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# BINDING OF NORFLOXACIN, ENOXACIN AND ENROFLOXACIN TO CALF THYMUS DNA

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A number of drugs exert their influence by interaction with nucleic acids, having as effect the inhibition of replication and transcription. The precise mechanism by which these functions are inhibited is unique to each clinically important agent and may involve inhibition of DNA polymerase activity, topoisomerase II activity, etc. A fundamental step in understanding the molecular mechanism of drugs' action is the characterization of their binding to DNA and/or RNA. Fluoroquinolones are a class of antimicrobial antibiotics that destroy bacteria by inhibiting enzymes DNA gyrase and DNA topoisomerase. They are useful in the treatment of many infections, including urinary tract infections, respiratory infections, sexually transmitted diseases, acute bronchitis and sinusitis. In the present study, the interaction of three fluoroquinolones, norfloxacin, enoxacin and enrofloxacin, with calf thymus DNA has been studied. The study has been done by means of using various techniques such as UV-Vis absorption spectroscopy and viscosity measurements. The viscosity experiments have revealed how these drugs bind to DNA while the spectroscopic experiments have allowed the determination of the binding parameters. The results have been processed in terms of Benesi-Hildebrand, Scott and Scatchard models, assuming that binding of drugs to DNA is in 1:1 system and they also have been fitted through the linear or non-linear regression, assuming that there is one single class of non-interacting binding sites that do not exhibit cooperative behaviour.

## INTRODUCTION

Quinolones are a group of synthetic antibacterial agents containing a skeleton 4-oxo-1,4-dihidrochinolinic.

Since the introduction of nalidixic acid in clinical practice in 1960, many antibacterial agents with broad-spectrum of action and related structure have been isolated. It was found that a fluorine atom in position 6 and a piperazine nucleus in position 7 greatly increased the spectrum of activity against gram-negative bacilli. In addition, fluoroquinolones are used to treat respiratory infections, urinary tract, soft tissue of joints, typhoid fever, sexually transmitted diseases, pneumonia, bronchitis and acute sinusitis.[1-3]

Fluoroquinolones have a special mechanism of action based on blocking of bacterial gyrase, an enzyme with an important role in DNA replication. Inhibition of this enzyme causes major cellular dysfunction, the blocking of the numerous stages of the protein synthesis, followed by death of bacterial cell.[1-3]

In this paper, we proposed to study the interaction of three fluoroquinolones, norfloxacin, enoxacin and enrofloxacin (Fig. 1), with calf thymus DNA through UV-Vis absorption spectroscopy and viscosity measurements for the determination of the binding parameters. The results have been interpreted in terms of Benesi-Hildebrand, Scott and Scatchard models, assuming that binding of drug to DNA is in 1:1 system and in terms of linear and non-linear regression, assuming that there is one single class of non-interacting binding sites that do not exhibit cooperative behaviour.

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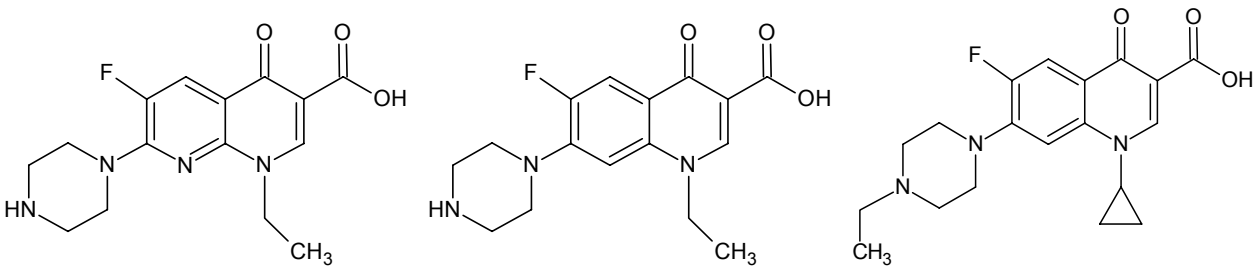


(a) (b) (c)

Fig. 1 – The formula of norfloxacin (a), enoxacin (b) and enrofloxacin (c).

## RESULTS AND DISCUSSION

Fig. 2 shows two families of absorption spectra in fluoroquinolone – DNA system, at different polymer to drug ratios (P/D). One observed: the decreasing of bands' intensity with increasing P/D ratios, a hypochromism marked (up to ~15%) in the absorption bands of fluoroquinolone – DNA complexes, an isosbestic point at 263 nm and a hypsochromic shift in the position of absorption maximum with ~8 nm in the enoxacin - DNA system, which proves that the system consists only of free DNA and DNA bound to enoxacin.

The binding constant of fluoroquinolones to DNA was determined, considering that it forms a 1:1 complex drug – DNA, by using several methods, such as:

- Benesi-Hildebrand:[4]

$$\frac{l}{\Delta A} = \frac{1}{C^0 \cdot K \cdot \Delta\varepsilon \cdot [DNA]} + \frac{1}{C^0 \cdot \Delta\varepsilon} \qquad (1)$$

- Scott:[5]

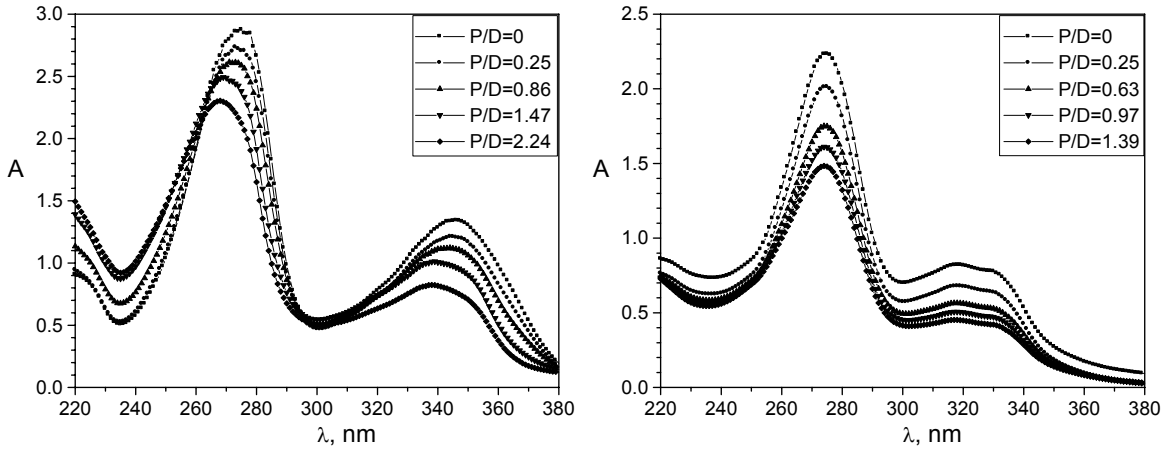
$$\frac{l \cdot [DNA]}{\Delta A} = \frac{1}{C^0 \cdot \Delta\varepsilon} \cdot [DNA] + \frac{1}{C^0 \cdot K \cdot \Delta\varepsilon} \qquad (2)$$

- Scatchard:[6]

$$\frac{\Delta A}{l \cdot [DNA]} = -\frac{K}{l} \cdot \Delta A + C^0 \cdot K \cdot \Delta\varepsilon \qquad (3)$$

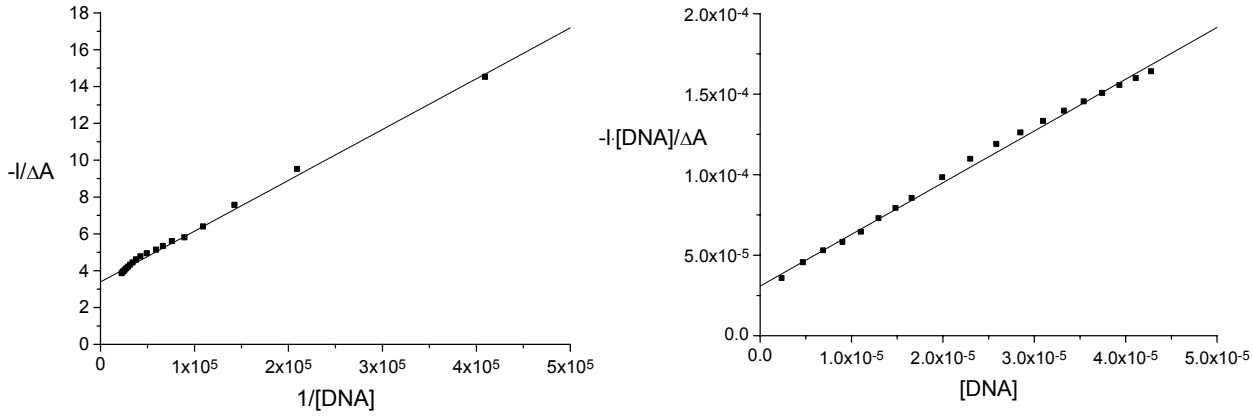
where $l$ is the path length, $\Delta A$ is the observed absorbance change, $C^0$ the total concentration of the drug, $[DNA]$ is DNA concentration, $\Delta\varepsilon=\varepsilon_B-\varepsilon_F$, $\varepsilon_F$ and $\varepsilon_B$ are the free and bound drug absorption coefficients and $K$ is the binding constant.

The linear Benesi-Hildebrand, Scott and Scatchard plots for all fluoroquinolone – DNA systems have been obtained. In Fig. 3 there are presented a Benesi-Hildebrand plot and a Scott plot for enoxacin – DNA system. The obtained results are summarized in Table 1. As we can notice, the values for the binding constant of these drugs to DNA obtained by the three methods do not differ too much. However, the binding constant of enrofloxacin to DNA is with an order of magnitude higher than the binding constant from other two fluoroquinolone – DNA systems.




(a) (b)

Fig. 2 – The absorption spectra for enoxacin (a) and enrofloxacin (b) in presence of DNA.




(a) (b)

Fig. 3 – The Benesi-Hildebrand (a) and Scott (b) plots for the enoxacin – DNA system.

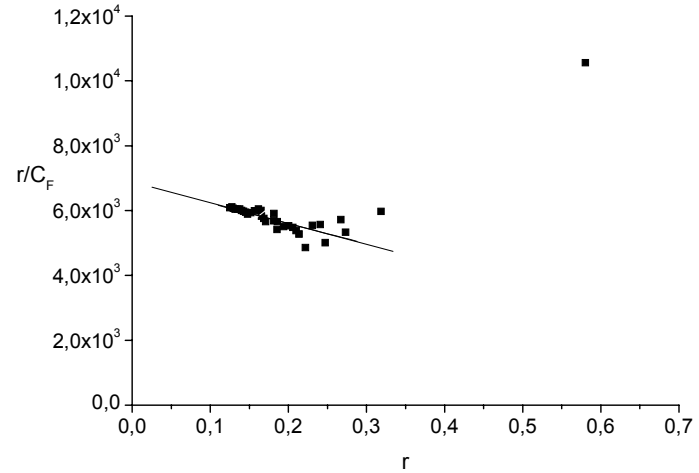



Fig. 4 – The Scatchard plot for norfloxacin – DNA system (the straight line of this plot corresponds to values of P/D ratios between 0.5 and 3.5 and was obtained by linear regression of experimental data, after elimination points that correspond at P/D<0.5).

In the absence of the presumption that a 1:1 drug – DNA complex is formed and assuming that there is one single class of non-interacting binding sites that do not exhibit cooperative behaviour, the experimental data were fitted through the linear Scatchard plot,

$$\frac{r}{C_F} = (n - r) \cdot K \tag{4}$$

or through a non-linear regression:

$$r = \frac{n \cdot K \cdot C_F}{1 + K \cdot C_F} \tag{5}$$

where $r$ is the binding ratio ($r = \frac{C_B}{[DNA]}$), $C_F$ and $C_B$ are the concentrations of free, respectively bound drug, *[DNA]* is DNA concentration, $n$ is the number of binding sites and $K$ is the drug – DNA binding constant.[1,3,7]

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

$$C_B = C^0 \cdot \frac{A_0 - A}{A_0} \tag{6}$$

$$C_F = C^0 - C_B \tag{7}$$

The Scatchard plot showed in Fig. 4 attest the presence of two processes of binding in the norfloxacin – DNA system. The linear part of this plot with a negative slope (corresponding to P/D ratios in the range 0.5 – 3.5) is characteristic for non-cooperative binding to one class with $n$ equivalent sites. Considering only this linear segment, the binding constant and the number of binding sites have been obtained. At low values of ratio *P/D* (*i.e.* high levels of binding ratio $r$), the large deviations from the linearity have been observed, the deviations indicating the existence of cooperative interactions, the presence of different classes of binding sites or multiple contacts of binding. In all fluoroquinolone – DNA systems, two binding processes have been observed.

Fitting the values of r that correspond to the linear part of Scatchard plot according to the non-linear regression equation allowed the determination of the binding constant and the number of binding sites for process (II). In Fig. 5 there is presented the non-linear regression of data for enoxacin – DNA system. The results obtained for the binding parameters by the linear and non linear models are summarized in Table 1.

The binding constant for drug – DNA interaction was also evaluated in accordance with the method proposed by Wolfe *et al.,*[8] using the equation:

$$\frac{[DNA]}{\Delta\varepsilon_{app}} = \frac{[DNA]}{\Delta\varepsilon} + \frac{1}{K \cdot \Delta\varepsilon} \quad (8)$$

where $\Delta\varepsilon_{app}=\varepsilon_{app}-\varepsilon_F$, $\Delta\varepsilon=\varepsilon_B-\varepsilon_F$, $\varepsilon_{app}$, $\varepsilon_F$ and $\varepsilon_B$ are the apparent, free and bound drug absorption coefficients. The obtained results are summarized in Table 1.

It is noted that the binding process (I), which occurs at low levels of P/D ratios, is analyzed in terms of Benesi-Hildebrand, Scott and Scatchard (linear and non-linear) methods and it is characterized by a binding constant lower than the binding process (II), which occurs at medium levels of P/D ratios and which is analyzed by Wolfe method.

In order to clarify the mode of binding of these fluoroquinolone to DNA, the viscosity measurements were carried out on DNA by varying the concentration of drug at 22°C, using an Ubbelohde viscometer. It has already been proven that the binding of a classical intercalation demands a space of adjacent base pairs, large enough to accommodate the bound ligand,[9] causing the elongation to DNA polymer and, therefore, an increase in viscosity. In contrast, drugs that bind in the grooves of DNA, under the same conditions, cause lower growth or no changes in DNA solution viscosity.[10]

Groove binding typically lead to subtle changes in the structure and the DNA remains in an unperturbated B form. In contrast, intercalation, in which a planar ligand moiety is inserted between adjacent base pairs, results in a substantial change in DNA structure and causes lengthening, stiffening and unwinding of the helix. Viscosity is proportional to $L^3$ for rod-like DNA of length L. The intercalator dramatically increases the lenght of DNA, resulting in an increased viscosity. The groove binder, in contrast, does not lengthen the DNA helix and does not increase the viscosity of DNA solutions.[11]

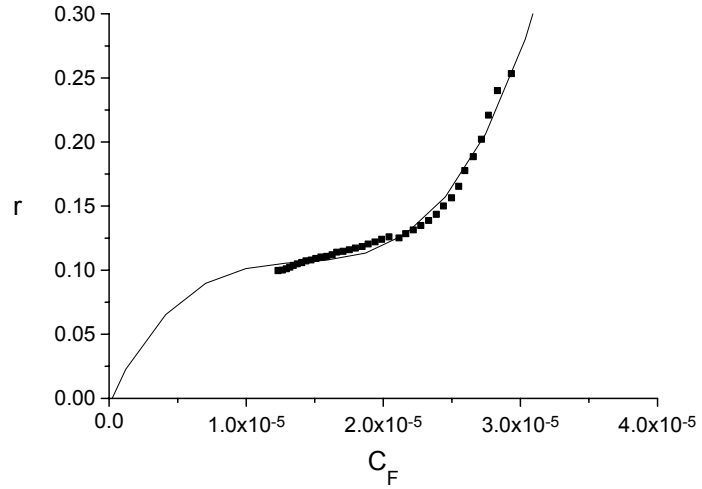



Fig. 5 – Fitting of the binding data of enoxacin – DNA system from non-linear regression.

*Table 1*

The results of the binding parameters in fluoroquinolone – DNA system

| Method \ Compound | enoxacin | | norfloxacin | | enrofloxacin | |
|---|---|---|---|---|---|---|
| | K, $M^{-1}$ | n | K, $M^{-1}$ | n | K, $M^{-1}$ | n |
| Benesi-Hildebrand, eqn. 1 | $1.66 \cdot 10^4$ | - | $1.16 \cdot 10^4$ | - | $1.23 \cdot 10^5$ | - |
| Scott, eqn. 2 | $2.04 \cdot 10^4$ | - | $1.07 \cdot 10^4$ | - | $1.04 \cdot 10^5$ | - |
| Scatchard, eqn. 3 | $1.81 \cdot 10^4$ | - | $0.89 \cdot 10^4$ | - | $1.15 \cdot 10^5$ | - |
| linear Scatchard, eqn. 4 | $4.71 \cdot 10^4$ | 0.44 | $0.94 \cdot 10^4$ | 0.98 | $0.92 \cdot 10^4$ | 1.51 |
| non-linear Scatchard, eqn. 5 | $4.56 \cdot 10^4$ | 0.45 | $0.98 \cdot 10^4$ | 0.97 | $0.89 \cdot 10^4$ | 1.48 |
| Wolfe, eqn. 8 | $9.29 \cdot 10^5$ | - | $4.27 \cdot 10^5$ | - | $2.86 \cdot 10^5$ | - |

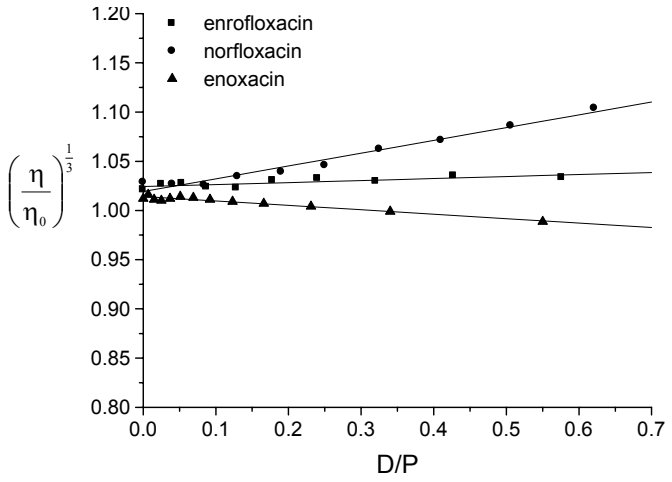



Fig. 6 – The effect of the amount of drug on the reduced relative viscosity of DNA solution at 22°C.

The reduced relative viscosity values were calculated on the basis of the flow time of DNA – drug mixtures (t) and the flow time of aqueous solution ($t_0$), using the following equation:

$$\eta = \frac{t}{t_0} \tag{9}$$

in accordance with the theory of Cohen and Eisenberg.[11] The viscosity data were plotted as $\left(\frac{\eta}{\eta_0}\right)^{\frac{1}{3}}$ versus the binding ratio $\left(r=\frac{D}{P}\right)$, where η represents the viscosity of DNA – drug mixtures and $\eta_0$ represents the viscosity of DNA alone. As shown in Fig. 6, the addition of norfloxacin, enoxacin and enrofloxacin to DNA caused no appreciable viscosity change in our experiments. In these conditions, the major binding mode of these drugs with DNA was evaluated to be the groove binding.

## EXPERIMENTAL

Norfloxacin, enoxacin, enrofloxacin and calf thymus DNA have been obtained from Sigma Aldrich, Germany. The stock solutions of drugs and DNA have been prepared in doubly distilled water. The concentrations of these solutions of reagents have been determined by means of the molar absorption coefficients: $\varepsilon_{275nm}$=37500$M^{-1}cm^{-1}$ for drugs and $\varepsilon_{260nm}$=6600$M^{-1}cm^{-1}$ for DNA.

The absorption spectra were recorded on a Lambda 25 UV-Vis spectrophotometer from Perkin-Elmer, USA, at room temperature, using the quartz cell. The absorption titrations with DNA were conducted by fixing the concentration of drugs while varying the DNA concentration.

The viscosity studies were performed with an Ubbelohde viscometer at a temperature of 22°C. The samples were prepared by adding an appropriate amount of drugs to stock solution of DNA so as to obtain the D/P ratios in the range of 0.05 – 0.7. The flow times of samples were repeatedly measured by using an electronic stopwatch and each point measured was the average of at least five readings.

## CONCLUSIONS

There have been determined the binding parameters of norfloxacin, enoxacin and enrofloxacin to calf thymus DNA. The binding data have been processed using the Benesi-Hildebrand, Scott, Scatchard and Wolfe methods.

The analysis of the results of the binding data of these drugs to DNA indicated two types of processes: an internal binding process, which would imply the drug intercalation between base pairs of DNA and an external binding process, which would imply the binding of drugs in the grooves from the nucleic acid structure. Predominant is the external binding, the binding constant of this process being with an order of magnitude greater than the binding constant calculated by the Benesi-Hildebrand, Scott and Scatchard methods, which require that forms a 1:1 drug – DNA complex.

## REFERENCES

1. L.E. Vîjan and M. Conci, *Macromol. Symposia*, **2008**, *265*, 260-267.
2. L.E. Vîjan, *OAM-RC,* **2009**, *3*, 60-64.
3. L.E. Vîjan and I. Puică, *OAM-RC,* **2010**, *4*, 2129-2131.
4. H. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc*., **1949**, *71*, 2703-2707.
5. R.L. Scott, *Rec. Trav. Chim*., **1956**, *75*, 787-789.
6. G. Scatchard, *Ann. N. Y. Acad. Sci*., **1949**, *51*, 660-672.
7. E. Volanschi and L.E. Vijan, *Rev. Roum. Chim.*, **2001**, *46*, 163-173.
8. A. Wolfe, G.H. Shimer and T. Meehan, *Biochemistry,* **1987**, *26*, 6392-6396.
9. L.S. Lerman, *J. Mol. Biol.*, **1987**, *3*, 18-30.
10. D. Dongchul and J.B. Chaires, *Bioorgan. Med. Chem.*, **1995**, *3*, 723-728.
11. G. Cohen and H. Eisenberg, *Biopolymers,* **1969**, *8*, 45-55.