Journal of Natural Sciences and Mathematics
Qassim University, Vol. 13, PP 24-41 (January 2020)

Binding of some antibacterial fluoroquinolones with ds-DNA, β-cyclodextrin and hemoglobin: spectroscopic and voltammetric studies

Ibrahim A. Alhagri[1,2]*, Yassien M. Temerk[3], Sadeq M. Al Hazmy[1], Mustafa M. Kamal[3], Mohamed S. Ibrahim[3], Nabil A. Alhemiary[2,4], Ahmed N. Al-Hakemi[1,2] and Hassan Elobeid. Ahmed[1]

[1]Department of Chemistry, College of Science, Qassim University, Buraidh, Saudi Arabia.
[2]Department of Chemistry , Faculty of Science , Ibb University, P. O. Box 70270, Ibb, Yemen
[3]Chemistry Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
[4]Department of Chemistry, College of Science & Arts – Sharurah, Najran University, Sharurah, Saudi Arabia

Email (I.A. Alhagri): EB.AHMED@qu.edu.sa , ialhagri1@gmail.com

Abstract: The interaction of some antibacterial fluoroquinolones (FQ), Difloxacin (DIX), Danofloxacin (DANO) and Orbifloxacin (ORBX) drugs, with ds-DNA, β-Cyclodextrin (β-CD) and hemoglobin was investigated by UV-Vis spectroscopy, cyclic voltammetric and differential pulse voltammetry. The absorption spectral and electrochemical data show that DIX or DANO or ORBX, acting an intercalator**,** is inserted into the base-stacking domain of the ds-DNA. Structural effects of investigated fluoroquinolones on their binding to ds-DNA were discussed. The interaction of inclusion complex of DIX- β-CD, DANO- β-CD and ORBX- β-CD with ds-DNA is reported. The nature of the process of hemoglobin and DIX, DANO and ORBX was clarified. The binding affinity and thermodynamic parameters of DIX, DANO and ORBX with the host molecule were computed. The calibration graph for the determination of ds-DNA, β-CD and hemoglobin was obtained by the decrease in the differential pulse peak current of the investigated fluoroquinolones in the presence of the host molecule.

Keywords: Antibacterial Fluoroquinolones, ds-DNA, β-cyclodextrin, UV-Vis spectroscopy, Voltammetry.

1. Introduction. Fluoroquinolones are important antibacterials developed in recent years which have wide applications in veterinary and human medicine especially for the treatment of respiratory diseases, urinary tract infections and enteric diseases [1-3]. Fluoroquinolones develop its pharmacological activity via specific inhibition of sub-unit A of the bacterial gyrase, an enzyme that controls DNA shape [4]. Although the exact mechanism of this action is still unclear, there is evidence that fluoroquinolones interact directly with DNA in synergy with gyrase enzyme [5,6]. Such interaction undoubtedly contributes to the desired antibacterial activity but it can

also be responsible at least in part, for the unwanted toxic effects. Contributions to deeper insight into the mechanism of interaction of this class of antibiotics with DNA might be important for a better understanding of their therapeutic efficacy. Electrochemical and spectroscopic methods can provide useful information on drug-DNA interaction and elucidate some mechanisms of the drug action in Vivo [7]. In this context several methods have been reported only for the determination of fluoroquinolones such as spectrophotometry and spectrofluorimetry [8-10], fluorescence, capillary electrophoresis, high performance liquid chromatography [11-16] and electroanalytical methods [17-19]. As well known, cyclodextrins (CD) are a kind of polysaccharides made up of six to eight D-glucose monomers connected at 1 and 4 carbon atoms, and they can provide a hydrophobic cavity in aqueous solution for the hydrophobic molecules or groups to form inclusion complexes [20]. This important characteristic makes it possible for cyclodextrines to be used as biomimetic enzyme models [21], drug delivery systems [22] and electrode reaction modifiers [23]. Both DNA and cyclodextrin are common in having hydrophobic coat / hydrophobic core structure. Thus, an aromatic ring either stretching between nucleobase pairs or incorporated in CD cavity is the main driving force for the binding of an intercalator into double-stranded DNA and a guest molecule to CD, respectively. These stimulate us to investigate the complexing properties of CD and DNA as hosts for antibacterial drug substances, DIX, DANO and ORBX (Fig. 1) as guest models.

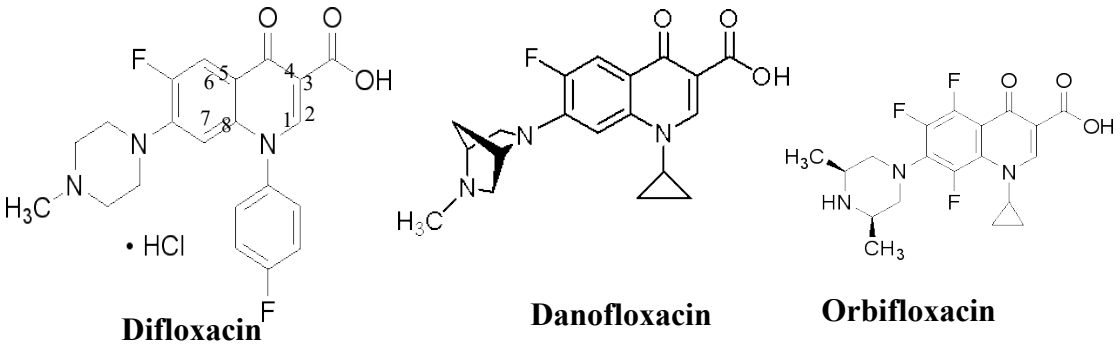

Fig.1. Chemical structure of antibacterial fluoroquinolones.

Therefore, and in continuation of our work [24,25] on the binding of small molecules with biomacromolecules, the present paper is concerned with the spectroscopic and voltammetric studies of the interaction of some antibacterial fluoroquinolones, DIX, DANO and ORBX with β-CD and ds-DNA. Furthermore, the binding of hemoglobin, an oxygen carrier, with the investigated fluoroquinolones as guest molecules was also investigated. The binding constant (k_b) and thermodynamic

parameters (ΔG^0, ΔH^0 and ΔS^0) for the interaction of DIX, DANO and ORBX with the host molecules were determined.

2. Experimental

2.1. Instrumentation. The ultraviolet and visible absorption spectra were obtained using a Perkin Elmer (Lambda 35) spectrophotometer. Differential pulse stripping voltammetry (DPSV) and cyclic voltammetry (CV) were carried out using a PAR Model 264A polarographic analyzer-stripping voltammetry in conjunction with a PAR Model 303A hanging mercury drop electrode (HMDE) and a PAR Model 305 magnetic stirrer. An Ag/AgCl saturated KCl as a reference electrode and Pt wire auxiliary electrode were used.

2.2. Chemicals and reagents

Salmon double strand deoxyribonucleic acid (ds-DNA), β-Cyclodextrin (β-CD), Hemoglobin, DIX, DANO and ORBX were obtained from Sigma and were used without further purification. Stock solution of ds-DNA was prepared by dissolving 0.1g of ds-DNA in 100 ml autoclaved double distilled water and stored at 4ºC [26] and discarded after no more than 4 days. The concentration of the stock solution of ds-DNA (1.86×10^{-3}M in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient (ε) as 6600 $M^{-1}cm^{-1}$. Stock solution of β-CD was prepared by dissolving the desired weight in the solution of Britton- Robinson (BR). Stock solutions of hemoglobin were stored at temperature of 5ºC. Solutions containing different concentrations of DIX, DANO and ORBX were prepared by dissolving a required weight of the investigated compound in twice distilled water and stored in the dark at 4 ºC. More dilute solutions were prepared daily with twice distilled water just before use. The supporting electrolyte was Britton-Robison (BR) buffer prepared in the usual way, by adding appropriate amount of sodium hydroxide (0.4M) to an orthophosphoric acid, boric acid and acetic acid mixture (0.08M). All chemicals were reagent grade (Merck, Darmstadt). Double distilled deionized water was used to prepare the solutions.

2.3. Voltammetric DPSV and CV measurements

For voltammetric measurements the test solution was placed in a polarographic cell of volume 10 ml and deoxygenated by passing nitrogen for 15 min. Instruments setting for DPSV (unless otherwise stated) were: scan rate 5 mVs^{-1}, modulation amplitude100 mVpp, adsorption time (with stirring) 60 sec (unless otherwise stated) plus a 15 sec as quiescent period. Cyclic voltammetric response was obtained using scan rate 100 mVs^{-1} (unless otherwise stated). Keeping both the concentration of the investigated fluoroquinolones and the total volume of solution constant (10 ml) then DPSV and CV procedures were carried out, while varying the ds-DNA or β-CD or hemoglobin concentration.

2.4. Spectrophotometric measurements

The spectral shifts of DIX, DANO and ORBX were studied upon addition of various ds-DNA. A Perkin Elmer (lambda 35) spectrophotometer was used for all experimental.

3. Results and Discussion

3.1. Interaction antibacterial fluoroquinolones with ds-DNA:

A systematic study on the interaction of antibacterial fluoroquinolones DIX, DANO and ORBX with ds-DNA was studied in Britton buffer solution using spectroscopic method. The absorption spectra of the investigated fluoroquinolones in absence and presence of ds-DNA were represented in Fig. 2. Some important changes in the adsorption spectra were observed upon the addition of ds-DNA. The absorption bands at 210.17 nm, 279.76 nm and 316 nm showed decrease in the peak intensities on increasing concentration of ds-DNA. Also the bands at 210 nm and 279 nm shifted to longer wavelength. Such pronounced hypochromism and bathchromism were suggested to be due to a strong intercalation of the investigated fluoroquinolones into ds-DNA base pairs.

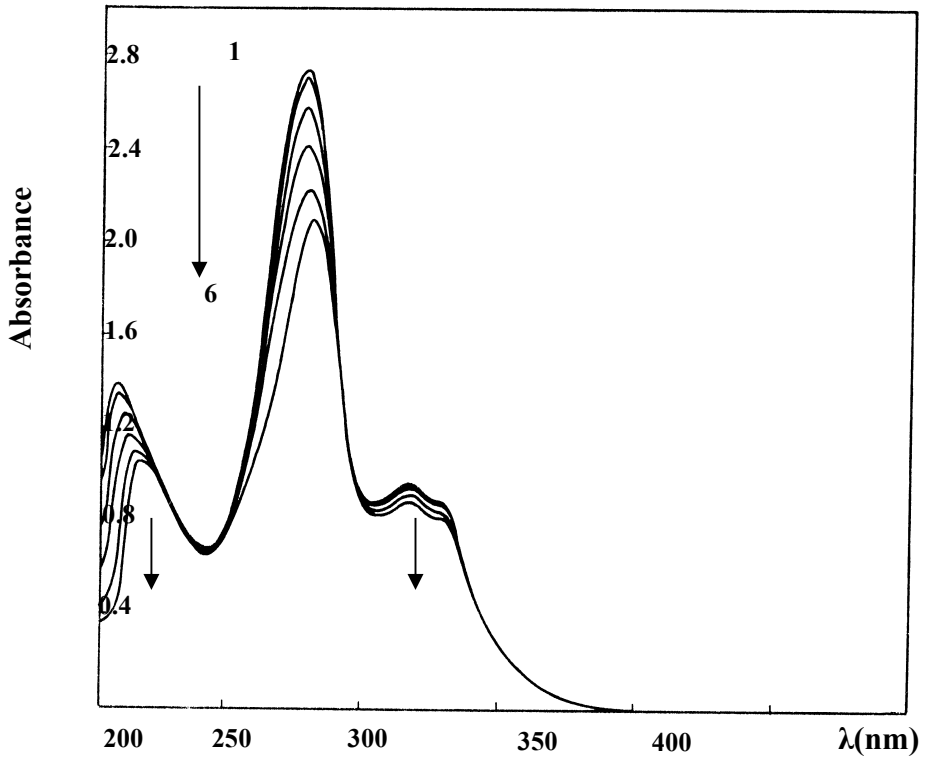

Fig.2. UV-Vis spectra of 7.14×10^{-5}M DIX in B.R buffer (pH 5) in the absence (1) and presence of (2) 0.51, (3) 1.02, (4) 2.05, (5) 3.08 and 3.60×10^{-4}M ds-DNA.

This implies a close proximity of fluoroquinolones chromophore to the ds-DNA base pairs i.e a strong overlap between the electronic states of the intercalating chromophore and that of the ds-DNA bases occurs. The association of the ds-DNA

and guest fluoroquinolones molecule is governed by the following thermodynamic equilibrium:

$$FQ + \text{ds-DNA} \rightleftharpoons \text{ds-DNA.FQ} \quad (1)$$

and thus the binding constant is defined as:

$$K_b = [\text{ds-DNA.FQ}]/ [FQ][\text{ds-DNA}] \quad (2)$$

assuming the most common host guest ratio of 1:1.

The binding constant was determined from the differences in absorbance (A) due to ds-DNA intercalation using the following equation [27]:

$$\frac{A}{A-A^0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} X \frac{1}{K_b[\text{ds-DNA}]} \quad (3)$$

In which A^0 and A are the absorbances at λ_m of the free and complex respectively, ε_G and ε_{H-G} are the absorption coefficients of the guest and complex respectively. The value of k demonstrated that DIX, DANO and ORBX bind to ds-DNA with a high association constant (Table 1).

The cyclic voltammetry and differential pulse voltammetry were also employed in examination of the mode of three fluoroquinolones with ds-DNA. In this context CV behaviour of three fluoroquinolones in the absence and presence of ds-DNA are represented in Fig. 3. When ds-DNA is added to a solution of the investigated fluoroquinolone, marked decreases in the peak current heights occur. In terms of this result, it seems that binding of three fluoroquinolones to the large , slowly diffusing ds-DNA, caused the decrease in the peak current of DIX, DANO and ORBX in the presence of ds-DNA, which results in the considerable decrease in the apparent diffusion coefficient of the FQ–ds-DNA adduct. This is emphasized from the decrease in the slope of the linear i_p-$\upsilon^{1/2}$ plots ($R \geq 0.991$). From the values of slopes, the diffusion coefficient (D_f) of the free fluoroquinolones and the bound fluoroquinolone were found (Table 1). The changes in current upon addition of ds-DNA can be explained in terms of diffusion of an equilibrium mixture of free and bound fluoroquinolone to the electrode and that can be used to quantify the binding of investigated fluoroquinolone to ds-DNA. In this context current titrations were performed by keeping the concentration of investigated fluoroquinolone constant while varying the concentration of ds-DNA using DPSV (Fig.4). The current titration was described by the following equation:

$$\text{Log}\frac{1}{[\text{ds-DNA}]} = \text{Log } K_b + \text{Log}\frac{I_{H-G}}{I_G - I_{H-G}} \quad (4)$$

Where K_b is the apparent binding constant, I_G and I_{H-G} are the peak current of the free guest (G) and the complex (H-G) respectively.

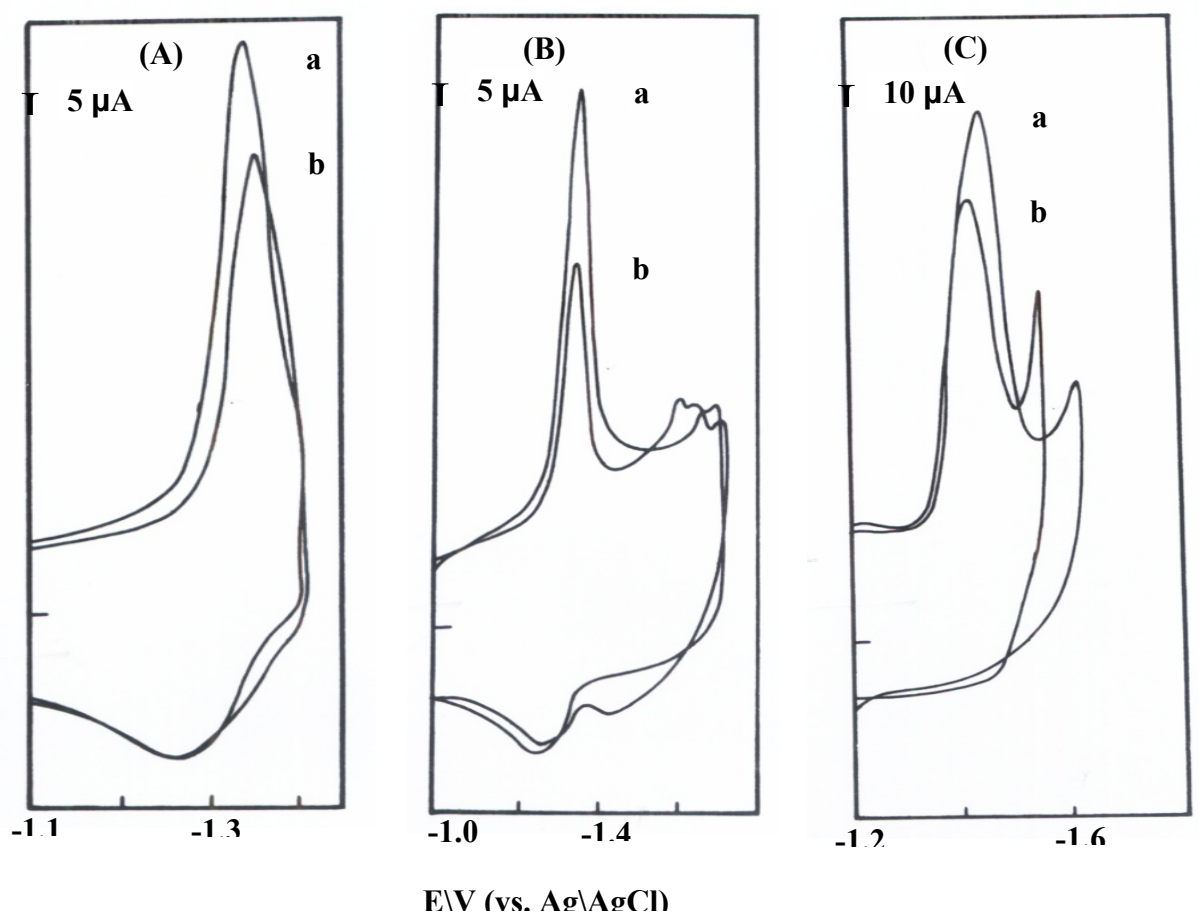

Fig.3. (A): Cyclic voltammograms of (a): 3.98×10^{-6}M DIX and (b): a + 3.57 x 10^{-5}M of ds- DNA at pH 9 and scan rate of 200 mVs^{-1}, (B): Cyclic voltammograms of (a): 9.9×10^{-7}M DANO and (b): a + 3.57×10^{-5}M of ds-DNA at pH 10 and scan rate of 200 mVs^{-1}, (C): 3.98×10^{-6}M Orbx and (b): a + 1.07×10^{-5}M DNA of ds- DNA at pH 7 and scan rate of 200 mVs^{-1}.

The plot of log1/[DNA] versus log[I_{H-G} / (I_G-I_{H-G})] is linear with intercept of log K_b. The binding constant of this complex was evaluated according to Eq(4) and cited in Table 1. The values of k are in good agreement with those obtained from absorption spectra. It is easily found that the result of absorption spectra is consistent with voltammetric technique.

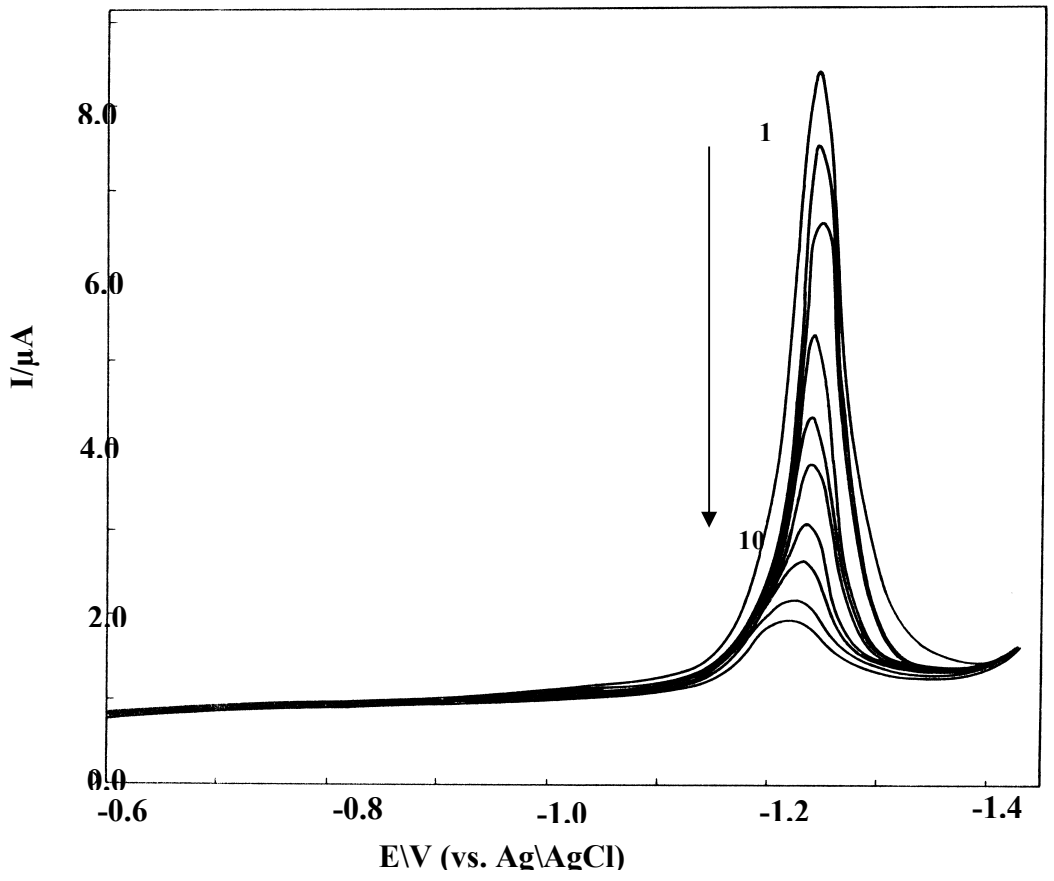

Fig. 4. DPSV of 0.69 × 10^{-6}M DIX in the absence (1) and presence of (2) 2.63, (3) 4.34, (4) 7.67, (5) 9.29, (6) 10.80, (7) 11.49, (8) 12.52, (9) 13.46 and (10) 14.31× 10^{-6}M ds-DNA, pH 9, scan rate 5 mVs^{-1} and pulse amplitude 100 mVpp.

The binding constants of FQ-ds-DNA system at different temperatures were studied using DPSV. It can be observed that the values of K increase as the temperature increases, revealing the influence of temperature on stability of the complex of FQ-ds-DNA. The integrated form of Vant Hoff equation (5) permits to calculate the values of enthalpy and entropy changes depending on variation of the binding constant with temperature.

$$\ln K_b = -\Delta H^o/RT + \Delta S^o/R \qquad (5)$$

The Vant Hoff plots for the complex of FQ-ds-DNA shows a linear behaviour. The relative thermodynamic parameters were calculated and cited in Table 1. The positive value of enthalpy change indicates that the interaction process of DIX, DANO and ORBX with ds-DNA is endothermic. The change of entropy is also positive. This behaviour indicates that the complexation causes an increase in transitional and rotational degree of freedom of the complexed molecular.

In the binding system of intercalations, Gibbs free change ΔG^0 is a very important parameter, which reflects the binding degree and the stability of the formed complex. ΔG^0 can be calculated according the following equation.

$$\Delta G^0 = -RT \ln K_b \quad (6)$$

Where the R is the gas constant with the value 8.31 J K^{-1} mol^{-1}, T the absolute temperature, K_b the binding constant. The free energy change for the interaction involved in the complex is also computed using the values of Helmholtz free energy ΔH^o, and entropy change ΔS^o, of the intercalation of investigated fluoroquinolones and ds-DNA.

$$\Delta G^0 = \Delta H^o - T\Delta S^o \quad (7)$$

The calculated value of the change in Gibbs free energy using Eqs 6 and 7 are of the same order of magnitude (Table 2). The value of ΔG^0 was found to be negative, revealing that the binding process is favorable and spontaneous.

On comparison of the binding affinity and thermodynamic parameters of DIX-ds-DNA, DANO-ds-DNA and ORBX-ds-DNA systems we observed in the following order of tendencies. Difloxacin and Danofloxacin compounds displayed high binding affinity to ds-DNA than Orbifloxacin (Tables 1 and 2). At the same time the magnitudes of the changes in ΔG^0, ΔH^o and ΔS^o decrease in the same order: DIX-ds-DNA ≅ DANO-ds-DNA > ORBX-ds-DNA. On the other hand the calculated values of k and ΔG^0 at different temperatures indicated that the binding process of Difloxacin and Danofloxacin with ds-DNA is more favorable and spontaneous than Orbifloxacin (Table 2).The significant differences of the binding constant and thermodynamic parameters for the interaction of the investigated fluoroquinolones with ds-DNA are highly interesting considering very small structural difference between these antibacterial compounds. This relates to the position of fluorine atom of the quinolone moiety. Fluoroquinolones having only C-6 fluorine atom (Difloxacin and danofloxacin) is more efficient in enhaning the hydrophobic interaction with ds-DNA than those having fluorine substituents at C-5, C-6 and C-8 positions (Orbifloxacin). The obtained results showed that different chemical substituents of fluorine on quinolone yield distinct changes in the binding behaviour of fluoroquinolones with ds-DNA. The results indicated also that a slight change in chemical structure causes significant changes in the thermodynamic parameters of FQ –ds-DNA systems and in their biological and clinical properties.

3.2. Interaction of the inclusion complex of FQ-β-CD with ds-DNA

UV-Vis spectra were used to testify the formation of the inclusion complex of fluoroquinolone- β-CD. The absorption spectra of investigated fluoroquinolone upon increasing the concentration of β-CD showed an increase in the absorbance (Fig. 5).

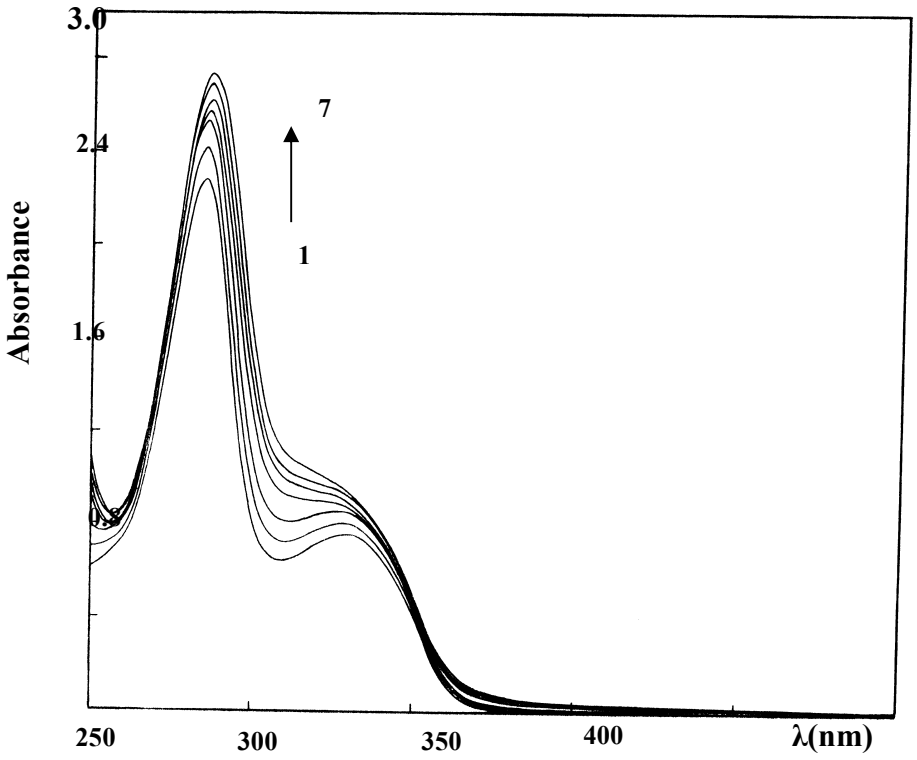

Fig. 5. UV-Vis spectra of 5.71 x 10^{-5}M Orbx in BR buffer (pH 7) in the absence (1) and presence of (2) 0.57, (3) 1.11, (4) 2.28, (5) 2.85, (6) 3.42 and (7) 3.71 × 10^{-3}M β-CD.

Similar behaviour was observed for a hypatoprotectant drug thioctic and β-CD system [28] as well as for anthracene phenothiazine and thianthren- β-CD system [29]. These changes in the absorbance values are believed to result from changes in the solvent micro-environment upon inclusion of the solute. The changes in molar absorbance values may result from the loss of hydrogen binding that accompanies the transfer of the guest molecule from the solution of cyclodextrin cavity.

The nature of the electrochemical process of the investigated fluoroquinolones in presence of β-cyclodextrin was also investigated. In presence of different concentrations of β-CD, the reduction peak current of the investigated fluoroquinolones is strongly affected revealing that an interaction has occurred between fluoroquinolone and β-CD (Fig. 6).

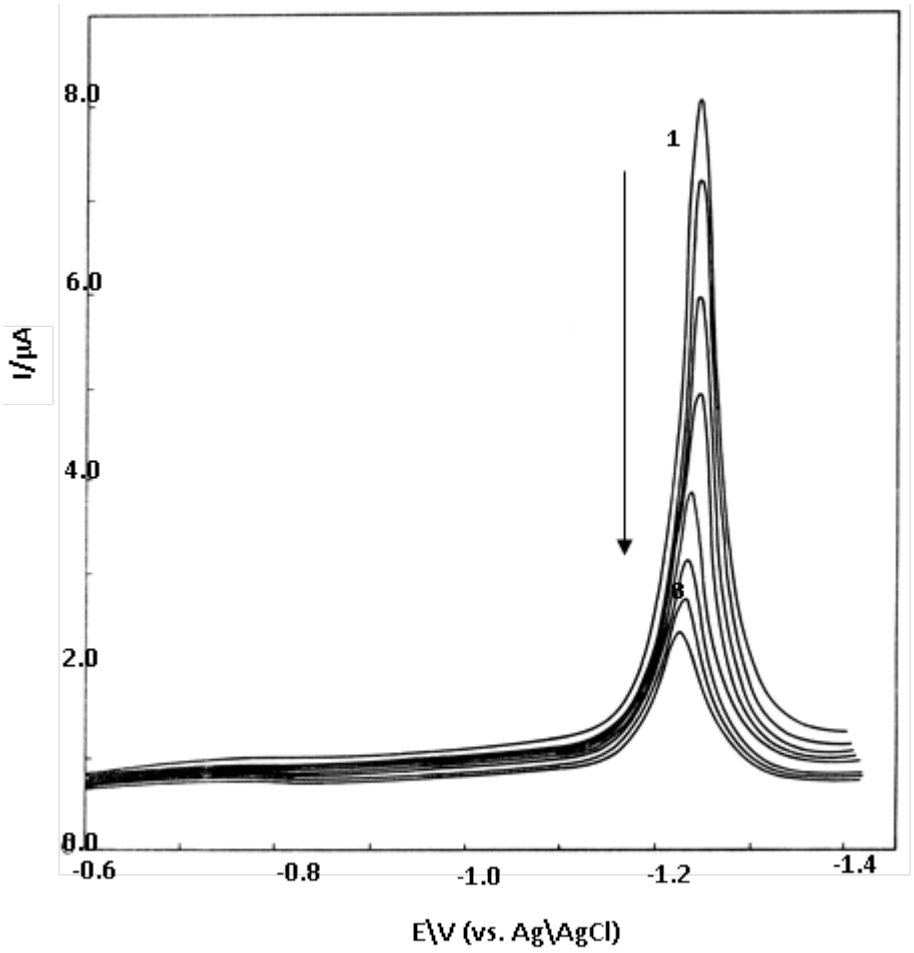

Fig. 6. DPSV of 6.95x10^{-7}M DIX in BR buffer (pH 9) in the absence (1) and presence of (2) 1.98, (3) 3.92, (4) 5.82, (5) 7.60, (6) 8.96, (7) 10.24 and (8) 11.48 × 10^{-4}M β-CD, pH 9, scan rate 5 mVs^{-1} and pulse amplitude 100 mVpp.

The decrease of the peak current observed upon addition of β-CD is due to the lower diffusion coefficient of FQ- β-CD complex compared to that of the free guest. This is emphasized from the decrease in slope of the linear i_p-$\upsilon^{1/2}$ plots. From the value of slope, the diffusion coefficient of the free fluoroquinolone and the bound fluoroquinolone with β-CD was determined (Table 1). The formation constant and Gibbs free energy of the inclusion complex of FQ- β-CD were also determined from spectrophotometric and voltammetric results using Eqs 3, 4 and 6 respectively (Table 1). There is fair agreement, within experimental errors, between Kand ΔG^o values of the inclusion complex obtained from both voltammetric and spectrophotometric techniques.

The DPSV of the inclusion complexes of FQ-β-CD in presence of ds-DNA is interesting. The addition of ds-DNA to the inclusion complex of FQ-β-CD resulted in the decreasing of the cathodic reduction peak of fluoroquinolone-β-CD and shifted it to a less negative value (Fig.7). If the inclusion complex does not decompose, the reduction potential would have not change by adding ds-DNA. But in fact the redox potential shifted to the potential at which the three fluoroquinolones were oxidized or

reduced in absence and presence of ds-DNA as shown in Fig. 7. This suggested that the inclusion complex of FQ-β-CD decomposed when it interacted with ds-DNA. Accordingly, the interaction of the investigated fluoroquinolone with ds-DNA is more favored and thus the β-CD is replaced by ds-DNA to form intercalates with three fluoroquinolones. In this context the binding affinity increases in the sequence: FQ-ds-DNA ≅ FQ-β-CD-ds-DNA > FQ-β-CD. On comparing the change in Gibbs energy values (ΔG^0), enthalpy and entropy for the binding of the fluoroquinolone complexes, we observed that the different values of thermodynamics parameters decrease in the same order (Tables 1 and 2). This confirmed the experimental phenomena that the inclusion complex of FQ-β-CD decomposed when it binds with ds-DNA.

3.3. Interaction of Difloxacin, Danofloxacin and Orbifloxacin with Hemoglobin

The interaction of DIX, DANO and ORBX as a kind of biochemical drug with hemoglobin at a HDME is reported for the first time. An addition of different concentrations of hemoglobin to the investigated fluoroquinolone and subsequent scanning over the potential range -0.6 to -1.6V produced no new waves but only a decrease in peak current for the reduction of the investigated fluoroquinolone (Fig. 8).

Under the above conditions hemoglobin alone shows no electrochemical response. In this context, hemoglobin causes the equilibrium concentration of the three fluoroquinolones to decrease, which results in the decrease of peak current. The decrease of the peak current observed upon addition of hemoglobin can be explained in terms of diffusion of an equilibrium mixture of free and bound fluoroquinolone to the electrodes which can be used to quantify the binding of the three fluoroquinolones to hemoglobin. This is emphasized from the decrease in the slope of the linear i_p-$\upsilon^{1/2}$ plots. From the values of slope, the diffusion coefficients of the free fluoroquinolone and the bound fluoroquinolone with hemoglobin were computed and cited in Table 1. The decrease in the DPSV current enables us to calculate the binding constant of the complex formed between the investigated fluoroquinolones and the studied host molecules. In this context the plot of log 1/ [hemoglobin] versus log $[I_{H\text{-}G}/I_G\text{-}I_{H\text{-}G}]$ is linear with the intercept of log K. The calculated values of ΔG^o were found to be negative, indicating that the binding process is favorable and spontaneous (Table 1). Based on the above mentioned results of CV and DPSV that the addition of hemoglobin to the investigated fluoroquinolone produced no new reduction peaks. The conclusion that can be drawn is that the three fluoroquinolones interacting with hemoglobin form an electrochemically inactive complex, which should be called a supramolecular complex and it cannot be reduced at the electrode surface.

3.4. Analytical Applications

3.4.1. Analytical aspects of the FQ-ds-DNA- interaction

We have shown in this paper that interactions of DIX, DANO and ORBX with ds-DNA can be conveniently studied by DPSV. In this context the decrease in peak current of DIX, DANO and ORBX resulted from the addition of ds-DNA into the investigated fluoroquinolones can be employed to determine the lower concentration

of ds-DNA. The peak current of the DPSV of the investigated fluoroquinolone was used as detection signal under conditions when free ds-DNA does not show any signal. Under the optimum conditions**,** the decreases in the DPSV peak current were linearly related to ds-DNA concentration in the range of 2.63 x 10^{-6}M to 1.08 x 10^{-5} M when the investigated fluoroquinolone concentration was fixed at 6.95 x 10^{-7}M. The limits of detection and quantitation of ds-DNA were cited in Table 3. The proposed method is simple, sensitive and rapid, and hence, can be applied to the determination of many kinds of ds- DNA.

3.4.2. Calibration graph for the determination of β-CD

The dependence of the DPSV peak current of DIX, DANO and ORBX upon successive additions of β-CD at HMDE can be employed to determine the concentration of β-CD. The variation of the decrease in peak current of investigated fluoroquinolones versus the β-CD concentration was represented by a straight line**.** The obtained LOD and LOQ values are cited in Table 3. The proposed method is simple, sensitive and rapid and hence can be applied to the determination of β-CD.

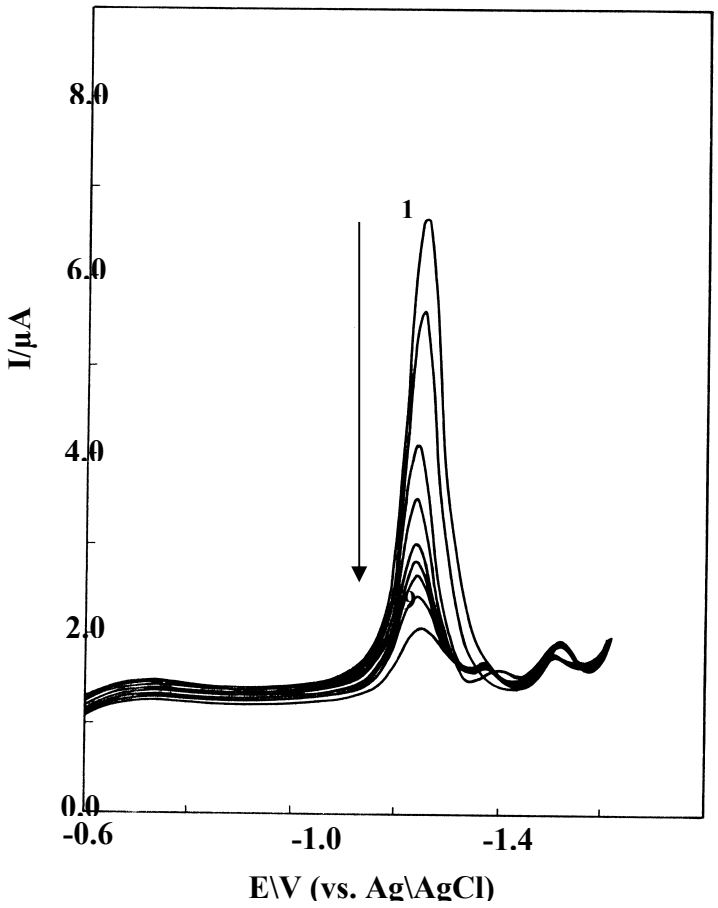

Fig. 7. DPSV of DANO (curve 1) in presence of β-CD (curve 2) and ds-DNA (curve 3-9), (1) 9.9 x 10^{-7}M DANO (2) 9.9 x 10^{-7}M DANO + 6.88 x 10^{-5}M β-CD, DANO-β-CD complex in presence of ds-DNA (3) 1.74, (4) 2.78, (5) 3.47, (6) 4.31, (7) 5.15, (8) 5.98 and (9) 6.81 × 10^{-6}M DNA, pH 10, scan rate 5 mVs^{-1} and pulse amplitude 100 mVpp.

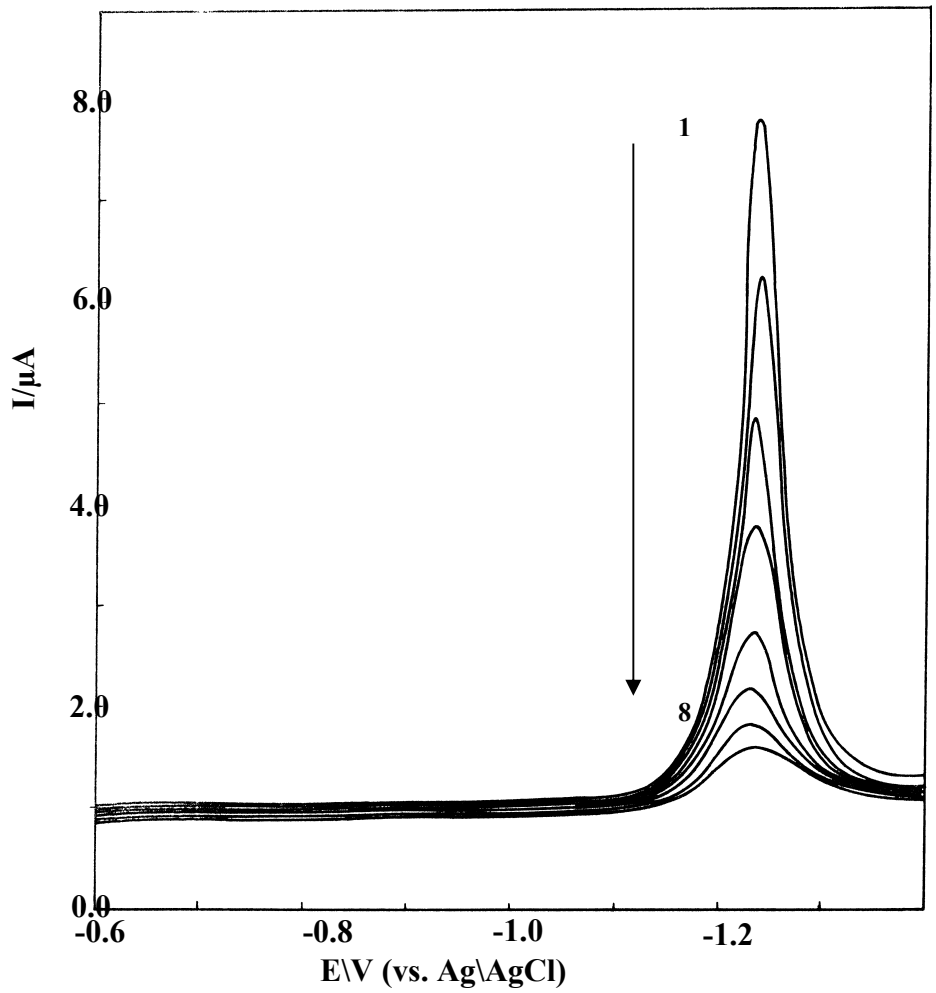

Fig. 8. DPSV of 6.9 x 10^{-7}M DIX in the absence (1) and presence of (2) 0.36, (3) 0.61, (4) 0.85, (5) 1.08, (6) 1.26, (7) 1.38 and (8) 1.50 × 10^{-6}M Hemoglobin, pH 9, scan rate 5 mVs^{-1} and pulse amplitude 100 mVpp.

3.4.3. Linear range of Hemoglobin determination

The calibration graph for the determination of hemoglobin was obtained by the decrease in the DPSV peak current of investigated fluoroquinolones in the presence of hemoglobin. There is linear range between reduction peak current of the investigated fluoroquinolones and the concentration of hemoglobin. For a simple sample, there is no problem to obtain result by directly applying the calibration plot. When the concentration of difloxacin is 6.95 x 10^{-7}M, we can directly determine 3.60 x 10^{-7}M hemoglobin concentration (Table 3). The proposed method may be useful for analysis of clinical samples.

4. Conclusion

In this paper, the electrochemical and UV-Vis spectroscopic results demonstrated the intercalation of DIX, DANO and ORBX, antibacterial drugs, with ds-DNA. The obtained results showed that different chemical substituents of fluorine on quinolone moiety yield distinct changes in the binding behaviour of fluoroquinolones with ds-DNA. The inclusion complex of FQ-β-CD decomposed when it interacted with ds-DNA. Hemoglobin interacts with the investigated fluoroquinolones and forms a non-electroactive supramolecular complex. This investigation may play an active part in biological and biomedical research in the future.

Acknowledgement

The authors gratefully acknowledge the financial support to this investigation by Alexander von Humboldt Stiftung, Germany.

Table 1: Diffusion coefficients, binding constants, standard Gibbs free energy, enthalpy and entropy changes for the binding of DIX, DANO and ORBX with ds-DNA, β-CD and hemoglobin at 298 K.

system	$D_f (10^{-6})$ ($cm^2.s^{-1}$)	$D_b (10^{-6})$ ($cm^2.s^{-1}$)	log K_b	log K_b	$-\Delta G^o$ ($k.J.mol^{-1}$)	$-\Delta G^o$ ($k.J.mol^{-1}$)	ΔH^o ($kJ.mol^{-1}$)	ΔS^o ($J.mol^{-1}K^{-1}$)
			a	b	a	b		
DIX-DNA	7.65*	5.40	5.250	4.320	29.964	24.660	51.004	274
DANO-DNA	4.21*	1.05	5.352	3.998	30.546	22.822	46.233	257
ORBX-DNA	0.88*	0.49	4.637	3.258	26.465	18.599	39.680	222
DIX-β-CD	----	5.47	3.660	3.024	20.889	17.263	44.814	222
DANO- β-CD	----	3.47	3.281	2.733	18.726	15.601	26.259	152
ORBX- β-CD	----	0.53	2.578	2.629	14.713	15.008	19.204	113
DIX- β-CD -DNA	----	----	5.291	----	30.198	----	52.202	276
DANO- β-CD - DNA	----	----	5.336	----	30.455	----	77.934	364
ORBX- β-CD - DNA	----	----	4.379	----	25.830	----	26.722	170
DIX-hemoglobin	----	5.90	6.191	----	35.381	----	----	----
DANO-hemoglobin	----	3.53	6.150	----	35.111	----	----	----
ORBX-hemoglobin	----	0.51	5.630	----	32.140	----	----	----

a: from the result of voltammetry, b: from the result of spectroscopy and *: diffusion coefficient for free fluoroquinolone

Table 2: Binding constants (K_b) and standard Gibbs free energy (ΔG^o) of fluoroquinolone systems calculated from the results of DPSV at different temperatures

System	Log K_b				-ΔG^o (kJ mol^{-1}) using equation (6)				-ΔG^o (kJ mol^{-1}) using equation (7)			
	278 K	288K	298K	308K	278 K	288K	298K	308K	278 K	288K	298K	308K
DIX-DNA	4.846	4.958	5.250	5.795	22.777	27.348	29.964	34.184	25.209	27.949	30.689	33.429
DANO-DNA	4.689	5.132	5.352	5.552	24.967	28.308	30.546	32.750	25.251	27.821	30.391	32.961
ORBX-DNA	4.138	4.440	4.637	4.641	22.033	24.491	26.465	27.376	22.069	24.289	26.509	28.729
DIX-β-CD	3.205	3.513	3.660	4.069	15.064	19.377	20.889	24.002	16.935	19.155	21.375	23.595
DANO- β-CD	3.068	3.139	3.281	3.582	16.335	17.314	18.726	21.129	16.019	17.539	19.059	20.579
ORBX- β-CD	2.329	2.481	2.578	2.676	12.401	13.685	14.713	15.785	12.222	14.221	14.486	15.616
DIX-β-CD-DNA	4.638	4.894	5.291	5.566	21.799	26.995	30.198	32.833	24.567	27.327	30.087	32.847
DANO- β-CD-DNA	4.238	5.138	5.336	5.744	22.565	28.341	30.455	33.883	23.314	26.954	30.594	34.234
ORBX- β-CD-DNA	3.873	4.125	4.379	4.365	20.622	22.753	25.830	25.748	20.563	22.263	23.963	25.663

Table 3: The limit of detection (LOD) and limit of quantitation (LOQ) for ds-DNA, β-CD and Hemoglobin using DPSV of DIX, DANO and ORBX as detection signals.

System	DNA		-CDβ		Hemoglobin	
	LOD (M)	LOQ (M)	LOD (M)	LOQ (M)	LOD (M)	LOQ (M)
Difloxacin	9.43×10^{-7}	3.14×10^{-6}	5.81×10^{-5}	1.93×10^{-4}	1.49×10^{-7}	4.96×10^{-7}
Danofloxacin	5.96×10^{-7}	1.98×10^{-6}	4.70×10^{-6}	1.56×10^{-5}	1.15×10^{-8}	3.83×10^{-8}
Orbifloxacin	7.81×10^{-7}	2.60×10^{-6}	5.88×10^{-5}	1.96×10^{-4}	3.33×10^{-8}	1.11×10^{-7}

References

[1] D.T.W. Chu, P.B. Fernandes in : B. Testa (Ed), Advances in Drug Research, Vol. 21, London, Academic Press, (1991) 39-144.

[2] J.E.F. Reynolds (ED) Martindale, The Extra Pharmacopeia, 30 th ed., The Pharmaceutical Press, London (1993) 145-147.

[3] H.C. Neu, Ciprofloxacin: an overview and prospective appraisal, Am.J. Med. 82 (1987) 395-404.

[4] A. Maxwell, The molecular basis of quinolone action, J. Antimicrob. Chemother. 30 (1992) 409-414.

[5] *G.S. Son, H.A. Yeo, M.S. Kim, S.K. Kim, A. Holmen, B. Akerman, B. Norden, Binding Mode of Norfloxacin to Calf Thymus DNA, J. Am. Chem. Soc. 120 (1998) 6451-6457.*

[6] C.Bailly, P. Colson, C. Houssier, The orientation of norfloxacin bound to double-stranded DNA, Biochm. Biophys. Res. Commun. 243 (1998) 844-848.

[7] I. S. Shehatta and M. S. Ibrahim, Binding of anti-inflammatory drug indomethacin with cyclodextrin and DNA: solubility, spectroscopic, and voltammetric studies, Can. J. Chem. 79 (2001) 1431–1438.

[8] M.E. El-Kommos, G.A. Saleh, S.M. El-Gizawi, M.A. Abou-Elwafa, Spectrofluorometric determination of certain quinolone antibacterials using

metal chelation Talanta. 60 (2003) 1033-1050.

[9] M.I. Pascual-Reguera, G.P. Parras, A.M. Dıaz, Solid-phase UV spectrophotometric method for determination of ciprofloxacin, Microchemical. J. 77 (2004) 79-84.

[10] H.R.N. Marona, E.E.S. Schapoval, Spectrophotometric determination of sparfloxacin in pharmaceutical formulations using bromothymol blue, J. Pharm. Biomed. Anal. 26 (2001) 501-504.

[11] C. M. Rasheed, N.A. Fakher, M. Ibrahim, Simultaneous Determination of Enrofloxacin and Tylosin in Chicken Samples by Derivative Spectrophotometry, Arabian Journal for Science and Engineering. 42 (2017) 4453–4463.

[12] P. Ewelina, K. Krzysztof, Determination of Fluoroquinolones in Animal Feed by Ion Pair High-performance Liquid Chromatography with Fluorescence Detection, Liquid Chromatography. 50 (11) (2017) 1711-1720.

[13] F. Dong-Mei, W. Hai-Long, D. Yu-Jie, H. Le-Qian, X. A-Lin, Y. Ru-Qin, Interference-free determination of fluoroquinolone antibiotics in plasma by using excitation–emission matrix fluorescence coupled with second-order calibration algorithms, Talanta. 70 (2006) 58-62.

[14] C. Ching-Ling, F. Chia-Hung, C. Chen-His, Determination of norfloxacin in rat liver perfusate using capillary electrophoresis with laser-induced fluorescence detection, J. Chromatogr. B. 856 (2007) 381-385.

[15] S. Han-wen, H. Pan, L. Yun-kai, L. Shu-xuan, Effective separation and simultaneous determination of seven fluoroquinolones by capillary electrophoresis with diode-array detector, J. Chromatogr. B. 852 (2007) 145-151.

[16] L. Liu, X. Pang, D. Zhang, X. Xu, H. Liu,Y. Liang, L. Xie, X. Liu, Determination of caderofloxacin lactate in rat plasma by high-performance liquid chromatography–mass spectrometry and its application in rat pharmacokinetic studies, J. Pharm. Biomed. Anal. 45 (2007) 799-803.

[17] A. G. Cabanillas, M.I. R. C´aceres, M.A. M. Ca˜nas, J. M.O. Burguillos, T. G. D´ıaz, Square wave adsorptive stripping voltametric determination of the mixture of nalidixic acid and its main metabolite (7-hydroxymethylnalidixic acid) by multivariate methods and artificial neural network, Talanta. 72 (2007) 932-940.

[18] K. Serge, N. Yongnian, W. Yuerong. Simultaneous Determination of Three Fluoroquinolones by Linear Sweep Stripping Voltammetry With The Aid of Chemometrics. Talanta, 69(1) (2017) 216-225.

Contact Author

[19] M.A.G. Trindade, G.M. Silva, V.S. Ferreira, Determination of moxifloxacin in tablets and human urine by square-wave adsorptive voltammetry,

Microchemical. J. 81 (2005) 209-216.

[20] H.J. Buschmann, E. Cleve, E. Schollmeyer, The Interactions Between Nonionic Surfactants and Cyclodextrins Studied by Fluorescence Measurements, J. Inclusion Phenomena and Macrocyclic Chemistry, 33 (1999)233-241.

[21] F. Gramer, W. Kampe, nclusion Compounds. XVII.1 Catalysis of Decarboxylation by Cyclodextrins. A Model Reaction for the Mechanism of Enzymes, J. Am. Chem. Soc. 87 (1965) 1115-1120.

[22] W. Saenger, Cyclodextrin Inclusion Compounds in Research and Industry, Chem. Int. Ed. Engl. 19 (1980) 344-362.

[23] T. Matsue, M. Fujhira, T. Osa, Selective Electrosyntheses on Chemically Modifies Electrodes. IV. Selective reduction of o-Nitrophenol in the Presence of p-Nitrophenol with β-Cyclodextrin in Solution and on Electrode Surfaces, J. Electrochem. Soc. 129 (1982) 1681-1685.

[24] M.S. Ibrahim, M.M. Kamal, Y.M. Temerk, Comparison of the voltammetric studies at mercury and glassy carbon electrodes for the interaction of lumichrome with DNA and analytical applications, Anal. Bioanal Chem 375 (2003) 1024-1030.

[25] Y.M. Temerk, M.S. Ibrahim, M. Kotb, Voltammetric and spectroscopic studies on binding of antitumor Morin, Morin–Cu complex and Morin–β-cyclodextrin with DNA, Spectrochimica Acta Part A. 71 (2009) 1830-1836.

[26] J. Kang, X. Lu, Z. Li, Electrochemical study on the behavior of Morin and its interaction with DNA, J. Pharm. Bipmed. Anal. 40 (2006) 1166-1171.

[27] X.J. Dang, M.Y.Nie,J. Tong,H.L.Li,J.Electroanal.Chem.
437 (1997) 53.

[28] X.J. Dang, M.Y. Nie, J.Tong, H.L.Li, Inclusion of the parent molecules of some drugs with β-cyclodextrin studied by electrochemical and spectrometric methods, J.Electroanal. Chem. 448 (1998) 61-67.

[29] E. Junquera, E. Aicart, Thermodynamic analysis of the binding of a hepatoprotectant drug, thioctic acid, by β-cyclodextrin, J.Pharm. Sci. 88 (1999) 626-631.

الملخص.

ارتباط بعض الفلوروكوينولونات المضادة للبكتريا مع دي إن أي، بيتا سيكلو دكسترين والهيموجلوبين: دراسات طيفية فلتامترية

إبراهيم الحجري[١،٢،*]، يس تميرك[٣]، صادق الحزمي[١]، مصطفى كمال[٣]، محمد سيد[٣]، نبيل الحميري[٢،٤] ، أحمد الحكيمي[١،٢] ، حسن العبيد[١]

١- قسم الكيمياء، كلية العلوم، جامعة القصيم، بريدة، المملكة العربية السعودية.
٢- قسم الكيمياء، كلية العلوم، جامعة إب، إب، الجمهورية اليمنية.
٣- قسم الكيمياء، كلية العلوم، جامعة أسيوط، أسيوط، جمهورية مصر العربية.
٤- قسم الكيمياء، كلية العلوم والاداب-شرورة، جامعة نجران، شرورة، المملكة العربية السعودية.

تفاعلات المضادات البكتيرية دايفلوكساسن ، دانوفلوكساسين واربوفلوكساسين مع الجزيئات المضيفة(دي إن أي، بيتا سيكلو دكسترين والهيموجلوبين) دُرست بواسطة التحليل الطيفي، الفلتامتري الدائري والفلتامتري النبضي التفاضلي. أظهرت بيانات التحليل الكهروكيميائي والطيفي أن دايفلوكساسن ، دانوفلوكساسين واربوفلوكساسين تدخل بين القواعد في شريط الحمض النووي دي إن أي. نُوقشت تأثير تركيب الفلوروكوينولات على الارتباط مع دي إن أي. درست أيضا تفاعلات متراكبات الفلوروكوينولونات -بيتا سيكلودكسترين مع الحمض النووي دي إن أي. وضُحت طبيعة العمليات بين الهيموجلوبين والفلوروكوينولونات . حُسبت ثوابت الارتباط والمعاملات الثرموديناميكية بين الفلوروكوينولونات والجزيئات المضيفة. تم الحصول على المنحني القياسي لتقدير كلاً من الحمض النووي دي إن أي، بيتا سيكلو دكسترين والهيموجلوبين من الإنخفاض في التيار النبضي التفاضلي للفلوروكوينولونات بتاثير إضافة الجزيئات المضيفة.