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DNA influence on norfloxacin fluorescence

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ABSTRACT

The emission properties of norfloxacin, a quinolone antibiotic, in presence of salmon sperm DNA were studied at room temperature and in conditions of acid, alkaline and neutral pH. It was found that norfloxacin molecules are inserted between the DNA base pairs, as evidenced by the emission spectra features and the significant increases in relative viscosity of DNA by the addition of norfloxacin. The fluorescence quenching process was characterized by Stern–Volmer plots which display a positive deviation from the linearity. The analysis was performed in terms of the Stern-Volmer modified equations including both dynamic and static quenching. The use of the finite sink approximation model showed that the process of quenching of the norfloxacin fluorescence with DNA was diffusion limited, irrespective to the pH of the work solution. At the same time, relying on the formation of the ground state complex model and the sphere of action static quenching model, we concluded that the quenching reaction from the norfloxacin - DNA system is due to the combined effect of both dynamic and static quenching processes.

1. Introduction

The fluorescence probes are widely used for obtaining information on the molecular interactions, especially in the drug - biopolymers system. Among other compounds, the quinolone antibiotics were intensively studied for their wide applications. These antibiotics have a broad antibacterial spectrum, good oral absorption and have been used extensively in the treatment of infectious diseases [1–3].

Quinolone antibiotics structures (Fig. 1a) are based on two types of ring: a naphthyridine nucleus, with two nitrogen atoms at the positions 1 and 8 and a quinoline nucleus, with only one nitrogen atom in the position 1. Both quinolones and naphthyridones contain keto oxygen at C-4 and a carboxylic acid side chain at C-3, both of them being essential to their activity. A number of quinolones, including norfloxacin, have a piperazinyl group at 7-carbon atom.

Because these drugs contain potentially ionisable functional groups (carboxyl and/or amine groups), they are fully or partially ionized at different pH values. At the same time their antibacterial activity is pH-dependent, knowing that the quinolone antibiotics act by inhibition of bacterial DNA gyrase, a process that depends on the pH and acid concentration [3–5]. It has been shown that the presence of charged groups is responsible for quinolones biological activity and solubility [3,4,6].

Norfloxacin (Fig. 1b) has two relevant ionisable functional groups, the 3-carboxyl group and N4 of the piperazinyl group. It is primarily cationic below pKa1 = 6.23 (N4 in the piperazinyl group), anionic above pKa2 = 8.55 (3-carboxyl group) and zwitterionic (net neutral) between pKa1 and pKa2 [3,5,7].

In this paper, we have used the steady-state fluorescence spectroscopy in order to investigate the fluorescence quenching of norfloxacin with salmon sperm DNA in aqueous solution, at room temperature, 25 °C and in conditions of acid, alkaline and neutral pH. The various parameters responsible for fluorescence quenching have been determined by different models (the formation of ground state complex, the sphere of action static quenching and the finite sink approximation). Based on these parameters, the possible quenching mechanisms are discussed. In addition, based on our previously experimental absorption data [8] and various literature spectroscopic data [9–14], by viscosity measurements, the binding mode of norfloxacin to DNA is estimated.

2. Materials and Methods

Norfloxacin (CAS number 70458-96-7) with purity ≥98% was obtained from Sigma Aldrich, Germany and was used without further purification. Salmon sperm DNA with purity ≥96% was obtained from Ogata Research Laboratory, Chitose, Hokkaido, Japan and was used without further purification.

The stock solutions of norfloxacin and DNA were prepared in 0.15 M NaCl aqueous solution. The concentrations of these solutions have been determined by means of the molar absorption coefficients: ε_{275nm} = 37,500 M^{-1} cm^{-1} for norfloxacin and ε_{260nm} = 6600 M^{-1} cm^{-1} for salmon sperm DNA [8,15].

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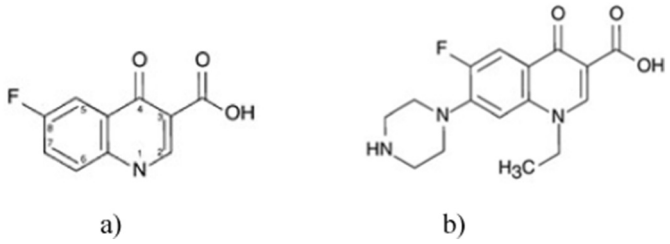

Fig. 1. The basic quinolone structure, with the ring atoms numbered (a). The structure of norfloxacin (b).

For measurements at acid and alkaline pH, the neutral pH of the stock solutions was adjusted by adding the appropriate amount of 0.1 M HCl or 0.1 M NaOH.

The absorption spectra were recorded on a Lambda25 UV–vis spectrophotometer from Perkin-Elmer, USA, at room temperature (25 °C), using 1 cm optical path length quartz cells.

The fluorescence spectra were recorded by exciting the samples at 330 nm using a Jasco FP-6500 spectrofluorometer. It was avoided the excitation on 270 nm (the maximum of absorption for norfloxacin) because this region corresponds to the nitrogenous bases from the nucleic acid structure.

The absorption and fluorescence spectra were performed by adding an appropriate amount of the DNA solution to a constant volume of the drug solution (2 mL), the pH modifications during these experiments being insignificant because the stock solutions of NaCl, DNA and norfloxacin at the same pH value were used.

The viscosity studies were performed with an Ostwald viscometer at room temperature (25 °C), by adding an appropriate amount of drug to the stock solution of DNA in order to obtain the ratios of drug concentration to polymer DNA concentration in the range of 0.05–0.7. The flow times of NaCl, DNA and norfloxacin - DNA solutions were repeatedly measured by using an electronic stopwatch and each point used in the plots represent the average of at least three readings. The experimental data are presented as plots $(\eta/\eta_0)^{1/3}$ versus the polymer DNA/drug concentration ratios, where η and η_0 represent the viscosity of DNA in the presence and in the absence of norfloxacin. Viscosity values were calculated based on the difference between the flow times of norfloxacin - DNA solutions (t_i) and the flow time of NaCl solution (t_0).

All experimental data are reproducible within 5–7% of experimental error.

3. Results and Discussion

The absorption spectra for norfloxacin - DNA system, at different polymer DNA/drug concentration ratios and acid, alkaline or neutral pH are shown in Fig. 2.

It can be seen that by increasing the DNA content, we obtained a gradual increase in absorbance of the strong peaks from 275 nm (pH 4), 271 nm (pH 7) or 266 nm (pH 10.7), accompanied with a decrease in absorbance of the small peak from 330 nm. In all cases, one can observe an isosbestic point at ~300 nm and a hypsochromic shift of ~10 nm, which prove that the system consists in free DNA and DNA bound to norfloxacin. These observations represent the evidence for the norfloxacin - DNA complex formation, similar results being presented in numerous studies [3,16,17].

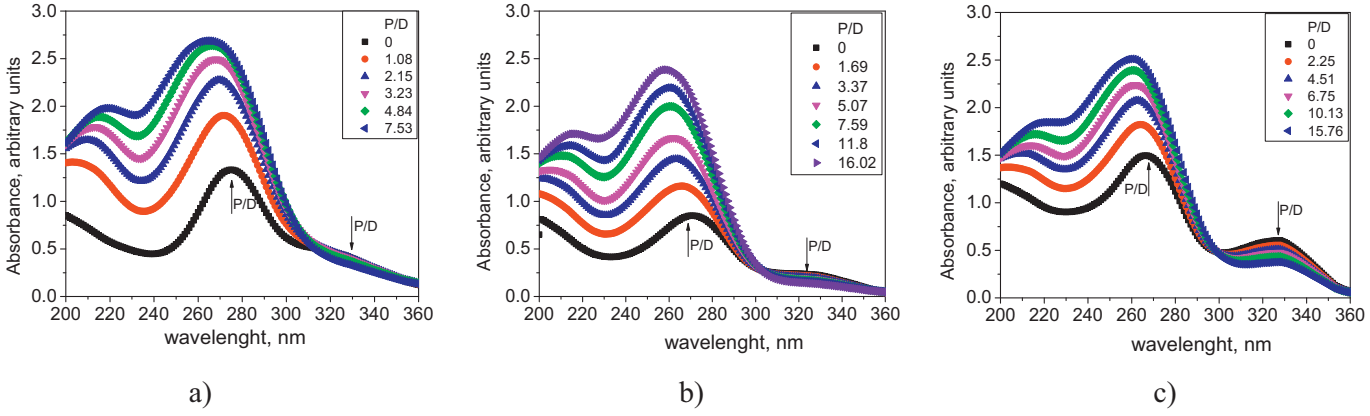

Fig. 2. Absorption spectra of norfloxacin, at different polymer DNA/drug concentration ratios and pH 4 (a), 7 (b) and 10.7 (c).

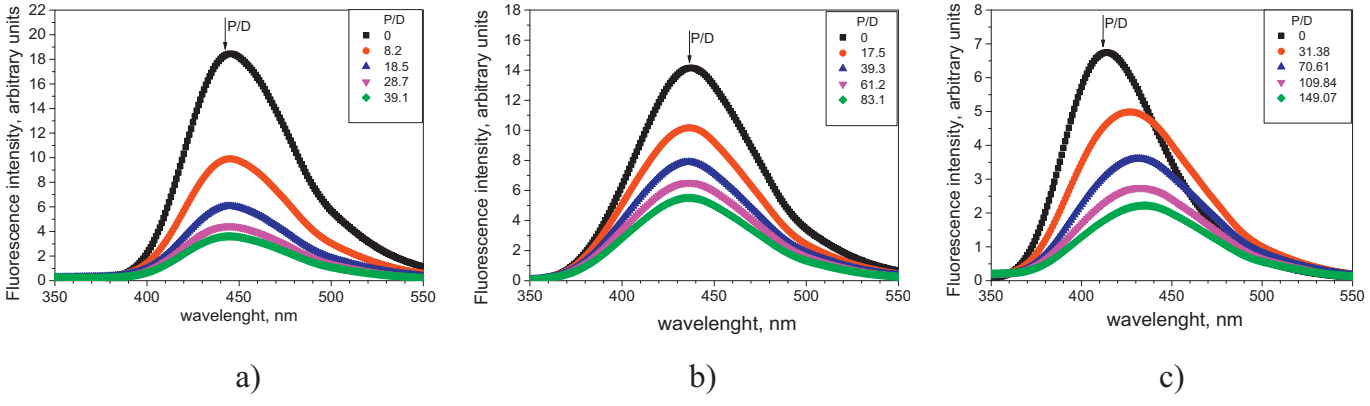

Fig. 3. Emission spectra (λ_{ex} = 330 nm) of norfloxacin, at different polymer DNA/drug concentration ratios and pH 4 (a), 7 (b) and 10.7 (c).