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Metal Complexes and Determination of Nalidixic Acid by Potentiometric and Conductometric Methods

O. A. Farghaly[1,*], *H. M. Al–Saidi*[2], *A. H. Naggar*[1], *I. M. El–Mabrouk*[3]

[1] Chemistry Department, Faculty of Science, Al–Azhar University, Assiut Branch, 71524, Assiut, Egypt.
[2] Department of Chemistry, University College in Al–Jamoum, Umm Al–Qura University, 21955, Makkah, Saudi Arabia
[3] Chemistry Department, Faculty of Science, Sebha University, Sebha, Libya
[*]E–mail: Othman15@yahoo.com

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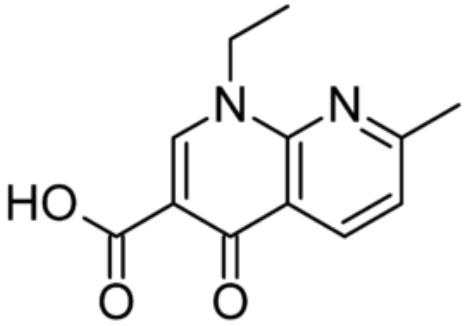
Ten metal ions viz, Fe(III), Cr(III), La(II), Sn(II), Co(II), Ba(II), Pd(II), Ti(II), Sr(II) and Zr(IV), were selected to elucidate their interaction with nalidixic acid (NA) using potentiometric and conductometric methods. The ligand ionization and the complexes stability constants have been obtained at 25±1.0 ºC and 0.01 M ionic strength of NaCl in 25 % (v/v) aqueous–ethanol solution. Complexes of 1:1, 1:2 and/or 1:3 metals to ligand ratios were formed depending on the nature of the ligand or metal ions. As well as, the stoichiometry of complexes confirmed by the conductometric method. Also simple, precise, rapid and low–cost potentiometric and conductometric methods for NA determination and tablets are proposed. NA present in tablets containing known quantity of drug was potentiometrically titrated by 0.1 M of NaOH using a combined glass pH electrode. The detection limit was 0.19 mg L^{-1}. The calibration graph was found to be linear in the range of 0.23–2.55 mg L^{-1}. The correlation coefficient (r) was calculated to be 0.9952. The standard deviation (SD) was < 1.0. No interferences were observed in the presence of common components of the tablets. The percentage recoveries of NA in tablet dosage formulations by potentiometric and conductometric methods were (95.8–98.68) %, with standard deviations (SD) were within (0.18–0.4) (n=5).

Keywords: Nalidixic acid, metal complexes, potentiometric and conductometric determination, tablets.

1. INTRODUCTION

Quinolones are gyrase inhibitors that are widely used clinically as antibiotics. They are active against many gram–positive and gram–negative bacteria. Nalidixic acid (1–ethyl–1,4–dihydro–7–methyl–4–oxo–1,1,8–naphthyridine–3–carboxylic acid) (NA) is widely used to treat infections of the urinary tract. It is effective against most Proteus strains, Klebsiella, Enterobacter, some Salmonella and

Shigella strains, and Escherichia coli. It is rapidly and almost completely absorbed from the gastro-intestinal tract and about 93 % of NA is bound to plasma protein [1–4]. NA has the following structure:



Several types of analytical procedures have been proposed for the analysis of NA in pure form, pharmaceuticals formulations and biological fluids. These procedures include high–performance liquid chromatograph (HPLC) [5–7], voltammetry [8–10], fluorometric [11–13], spectrophotometric [14, 15] and potentiometric method [16]. Although all these method showed an excellent recovery but they still required special instruments, reagents and experience.

The use of Gran plot [17] for finding the equivalence point of a potentiometric titration has several advantages over the more commonly used procedure of inflection point determination of a sigmoidal logarithmic plot such as a pH titration curve. Several applications of Gran plot have appeared in the literature like fluoride concentration by use of a specific ion electrode [18], acid rain analysis [19] and the measurement of percent strong acid found in atmospheric aerosols over the northeastern United States [20].

Recently, great attention has been paid to use potentiometric methods in study of binary and ternary complexes of transition metals with molecules of biological and pharmaceutical interest [21–30]. The significance of potentiometric methods as the most accurate and widely applicable technique in studies related to ionic equilibria of different complexes [31]. It should be noted that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of such organic compounds [32]. Such metal complexes were produced also from reactions between NA and several metal complexes [33–39].

The objective of this work was study of the complexation equilibrium between NA and metal ions such as Pb (II), Cr (III), Fe (III), La (III), Sn (II), Co (II), Ti (II), Zr (IV), Ba (II) and Sr (II), by accurate and widely applicable potentiometric and conductometric methods. Also, potentiometric and conductometric methods (for the first time) were development to the determination of NA of pure and tablet dosage formulations. Although, several method were used to determination of NA but it still required high cost instruments, reagents and experience, but these methods are simple, precise, rapid, low–cost and showed an excellent recovery. These methods are based on the potentiometric and conductometric titrations of NA (carboxyl group) in aqueous solutions with sodium hydroxide solution.

2. EXPERIMENTAL

2.1. Apparatus

All pH measurements were carried out on pH–meter model ELE international, using combined glass electrode (accurate total 0.01 pH units). Conductometric titration measurements were carried out using conductivity meter model 4320, Jenway, using an immersion cell. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities; thus all constants determined in this work are concentration constants. The stoichiometry and stability constants were calculated using numerical and computerized programs (Excel) [21].

2.2. Materials

Nalidixic acid (NA) was purchased from Sigma (St Louis, MO, USA) and sodium hydroxide (BDH) were used as such. All other metal ions solutions (as nitrates and chlorides salts) were used as purchased with an analytical grade from (BDH, UK, GENEVA or INDIA).

Pharmaceutical formulations: Nalidram (Tablet) (Memphis Chemicals, Egypt), Nalidixic acid (Tablet) (Dar Al–daoa–Jordan) and Uroneg (Tablet) (Micro Labs Limited–India). Each of them labeled to contains 500 mg NA per tablet.

2.3. Procedure

2.3.1. Metal complexes of NA

A stock standard solution of NA (1.0×10^{-2} mol L^{-1}) was prepared by dissolving the product from Sigma in 25 % (v/v) aqueous ethanol medium. Standard solutions were prepared from the stock solution by appropriate dilution with double distilled water. Working solutions have shown enough stability during all time of storage. Generally, the following solutions: (a) 0.001 M HCl + 0.009 M NaCl; (b) solution (a) + 0.001 M NA; and (c) solution (b) + 0.001 M metal ion, were prepared and titrated against standared CO_2–free NaOH solution [40] at room temperature.

In all titrations, the total volume was maintained constant at 50 mL with ionic strength 0.01 M NaCl at 25±1.0 ºC. 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.

Conductometric titration were carried out at room temperature by titration 25 mL of 1×10^{-3}M of each metal ion with 1×10^{-2} M of NA solution in 0.5 mL increments. Correction for the dilution effect is performed by multiplying the values of specific conductance by factor (25 + v) / 25, where V is the volume of titrant added.

2.3.2. Determination of NA

2.3.2.1. In pure form

A standard solution (25 mL) of NA (1×10^{-2} M) (ionic strength (I) adjusted to 0.5 M with $NaNO_3$) was prepared by suitable dilution of the stock solution. Then, an aliquot of 15 mL of NA solution was transferred to a thermostated glass cell (25±1.0 ºC) then potentiometrically and conductometrically titrated with a standard solution of NaOH 0.1 M adjusted of ionic strength.

2.3.2.2. In Tablets

Ten tablets of dosage form were weighed to calculate the average tablet weight. They were finely powdered and homogenized. A portion of the powder equivalent to 100 mg of NA was accurately weighed and dissolving in ethyl alcohol and filtered. The filtrate was diluted with double distilled water and its ionic strength was adjusted to 0.5 M with $NaNO_3$. Finally, a portion of this solution was diluted with double distilled water in a 25 mL flask and analyzed under the same procedure described in pure form. The quantity per tablet was calculated from the standard calibration curve.

3. RESULTS AND DISCUSSION

3.1. Formation constants of NA complexes:

3.1.1. Potentiometric studies of NA with some metal ions:

The interaction of NA with selected ten metal ions viz; Pb (II), Cr (III), Fe (III), La (III), Sn (II), Co (II), Ti (II), Zr (IV), Ba (II) and Sr (II), was studied using potentiometric and conductometric methods. In the potentiometric method, the ionization constant of NA and stability constants of the formed complexes have been tabulated at 25±1.0 ºC, ionic strengths (0.01 M) NaCl in 25 % (v/v) aqueous ethanol medium.

3.1.2. Determination of the proton–NA stability constant:

Potentiometric titration of nalidixic acid (NA) in the presence of 0.001 M hydrochloric acid was carried out with ionic strength, of I = 0.01 M NaCl at 25±1.0^{o}C and using carbonate-free CO_2 sodium hydroxide as a titrant. The obtained titration curve is shown in Fig.1.

The values of ńA (average number of proton attached per ligand) as determined according to Irving and Rossotti [41.42] were obtained from the titration curves data using solutions (a) and (b). Calculation of proton-ligand dissociation constants were carried out by plotting ńA against pH (Fig.2).

The value of $\log K_1^H$ (the first proton dissociation constant of NA) is the pH value corresponding to ńA = 0.5. The average number of proton attached per ligand (ńA) was calculated at different pH values using Irving and Rossotti[41.42], as shown in eq. (1).

$$\bar{n}A = y + \frac{(V_1 - V_2)(N^o + E^o)}{(V_0 + V_1)\cdot T_c L^o} \quad \text{.....1}$$

Where: Y = 2 (number of dissociable protons in the ligand), V_0 is the initial volume, V_1 and V_2 are the volume of alkali required to reach the same pH in mineral acid (HCl) and (HCl + NA) solutions, respectively. T_cL^0 is the total concentration of the ligand, N^0 is the normality of the alkali and E^0 is the initial concentration of free acid.

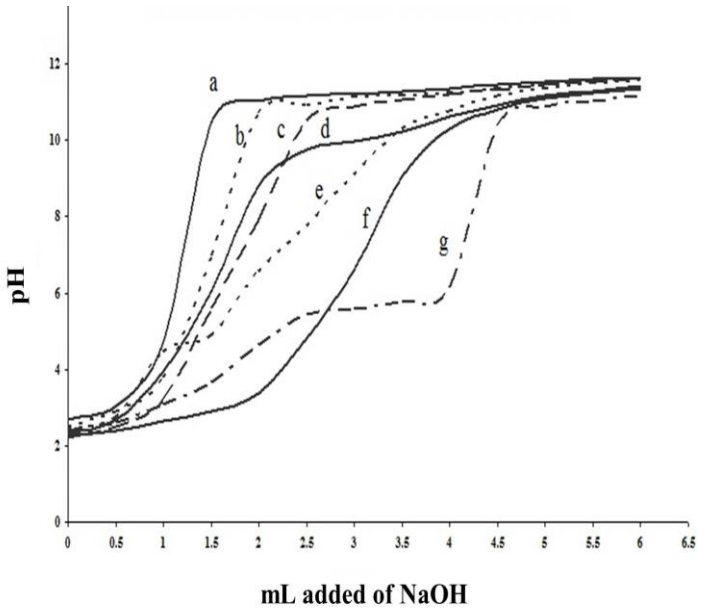


Figure 1. Potentiometric titration curves of NA: (a) HCl, (b) NA, (c) Ba (II), (d) Co (II), (e) Pb (II), (f) Sn (II) and (g) Cr (III) with I = 0.01 M NaCl at 25±1.0 °C.

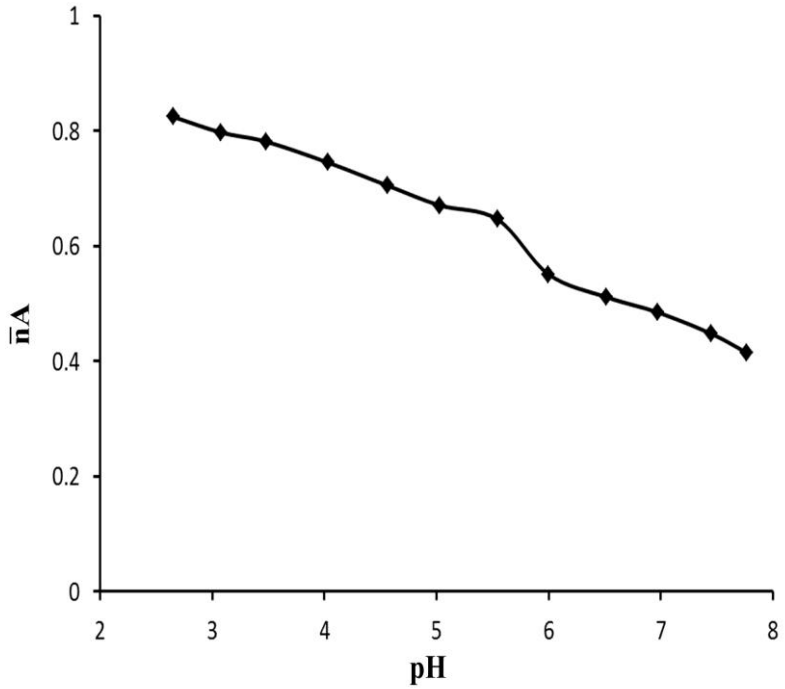


Figure 2. Proton–ligand formation curve of NA, I = 0.01 M NaCl at 25±1.0 °C.

The values obtained of $\log K_1^H$ (the first proton dissociation constants of the NA) was 6.4. The pK_a value obtained by treatment was good agreement with literatures [16, 33] for several sets of potentiometric data as we can see in Table 1.

However, the reaction mechanism is shown as follow:

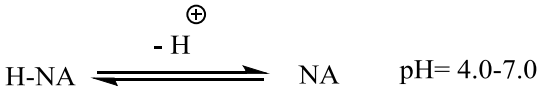


Table 1. Protonation constants of NA and stability constants of metal ions complexes at I = 0.01 M NaCl and 25±1.0 °C.

Metal ion	$LogK_1$ (M:L)*	LogK (M:L)*	$LogK_3$ (M:L)*	Reference
H+	6.4			Present work
	(6.1)			[33]
	(6.15)			[16]
Pb (II)	9.7	6.14	3.88	Present work
	(1:1)	(1:2)	(1:3)	
Sr (II)	9.11	5.02	3.12	Present work
	(1:1)	(1:2)	(1:3)	
Fe (III)		7.46	4.99	Present work
		(1:2)	(1:3)	
Co (II)	9.41	5.67	2.73	Present work
	(1:1)	(1:2)	(1:3)	
Ti (II)	9.96	5.46	3.08	Present work
	(1:1)	(1:2)	(1:3)	
Cr (III)	9.23	5.46	3.65	Present work
	(1:1)	(1:2)	(1:3)	
Ba (II)	10.21	5.91	1.34	Present work
	(1:1)	(1:2)	(1:3)	
Sn (II)		8.21	5.46	Present work
		(1:2)	(1:3)	
La (III)	9.83	5.27	3.38	Present work
	(1:1)	(1:2)	(1:3)	

(*) These ratios are from potentiometric and conductometric methods

3.1.3. Determination of formation constants of metal–NA complexes:

As shown in Fig. 1, the titration curves of the metal–ligand solutions (curve c) differ and well separated from those of free metal ions solutions (curve b). Fig. 1, demonstrating of H^+ ion due to

complexation. The values of $\bar{n}$ (average number of ligand molecules per metal ion) and *pL* (free ligand exponent) were calculated using Irving and Rossotti [41, 42], Eq. 2, 3:

$$\bar{n} = \frac{(V_3 - V_2)(N^{\circ} + E^{\circ})}{(V_0 + V_2)\bar{n}HT_C M^{\circ}} \quad (2)$$

$$pL = Log\left[\frac{1+\beta_1[H^+]+\beta_2[H^+]^2}{(T_C l^{\circ} - \bar{n}T_C M^{\circ})} \times \frac{V_0 + V_3}{V_0}\right] \quad (3)$$

The $\bar{n}$ values were plotted against the corresponding *pL* values to obtain the formation curves of the complexation equilibria Fig. 3. From these curves the values of the stability constants were computed using standard procedures based on the calculations of $\bar{n}$ and *pL* as described previously [21]. The stoichiometry of the chelates depends on the nature of the metal ion and ligand. As shown in Table 1, we observe that the most metal ions investigated form complexes with NA in the molar ratio metal to ligand 1:1, 1:2 and 1:3. On the other hand, Fe (III), Sn (II) and Zr (IV) metal ions form complexes with stoichiometric ratios 1:2 and 1:3. This is due to the nature of metal ions. The stability constants of complexes formed between NA and metal ions investigated in this work for 1:2 (metal:ligand) follow this order [43, 44]:

Zr (IV) > Cr (III) > Fe (III) > Sn (II) > Pb (II) > Ba (II) > Co (II) > La (III) > Ti (II) > Sr (II)

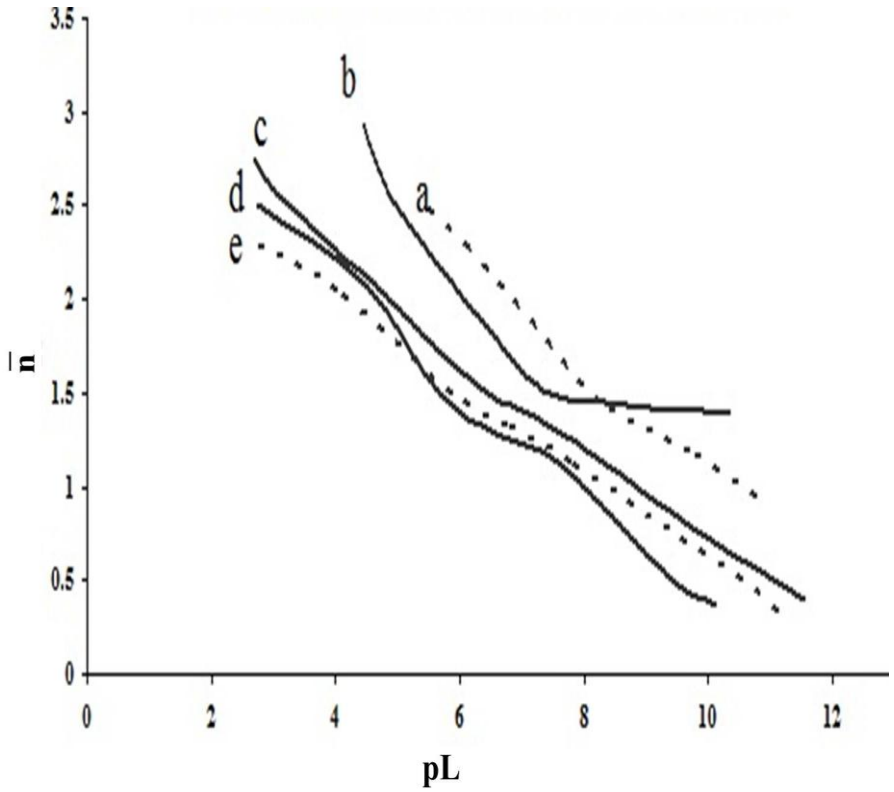


Figure 3. Metal ion–NA formation curves: (a) Sn (II), (b) Fe (III), (c) Ti (II), (d) Co (II), (e) Ba (II), I = 0.01 M NaCl at 25±1.0 °C.

The effect of concentration of medium (ionic strength) on stability constant of nalidixic acid with metal ions; Pb(II), Co(II), Ti(II), Cr(III) and La(III) was studied viz; I = 0.01, 0.05 and 0.1 M NaCl at 25 ± 1.0 ºC. From this study it can concluded that the stability constant of metal -ligand complex (1:1) decreased as the concentration of medium increased, as we can see in Fig. (4).

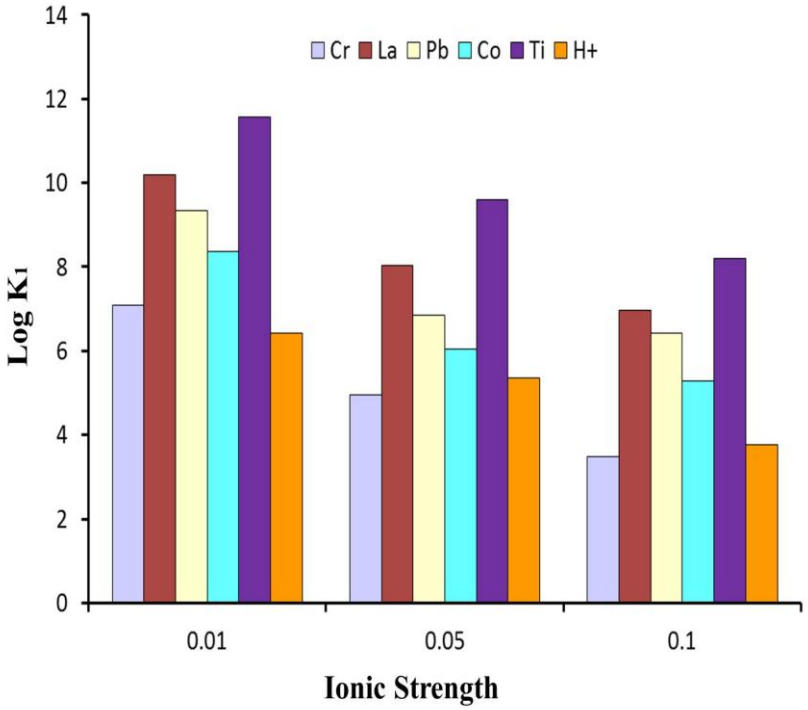


Figure 4. Effect of ionic strength on stability constants of NA with some metal ions.

3.1.4. Species distribution diagrams of NA:

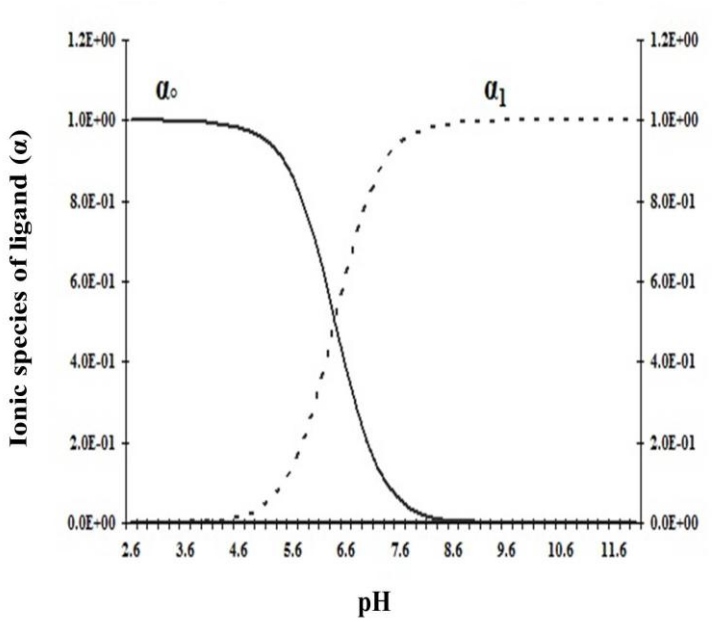


Figure 5. Ionic equilibria of NA in different pH`s ranges.

The species distribution curve of NA is shown in Fig. 5. All species have broad protonation space between pH ≈ 2.6–11.6. When pH increases, the protonated ligands lose the protons and convert to the other forms as seen in Fig.5.

The HL species starts to form at pH ≈ 2.6 and decrease up to pH ≈ 8.6. The free ligand (L^-) tends to starts from pH ≈ 3.8 and reaches its maximum at pH ≈ 8.8. The data obtained from M–NA complexes have been evaluated using Excel program [21,45] and the species distribution curves obtained from calculations is given in Fig. 6. In this figure, the concentration of various complexes formulated as ML and ML_2 between the ligand and the metal ions under investigation are depending on pH and the type of medium.

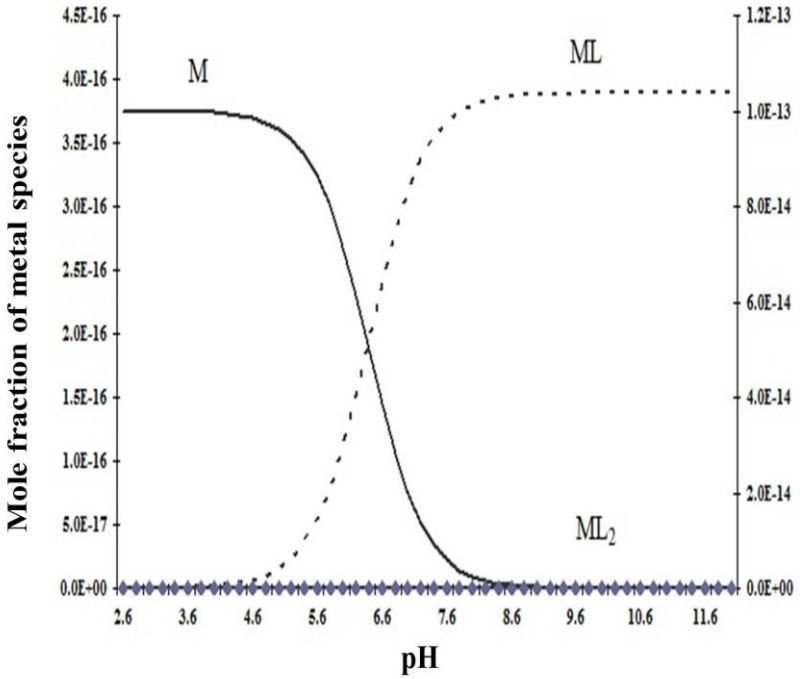


Figure 6. Ionic equilibria of Co–NA complex in different pH`s range.

3.1.5. Conductometric measurements:

Conductometric measurements can be applied for tracing complex formation in solution. This method has useful application as a sensitive tool to test for decimal variations in ionic radii of transition metal ions investigated. The conductometric analysis is based on changes in the electrical conductivity values of solutions as a result of complex formation. These changes depend upon the number of ions present, and their mobilities. In this work, conductivity measurements were employed to trace the different types of complexes formed between some metal ions Pb (II), Cr (III), Sn(II), La(III) and Fe(III) and the subject ligand.

The conductometric titrations are performed by titrating of 25 ml (1×10^{-3}M) of each metal ion with the successive volume of 1×10^{-2} M NA solution. Generally, on plotting the specific conductance values as a function of the volume of each ligand added, the graphs shown in Fig. 7 is gathered.

The relationships show well defined breaks corresponding to the stiochiometric ratios 1: 1, 1: 2 and/ or 1: 3, M: L. These results are in agree with those obtained by potentiometric method (Table 1).

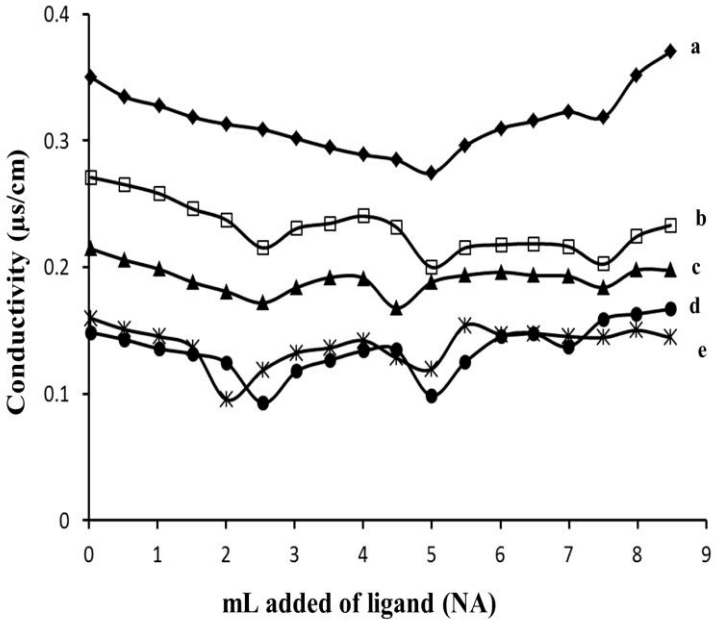


Figure 7. Conductometric titration curves of 25 mL metal ions (1×10^{-3} M) with NA (1×10^{-2} M): (a) Fe (III), (b) Cr (III), (c) La (III), (d) Co (III), (e) Pb (II).

3.2. Potentiometric determination of NA:

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

In current work, we aimed to determine NA using the neutralization reaction involving the acidic property of NA. The proposed methods depend on the principle that substance, which is weakly acidic in an aqueous medium, exhibit enhanced acidity in non–aqueous media thus allowing their easy determination. In the present titrimetric methods, the weakly acidic property of NA was titrated with NaOH using potentiometric and conductometric end point detection.

The main concept of a Gran plot is that a linear relationship can be found between the analyte concentration (NA) during titration and the volume of base added as titrant (NaOH). Such that a plot of this relationship versus the volume of titrant will yield a straight line that intercepts the titrant volume axis at the equivalence volume for the analyte [17, 46].

NA is one basic acid having dissociation constant $pK_1 = 6.4$ (carboxyl group). Observing the value of NA, it can be foreseen that the titration curve presents a clear inflection for the first point of equivalence since $K_1 = 1\times10^{-6.4}$.

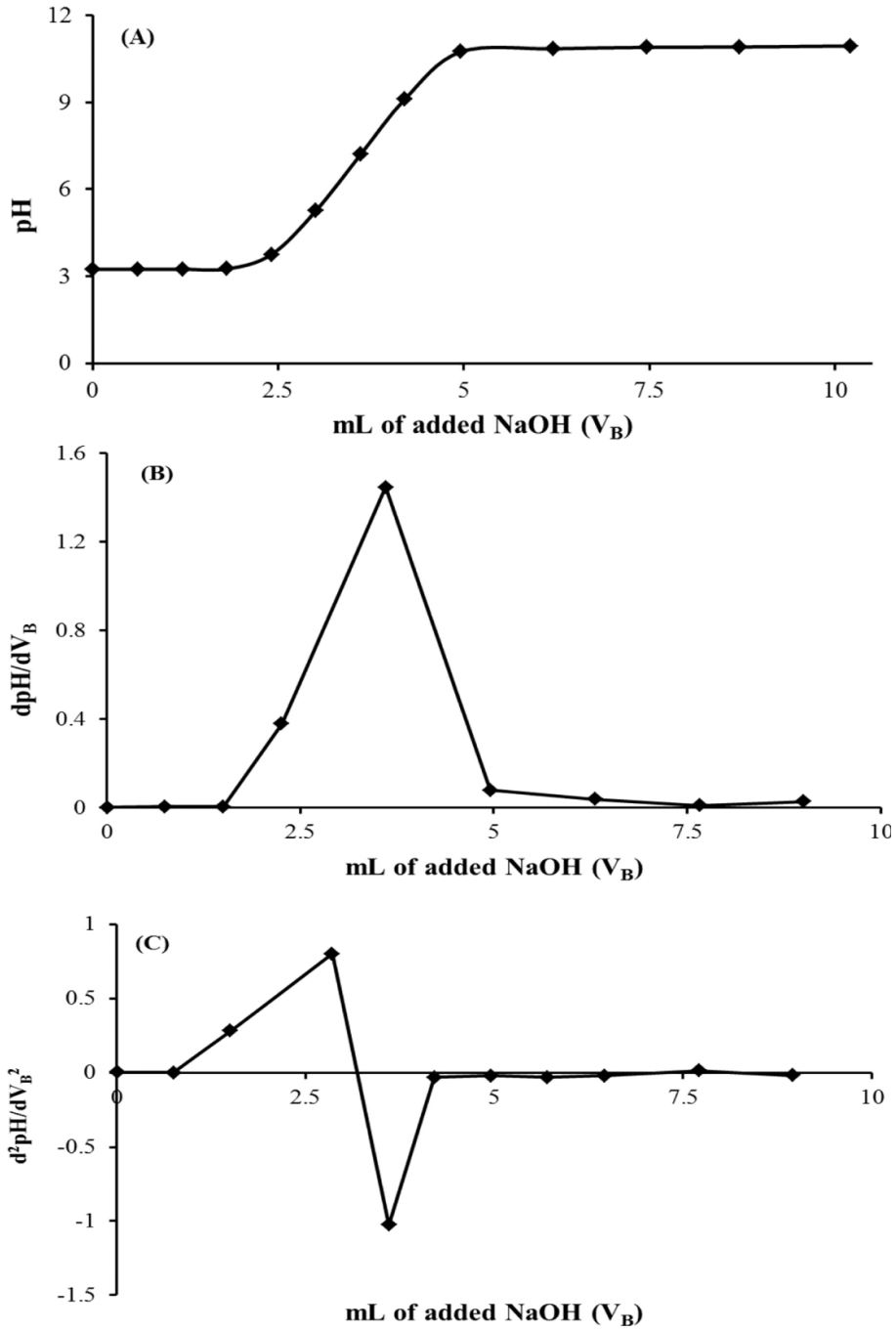


Figure 8. Typical potentiometric titration curves of NA (pure): (a) normal titration curve, (b) first derivative, (c) second derivative with I = 0.5 M $NaNO_3$ at 25±1.0 °C.

Herein, potentiometric titration for determination of NA of pure form was performed with NaOH as the titrant, I = 0.5 M $NaNO_3$ at 25±1.0 ºC. The steep rise in the pH was observed in Fig. 8 (curve a) at the equivalence point with potentiometric end point detection. The first and second derivative of potentiometric titration curve as shown in Fig. 8 (curves b and c) methods were applied to ascertain the equivalence point.

Also, the conductometric analysis is based on changes in the electrical conductivity values of solutions as a result of neutralization by the base. These changes depend upon the number of ions present, and their mobilities. In this work, conductivity measurements were employed to determine the weakly acidic NA by titration with sodium hydroxide.

Generally, on plotting the specific conductance values as function of the volume of 0.1 M sodium hydroxide. This relationships show a well-defined break corresponding to the end point of neutralization.

3.2.1. Effect of ionic strength on the determination of pure NA:

To study the effect of ionic strength on the determination of nalidixic acid of pure form using potentiometric method, aliquots equivalent 5×10^{-3}M of the drug was determined by the varying the ionic strength of medium in range (0.05-1.0) M $NaNO_3$. The recovery increase with increasing concentration of the $NaNO_3$ solution, and it was found that 0.5M $NaNO_3$ gave the best value of the recovery (in closed 100 %). Thus, it was used in the determination of nalidixic acid of pure and dosage forms. The results are listed in table 2.

Table 2. Effect of ionic strength on the percentage recovery for pure NA.

Ionic Strength (M)	Add from pure (mg)	Found (mg)	Percentage recovery ± SD (%)
0.05	500	405.5	81.1 ± 0.35
0.1	500	449.5	89.9 ± 0.25
0.5	500	494.0	98.8 ± 0.23
0.75	500	574.5	114.9 ± 0.42
1	500	655.5	131.1 ± 0.19

3.2.2. Determination of pure NA:

The results for the determination of NA of the pure form using potentiometric and conductometric methods are shown in Table 3, which shows the sensitivity, validity and repeatability of the methods. The recoveries of both two methods were found to be close to 100 %, the relative standard deviation does not exceed of 0.52 % (n=5) with confidence limit (at 95 % confidence level) in the range from (0.20–0.46). The detection limit (as 3σ/b, where b is the slope and σ = SD) [21] was 0.19 mg L^{-1}. The calibration graph is linear in the range of 0.23–2.55 mg L^{-1}. The correlation coefficient of determination (r) comes out to be 0.9952. The standard deviation (SD) was < 1.0 as shown in Fig. 9.

Table 3. Determination of NA in pure form by using proposed methods with I = 0.5 M $NaNO_3$ at 25±1.0 °C.

Add of pure (mg/L)	Found (mg/L)	Recovery (%)	SD (n=5)	Confidence (n=5) α =0.05
0.232	0.222	95.5	0.45	0. 39
	(0.221)	(95.1)	(0.52)	(0. 46)
0.697	0.671	96.34	0.38	0.33
	(0.668)	(95.8)	(0.46)	(0.40)
1.161	1.142	98.4	0.35	0.31
	(1.135)	(97.8)	(0.42)	(0.37)
1.625	1.628	100.2	0.28	0.25
	(1.615)	(99.4)	(0.38)	(0.33)
2.09	2.142	102.5	0.23	0.20
	(2.115)	(101.2)	(0.31)	(0.27)
2.55	2.657	104.2	0.22	0.19
	(2.64)	(103.5)	(0.33)	(0.29)

The data between brackets were from conductivity method

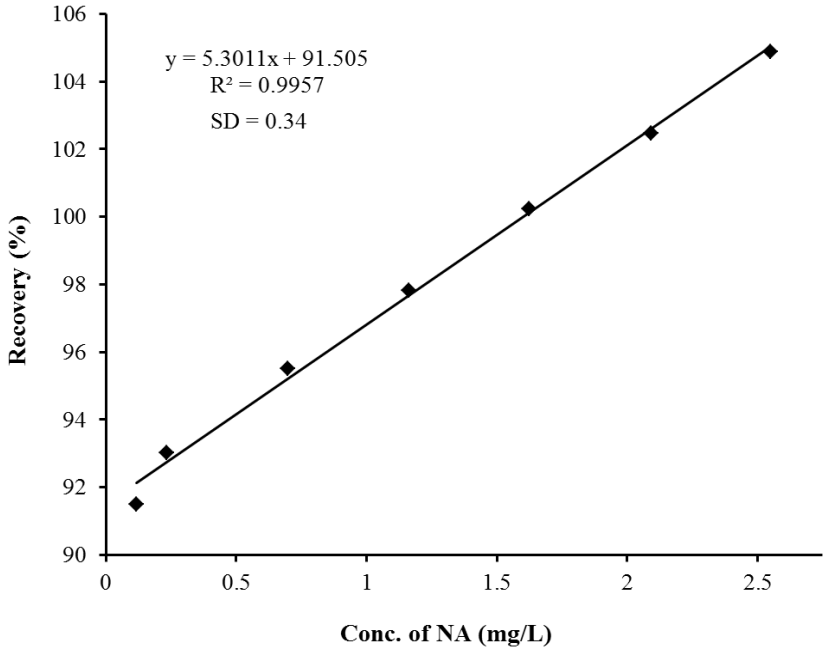


Figure 9. Linearity range of NA.

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 NA of the pure form, viz; (D(+) lactose monohydrate, sodium chloride and sodium acetate) were investigated in a concentration range at least 100 times higher than that of NA. No interferences observed in this concentration range.

3.2.4. Analytical application:

The proposed methods were successfully applied for NA determination in tablet formulation. In Fig. 10, curves a, b and c are: the typical potentiometric titration curve with only one inflection point, the first and the second derivative of the potentiometric curve, respectively.

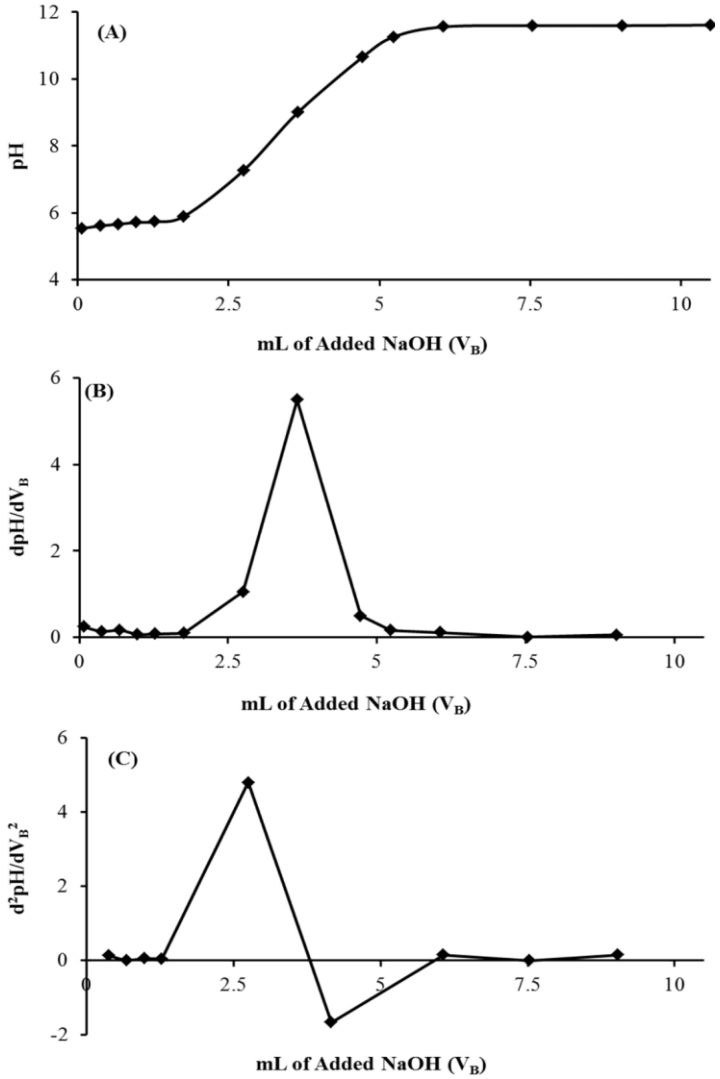


Figure 10. Typical potentiometric titration curves of NA (tablet): (a) normal titration curve, (b) first derivative, (c) second derivative with I = 0.5 M $NaNO_3$ at 25±1.0 °C.

Fig. 11 shows conductometric titration curves of determination of NA of pure form and its dosage formulations.

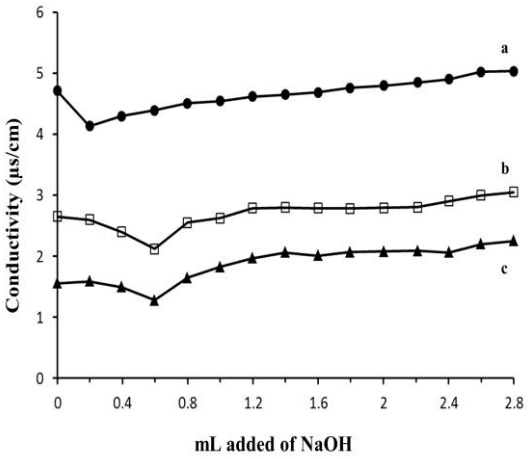


Figure 11. Typical Conductometric titration curves for the determination of NA of pure form and pharmaceutical preparations at 25±1.0 °C: (a) NA (pure), (b) NA tablet, (c) Uorng (tablet).

The data in Table 4 shows that the NA contents measured by the proposed methods were in good statistical agreement with the values supplied by the manufacturers. The percentage recoveries by potentiometric and conductometric methods were ranging (95.8–98.68) %, with standard deviations (SD) were within (0.18–0.4) (n=5). These results point out the accuracy and precision of the methods and the absence of significant matrix effects on proposed measurements at least for the samples analyzed.

Table 4. Determination of NA in pharmaceutical preparations

Sample	Manufacturer	Label to content (mg)	Proposed methods		
			Found (mg)	Recovery (%)	SD (%) n=5
Nalidixic acid (Tablet)	(Life Pharma italfarmaco–group Italy)	500	493.4 (489)	98.68 (97.8)	0.32 (0.4)
Nalidixic acid (Tablet)	(Dar Al–daoa–Jordan)	500	484 (481)	97. 5 (95. 8)	0.2 (0.15)
Uroneg (Tablet)	(Micro Labs Limited–India)	500	493.2 (484.3)	98.64 (96.86)	0.18 (0.2)

The data between brackets were from conductivity method

4. CONCLUSION

Potentiometric and conductometric methods are excellent methods for calculation of stability constant of metal ligand complexes. NA has one dissociation constant $\log K^{1}_{H}$ = 5.0. However, NA forms complexes with metal ions of types 1:1, 1:2 and/or 1:3 (M: L) complexes. The stoichiometric ratio that obtained from the potentiometric method is well agreement with the results of conductometric method. Finally, the species distribution of ligands and its metal complexes under investigated are variables during the pH's ranges.

Compared with many of already existing methods used for the determination of NA, which required special instruments, reagents and experience, our method exhibited the advantages of simple operations, fast response, low cost and sufficient accuracy in determination of NA in pharmaceutical formulation with high recovery ranging 95.8 – 98.68 % and no interferences observed.

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