Potentiometric and Conductometric study of complex formations between Norfloxacin and Some metal ions and Norfloxacin determination in Dosage Forms

Badriah . S. Al–Farhan[1], A. H. Naggar[2],O. A. Farghaly[2]

[1] Chemistry Department, Faculty of Girls for Science, King Khalid University, Abha, King Saudi Arabia.KSA
[2] Chemistry Department, Faculty of Science, Al–Azhar University, Assiut Branch, 71524, Assiut, Egypt.
*E-mail: shahd|_bb@hotmail.com

The determination of proton ligand association constants of Norfloxacin(NFX) with different metal ions viz; Ti(II), Ba (II), Sr(II), Co(II), Zr(IV), La(II), Pb(II), Cr(III),Fe(III) and Sn(II) by potentiometric and conductometric methods, at ionic strength (μ= 0.01 M NaCl) was investigated and the two logarithmic association constant values which calculated by the half–$\bar{n}$method were 6.2and 8.0. The stoichiometric of NFX–metal ion formed complexes was calculated to be 1:1, 1:2 and/or 1:3 metal to ligand ratios are formed depending on the nature of the ligand and/or metal ions. The effect of ionic strength on stability constant of NFX, with some different metal ions was studied.The stoichiometry of complexes formed in solution was confirmed by conductometric method.As well as, the species distribution (α) diagrams for NFX and their metal complexes which calculated as mole fraction α_{ML} andα_{ML2}, were discussed.Also simple, precise, rapid and low–cost potentiometric and conductometric methods for determination of NFX(in pure form) were performed using sodium hydroxide as titrant, at 25±1.0ºC. The calibration graph was linear from 0.32–2.87 mg L^{-1} with detection limit of 0.27 mg L^{-1}, at SD was < 1.0, and correlation coefficient (r) was calculated to be 0.9952.The proposed methods were successfully applied for NFXdetermination in pharmaceutical formulations (tablets and eye drops) with no interferences from usual excipients. The analytical results obtained by applying the proposed methods compared very favorably with those obtained by the official method such as United States Pharmacopoeia and British Pharmacopoeia. The Recoveries obtained of the proposed methods for determination of NFX in various tablet dosage forms were found to be in the range of 95.8 to 103.3%, with standard deviations (SD) were within 0.16–0.24 (n=5).

1. INTRODUCTION

Norfloxacin (NFX) [1–ethyl–6–fluoro–1,4–dihydro–4–oxo–7–(1–piperazinyl) –3–quinolone carboxylic acid] is a broad–spectrum fluoroquinolone antibacterial agent that is structurally related to nalidixic acid. It is one of the third–generation members of quinolone antibiotics, fluorinated in position 6 and bearing a piperazinyl moiety in position 7. It exhibits greater antibacterial activity against both gram–positive and gram–negative bacteria than other nalidixic acid analogs and it's extremely useful for the treatment of a variety of infectious diseases [1–4]. Generally, fluoroquinolones was also introduced as antitumor agent [5].NFX received approval by the Food and Drug Administration in 1986 [6]. NFX has been prepared by displacing the chlorine atom in l–ethyl–6–fluoro–7–chloro–1,4–dihydro–4–oxoquinoline–3–carboxylic acid under the action of anhydrous or hexahydrate piperazine[7].

Norfloxacin (NFX)
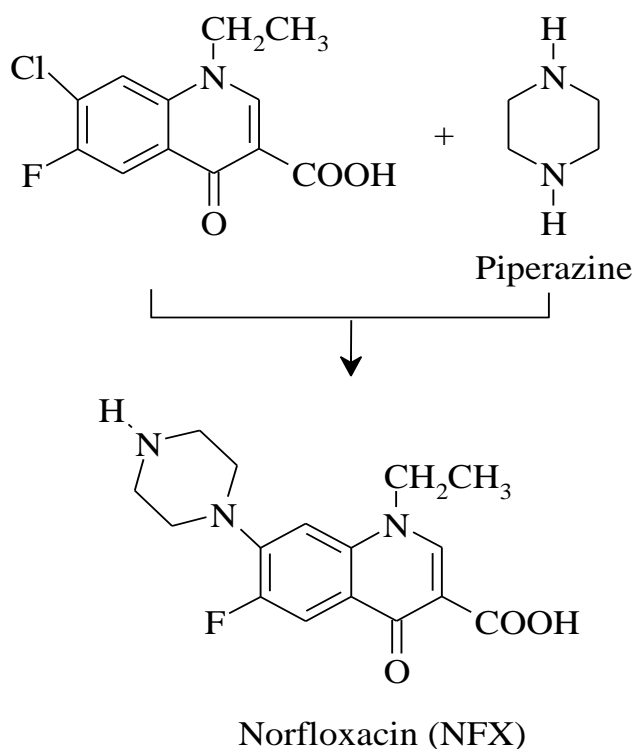
NFX (as a member quinolones) can be considered as effective complexing agents for a variety of metal ions including alkaline earth metal ions. This can be attributed to the ring carbonyl group at position 4 and one of the oxygen atoms of carboxylato group at position 3. NFX acts as bidentate ligand and it can also act as bridging ligands and hence they are capable of forming polynuclear.The formed complexes are biologically active complexes and are especially important [6]. The synthesis, characterization and antibacterial activity of NFX with different metal ions had a great attention[8–17].As complexing reagent, NFX was used for the spectrophotometric determination of Fe(III) [18], its complexation with Al(III) ions was studied using NMR [19] while the crystal structure of Mg^{2+} and Ca^{2+} dimers with NFX was studied using X–Ray [20].

Extremely much had been paid to the study of binary and ternary complexes of transition metals with molecules of biological and pharmaceutical interest. Furthermore, it had been suggested

that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of drugs [21–27].

Several analytical methods have been developed for the determination of NFX. These methods includecapillary electrophoresis [28, 29], flow injection analysis [30–32], chromatography [33–37], spectrofluorometric [38–42], spectrophotometric [43–49], and electrochemical methods [50–56].

Potentiometric techniques were used for the determination of metal ions using ion–selective electrodes [57–60]. Also, from analytical point of view, Gran plot [61] was suggested for determine the equivalence point of a potentiometric titration curves, and it was utilized successfully for the determination of several analytes [62–65]

Thus, the present work concerns study on the possibility of interaction of NFXwith different metal ions in solution using potentiometric and conductometric techniques. The effect of ionic strength on the stability constants was discussed. Also, study of the species distribution of ionic equilibria for the NFXand its metal complexes in solution were carried out.

Moreover, the work includes(for the first time), the development of rapid, simple, sensitive and selective method for the determination of NFX in pure and dosage forms, with the study of interferences for some excipients on the accuracy of the proposed methods.

2. EXPERIMENTAL

2.1. Instrumentation

Potentiometric titrations were performed using pH meter model JENWAY. Conductometric titration and molar conductance measurements were carried out by JENWAY model, using on immersion cell. The stoichiometry and stability constants were calculated using numerical and computerized programs (Excel) [65, 66].

2.2. Preparation of solutions:

2.2.1. Sodium chloride (NaCl):

0.5 M of sodium chloride (Riedel–de Haen) was prepared in bidistilled water.

2.2.2. Hydrochloric acid (HCl):

0.1 M hydrochloric acid (BDH) solution was prepared and standardized by sodium hydroxide solution. The latter was standardized against standard oxalic acid.

2.2.3. Sodium hydroxide (NaOH):

A 1:1 (w/v) sodium hydroxide (BDH) solution was prepared and well stored in a well steamed waxed tall glass tube for some days to obtain a carbonate–free NaOH solution [72]. The required molarity was prepared by dilution from such solution then standardized against standard oxalic acid.

2.2.4. Sodium nitrate (NaNO₃):

0.5 M solution of sodium nitrate (BDH) was prepared by dissolving the appropriate amount of the substance in bidistilled water.

2.2.5. Norfloxacin (NFX):

Norfloxacin (standard substance) was purchased from Sigma (St Louis, MO, USA). The norfloxacin as tablets (400mg) and solutions drops (0.3% w/v) were Norflox (tablet) (Al'shahba Labs–Syria), Noracin (tablet) (Memphis Co.–Egypt), Norflox (tablet) (Cipla. LTD–India), Floxamed (drops) (Unimed–Tunisia),Norflox (drops) (Cipla. LDT–India).

Stock standard solution of NFX (1×10^{-2}M) was prepared by exact weighing of the product from Sigma in bidistilled water, at pH below 5 adjusted by 0.012 M of HCl. The mixture was diluted to required volume with water. The solution was stored in the dark at 4 °C. Working standard solutions were prepared from the stock solution by appropriate dilution with water.

Also, in case of pharmaceutical preparations, ten tablets were weighed and powdered. The powder equivalent to 100 mg of NFX was shaken with 5 mL of HCl and about 100 mL of bidistilled water in a water–bath at 50 °C for 10 min. After cooling, the solution was filtered into a 250 mL calibrated flask, the residue was washed several times with water and the solution diluted with water to the mark.

For the determination of the NFX in eye/ear drops samples, the mass of the NFX per mL was determined. An amount of eye/ear drops equivalent to 10 mg of NFX was transferred into a 25 mL calibrated flask and diluted to the mark with bidistilled water.

2.2.6. Metal solutions:

1×10^{-1} M of the metal nitrate or chlorides salts (BDH, UK, GENEVA or INDIA), was prepared. The metal ions are: Pb(II), Co(II), Ba(II), Ti(II), Sr(II), Cr(III), Fe(III), La(III), Zr(III) and Sn(II) were prepared by dissolving the appropriate amounts of each metal ion in bidistilled water.

2.3. Procedures

2.3.1. Potentiometric measurements for complexes:

This experimental method consisted of making potentiometric measurments of hydrogen ion concentration solution of NFX in the presnce and absence of the metal ions [68]. The titration was performed in the presence of 0.01 M NaCl. Generally, the following solutions were prepared and titrated against standared CO_2–free NaOH solution at room temperature.
   (a)   0.001MHCl + 0.009 M NaCl.
   (b)   Solution (a) + 0.001 M NFX.
   (c)   Solution (b) + 0.001 MNFX.

In all titrations , the total volume was maintained constant at 50 mL with ionic strength 0.01 M NaCl and 25±1.0°C. Multiple titrations were carried out for each system. The pH–meter was calibrated before and after each titration using three standard buffer solutions at pH 4, 7 and 10.

2.3.2. Conductometric measurments for NFX complexes:

Conductometric titration were carried out[65, 66] at room temperature (25±1.0°C)by titrating 25 mL of 0.001 M of each metal ion with 0.01 M of NFX solution in 0.5 mLincreament. Correction for the dilution effect is performed by multiplying the values of specific conductonce by factor$\frac{(25+v)}{25}$, where V is the volume of added NFX.

2.3.3. Potantiometric and conductometric determination of NFX:

2.3.3.1. In pure form:

An aliquot of 15.0 mL of pure NFX solution(1×10^{-2} M)was transferred to athermostated glass cell (25.0±1.0°C) then potentiometrically and conductometrically titrated with a standard solution of NaOH 0.1 M adjusted of ionic strength.

2.3.3.2. In pharmaceutical preparations:

The obtained NFX solution form powdered tablets dosage forms (as described in experimental section) analyzed under the same procedure described in pure form. The quantity per tablet was calculated from the standard calibration curve.

For the determination of the NFX in eye/ear drops samples, the diluted solution of eye/ear drops samples was analyzed by standard solution of NaOH 0.1 M adjusted of ionic strength. The quantity per tablet was calculated from the standard calibration curve

3. RESULTS AND DISCUSSIONS

3.1. Potentiometric and conductometric studies of NFX with some metal ions:

3.1.1. Determination of the proton–ligand stability constants of NFX:

pH–metric titration of NFX was carried out with ionic strength, μ = 0.01 M NaCl at 25±1.0°C and using standared CO_2–free NaOH as a titrant. Titration curves obtained for NFX are shown in Fig.1. The values of $\bar{n}A$ (average number of proton attached per ligand)was determined according to Irving and Rossotti [68] were compiled from the titration data using solutions (a) and (b). Calculations of proton–ligand association constants were carried out by plotting$\bar{n}A$ against pH (Fig.2) and the obtained data was illustrated in Table 1.
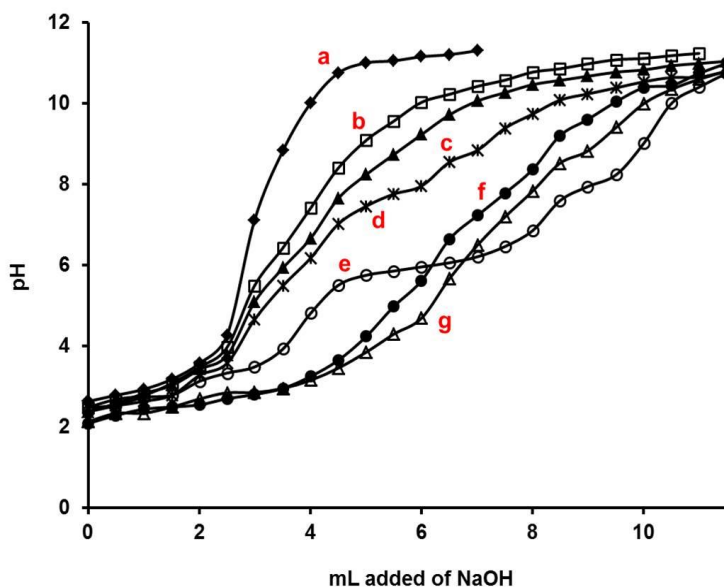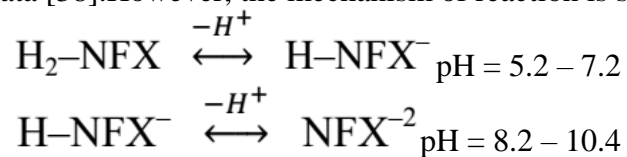
Figure 1. Potentiometric titration curves of NFX: (a) HCl, (b) NFX, (c) Ti(II), (d) Pb(II), (e) Cr(III), (f) Sn(II) and (g) Fe(III), with $\mu = 0.01$ M NaCl at 25 ± 1.0 ºC.

Table 1. Protonation constants of NFX and stability constants of metal ion complexes at $\mu= 0.01$ M NaCl and 25 ± 1.0 ºC.

Metal ion	Log K_1 (M:L)[*]	Log K_2 (M:L)[*]	Log K_3 (M:L)[*]	Ref.
H^+	8	6.2	– – –	Present work
	8.38	6.22	– – –	[38]
Pb (II)	11.23 (1:1)	4.84 (1:2)	– – –	– – –
Sr (II)	9.23 (1:1)	4.62 (1:2)	– – –	– – –
Fe (III)	– – –	6.89 (1:2)	4.17 (1:3)	– – –
Co (II)	10.84 (1:1)	5.13 (1:2)	– – –	– – –
Ti (II)	12.83 (1:1)	5.79 (1:2)	– – –	– – –
Cr (III)	12.43 (1:1)	8.85 (1:2)	6.33 (1:3)	– – –
Ba (II)	11.63 (1:1)	5.45 (1:2)	– – –	– – –
Sn (II)	– – –	9.65 (1:2)	5.94 (1:3)	– – –
La (III)	10.23 (1:1)	5.72 (1:2)	3.76 (1:3)	– – –
Zr (IV)	– – –	8.45 (1:2)	5.56 (1:3)	– – –

(*) These ratios are from potentiometric and conductometric methods

The values of $LogK_1^H$ and $LogK_2^H$ (the first and second proton association constants of NFX) at the pH corresponding to $\bar{n}A = 0.5$ and 1.5, were 8.0 and 6.2 respectively. The results were good agreement with literature data [38]. However, the mechanism of reaction is show as follow:

$$H_2\text{–NFX} \overset{-H^+}{\longleftrightarrow} H\text{–NFX}^- {}_{pH = 5.2 - 7.2}$$

$$H\text{–NFX}^- \overset{-H^+}{\longleftrightarrow} NFX^{-2} {}_{pH = 8.2 - 10.4}$$

3.1.2. Determination of formation constants of metal–NFXcomplexes:

The pH–metric titration of ten metal ions that mentioned previously was carried out to elucidate their interaction with NFXwith μ=0.01 M NaCl at 25±1.0°C. The stability constants of formed complexes were calculated using the titration curves of the metal–ligand solutions (c) as shown in Fig. 1.
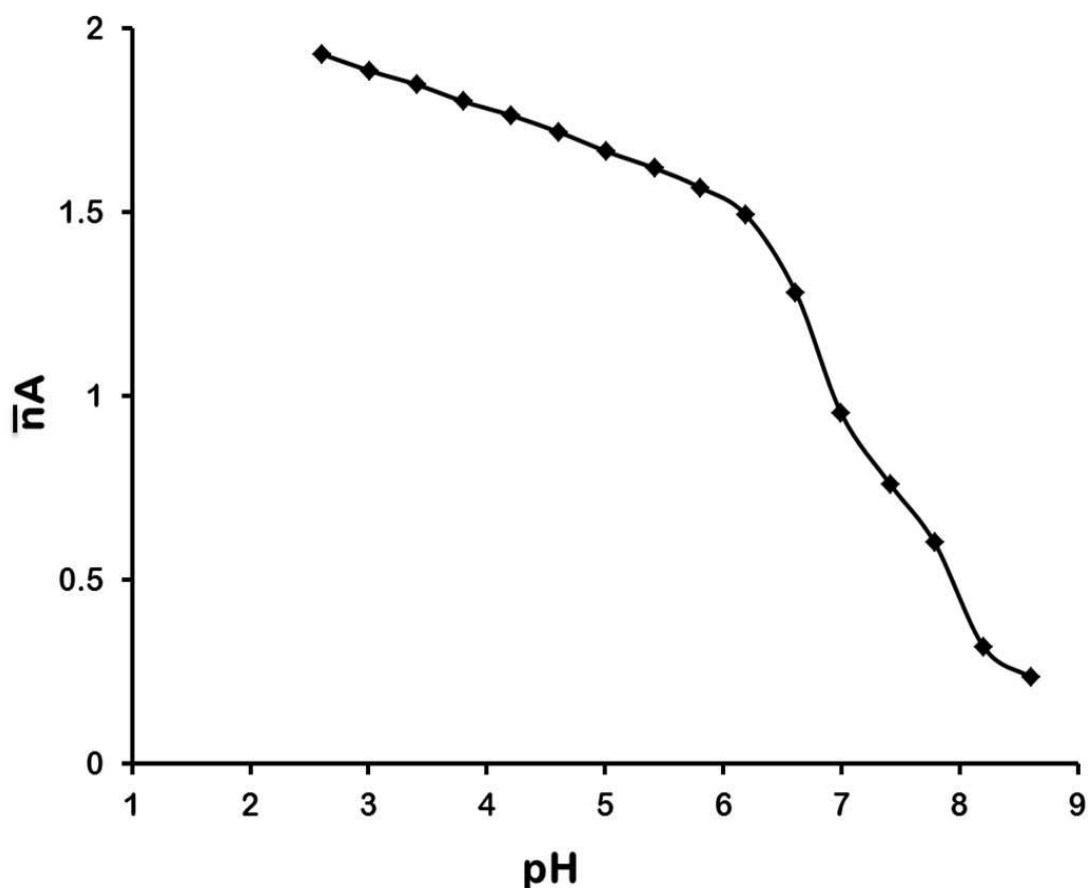

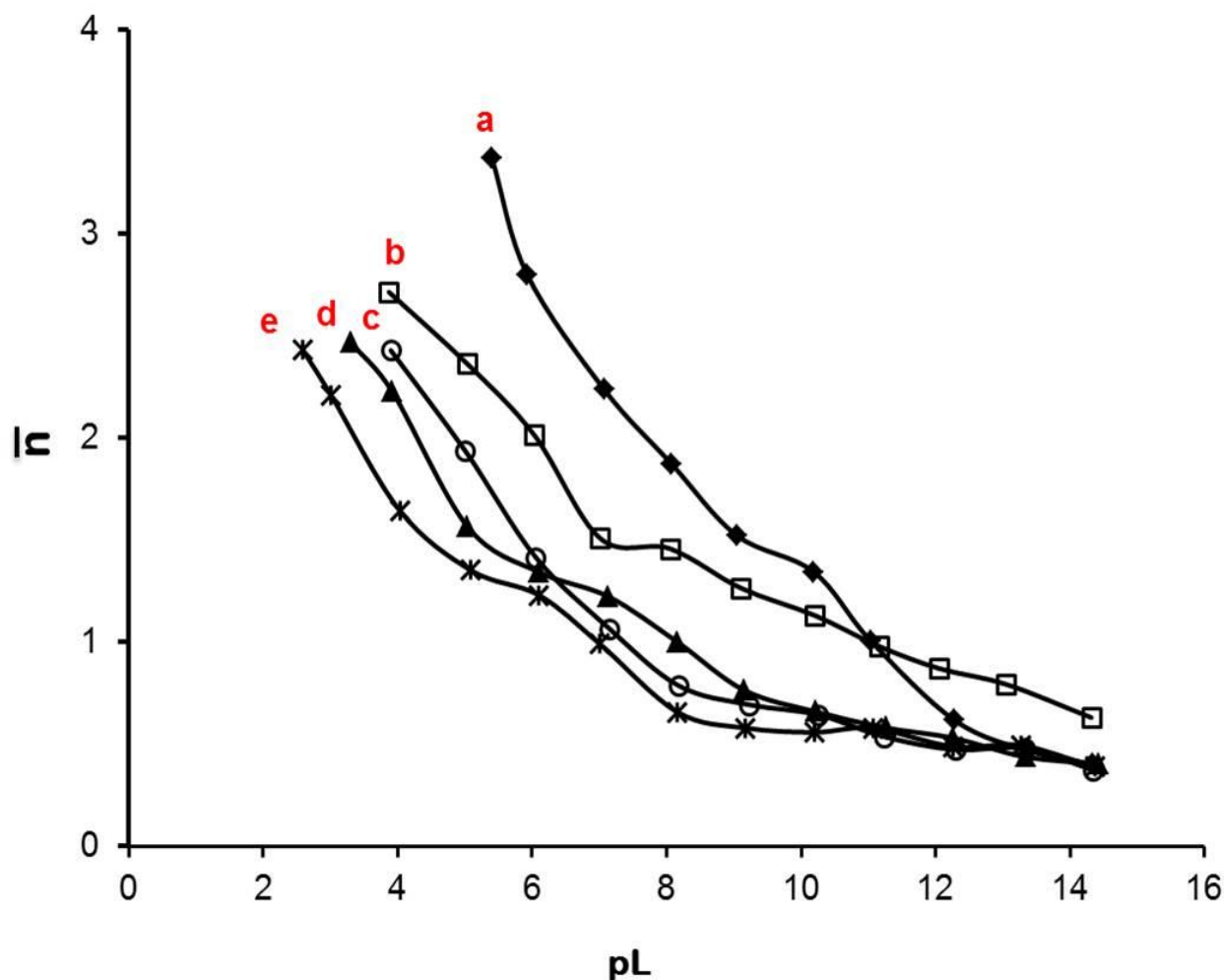**Figure 2.** Potentiometric constant curve of NFX, μ = 0.01 M NaCl at 25±1.0 °C.

Figure 3. Formation curves of binary metal ions with NFX at μ = 0.01 M NaCl at 25±1.0 ℃: (a) Fe(III), (b) La(III), (c) Co(II), (d) Pb(II), and (e) Cr(III).

The formation curves for the metal complexes as shown in Fig. 3 were obtained by plotting the average number of ligands attached per metal ions ($\bar{n}$)*vs.* the free ligand exponent (pL), according to Irving and Rossotti equations [68]. The values of stability constants at ionic strength 0.01 M NaCl listed in Table 1were determined using the half–integral method [68].

Looking at the Table 1 we concluded that, some metal ions viz; Cr (III) and La (III) react with NFX to form three types of metal–ligand complexes; 1:1, 1:2 and 1:3 at the ionic strength under investigation. Furthermore, the other metal ions Pb(II), Sr(II), Ti(II), Ba(II) and Co(II) tend to form two types of metal complexes 1:1 and 1:2 metal to ligand. On other hand, metal ions Fe(III), Sn(II) and Zr(IV) form complexes of type 1:2 and 1:3. It may be due to the concentration of ligand, ionic strength and the nature of metal ion.

In these complexes the quinolone acts as a bidentate ligand through the ring carbonyl group and through one of the oxygen atoms of the carboxylato group. Quinolones can also act as bridging ligands and thus capable of forming polynuclear complexes [8,20]. These sites are shown as follow:

Ratio (1:2) (M:L)
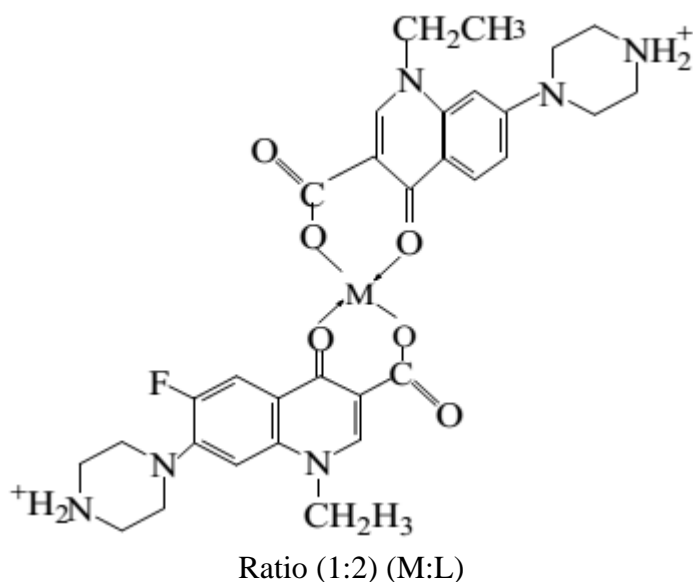
The order of stability constants of the different binary complexes formed between NFX and bivalent metal ions investigated in this study is in the expected Irving–Williams order [69] for (1:2)metal to ligand at $\mu = 0.01M$ NaCl:

Sn(II) > Cr(III) >Zr(IV) > Fe(III) >Ti(II) > La(III) > Ba(II) > Co(II) >Pb(II) >Sr(II)
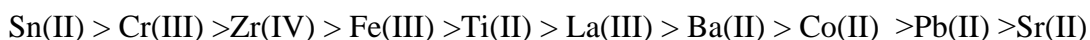
The effect of ionic strength on stability constants of NFX with different metal ions *viz* .Co(II), Ti(II), La(III), Cr(III) and Pb(II) has been discussed. The ionic strength's choosing was 0.01, 0.05 and 0.1M NaCl at 25±1.0 ℃. By plotting the relation between the ionic strength under investigation and the first stability constants $LogK_1$, we can conclude that the stability constants of metal–NFX complex (1:1) were decreased as the ionic strength increased Fig. 4.
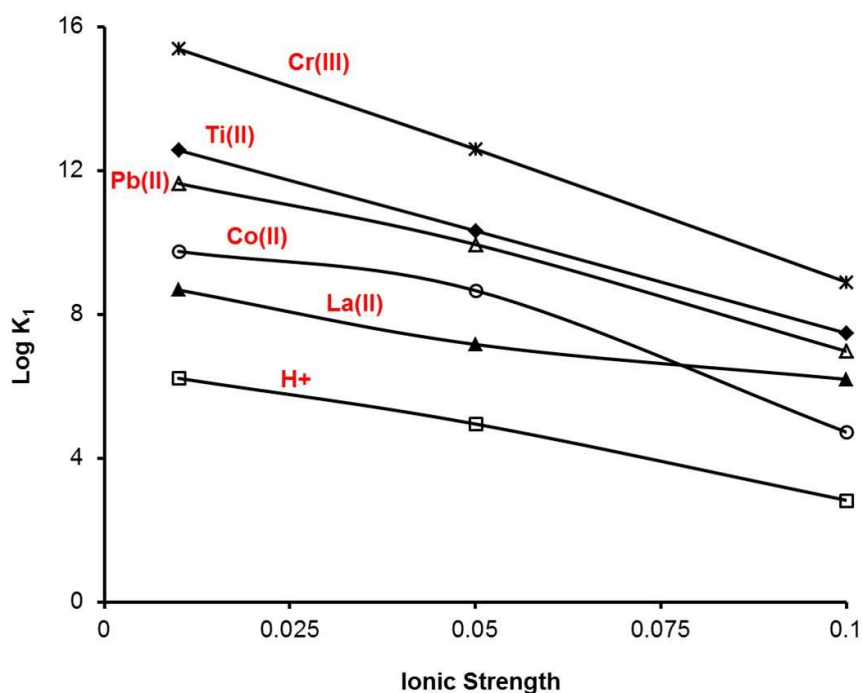

Figure 4. Effect of ionic strength on stability constants of NFX with some metal ions.

3.1.3.Conductometric studies on the metal complexes of NFX:

The conductometric titrations are performed by titrating of 25 mL (1×10^{-3} M) of each metal ion with successive volumes of 1×10^{-2} M NFX solution.
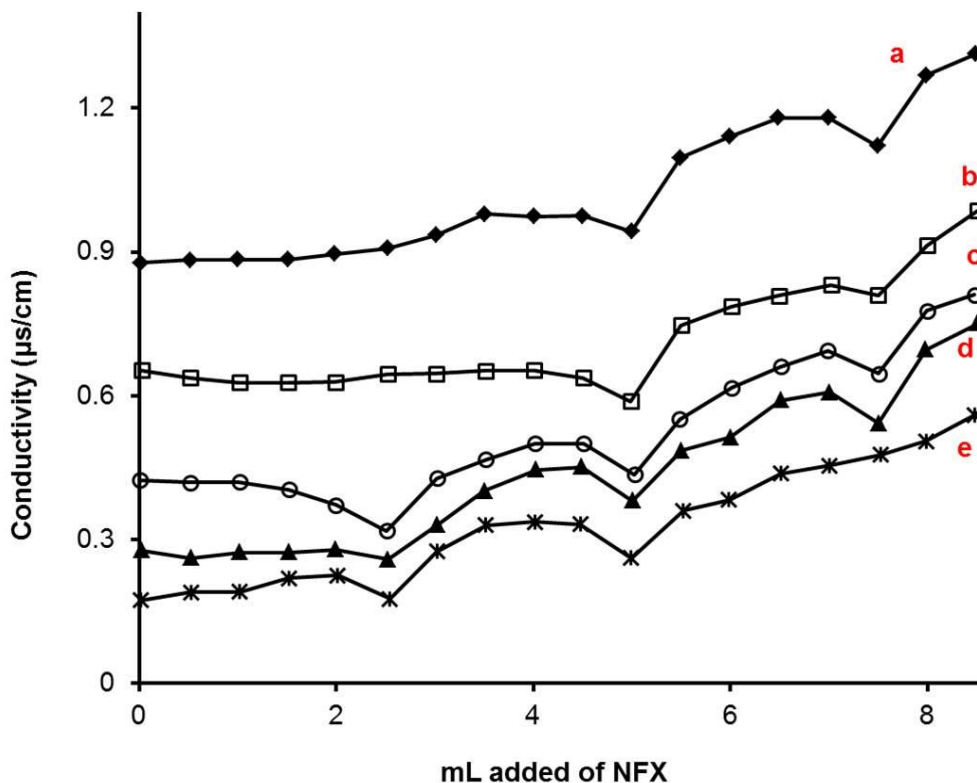

Figure 5. Conductometric titration curves of 25 mL metal ions (1×10^{-3} M) with NFX (1×10^{-2} M): (a) Sn(II), (b) Fe(III), (c) Cr(III), (d) La(III) and (e) Pb(II)

The graphs shown in Fig.5 were obtained. The relationship shows a well–defined breaks corresponding to the stoichiometric ratios 1: 1, 1:2 and 1:3 (M:L). These results are in agreement with those obtained by potentiometric method (Table 1). The observed increase in conductivity during the range titration of metal ion with NFX ligand during the complex formation, clearly indicate liberation of high ionic mobile H^+ ions.

3.1.4. Species distribution diagrams of NFX complexes:

Looking at Fig.6 for NFX (pure), at pH below 5.2, the neutral form (H_2–NFX) (α^o) of the molecule was dominant. At pH between 6.2 and 8.2, (H–NFX$^-$) (α^1) being dominant. But at pH higher than 8.5, the negatively charged form (NFX^{-2}) (α^2) of NFX is the dominated one.
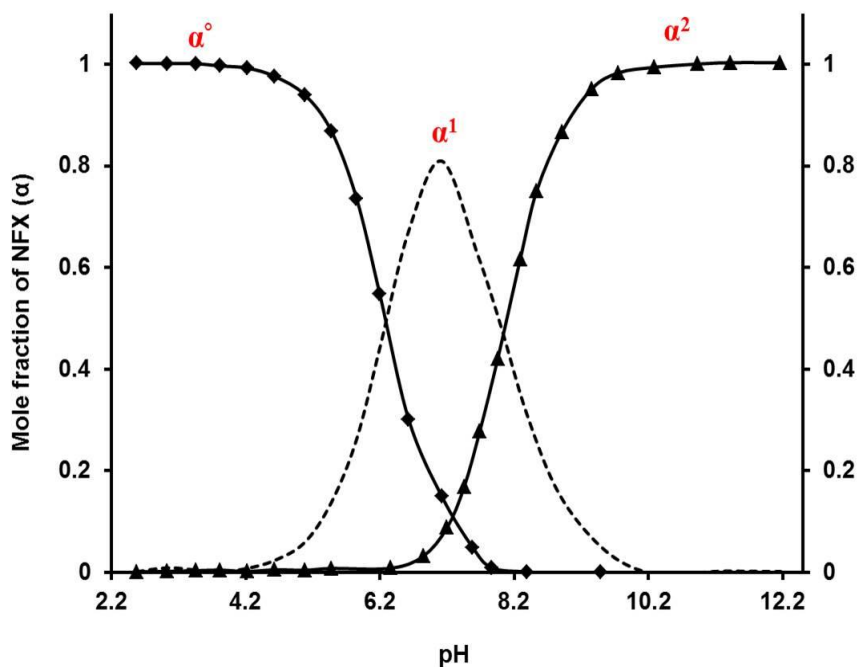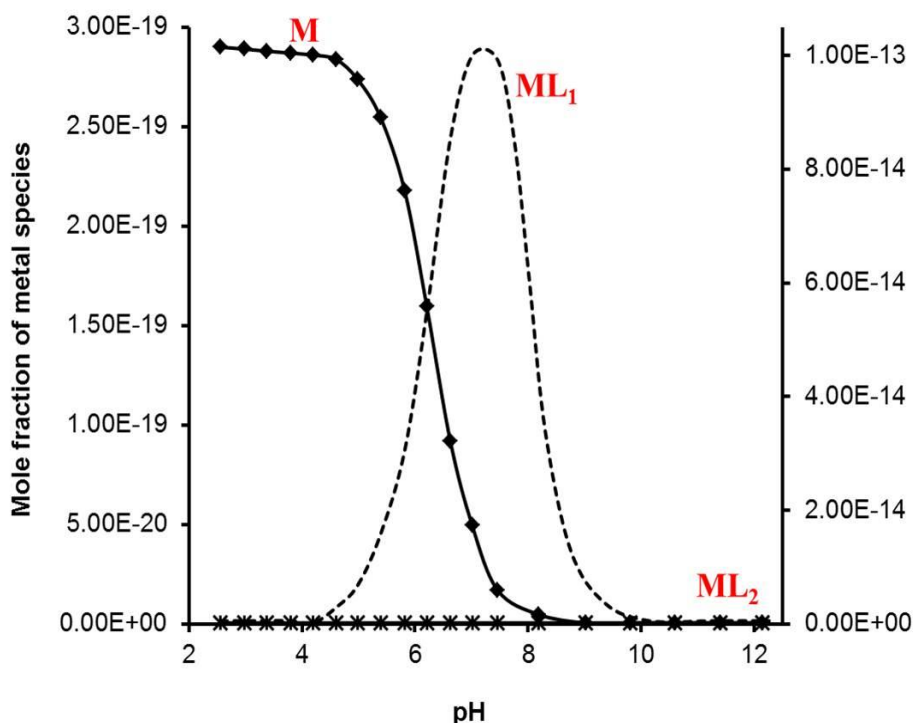
Figure 6. Ionic equilibria of NFX in different pH's range.

Figure 7. Ionic equilibria of Cr–NFX in different pH's range.

The mole fraction α_{ML} and α_{ML2} can be calculated from potentiometric data using the obtained stability constants for ML, and ML_2 complexes and the pH [70]. The species distribution curves can be obtained by plotting α (α= mole fraction of the species) *vs*.pH. Representative closely related plots were obtained for other metal-ligand complexes as shown in Fig. 7.

The values of α_M, α_{ML} and α_{ML2} present in solution depends mainly on the pH of the medium. The plots reveal that at low pH value, the metal ions are often present as free ions. This indicates no

complex formation in the acidic medium. On increasing the pH of medium, the concentration of metal ion tends to decrease, while that of ML species tends to developed at moderately acidic media (pH≈5.5–8.2), and reaches to the value maximum at pH value ≈ 7.4 for all metal ions complexes, except for Fe(III), Sn(II) and Zr(IV) ions the formed complexes as ML and ML_2 are very little.

At pH above 7.4 the essential change is the increase in the concentration of ML_2 with decrease in ML. Above this region almost of all metal ions remains in the form of ML and ML_2 species and their concentration increases on increasing the pH of solution. This demonstrates clearly that ML species are much more stable than ML_2 in their solutions.

3.2. Potentiometric and conductometric determination of NFX:

Although NFX were determined previously by several analytical techniques, the proposed method in current article is still characterized as simple, low cost and do not involve laborious time–consuming sample preparation.
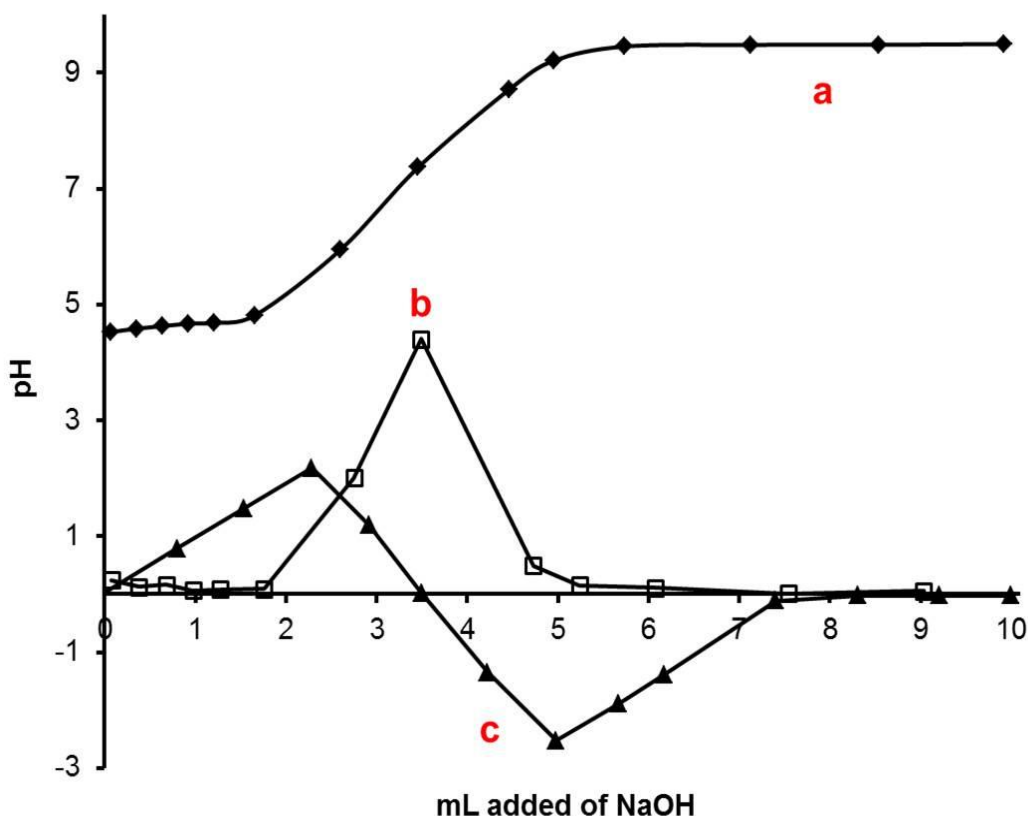

Figure 8. Typical potentiometric titration curves of NFX (pure), $\mu = 0.01$ M $NaNO_3$ at 25±1.0 °C: (a) titration curve, (b) first derivative and (c) second derivative.

Potentiometric and conductometric titration for the determination of NFX in pure form were performed with NaOH as titrant, at 25±1.0°C. The Fig.8 shows potentiometric titration curves for determination of NFX in pure form, where (a) show normal titration curve of NFX, (b) and (c) show

first and second derivative of titration curve for the determination of NFX, respectively. These derivatives were applied to ascertain the equivalence point.

3.2.1 Effect of ionic strength on determination of pure NFX:

In trials to elucidate optimum ionic strengths of aqueous solution medium for the quantitative determination of NFX in pure form, several ionic strengths in range from 0.05–0.5M $NaNO_3$ by using potentiometric method, were tested at 25±1.0°C. It was observed that recovery values increase with ionic strength increase, and it was found that 0.1M $NaNO_3$ gave the best recovery value (around 100 %) as listed in Table 2.

Table 2. Effect of the ionic strength ($NaNO_3$) using potentiometric method at 25±1.0 °C.

Ionic strength (M)	Add from pure NFX (mg)	Found (mg)	Percentage Recovery ± SD (%)
0.05	8.1	7.5	92.6 ± 0.5
0.1	8.1	8.2	101.2 ± 0.29
0.2	8.1	8.8	108.6 ± 0.35
0.3	8.1	11.1	136.8 ± 0.21
0.4	8.1	13.4	165.4 ± 0.19
0.5	8.1	15.36	189.6 ± 0.42

3.2.2. Determination of pure NFX:

Table 3. Determination of NFX in pure form by using proposed methods with $\mu = 0.1$ M $NaNO_3$ at 25±1.0 °C.

Add of pure NFX (mg/L)	Found (mg/L)	Recovery (%)	SD (n=5)	Confidence (n=5) α=0.05
0.32	0.31	95.8	0.36	0.32
	(0.3)	(95.1)	(0.32)	(0.28)
0.96	0.94	97.8	0.23	0.2
	(0.93)	(96.8)	(0.26)	(0.23)
1.6	1.58	98. 7	0.24	0.21
	(1.57)	(98.3)	(0.27)	(0.24)
2.24	2.25	100.4	0.45	0.39
	(2.23)	(99.6)	(0.32)	(0.28)
3.01	3.07	102.1	0.19	0.17
	(3.06)	(101.7)	(0.21)	(0.18)

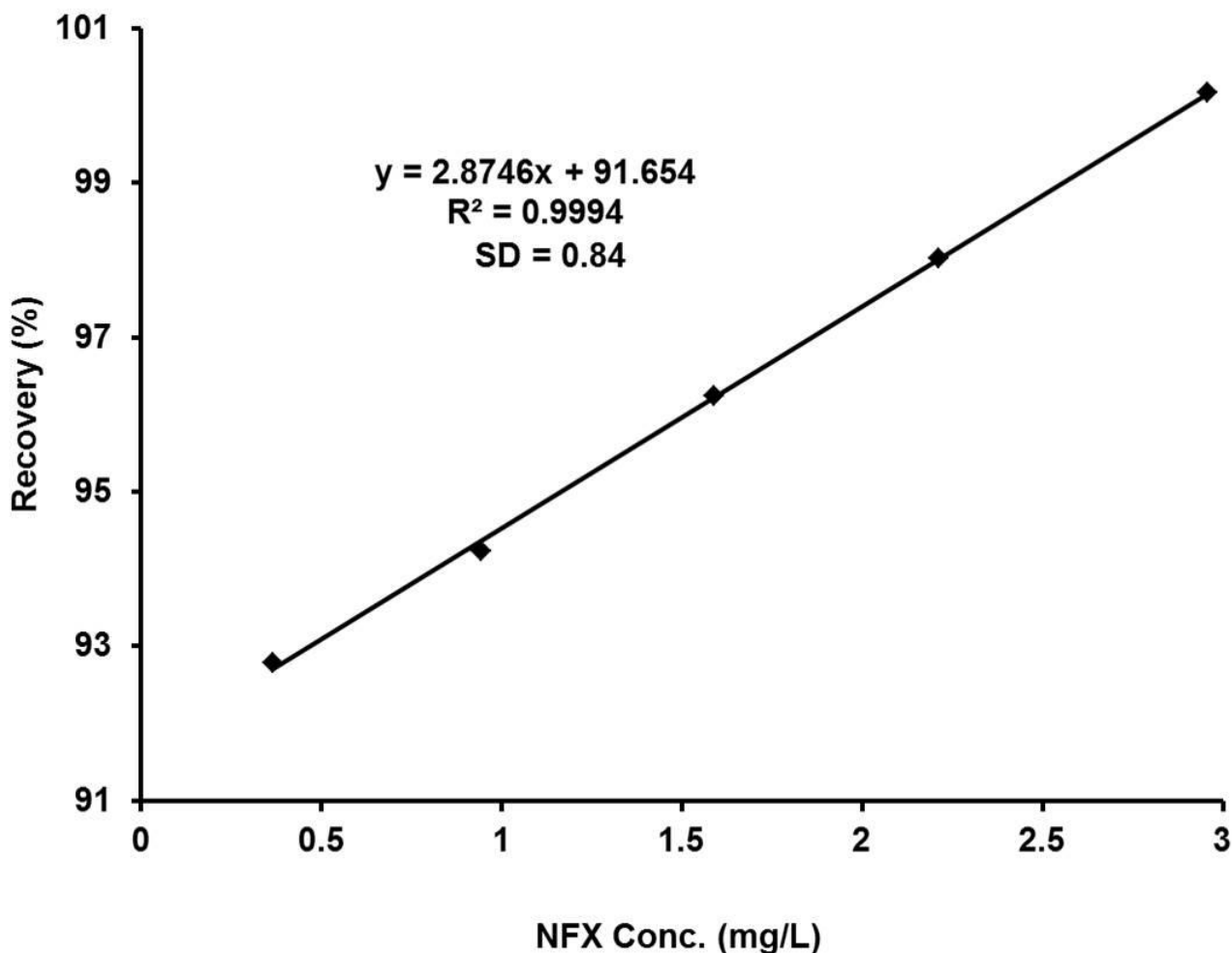The data between brackets were from conductivity method

Figure 9. Linearity range of NFX (pure) $\mu = 0.01$ M $NaNO_3$ at 25 ± 1.0 ℃.

The five independent analysis of NFX sample in pure form in concentration range of 0.32–2.87 mg L^{-1} were performed using the proposed methods. The recoveries of proposed methods were from 95.1–102.1% with the standard deviation (SD) within 0.19–0.45mg L^{-1}(n=5) and confidence was in the range 0.17–0.39 mg L^{-1}. The obtained results are compiled in Table 3.

The results presented agreed fairly well with those obtained by the standard procedure [71,72] (iodimetric titration). The detection limit (as $3\sigma/b$, where b is the slope and σ = SD) [70, 71] was 0.27 mg L^{-1}. The calibration curve was linear(r =0.9891) in the concentration range of0.32–2.87 mg L^{-1}. The standard deviation (SD) was less than 1.0 % (n=5), as we can see in Fig.9.

3.2.3. Effect of interferences:

To assess the usefulness of the proposed method, the effect of the common components (additives and excipients), which often accompany NFX in dosage forms (lactose, sodium chloride, and magnesium stearate) were investigated over a concentration range at least 100 times higher than that of NFX. No interferences were observed in the presence of the substances tested.

3.2.4. Analytical applications:

The proposed methods were successfully applied for NFX determination in dosage forms. Fig.9 and Fig. 10 show the potentiometric titration curve used for the determination of NFX in tables and eye/ear drops, respectively. In both of Fig. 9 and Fig. 10, curves *a*, *b* and *c* are: the typical potentiometric titration curve based on with only one inflection point, the first and the second derivative of potentiometric curve, respectively.
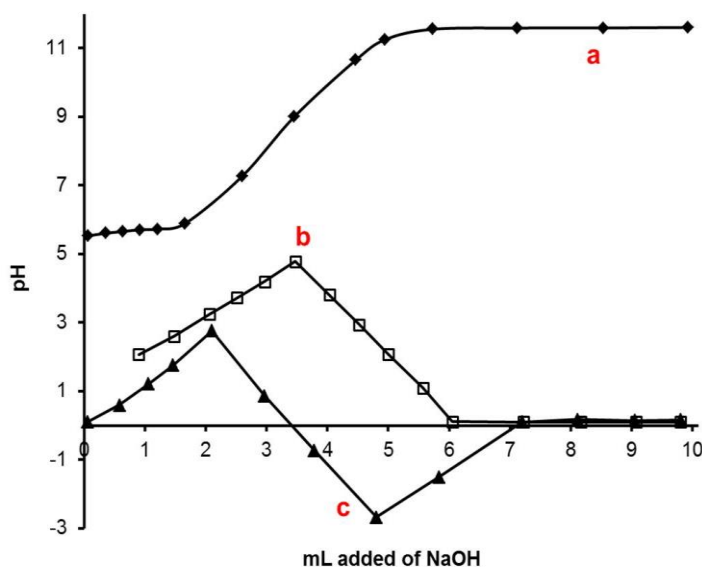

Figure 10. Typical potentiometric titration curves of Norflox tablet (400 mg): $\mu = 0.01$ M $NaNO_3$ at 25±1.0 ℃: (a) titration curve, (b) first derivative and (c) second derivative.
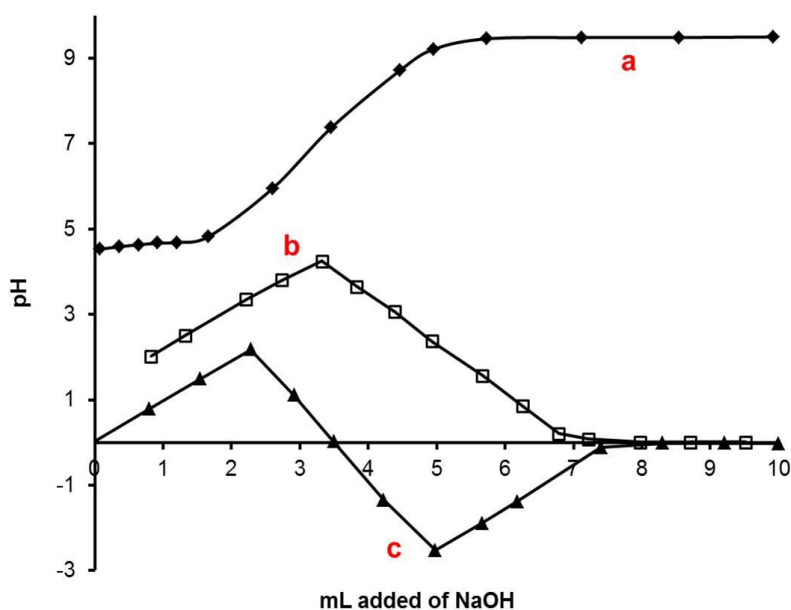

Figure 11. Typical potentiometric titration curves of Floaxamid drops: $\mu = 0.01$ M $NaNO_3$ at 25±1.0 ℃: (a) titration curve, (b) first derivative and (c) second derivative.
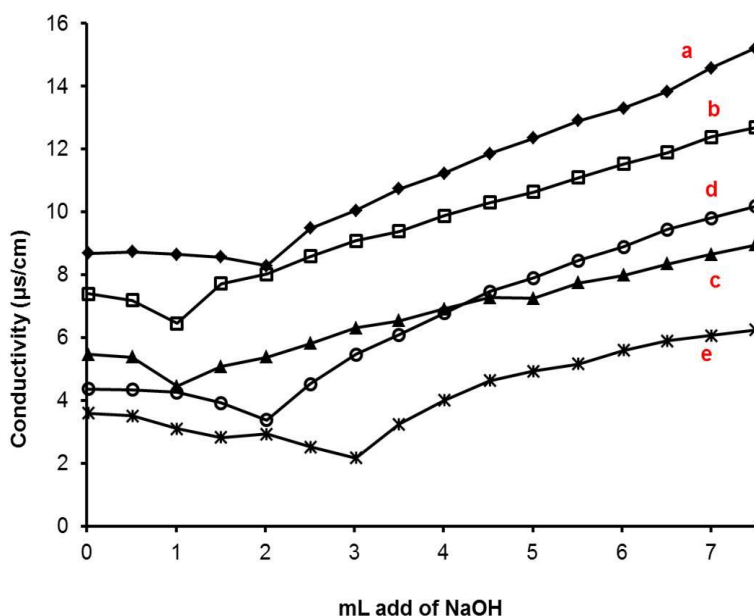
Figure 12. Conductometric titration curves for the detrmination of NFX in pure form and dosage forms: (a) Norflox (tablet), (b) Floaxamid (drops), (c) Norflox (drops), (d) Noracin (tablets) and (e) NFX (pure).

Conductometric titration for the quantitative determination of NFX in its dosage forms was carried out under ideal conditions of potentiometric titration. The produced conductometric titrationcurves are shown in Fig.11.

Table 4. Determination of NFX in pharmaceutical preparations

Sample	Manufacturer	Label to content (mg)	Proposed methods		
			Found (mg)	Recovery (%)	SD (n=5) (%)
Norfox (tablet)	(Al'shahba Labs– Syria)	400	398 (395.6)	99.5 (98.3)	0.16 (0.23)
Noracin (tablet)	(Memphis Co., Egypt)	400	390 (389.2)	97.5 (95.8)	0.2 (0.24)
Norflox (tablet)	(Cipla. LTD–India)	400	393 (391.5)	98.25 (98.1)	0.18 (0.19)
Floxamed (drops)	(Unimed– Tunisia)	300	301 (289)	103.3 (98.8)	0.16 (0.21)
Norflox (drops)	(Cipla. LDT–India)	300	295 (294.3)	98.3 (97.2)	0.19 (0.2)

The data between brackets were from conductivity method

The results presented in Table 4 show that the percentage recoveries of potentiometric and conductometric methods were 98.3–103.3 % and 95.8–98.8 %, the standard deviations (SD) were within 0.16–0.2 and 0.2–0.24 mg L^{-1}(n=5) respectively. These results indicate the accuracy and

precision of the methods and the absence of significant matrix effects on potentiometric and conductometric measurements at least for the samples analyzed, and they were agreement with the recoveries of flow injection chemiluminescence method (95–102%) [29].Table 5 shows a comparison of recovery and RSD calculated values of the proposed methods and other methods used for NFX determination. The tabulated results of recovery values shows a good agreement or higher than recovery values of other analytical methods used for the determination of NFX. Also, the RSD values are lower than those values of other analytical methods used for the NFX determination.

Table 5. Comparison of recovery and RSD of the proposed methods and other methods used for NFX determination.

Method	Recovery (%)	RSD (%)	Ref.
Capillary Zone Electrophoresis	35.40–85.60	3.90–6.80	27
Flow–injection	98.20–100.70	0.70–1.50	30
Chromatographic	100.7	0.55	31
	99.74	1.278	32
	97.92–103.95	0.40–0.74	33
	82.50–92.70	6.00–17.30	35
	100.51–101.96	1.53–1.89	36
Spectrofluorimetric	95.00–103.00	2.40	37
	96.00–102.00	1.40	38
	96.50–106.20	3.77–5.67	39
	95.20–100.20	5.40–9.90	40
Spectrophotometric	95.80–103.80	1.00–1.70	41
	97.65–100.73	0.66–1.26	42
	99.15	0.81	47
	97.50–101.80	1.02–1.42	48
Electrochemical	98.37–101.30	0.69–1.51	50
	85.00	4.00	52
	97.20–103.80	2.80	53
	97.50–103.30	0.16–0.20	This Work
	95.80–98.80	0.19–0.24	This Work

4. CONCLUSION

Determination of proton ligand association constants of norfloxacin and their metal complexes with $\mu = 0.01$ M NaCl was investigated and the two logarithmic association constants values which

calculated by the half–$\bar{n}$method were 6.2and 8.0. The stoichiometric of metal complexes of norfloxacin was as following Cr(III) and La(III) ions with norfloxacin forms three types of metal–norfloxacin complexes 1:1, 1:2 and1:3, while Pb(II), Co(II), Ti(II), Ba(II) and Sr(II) ions tends to form two types of metal complexes 1:1 and 1:2 (metal: ligand). On other hand, Sn(II), Fe(III) and Zr(IV) ions formed complexes with norfloxacin of two types 1:2 and 1:3. So, the effect of ionic strength on stability constant of norfloxacin, with some different metal ions was studied. The stoichiometry of complexes formed in solution was confirmed by conductometric method. Also, the species distribution (α) diagrams for norfloxacin and their metal complexes which calculated as mole fraction α_{ML} andα_{ML2}, were discussed.

Norfloxacin was determined by potentiometric and conductometric methods using sodium hydroxide as titrant, at 25±1.0°C. The calibration graph was constructed over a concentration range of 0.32-2.87 mg L^{-1}, and the calculated value of detection limit was 0.27 mg L^{-1} at SD value < 1.0.. The proposed procedures were successfully applied for the determination of norflxacin in dosage forms (tablets and eye drops) with no interferences from common components usually exist. The Recoveries obtained of the proposed methods for determination of norfloxacin from various tablet dosage formulations in range from 95.8 to 103.3% with SD was in range 0.16-0.24mg L^{-1}.

References

1. C. Mazuel, K. Florey, Analytical Profiles of Drug Substances, Academic Press, New York, 20, 557 (1991).
2. I. Turel, *Coordin. Chem. Rev.,* 232 (2002) 27–47.
3. D. T. W. Chu and P. B. Fernandes in: B. Testa (Ed.), Advances in Drug Research, Vol. 21, London, Academic Press, pp. 39–144 (1991).
4. J. E. F. Reynolds (Ed.), Martindale, The Extra Pharmacopeia, 30th Ed., The Pharmaceutical Press, London, pp. 145–147 (1993)
5. Y. Xia, Z. Y. Yang, S. L. Morris–Natschke and K. H. Lee, *Curr. Med. Chem.*, 6 (1999) 179–194.
6. B. M. Lomaestro and G. R. Bailie, *Ann. Pharmacother.*, 25 (1991) 1249–1258.
7. G. A. Mokrushina, S. K. Kotovskaya, Z. M. Baskakova, G. M. Petrova, T. V.Kolmakova, V. N. Charushin, V. L. Rusinov and O. N. Chupakhin, *Pharm. Chem. J.*, 30 (1996) 540–542.
8. J. Al–Mustafa, *Acta Chim. Slov.*, 49 (2002) 457–466.
9. F. Gao, P. Yang, J. Xie and H. Wang, *J. Inorg. Biochem.*, 60 (1995) 61–67.
10. V. Uivarosi, S. Neagoe, V. Aldea and A. Nitulescu, Roum. Arch Microbiol. Immunol., 60 (2001) 267–277.
11. S. C. Wallis, B. G. Charles, L. R. Gahan, L. J. Filippich, M. G. Bredhauer and P. A. Duchworth, *J. Pharm. Sci.*, 85 (1996) 803–809.
12. R. Singh, A. Debnath, D. T. Masram and D. Rathore, Res. *J. Chem. Sci.,* 3 (2013) 83–94.
13. S. K. Upadhyay, P. Kumar and V. Arora, *J. Struct. Chem.*, 47 (2006) 1078–1083.
14. G. Palú, S. Valisena, G. Ciarrocchi, B. Gatto and M. Palumbo, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 9671–9675.
15. A. R. Shaikh, R. Giridhar and M. R. Yadav, *Int. J. Pharm.*, 332 (2007) 24–30.
16. Y. X. Li, Z. F. Chen, R. G. Xiong, Z. Xue, H. X. Ju and X. Z. You, *Inorg. Chem. Commun.*, 6 (2003) 819–822
17. M. S. Refat, *Spectrochim. Acta A*, 68 (2007) 1393–1405.
18. P. B. Issopoulos, *Analyst*, 114(1989) 627–630.
19. C. M. Riley, D. L. Ross, D. V. Velde and F. Takusagawa, *J. Pharm. Biomed. Anal.*, 11 (1993) 49–59.

20. Z. F. Chen, R. G. Xiong, J. L. Zuo, Z. Guo, X. Z. You, K. and H. Fun, *J. Chem. Soc. Dalton Trans*, 22 (2000) 4013–4014.
21. A. H. Amrallah, N. A. Abdalla and E. Y. El–Haty, *Talanta*, 46 (1998) 491–500.
22. P. G. Daniele, O. Zerbinati, V. Zelano and G. Ostacoli, *J. Chem. Soc., Dalton Trans.*, (1991) 2711–2715.
23. S. Kirschner, Y. K. Wei, D. Francis and J. G. Bergam, *J. Med. Chem.*, 9 (1966) 369–372.
24. A. H. Naggar, H. A. Mauof, A. A. Ekshiba and O. A. Farghaly, *Pharm. Chem. J.*, 3 (2016) 125–137.
25. W. M. Yousef, K. Alenezi, A. H. Naggar, T. M. Hassan, S. Z. Bortata and O. A. Farghaly, *Int. J. Electrochem. Sci.*, 12 (2017) 1146–1156.
26. H. M. El–Sagher and O. A. Farghaly, *International Journal of Engineering Science and Technology* (IJEST), 9 (2017) 215–225.
27. A. A. Al-Rashdi, A. H. Naggar, O. A. Farghaly, H. A. Mauofand A. A. Ekshiba, *American J. Anal. Chem.*, 9 (2018) 99-112.
28. S. Zhao, H. Zhao, H. Zhu, C. Li, X. Lu and S. Bi, *Journal of Chemistry*, Volume 2014, Article ID 610120.
29. B. Deng, C. Su and Y. Kang, *Anal. Bioanal. Chem.*, 385 (2006) 1336–1341.
30. H. W. Sun, L. Q. Li, X. Y. Chen, H. M. Shi and Y. K Lü, *Can. J. Anal. Sci. Spectros.*, 51 (2006) 100–107.
31. X. Yu and J. Bao, Journal of Luminescence, 129 (2009) 973–978
32. S. M. Sultan and F. E. O. Suliman, *Analyst*, 118 (1993) 573–576.
33. M. Córdoba–Borrego, M. Córdoba–Díaz and D. Córdoba–Díaz, *J. Pharm. Biomed. Anal.*, 18 (1999) 919–926.
34. N. M Kassab, A. K. Singh, E. R. M. Kedor–hackmam and M. I. R. M. Santoro, *Braz. J. Pharm. Sci.*, 41 (2005) 507–513.
35. S. J. Morton, V. H. Shull and J. D. Dick, *Antimicrob. Agents Chemother.*, 30 (1986) 325–327.
36. J. Lim, B. Park and H. Yun, *J.Chromatogr. B*, 772 (2002) 185–189.
37. R. N. Rao and V. Nagaraju, *J. Pharm. Biomed. Anal.*, 34 (2004) 1049–1056.
38. T. Pérez–Ruiz, Carmen Martínez–Lozano, V. Tomás and J. Carpena, *Analyst*, 122 (1997) 705–708.
39. J. L. Vílchez, O. Ballesteros, J. Taoufiki, G. Sánchez–Palencia and A. Navalón, *Anal. Chim. Acta*, 444 (2001) 279–286.
40. C. Tong and G. Xiang, *J. Fluoresc.*, 16 (2006) 831–837.
41. R. Duan, J. Jiang, S. Liu, J. Yang, M. Qiao, Y. Shi and X. Hu, *J. Sci. Food Agric.*, 97 (2017) 2569–2574.
42. L. Li, Y. Cheng, Y. Ding, S. Gu, F. Zhang and W. Yu, *Eur. J. Inorg. Chem.*, (2013) 2564–2570.
43. S. K. Bhowal and T. K. Das, *Anal. Lett.*, 24 (1991) 25–37.
44. S. Z. El Khateeb, S. A. Abdel Razek and M. M. Amer, *J. Pharm. Biomed. Anal.*, 17 (1998) 829–840.
45. O. Korkuna, T. Zhak and M. Smolinska, *Adsorption Science & Technology*, (2017) 1–11.
46. M. Keskar and R. Jugade, *Anal. Chem. Lett.*, 5 (2015) 319–328.
47. N. Rahman, Y. Ahmad and S. N. H. Azmi, *Eur. J. Pharm. Biopharm.*, 57 (2004) 359–367.
48. G. H. Ragab and A. S. Amin, *Spectrochim. Acta A*, 60 (2004) 973–978.
49. I. A. Darwish, M. A. Sultan and H. A. Al–Arfaj, *Talanta*, 78 (2009) 1383–1388
50. M. Devaraj, R. K. Deivasigamani and S. Jeyadevan, *Colloid Surface B*, 102 (2013) 554–561.
51. M. S. Rizk, F. Belal, F. A. Ibrahim, S. M. Ahmed and Z. A. Sheribah, *Electroanalalysis*, 12 (2000) 531–534.
52. A. A. J. Torriero, E. Salinas, J. Raba and J. J. Silber, *Biosens. Bioelectron.*, 22 (2006) 109–115.
53. H. da Silva, J. Pacheco, J. Pimenta, S. Viswanathan and C. Delerue–Matos, *Sens. Actuators, B*, 219 (2015) 301–307
54. K. J. Huang, X. Liu, W. Z. Xie and H. X. Yuan, *Colloid Surface B*, 64 (2008) 269–274.

55. Z. Liu, M. Jin, J. Cao, J. Wang, X. Wang, G. Zhou, A. van den Berg and L. Shui, *Sens. Actuators, B*, 257 (2018) 1065–1075.
56. Z. Ye, L. Wang and J. Wen, *Braz. J. Pharm. Sci.*, 51 (2015) 429–437.
57. V. K. Gupta, A. K. Jain, G. Maheshwari, Heinrich Lang, Z. Ishtaiwi, *Sens. Actuators, B*, 117 (2006) 99–106
58. V. K. Gupta, S.Juinand U.Khuranu, *Electroanalysis,* 9 (1997) 478–480.
59. V. K. Gupta, A. K. Singh, S.MehtabandB. Gupta, *Anal.Chim. Acta,* 566 (2006) 5–10.
60. V. K. Gupta, A. K. JainandP. Kumar, *Sens. Actuators, B*, 120 (2006) 259–265.
61. G. Gran, *Analyst*, 77 (1952) 661–671.
62. R. J. Barnhard, *J. Chem. Educ.*, 60 (1983) 679–680.
63. C. E. Ophardt, *J. Chem. Educ.*, 62 (1985) 257–258.
64. R. J. Ferek, A. L. Lazarus, P. L. Haagenson and J. W. Winchester, *Environ. Sci. Technol.*, 17 (1983) 315–324.
65. O. A. Farghaly, H. M. Al–Saidi, A. H. Naggar and I. M. El–Mabrouk, *Int. J. Electrochem. Sci.*, 12 (2017) 9865–9881.
66. H. A. Mohamed, H. M. A. Wadood and O. A. Farghaly, *J. Pharm. Biomed. Anal.*, 28 (2002) 819–826.
67. Vogel's Text Book of "Quantitative Inorganic Analysis" Revised by J.Bassett, R. C. Denny, G. H. Jeffery and J. Menoham; 4th Longman, London, 302 (1978).
68. H. Irving and H. S. Rossoti, Methods for computing successive stability constants from experimental formation curves, *J. Chem. Soc.*, (1953) 3397–3405. Ibid, *J. Chem. Soc.*, (1954) 2904–2910.
69. H. Irving, R. J. P. Williams, *Nature*, 162 (1948) 746–747; Ibid, *J. Chem. Soc.*, (1953) 3192–3210.
70. M. T. Beck, "Chemistry of Complex Equilibria", Van Nostrand Reinhold, 73 (1970).145
71. The United States Pharmacopoeia, 24 Revision, The National Formulary 19, USP Convention, INC. Rockville, MD, (2000).
72. The British Pharmacopoeia, Her Majesty's Stationery Office, London, (1998).