Spectral study of the interaction between enoxacin and calf thymus DNA

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The spectroscopic behavior of enoxacin has been investigated in aqueous solutions. The absorption spectra of enoxacin - DNA system were measured and interpreted. The binding of enoxacin to calf thymus DNA was considered in terms of Benesi-Hildebrand, Scott, Scatchard and Wolfe methods. The number of absorbing species presents in solution, the binding constant and the number of binding sites for each segment of DNA were determined. The modifications observed in the absorption spectra were interpreted in terms of the stacking of drug molecules, fixed by electrostatic interactions to the anionic groups from the biopolymer.

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1. Introduction

Enoxacin, 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, is a fluoroquinolone antibiotic, with broad antimicrobial spectrum, which includes most Gram-negative bacteria, many Gram-positive bacteria and some anaerobes [1].

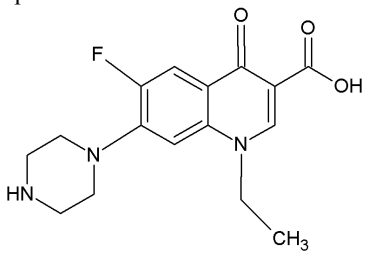

Fig. 1. Chemical structure of enoxacin.

Enoxacin is effective in the treating of nosocomial infections, has bactericidal action and inhibits bacterial enzyme DNA gyrase, the enzyme that catalyzes the condensation process of the helical chains of DNA in during division, so they fit into the cell. Although the mechanism of action of enoxacin is unclear, it can be argued that its action is determined by interaction directly with DNA in synergy with gyrase enzyme [2-3].

A number of analytical methods are reported for the study of quinolones based on mainly spectrofluorometry [4], spectrophotometry [5-6], liquid chromatography [7-8], atomic absorption [9] and voltammetric method [10].

The interaction between fluoroquinolones and different biopolymers has received much attention in the literature, but, in spite of the numerous studies in this topic, the nature of this interaction is not yet completely elucidated.

The binding of the drug molecules to biopolymers is accompanied by the modification of the physico-chemical properties of the drug. It is the aim of this paper to report absorption spectral experiments to study the interaction of enoxacin with calf thymus DNA, in order to determine the number of absorbing species present in solution, the binding constant (K) and the number of binding sites for each segment of DNA (n).

2. Experimental set-up and procedures, used substances

Enoxacin (Sigma-Aldrich, USA) and calf thymus DNA (Sigma-Aldrich, USA) were employed. The solutions aqueous of the enoxacin and DNA were prepared. The concentrations of the solutions were determined using the following molar absorption coefficient values: enoxacin (ε_{275nm}=37500 $M^{-1}cm^{-1}$) and DNA (ε_{260nm} = 6600 $M^{-1}cm^{-1}$).

The absorption spectra were recorded in a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer, with quartz cells, at room temperature.

3. Results and discussions

The absorption spectra of enoxacin are presented in Figure 2. They consist of two absorption bands: one major band at 275 nm and one minor band at 345 nm.

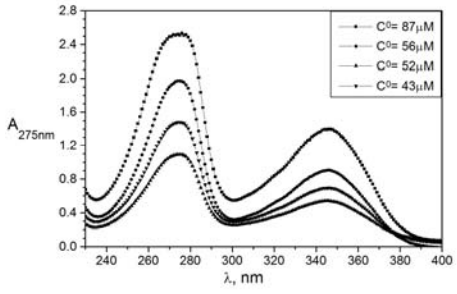

Fig. 2. Absorption spectra of enoxacin, at different concentrations of drug.

It may be noted that the relative intensities of those bands vary upon dilution and that the absorption in the range 230-400 nm is more pronounced at higher concentrations. On this basis and taking into account literature data on the aggregation of other fluoroquinolone antibiotic [11], the band at 275 nm was assigned to the monomer and that at 345 nm to the dimer.

Fig. 3 presents the absorption spectra of enoxacin-DNA system, at five values of the ratio of concentrations of polymer to drug (P/D).

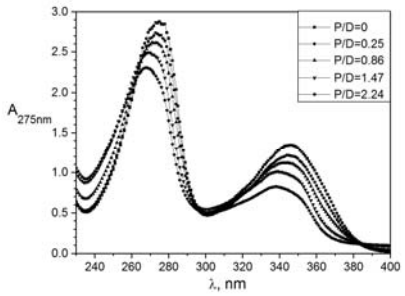

Fig. 3. Absorption spectra of enoxacin-DNA system, at different polymer/drug ratios.

One observes the relative increase of intensities of the bands centered at 275 nm and 345 nm with the increase of P/D ratios, an isosbestic point at 263 nm and a marked hypochromism. Therefore, on this basis and taking into account the binding data in other system [11], one may conclude that the enoxacin-DNA system consists only the DNA free and DNA bound enoxacin.

Assuming that is formed one complex 1:1 between enoxacin and DNA, the binding constant was evaluated in terms of the methods proposed by Benesi-Hildebrand, Scott and Scatchard [12-14].

The linear Benesi-Hildebrand, Scott and Scatchard plots for the enoxacin – DNA system were obtained. A Benesi-Hildebrand plot for this system is presented in Fig. 4.

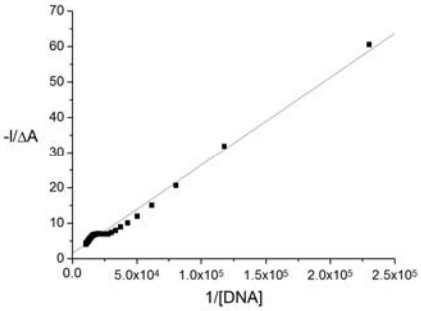

Fig. 4. Benesi-Hildebrand plot for enoxacin - DNA system.

In addition, the experimental data for the binding of enoxacin to calf thymus DNA were fitted either to the linear Scatchard plot or to a non-linear regression [11,15].

On the assumption of the absorption is due only to the free form of drug ($f_B = 0$), the concentrations of free (C_F) and bound (C_B) drug are given by:

$$C_B = C^0 \frac{A_0 - A}{A_0} \quad (1)$$

$$C_F = C^0 - C_B \quad (2)$$

and the binding ratio (r) is calculated by:

$$r = \frac{C_B}{[DNA]} \quad (3)$$

In Fig. 5 is presented a Scatchard plot for enoxacin-DNA system.

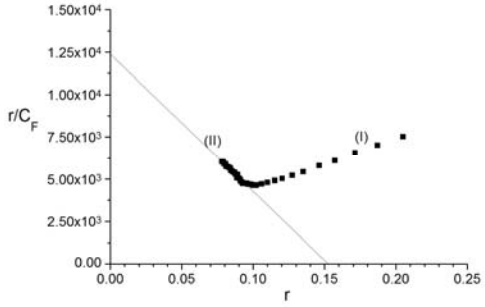

Fig. 5. Scatchard plot for enoxacin-DNA system.

The Scatchard plot attests the presence of two processes of binding:

- the process (I), at small values of polymer to drug (P/D) ratio and
- the process (II), at medium values of P/D ratio. From the linear segment of this plot, the binding constant ($K=4.71 \times 10^4\ M^{-1}$) and the number of sites (n=0.44) were obtained.

Fitting from non-linear regression the values of r corresponding the linear part of Scatchard plot, with equation (2) (Fig. 6), the binding parameters for process (II): $K=4.56 \times 10^4\ M^{-1}$ and n=0.45 were obtained.

At small values of P/D ratio, high deviation from estimated linearity was observed. This deviation attests the existence of the cooperative interactions, the different classes of the binding sites or the multiple contacts [16-18].

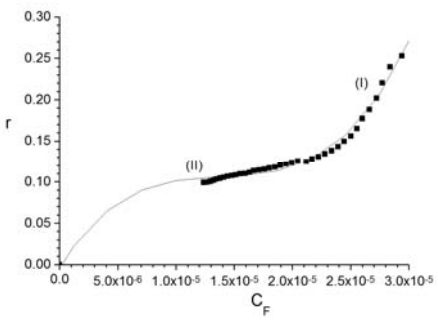

Fig. 6. Fitting of the binding' data from non-linear regression.

The binding constant for enoxacin - DNA interaction was also evaluated from the method proposed by Wolfe et al. [19] and the result obtained was summarized in Table 1.

Table 1. The results of the binding constants for enoxacin – DNA interaction.

Methods	Equations	K, M^{-1}	R
Benesi-Hildebrand	$\frac{l}{\Delta A}=\frac{1}{C^0K\Delta\varepsilon[DNA]}+\frac{1}{C^0\Delta\varepsilon}$	$1.66x10^4$	0.9943
Scott	$\frac{l[DNA]}{\Delta A}=\frac{1}{C^0\Delta\varepsilon}[DNA]+\frac{1}{C^0K\Delta\varepsilon}$	$2.04x10^4$	0.9917
Scatchard	$\frac{\Delta A}{l[DNA]}=-\frac{K}{l}\Delta A+C^0K\Delta\varepsilon$	$1.81x10^4$	0.9683
	$\frac{r}{C_F}=(n-r)K$	$4.71x10^4$	0.9956
	$r=\frac{nKC_F}{1+KC_F}$	$4.56x10^4$	0.9886
Wolfe	$\frac{[DNA]}{\Delta\varepsilon_{app}}=\frac{[DNA]}{\Delta\varepsilon}+\frac{1}{K\Delta\varepsilon}$	$6.75x10^4$	0.9893

where $\Delta\varepsilon_{app}=\varepsilon_{app}-\varepsilon_F$, $\Delta\varepsilon=\varepsilon_B-\varepsilon_F$, ε_{app}, ε_F and ε_B are the apparent, free and bound drug absorption coefficients, l is path length, ΔA - the observed absorbance change, C^0 - the total concentration of drug, C_F - the concentrations of free drug, $[DNA]$ - DNA concentration (concentration in moles per unit volume), r - the binding ratio, n - the number of binding sites and K - the binding constant.

It is noted that the binding process (I), analyzed by Benesi-Hildebrand, Scott and Scatchard methods presented a binding constant lower than binding process (II), analyzed by non-linear Scatchard and Wolfe methods.

4. Conclusions

The absorption data for the binding of enoxacin to calf thymus DNA were interpreted in terms of the stacking of drug molecules and points out two binding types: a non-electrostatic (internal) type consisting of the intercalation of the drug between the base-pairs from DNA and an external type where the electrostatic interactions with the anionic groups of DNA are predominant.

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