POTENTIOMETRIC AND SPECTRAL STUDIES ON MIXED METAL COMPLEXES OF SPARFLOXACIN

Hegazy, W. H.; Kamel*, R. M.; Ali R. S. and El-Sayed, A. A.

Chemistry Depart., Fac. of Sci.., Suez Univ., Suez 43518, Egypt
*Corresponding author: rashamoka@yahoo.com

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ABSTRACT

The potentiometric measurements for the interaction of sparfloxacin (SPFX) and metal ions Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with nucleosides (NS) adenosine, guanosine, cytidine and inosine were studied. The formation of various 1:1:1 ternary complex species are inferred from the potentiometric pH titration curves. The experimental conditions were selected such that self-association of the nucleosides and their complexes due to stacking interaction was negligibly small; that is the protonated ternary complexes, were studied. The formation of ternary complexes of some systems was confirmed by UV-Visible measurements in solution.

INTRODUCTION

Discoveries in the field of inorganic and bio-inorganic chemistry pose a significant impact on modern clinical medicine. These discoveries have predominantly emerged in the form of either metal-containing diagnostic imaging agents or metal-containing therapeutics (**Pillai *et al*., 2014).** Many metal-containing compounds have been utilized throughout history to treat a wide variety of disorders **(Chen *et al*.,2009)**. In medicinal chemistry traditionally dominated by organic chemistry metal complexes have gained favor as diagnostic tools and anticancer agents.

Sparfloxacin (SPFX) is potent third generation fluoroquinolone antibiotics. It is used in the treatment of wide range of gram positive and gram negative bacterial infections. The advantages of using this drug includes: rapid absorption after oral administration, excellent bioavailability, good tissue penetration, excellent wide spectrum of activity and long elimination half-life. SPFX is doubly fluorinated compound similar to ciprofloxacin in structure but has additional fluorine at C-8, an amine group at C-5 and two methyl groups at the C-3 and C5 of the piperazine group. The bactericidal activity of

the fluoroquinolone antibiotics including SPX is due to their inhibitory effect on the enzyme DNA gyrase that controls the replication of the bacterial DNA **(Al-Mustafa and Shinar, 2013).**

The partially filled *d* orbitals in transition metals impart interesting electronic properties that can act as suitable probes in the design of anticancer agents **(Hambley, 2007).** The oxidation state of a metal is also an important consideration in the design of coordination compounds, given that it allows the participation in biological redox chemistry and plays an influential role in optimal dose and bioavailability of the agent administered **Abrams and Orvig (1999)** and **Orvig and Thompson (2003).** Furthermore, the ability to undergo ligand exchanged reactions offers a myriad of opportunities for metals to interact and coordinate to biological molecules, as demonstrated by the widely used drug cisplatin **(Fricker,** 2007). Furthermore, when designing metal-based therapeutics, one is not restricted solely by metals selected by nature and can take advantage of the unique properties of nonessential metals, including other 1^{st} and 2^{nd} row transition metals and metals that can impart additional utility not found naturally **(Haas and Franz, 2009).**

In continuation of our previous work on ternary complexes containing biologically important ligands **(Azab *et al.*, 2016).** the mixed ligand complexes of the type M(II) + Sparfloxacin + Nucleosides have been investigated by potentiometric pH-titrations to determine the formation constants of the protonated mixed ligand complexes formed in solution.

MATERIALS AND METHODS

[4-amino-1-β-D-ribofuranosyl-2-(1H)-pyrimidine] $C_9H_{13}N_3O_5$ (Cytid- ine), [9-β-D-ribfuranosyl-9H-purine-6-amine) (9-β-D-ribofura-nosyl -adenine] $C_{10}H_{13}N_5O_4$ (Adenosine), [1,9-dihydro-9-β-D-ribfuranosyl-6H-purine-6-one) (Hypoxanthine-9-D-ribofuranoside] $C_{10}H_{12}N_4O_5$ (Inosine) and [2-amino-1,9-dihydro-9-β-D-ribfuranosyl-6H-purine-6-one) (9-β-D-ribofuranosyl-guanine] $C_{10}H_{13}N_5O_5$ (Guanosine), were purchased from Sigma chemical Co. and were used without further purification.

5-amino-1-cyclopropyl-7-[(3R,5S)3,5-dimethyl piperazine-1-yl]-6.8-difluoro-4-oxo-quinoline-3-carboxylicacid ($C_{19}H_{22}F_2N_4O_3$) (Sparfloxacin) (SPFX) was purchased from Sigma chemical Co. and was used without purification.

For a stock solution of 10^{-2} $molL^{-1}$ of SPFX, 0.3924 g of solid ligand was dissolved in 100 ml ethanol. The stock solutions of 10^{-2} $molL^{-1}$ of nucleosides were prepared by dissolving 0.267, 0.283, 0.243 and 0.268 g, (in respectively, ml in bi-distilled water for adenosine, guanosine, cytidine and inosine.

Metal salt $Co(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Cu(NO_3)_2.6H_2O$, $Zn(NO_3)_2.6H_2O$, and $Cd(NO_3)_2$ were of Sigma Chemical Co. Stock solutions (0.01 $molL^{-1}$) of metal salts were prepared by dissolving precisely weighed amount of the salt in bi-distilled water. The concentrations of the metal ion

stock solutions were determined complexometrically by ethylenediamine tetracetic acid dissodium salt (EDTA) using suitable indicators.

Instrumentation

Potentiometric-pH measurements were performed of the solutions in a double-walled glass vessel at (25.0 ± 1.0) ^{o}C with a commercial Fisher combined electrodes, and a magnetic stirrer was used. A Fisher pH meter model 325 MP was used. Purified nitrogen was bubbled through the solutions during titrations.

A Shimadzu-1601PC UV-Visible automatic recording spectrophotometer with 1 cm quartz cell was used for the absorbance and spectral measurements.

Procedure for Potentiometric Measurements

The test solution was titrated with standard KOH free from CO_2. The electrodes were calibrated, in both the acidic and the alkaline regions, by titrating 0.01 molL^{-1} nitric acid with standard potassium hydroxide under the same experimental conditions. The concentration of free hydrogen ion C_{H+} at each point of the titration is related to the measured electromotive force (emf) E^{o} of the cell by the Nernst equation.

$$\mathbf{E = E^{o} + Q \log C_{H+}} \quad \mathbf{(1)}$$

Where E^{o} is constant which included the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of E^{o} for the electrode was determined from a Gran plot derived from a separate titration of nitric acid with standard KOH solution under the same temperature and medium conditions as these for the solution titration.

The protonation constants were then determined by use of the Bjerrum function **(Marzilli, 1981).**

$$\mathbf{\bar{n} = (H_T - h + K_W / h) / A_T = (\beta_1 h + 2\beta_2 h^2) / (1 + \beta_1 h + \beta_2 h^2)} \quad \mathbf{(2)}$$

which is calculated from the experimental quantities, h. The total concentration of titratable hydrogen ion H_T and the total reagent concentration A_T. pKa values of the investigated ligands were determined in distelled water from the overall protonation constants β_1 and β_2 calculated by the linearization method of Irving and Rossotti **(Barton and Lippard, 1980).**

The results so obtained were analyzed by the nonlinear least squares computer program ESAB2M **(De Stefano *et al.*, 1987).** to refine E^{o} and the autoprotolysis constant of water K_w. During these calculations, K_w was refined until the best value of Q was obtained.

The solution titrated can be presented according to the following scheme:

(a) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX (ligand 1).

(b) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} nucleosides (adenosine, guanosine, cytidine or inosine) (ligand 2).

(c) Solution (a) + 0.001 molL^{-1} M (II).

(d) Solution (b) + 0.001 molL^{-1} M (II).

(g) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX + 0.001 molL^{-1} nucleosides + 0.001 molL^{-1} M (II).

A constant ionic strength was obtained using 0.1 molL^{-1} KNO_3 and the total volume was kept constant at 25 ml. At least four titrations were performed for each system. For both ligand protonation and metal complex formation equilibria, data were recorded over the largest possible pH interval, although a number of experimental points were frequently discarded for the final stability constant calculations, especially within the range where the complexation observed was insignificant.

Initial estimates of the formation constants of the resulting species and the acid dissociation constants of the primary ligand and secondary ligands have been refined with the HYPERQUAD computer program **(Wolfe *et al*.,1987).** This program is an extremely powerful general purpose computer program for stability constant work. It can handle data from all known systems of potentiometric titration. These include batch titration **(Stephanos, 1996).** , electrode readings in pH or milli-Volts, alkali added or generated coulometrically, and determinate systems where the number of electrodes is equal to the number of reactants. It can cater for ion-selective electrodes whose response slope are other than Nernstian, and in principle could be modified for other non-Nernstian responses. Titration curves of different types can be mixed together.

The constants were refined by minimizing U, defined by

$$U = \sum W_i (E_{obs} - E_{calc})^2 \quad \textbf{(3)}$$

where E_{obs} and E_{calc} refer to the measured and calculated potential. The weighting factor W_i is defined as the reciprocal of the estimated variance of measurement

$$W_i = 1 / \sigma^2 = 1 / [\sigma_E^2 + (\delta E / \delta V)^2 \sigma_V^2] \quad \textbf{(4)}$$

where σ_E and σ_V are the estimated variances of the potential and volum reading, respectively. The quality of fit was judged by the values of the sample standard deviations, S, and the goodness of fit, X^2 (Pearson's test). At σ_E = 0.1 mV (0.001 pH error) and (V = 0.005 ml, the values of S in different sets of titrations were between 1.0 and 1.8 and X^2 was between 12.0 and 13.0. The scatter of residuals (E_{obs} - E_{calc}) vs pH was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data.

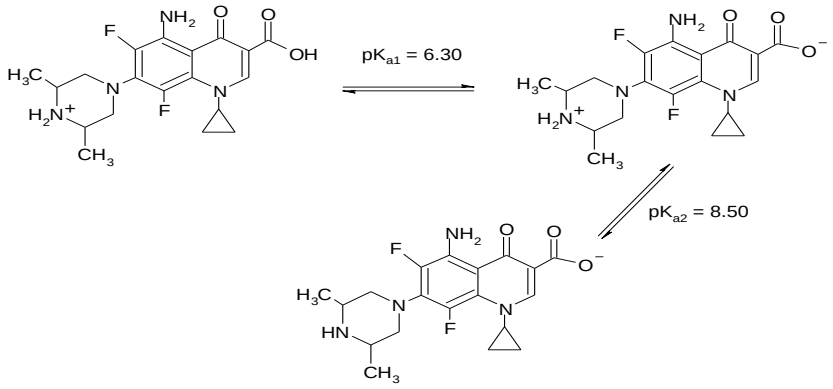
RESULT AND DISCUSSION

Potentiometric studies of metal (II) complexes of Sparfloxacin with nucleosides.

This section represents the result of the potentiometric measurements for the

interaction of sparfloxacin and metal ions Co(II), Ni(II), Cu(II), Zn(II) or Cd(II), with nucleosides adenosine, guanosine, cytidine and inosine. The formation of various 1:1:1 ternary complex species are inferred from the potentiometric pH titration curves. Initial estimates of the stability constants of the resulting species and the acid dissociation constants of sparfloxacin and nucleosides have been refined with the HYPERQUAD computer program **(Stephanos, 1996).** Furthermore, the formation constants values of the different 1:1 M(II)–sparfloxacin, or M(II)-nucleosides have been determined under identical conditions. This is made with the aim to compare the stability of the formed 1:1:1 ternary complexes with of the corresponding 1:1 binary metal complexes.

Sparfloxacin (SPFX) is a member of the fluoroquinolone family. It is a zwitterionic compound in which the carboxylic group is deprotonated while terminal nitrogen atom of piperazine is protonated. The acid-base properties of Sparfloxacin in 10 % (v/v) ethanol-water mixture and in ionic strength (0.1 $molL^{-1}$ KNO_3) indicate that one proton from carboxylic group was ionized in the pH range 3.50 - 6.90 (H-SPFX). HSPFX undergoes stepwise ionization on increasing the pH of solution. The transformation of the latter species (H-SPFX) to ($SPFX^-$) corresponding to the ionization of nitrogen atom of piperazine take place in the pH range 7.10-9.95. The dissociation constant of SPFX pK_{a1} corresponding to the ionization of carboxylic group was found to be 6.30 ±0.02 whereas the value of pK_{a2} for the nitrogen of piperazine proton was 8.50 ±0.02 [Scheme 1]. The values of the dissociation constants of SPFX in this work agree well with the values as reported in literature **(Dahloff, 1998).**



Scheme 1: Ionization of Sparfloxacin (HSPFX)

Although the pKa values of the nucleosides have already been reported. These values are determined for the sake of uniformity in experimental conditions. The dissociation constant values of adenosine (pK_a N1 = 3.60 ± 0.02), cytidine (pK_a N3 = 4.20 ± 0.02), guanosine (pK_{a1} N7 = 2.10 ± 0.02, pK_{a2} N1 = 9.20 ± 0.03) and inosine (pK_{a2} N1 = 9.20 ± 0.02). The pKa values of

nucleosides agree well with those reported in the literature **(Sigel, 1979).** The stability constants of the binary complexes of Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) with HSPFX were calculated from the titration graphs in which the metal to ligand ratio was 1:1, the data are listed in Table 1. The conditions for the measurements were the same as for the acidity constants.

The equilibria in the binary system containing SPFX is presented below:

$$M^{2+} + H_2SPFX \overset{K^{M}_{M\text{-}SPFX}}{\rightleftharpoons} [M(II)\text{-}SPFX] + 2H^{+} \qquad (5)$$

$$K^{M}_{M\text{-}SPFX} = \frac{[M(II)\text{-}SPFX]}{[M(II)]\,[H_2SPFX]} \qquad (6)$$

It is noticed that Cd(II)-SPFX complex is slightly less stable than Cu(II)-SPFX complex, this is a result of Cd (II) ion (0.97Å) has larger ionic radius than Cu(II) ion (0.57Å) **(Lippared, 1989).**

Table 1. The stepwise and overall formation constants for M(II) + Sparfloxacin (HSPPFX) (1:1) binary complexes, in 10% (v/v) ethanol-water mixture, I = 0.1 $molL^{-1}$ KNO_3 and at 25.0 ± 1.0°C.

Metal ion M(II)	$\log K_1$ M(II)(SPFX)	$\log K_2$ M(II)(SPFX)	$\log\beta$ M(II)(SPFX)
Co (II)	3.64 ± 0.02	5.57 ± 0.03	9.21 ± 0.03
Ni (II)	3.67 ± 0.04	5.64 ± 0.03	9.31 ± 0.02
Cu (II)	4.88 ± 0.03	7.06 ± 0.03	11.94 ± 0.02
Zn (II)	3.71 ± 0.03	5.70 ± 0.02	9.41 ± 0.02
Cd (II)	3.46 ± 0.02	5.16 ± 0.04	8.62 ± 0.02

± refers to three times standard deviation (3s).

The values of stability constants $\log K_1$ and $\log K_2$ of M(II)-SPFX, complexes have been evaluated at pK_{a1} (6.3) and pK_{a2} (8.5), respectively. the order of stability constant values of the binary complexes follows the following sequence:

Cu(II)-SPFX > Zn(II)-SPFX > Ni(II)-SPFX > Co(II)-SPFX > Cd(II)-SPFX

The titration curve of metal (II) ions with nucleosides showed that they form 1:1 complexes. The constants corresponding to the following equilibria were determined:

$$M^{2+} + NS \overset{K^{M}_{M\text{-}NS}}{\rightleftharpoons} [M\,(II)\text{-}(NS)] \qquad (7)$$

$$K^{M}_{M\text{-}NS} = \frac{[M(II)\text{-}(NS)]}{[M(II)]\,[NS]} \qquad (8)$$

The stability constants of M(II)–nucleosides complexes were found to agree well with those reported in the literature **(Smith *et al*., 1991).** The potentiometric titration curve of M(II)–SPFX or M(II)–nucleosides are shown in Figures [1,2].

In our study, we have three purine nucleosides adenosine, guanosine, inosine and one pyrimidine nucleoside cytidine. Metal ions have been found to play crucial role at some stages of gene expression (replication, transcription and translation) or ultimately are combined with the gene product to produce an active metallo-enzeme or other protein-metal complex. Binding studies of metal ions Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) to nucleosides as nucleic acid derivatives are currently of great interest, especially in view of the speculation that antitumor platinum complexes may act by cross-linking deoxyribonucleic acid (DNA) **(Connors and Roberts, 1974).**

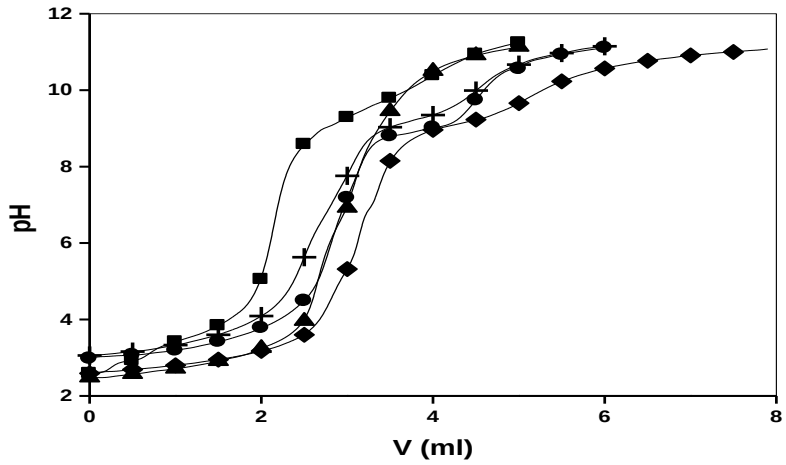


Fig. 1: pH against volume of 0.042 molL^{-1} KOH for Co (II) + Adenosine + Sparfloxacin system at 0.1 molL^{-1} KNO_3 in 10 % (v/v) ethanol water mixture and at 25 ^oC.

(a) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Adenosine (■)

(b) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Adenosine + 0.001 molL^{-1} Co (II) (●)

(c) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX (▲)

(d) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX + 0.001 molL^{-1} Co (II) (♦)

(e) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Adenosine+ 0.001 molL^{-1} SPFX + 0.001 molL^{-1} Co (II) (┼)

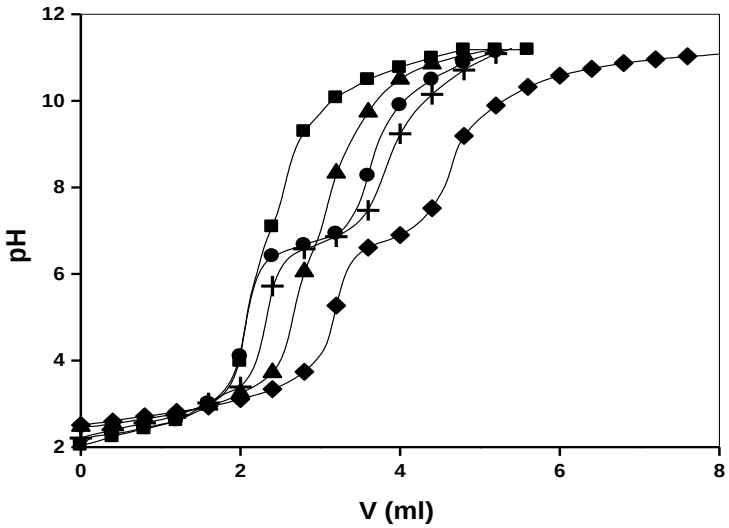


Fig. 2: pH against volume of 0.042 molL^{-1} KOH for Cu (II) + Guanosine + Sparfloxacin system at 0.1 molL^{-1} KNO_3 in 10 % (v/v) ethanol water mixture and at 25

(a) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Guanosine (■)

(b) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Guanosine 0.001 molL^{-1} Cu (II) (●)

(c) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX (▲)

(d) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} SPFX + 0.001 molL^{-1} Cu (II) (♦)

(e) 0.004 molL^{-1} HNO_3 + 0.001 molL^{-1} Guanosine + 0.001 molL^{-1} SPFX + 0.001 molL^{-1} Cu (II) (┼)

There are few examples of structure determination of metal complexes of nucleosides, as compared to those of nucleotides and of the purine and pyrimidine bases. In the structure of metal-purine complexes it has been found that the predominant mode of metal binding take place at the nitrogen atoms of the five-membered (imidazole) ring N7 and N9, also in some adenine complexes at the N3 and N1 positions of the six-membered (pyrimidine) ring. For purine nucleosides, however, the presence of the sugar ring reduces the number of coordination sites available. N9 is, of course blocked, and the N3 position, which is not a strong ligating position to begin with, it is made even less attractive by the bulk of the sugar moiety. This leaves N7 and O6 of guanine and N7 and N1 of adenine, as possible metal-binding sites.

In the purine nucleosides binding may occur at N1 or N7. The former nitrogen is protonated in neutral solutions of inosine (pK_a = 8.8) and guanosine (pK_a = 9.2), so that a metal ion may coordinate the weakly basic N7 or compete with the proton for the more basic N1. For weakly basic adenosine with pK_a 3.6 for N1, in neutral solutions both the N1 and N7 sites are free to bind metal ions **(Martin and Mariam, (1979)** and **Martin (1985).**

Generally, there is direct involvement of nucleobase moiety in the metal coordination sphere in the metal nucleoside complexes.

Metal complexes of pyrimidine nucleosides have been studied less extensively than those of purine nucleosides. This may be partly due to the fact that the pyrimidine nitrogen N3 is a weaker ligating atom than the imidazole nitrogen N7 of purines. Metal complexes of the pyrimidine bases themselves however are more plentiful and are thoroughly covered in Hodgson's review **(Hodgson, 1977).**

The complex formation between certain nucleosides and M(II) ions found that guanosine forms the strongest and adenosine the weakest complexes, which points to stronger complexing by the OH group and N-7, than by NH_2 and N-7. That inosine is the second most strongly absorbed compound is consistent with this view.

It is quite clear from potentiometric curves, that the 6-oxo group of guanosine and inosine nucleoside has an enhancement effect for the binding to metal ions. The order of stability constant values of the binary complexes follows the following sequence:

M(II)-Guanosine > M(II)-Inosine > M(II)-Cytidine > M(II)-Adenosine

Where, M(II) = Cu(II), Co(II), Cd(II), Zn(II) or Ni(II)

The weak complexing of adenosine in relation to the strong complexing of adenine is to be attributed to the role of the available N-3 and N-9 positions in adenine and the non-availability of the N-9 position in adenosine. Due to the lake of oxo-group enhancement, we can state that adenosine, as shown in Table 2, binds Cu(II), Co(II), Cd(II), Zn(II) or Ni(II) weakly. The order of the logK N (7) of adenosine for the different metal ions is:

Zn(II)-adenosine > Cu(II)-adenosine > Co(II)-adenosine > Ni(II)-adenosine > Cd(II)-adenosine.

Comparison of the nucleoside structures and the pK_a values reported previously reveals that for this select group of nitrogen heterocycles the nature of 2-substituent is relatively unimportant in determining pK_a. On the other hand, replacement of the amino group at position 4 in a pyrimidine or the corresponding position 6 in a purine with an oxo-group (or more accurately the replacement of an amino-pyridine with an amide function) increases the basicity of pK_a of N3 in the pyrimidine and N1 in the purine by 5 log units.

The stability constants for the ternary systems were computed from the titrations in which the concentrations of M(II): SPFX: NS were kept in the ratio 1: 1: 1, listed in Table 2. The data collected in the pH range 3.0 – 11.0 were used for the calculations and refinements. Representative titration curves for some ternary systems under investigation are given in Figures 1-2.

The formation constants for M(II)-SPFX-NS in 1:1:1 ratio are calculated based on that the titration curve lies between the corresponding binary curves

for M(II)-SPFX and M(II)-Nucleosides, i.e. the formed complex species is of monoprotonated type M(II)(SPFX)(HNS) where the nucleoside reacts as a secondary ligand in its protonated form.

$$[M\text{-}SPFX] + HNS \rightleftharpoons [M\text{-}SPFX\text{-}HNS] \qquad (9)$$

To the author's knowledge no data for the ternary complexes containing SPFX with the nucleosides guanosine, adenosine, inosine, and cytidine are available in the literature for comparison.

To calculate the initial estimates of the formation constants of the ternary complexes of Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) with sparfloxacin (HSPFX) and nucleosides (NS) adenosine, guanosine, cytidine and inosine, the following equilibria were used:

$$K_{M(SPFX)(NS)} = \frac{[M_p(SPFX)_q\,(NS)_r]}{[M_p(SPFX)_q]\,[NS]^r} \qquad (10)$$

Which refers to the addition of NS to the binary complex $M_P(SPFX)_q$

The overall complexation reaction involving protonation is

$$p\,M + q\,SPFX + r\,NS + s\,H \rightleftharpoons M_p\,(SPFX)_q\,(NS)_r\,(H)_s \qquad (11)$$

$$\beta_{pqrs} = \frac{M_p\,(SPFX)_q\,(NS)_r\,(H)_s}{[M]^p\,[(SPFX)]^q\,[(NS)]^r\,[(H)]^s} \qquad (12)$$

In which HSPFX = Sparfloxacin, NS = Nucleoside ligand Guanosine, adenosine, inosine and cytidine and M = Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) 10% ethanol-water mixture solvent.

Table 2. Formation constants for M (II) +Sparfloxacin + Nucleosides (NS) (1:1:1) ternary complexes, in 10% (v/v) ethanol-water mixture, I = 0.1 $molL^{-1}$ KNO_3 and at 25.0 ± 0.1°C. ± refers to three times standard deviation (3s)

Metal ion M(II)	Logk M(II)-SPFX-Adenosine	Logk M(II)-SPFX-Guanosine	Logk M(II)-SPFX-Inosine	Logk M(II)-SPFX-Cytidine
Cu (II)	9.57 ± 0.02	15.33 ± 0.02	9.19 ± 0.02	15.02 ± 0.04
Ni(II)	7.20 ± 0.03	15.27± 0.04	--	14.01± 0.02
Co(II)	--	--	--	16.14 ± 0.02
Zn(II)	11.18 ± 0.02	15.34 ± 0.03	10.36 ± 0.03	--
Cd (II)	--	11.03± 0.03	12.71 ± 0.03	--

The higher value for the stability constants of ternary complexes compared with those of the binary systems may be attributed to the interligand interactions or some cooperatively between the coordinated ligands, possibly H-bond formation. This also may be explained on the basis of the π-electron donating tending of the M(II) ion to the antibonding π* orbital of the hetero-aromatic N base, strengthens of the M(II)-N bond. The interaction of the electrons of the secondary ligands with the metal will increase to a greater extent and consequently enhance the formation of the mixed ligand complexes.

From Table 2 the formation constant values for the mixed ligand 1:1:1 systems can be arranged using the following comments: The interaction of guanosine nucleoside to the binary M(II)-SPFX complex is the most preferable one. We can conclude in general that the interaction of guanosine with M(II)-SPFX complex follow the order: Zn(II) > Cu(II) > Ni(II) > Cd(II). The formation constant of M(II)-SPFX-Adenosine follow the order: Zn(II) > Cu(II) > Ni(II). The formation constant of M(II)-SPFX-inosine follow the order: Cd(II) > Zn(II) > Cu(II). The formation constant of M(II)-SPFX-cytidine follow the order: Co(II) > Cu(II) > Ni(II).

The observed different orders may be attributed to different types of interactions depending on metal ion or different geometrical behavior during formation of binary and ternary complexes in solution.

Spectroscopic confirmation for the formation of ternary complexes of M(II)-Sparfloxacin-Nucleosides

In order to confirm the formation of binary and ternary complexes under study, UV-Visible spectroscopic measurements have been carried out under the same experimental conditions.

The absorption spectrum of Sparfloxacin ($1x10^{-4}$ $molL^{-1}$) in 10% (v/v) ethanol-water mixture shows a maximum absorption band at 223 and 290 nm due to π-π* transition, these bands belongs to fluoroquinolone nucleus and absorption band around 366 nm due to the n-π* transition, this band may be attributed to the overlapping Me-carbonyl and Me-carboxyl bonds absorption **(Urbaniak and Kokot, 2013).**

Addition of metal ion solution of Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) to the solution contains Sparfloxacin causes a red shift to the absorption spectrum of sparfloxacin, following the order Cu(II) > Ni(II) > Zn(II) ≈ Co(II) ≈ Cd(II) revealing the binding between sparfloxacin and M(II) ions, [Figure 3].

The large red shift is assigned for Cu-SPFX complex by about 9 nm which may be attributed to the high degree of binding between SPFX and Cu(II) ions which agree with the high stability constant of Cu-SPFX complex over other complex under study.

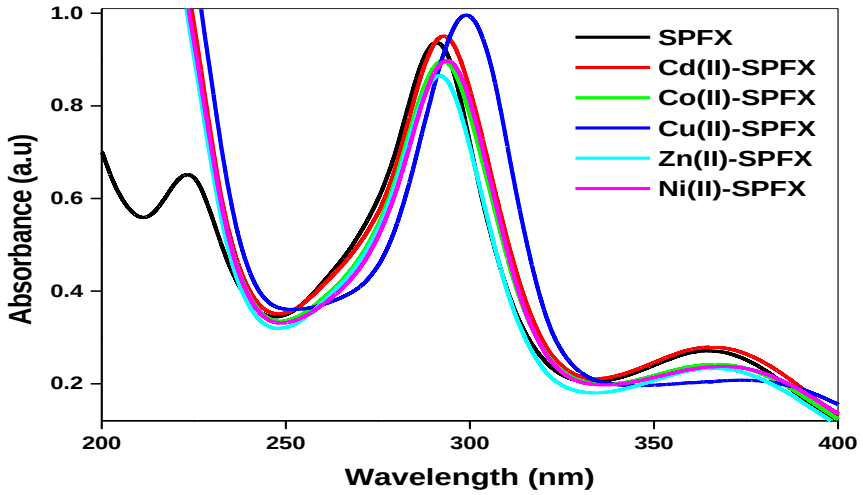


Fig. 3 Electronic absorption spectra of SPFX and M(II)-SPFX complexes, $[M^{2+}] = 1\times10^{-4}$ molL^{-1} and [SPFX] = 1×10^{-4} molL^{-1}.

Figures 4,5 show the UV-Visible spectra for some ternary systems of M(II)-SPFX-NS.

For each ternary system of M(II)-SPFX-NS is similar for all metal Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) which means the similar behavior of complexation between all metal ions with SPFX and nucleosides.

For all ternary complexes under study, show band around 290 nm corresponding to sparfloxacin moiety, except the absorption spectra of M(II)-SPFX-Cytidine ternary complexes show a broad around 292 nm – 260 nm due to the overlap between two peaks 292 nm and 270nm corresponding to sparfloxacin and cytidine moieties, respectively.

M(II)- SPFX-Adenosine systems show a new band around 260 nm corresponding to adenosine when compared to M(II)-SPFX binary complex. While for the ternary systems of M(II)-SPFX-Guanosine, a new band refers to guanosine appeared at 253 nm.

M(II)-SPFX-Inosine ternary systems, a new band refer to inosine appear around 250 nm when compared with the absorption spectra of M(II)-SPFX binary complexes.

Co(II)-SPFX-Adenosine ternary system exhibit hypochromic effect at 260 nm more than other M(II)-SPFX-Adenosine where, M(II) = Ni, Cu, Zn or Cd.

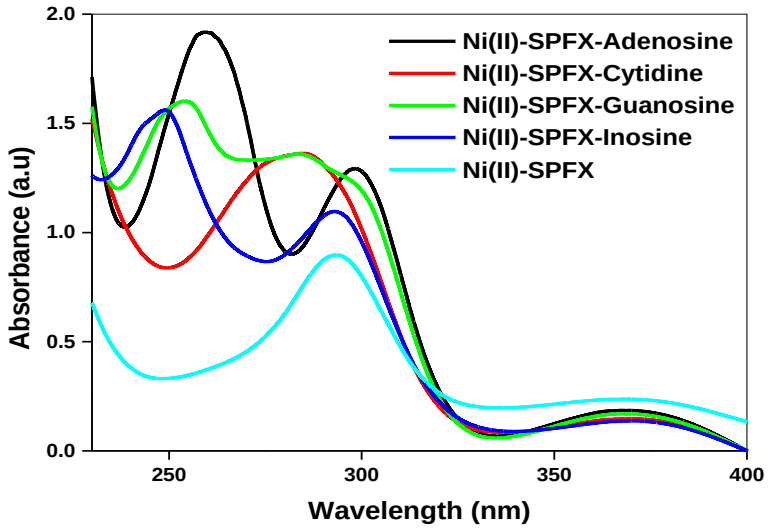


Fig. 4 Electronic absorption spectra of Ni(II)-SPFX complex with nucleosides (NS) under study, $[Ni^{2+}] = 1\times10^{-4}$ molL^{-1}, [SPFX] = 1×10^{-4} molL^{-1} and [NS] = 1×10^{-4} molL^{-1}.

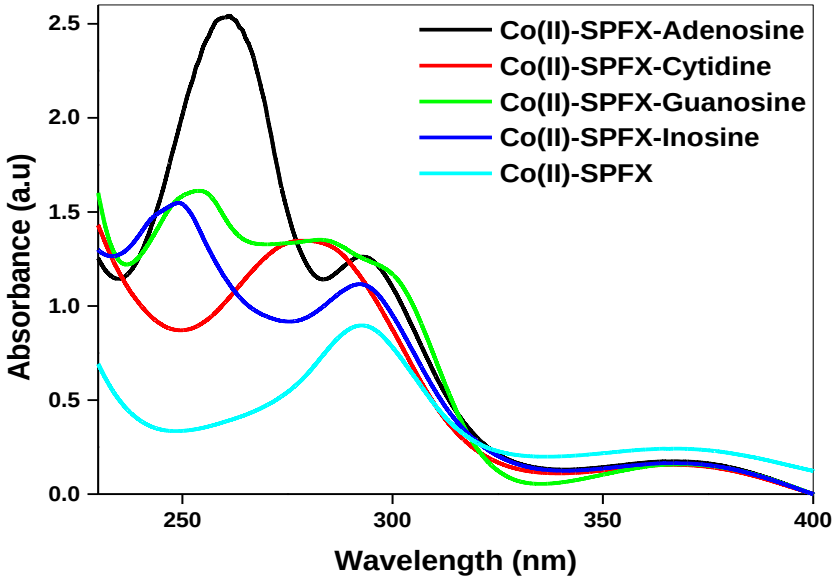


Fig. 5 Electronic absorption spectra of Co(II)-SPFX complex with nucleosides (NS) under study, $[Co^{2+}] = 1\times10^{-4}$ molL^{-1}, [SPFX] = 1×10^{-4} molL^{-1} and [NS] = 1×10^{-4} molL^{-1}.

CONCLUSION

We present here Spectral and potentiometric studies for the interaction of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) sparfloxacin complexes with some nucleosides (NS) adenosine, guanosine, cytidine and inosine. The stability constants for the ternary systems M(II)-SPFX-nucleosides were determined from potentiometric pH titration curves in which the ratio was kept in 1:1:1.

The formed ternary complex species is of monoprotonated type M(II)(SPFX)(HNS) where the nucleoside reacts as a secondary ligand in its protonated form. The ternary complexes M(II)-SPFX-guanosine have the highest stability constants. The formation of ternary complexes of some systems was confirmed spectroscopy with UV-visible measurements.

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دراسات جهدية و طيفية على المتراكبات المختلطة لبعض العقاقير الطبية

وائل حسين حجازى، رشا مصطفى كامل، ريهام سيد على و أ سماء عبد الفتاح

تتناول هذه الدراسه القياسات الجهدية والطيفية لتفاعل بعض الليجندات واولها المضاد الحيوى سبارفلوكساسين الذى يعتبر من ضمن الجيل الثالث لمركبات الفلوروكينولون فهو يستخدم فى علاج الالتهابات البكتيريه ايضا دراسه النيكلوسيدات من نوع البيورين (الادينوسين،الجوانوسين والاينوسين) والبيريمدين (سيتيدين) التى هى المركبات الاساسيه في النظم البيلوجيه مع ايونات بعض العناصر(النحاس، كوبلت،نيكل، كادميوم والزنك) وقد تم حساب قيم ثوابت الاستقرار للمتراكبات الثنائيه من ١:١(ايون الفلز: الليجند) بالاضافه الى المتراكبات الثلاثيه بنسبه ١:١:١ التي تمت في محاليل الكحول الايثيلى والماء المقطر بنسبه% ١٠ (حجم/حجم) وعند قيمة قوى ايونيه تساوى ١,٠ مول/ لترمن نترات البوتاسيوم وعند درجه حراره ٢٥م°. وقد اوضحت النتائج ان السبارفلوكساسين له قيمتين من ثوابت التفكك ٠,٠٢± ٦,٣٠ القابله لتأين مجموعه الكربوكسيل و ٠,٠٢±٨,٥٠ القابله لتأين النيتروجين وايضا تم تعين قيم ثوابت الاستقرار للمتراكبات الثنائيه من نوع ١:١ بالنسبه لايون العنصر- سبارفلوكساسين تتبع الترتيب التالي الكادميوم<الكوبلت<النيكل<الزنك < النحاس وكذلك تم حساب قيم ثوابت الاستقرار للمتركبات الثنائيه من نوع ١:١ بالنسبه لايون العنصر- نيكلوسيدات تتبع الترتيب التالى: الجوانوسين>الاينوسين>السيتيدين>الادينوسين وذلك نتيجه لوجود مجموعه ال٦ اوكسو الموجوده في الجوانوسين والاينوسين لها تأثيرو اضح في تعزيز قوى الترابط مع ايونات العناصر.

في حاله المتراكبات الثلاثيه من نوع ١:١:١ بالنسبه لايون العنصر سبارفلوكساسين و النيكلوسيدات اوضحت الدراسه انها تكون متراكبات ثلاثيه أحاديه الهيدروجين حيث تتحد النيكلوسيدات في صورتها المتعادله مع المتركبات الثنائيه لايون العنصر مع سبارفلوكساسين وقد يرجع انخفاض ثابت استقرار جميع المتراكبات الثلاثية لايون الكادميوم بالمقارنة مع المتراكبات الثلاثية الاخرى قيد الدراسة، نظرا لآن له أكبر نصف قطر ايونى مقارنة بايونات العناصرالأخرى قيد الدراسة وهى النحاس ، كوبلت ، النيكل و الزنك.

الجزء الثانى من الدراسه يشمل القياسات الطيفيه للسبارفلوكساسين التى تمت فى محاليل الكحول الايثيلى الماء المقطر بنسبه ١٠% (حجم/حجم) التى اظهرت حزم امتصاص عند ٢٢٣ و ٢٩٠ نانوميتر نتيجه حدوث انتقالات π^*-π و كذلك حزمه امتصاص عند ٣٦٦ نانوميتر نتيجه حدوث انتقالات من نوع π-n^* وقد لوحظ عند اضافه ايونات العناصر تحت الدراسة الى محلول يحتوى على سبارفلوكساسين يحدث انتقال الى طول موجى أعلى. كذلك تمت الدراسات الطيفية للمتراكبات الثلاثية لكل الانظمة تحت الدراسة.