromic effect and the red shift in the absorbance maxima of the studied drugs, upon binding to dsDNA. And it is concluded that electrostatic interaction with partial intercalation is the most probable mode of interaction between the studied quinolones and dsDNA.

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