Abstract:
Ten metal ions viz, Fe(III), Cr(III), La(II), Sn(II), Co(II), Ba(II), Pd(II), Ti(II), Sr(II) and Zr(II), were selected to elucidate the interaction of these metal ions with nalidixic acid (NAL) using potentiometric and conductometric methods. The ionization constant of the ligand and stability constants of the complexes formed have been tabulated at 25±1.0°C 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 are formed depending on the nature of the ligand or metal ions. As well as, the stoichiometry of complexes confirmed by conductometric method. Also simple, precise, rapid and low-cost potentiometric and conductometric methods for nalidixic acid determination in pure form and tablets are proposed. Nalidixic acid present in tablets containing known quantity of drug were potentiometrically titrated by 0.1M of NaOH using a combined glass pH electrode.

The detection limit was 2.20 mg /25ml. The calibration graph is linear in the range of 0.23– 2.55 mg/25ml. The correlation coefficient of determination (r) comes out to be 0.9972. The standard deviation (SD) was 2.77. No interferences were observed in the presence of common components of the tablets. The percentage recoveries of nalidixic acid 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).

Introduction:
Quinolones are gyrase inhibitors that are widely used as antibiotics in the clinic. They are active against many gram-positive and gram-negative bacteria [1]. Nalidixic acid (1-ethyl - 1, 4-dihydro - 7 - methyl - 4 - oxo - 1,8 - naphthyridine-3-carboxylic acid) is 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 [2].

Recently, much attention has been paid to the study of binary and ternary complexes of transition metals with molecules of biological and pharmacy-eutical interest [3,4]. Furthermore, it has been suggested that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of drugs [5,6]. The formation of complexes between nalidixic acid and several metal ions has been described [7-15]. However, several types of analytical procedures have been proposed for the analysis of nalidixic acid in pure form, pharma-ceuticals formulations and biological fluids. These procedures include liquid chromatographic (LC) [16], high-performance liquid chromatograph (HPLC) [17,18], voltammetry [19], fluorometric [20], gas
chromato-graphic [21,22] and potentiometric method [23].

The objective of this work was the study of the complexation equilibrium between nalidixic acid 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). Also, the development of potentiometric and conductometric methods for the determination of nalidixic acid in pure form and tablet dosage formulations.

Experimental:

A-Metal complexes of nalidixic acid

pH measurements were carried out using pH-meter JENWAY model 3205 Ltd, UK. Conductometric titration and molar conductance measurements were carried out by JENWAY model 4310, using on immersion cell. The stoichiometry and stability constants were calculated using numerical and comp-uterized programs (SUPERQUAD) [24]. All reagents were of the analytical-reagent grade and doubly distilled water was used in all experiments.

Nalidixic acid (standard substance) was purchased from sigma (St Louis, MO, USA). The nalidixic acid as tablets (500mg) Nalidixic acid (Life Pharma-Italy), Nalidixic acid (Dar Al-ddoa–Jordan) and Uroneg (Micro Labs LTD-India). Stock standard solution of nalidixic acid (pure) 1x10^{-2} M was prepared by dissolving the calculated amount of the regent in 25% (v/v) aqueous ethanol medium.

The aqueous solutions of metal ions (A.R; B.D.H) as nitrates or chlorides were standardized by the conventional methods[25]. Generally, the following solutions were prepared and titrated against standard CO₂ – free NaOH in 25% (v/v) aqueous ethanol medium at room temperature.

a- 0.001M HCl + 0.009 M NaCl.
b- Solution (a) + 0.001 M ligand.
c- Solution (b) + 0.001 M metal ion.

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.

Conductometric titration were carried out at room temperature by titration 25.0 ml of 1x10^{-3} M of each metal ions with 1x10^{-2} M of NAL 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 volume of titrant added.

B-Determination of nalidixic acid:

1-For pure form

A standard solution (25 ml) of nalidixic acid 1x10^{-2} M (I adjusted to 0.5 M with NaNO₃) was prepared by suitable dilution of the stock solution.

2-Tablets analysis:

For Pharmaceutical preparations, ten tablets containing (500mg) were weighed and powdered. The powder equivalent to 100 mg of NAL was dissolved in ethyl alcohol and was diluted with bidistilled water and filtered. The quantity per tablet was calculated from the standard calibration curve.

The resulting mixture was filtered and its ionic strength was adjusted to 0.5 M with NaNO₃.

RESULTS AND DISCUSSION:

A-Formation constants of nalidixic acid complexes:

A.1. proton- nalidixic acid dissociation constant:

The titration curves of nalidixic acid are shown in Fig. 1. The values of $\hat{n}_A$ as determined according to Irving and Rossotti [26] were complied from the titration data. Calculations of proton – ligand dissociation constant was carried out by plotting $\hat{n}_A$ against pH, Fig 2.
value of log\(K_{1}^{H}\) (the first proton dissociation constant of nalidixic acid) is the pH value corresponding to \(n_A = 0.5\). The \(pK_a\) value obtained by treatment of several sets of potentiometric data was found to be 6.4, and it was good agreement with literature [27] as shown in Table 1.

![Fig(1): Potentiometric titration curves of NAL: a) HCl, b) NAL, c) Ba(II), d) Co(II), e) Pb(II), f) Sn(II) and g) Cr(III) with \(\mu = 0.01\) M NaCl at 25±1.0°C.]

![Fig(2): Proton-ligand formation curve of NAL, \(\mu = 0.01\) M NaCl at 25±1.0°C.]

**A. 2. Binary metal – ligand systems:**

The titration curves of the metal – ligand solutions c differ well separated from those of solution b. Fig. (1), demonstrating of H\(^+\) ion due to complexation. \(\bar{n}\)(average number of ligand molecules per metal ion) and \(pL\) (free ligand exponent) values were calculated using Irving and Rossotti [26].

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 the average number of ligand bound per metal ion, \(\bar{n}\), and the free ligand exponent, \(pL\), as described previously [14].

![Fig(3): Metal ion-NAL formation curves; a) Sn(II), b) Fe(III), c) Ti(II), d) Co(II), e) Ba(II), \(\mu =0.01\) M NaCl at 25±1.0 °C.]

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 nalidixic acid in the molar ratio metal : ligand 1:1, 1:2 and 1:3. On the other hand, Fe(III), Sn(II) and Zr(IV) metal ions form complexes with stochiometric ratios 1 : 2 and 1 : 3. This is due to the nature of metal ions. The stability constants of complexes formed between NAL and metal ions investigated in this work for 1:2 metal to ligand follow the order:

\[
\text{Zr(IV)} > \text{Cr(III)} > \text{Fe(III)} > \text{Sn (II)} > \text{Pb(II)} > \text{Ba(II)} > \text{Co(II)} > \text{La(III)} > \text{Ti(II)} > \text{Sr(II)}.
\]

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 °C.

**Conductometric measurements:** The conductometric titration curve for the binary ligand system containing Pb (II),...
Cr (III), Sn(II), La(III) and Fe(III) ions associated with the complex that form were performed at room temperature. The raw conductometric titration curves are shown in Fig. (5). Conductivity vs. volume of titrant is proportional to the mobility of ions in the solution. This may be due to the neutralization of H+ ions resulting from the formation of the M (NAL) complex. The slow increase in conductance values on addition of NAL to metal ions may be ascribed to the formation of the highly charged NAL anions of the relatively weak acid.

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

(*) These ratios are from Potentiometric and conductometric methods.

The relationships show a well defined breaks corresponding to the stiochiometric ratios 1:1, 1: 2 and/or 1: 3, M: L, these results are in comparable with those obtained by potentiometric method as shown in Table 1.

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

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>LogK1 (M:L)</th>
<th>LogK2 (M:L)</th>
<th>LogK3 (M:L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H+</td>
<td>6.4</td>
<td>---</td>
<td>---</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>---</td>
<td>---</td>
<td>[23]</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>9.7 (1:1)</td>
<td>6.14 (1:2)</td>
<td>3.88 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Sr(II)</td>
<td>9.11 (1:1)</td>
<td>5.02 (1:2)</td>
<td>3.12 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>---</td>
<td>7.46 (1:2)</td>
<td>4.99 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Co(II)</td>
<td>9.41 (1:1)</td>
<td>5.67 (1:2)</td>
<td>2.73 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Ti(II)</td>
<td>9.96 (1:1)</td>
<td>5.46 (1:2)</td>
<td>3.08 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>9.23 (1:1)</td>
<td>5.46 (1:2)</td>
<td>3.65 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Ba(II)</td>
<td>10.21 (1:1)</td>
<td>5.91 (1:2)</td>
<td>1.34 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>---</td>
<td>8.21 (1:2)</td>
<td>5.46 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>La(III)</td>
<td>9.83 (1:1)</td>
<td>5.27 (1:2)</td>
<td>3.38 (1:3)</td>
<td>---</td>
</tr>
<tr>
<td>Zr(IV)</td>
<td>---</td>
<td>7.25 (1:2)</td>
<td>4.77 (1:3)</td>
<td>---</td>
</tr>
</tbody>
</table>

The species distribution curve of NAL is shown in Fig. (6). In weakly acidic solution (pH ~ 2.6), biguanide (NAL) exists in its dipronated form, H-NAL, and with rise of pH, undergoes successive depronation to form the free ligand (L'), and tends to starts from pH 4.6 and reaches its maximum at pH 8.6. All species have broad protonation space between pH 2.6–11.6. Various complexes formulated as M-L and M-L2 between the ligand and the metal ions are formed depending on pH. The data obtained from M-NAL complexes have been evaluated using SUPERQUAD program [24] and the species distribution curves obtained from calculations is given in Fig. 7. Complex formation equilibria in both (1 : 1) and (1 : 2) M : L systems start above pH 5. In M-L system, their concentrations ranges were about 90%. Whereas, M-L$_2$ complexes have also been observed but, it were at the lowest level (10-15%) perhaps, due to the solutions diluted ligand, and the nature of the metal ions.
Fig (6): Ionic equilibria of nalidixic acid in different pHs ranges

Fig (7): Ionic equilibria of Co-NAL complexes in different pHs range

Potentiometric determination of nalidixic acid:

Nalidixic acid is mono basic acid having dissociation constant $pK_1 = 6.4$ (carboxyl group). Observing the value of nalidixic acid, it can be foreseen that the titration curve presents a clear inflection for the first point of equivalence since $K_1 = 1 \times 10^{-6.4}$.

However, potentiometric titration for determination of nalidixic acid in pure form was performed with sodium hydroxide as titrant, $I = 0.5\, M\, NaNO_3$ at $25 \pm 1^\circ C$. The steep rise in the pH was observed in Fig (8a) at the equivalence point with potentiometric end point detection. The first and second derivative of potentiometric titration curve as shown in Fig. 8 (b, c) methods were applied to ascertain the equivalence point.

Table (2): Effect of ionic strength on the percentage recovery for pure nalidixic acid

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Add from pure (mg)</th>
<th>Found (mg)</th>
<th>Percentage recovery ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>500</td>
<td>405.5</td>
<td>81.1 ± 0.35</td>
</tr>
<tr>
<td>0.1</td>
<td>500</td>
<td>449.5</td>
<td>89.9 ± 0.25</td>
</tr>
<tr>
<td>0.5</td>
<td>500</td>
<td>494</td>
<td>98.8 ± 0.23</td>
</tr>
<tr>
<td>0.75</td>
<td>500</td>
<td>574.5</td>
<td>114.9 ± 0.42</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>655.5</td>
<td>131.1 ± 0.19</td>
</tr>
</tbody>
</table>

Effect of ionic strength on determination of pure nalidixic acid (NAL):

In table (2), ionic strength from 0.05 M to 1 M NaNO_3 using potentiometric method we can observe the high and best recovery percent (in closed 100 %) at 0.5 M NaNO_3. Thus, 0.5M ionic strength can be used for determination of pure and dosage forms of nalidixic acid.
3. A.5.2. Determination of pure nalidixic acid (NAL)

The results for the determination of nalidixic acid in 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 0.52% (n=5) with confidence limit (at 95% confidence level) in the range from (0.2-0.46). The detection limit (as 3σ/b, where b is the slope and σ=SD) [24] was 2.20 mg/25ml. The calibration graph is linear in the range of 23-2.55 mg/25ml. The correlation coefficient of determination (r) comes out to be 0.9972. The standard deviation (SD) was 2.77 as shown in Fig (9).

![Figure 9](image9.png)

**Fig(9): Linearity rang of nalidixic acid(pure), µ =0.5 M NaNO3 at 25±1.0 °C**

**Effect of interferents:**

To assess the usefulness of the proposed method, the effect of the common components(additives and excipients), which often accompany tenoxicam in pure form (D(+) lactose monohydrate, sodium chloride and sodium acetate) were investigated in a concentration range at least 100 times higher than that of nalidixic acid. No interferences observed in this concentration range.

**Analytical application:**

The proposed methods were successfully applied for nalidixic acid 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 derivative of potentiometric curve and the second derivative one, respectively.

**Fig 11** shows conductometric titration curves of determination of NAL in pure form and its dosage formulations.

![Figure 10](image10.png)

**Table (3): Determination of NAL in pure form by using proposed methods with µ =0.5 M NaNO3 at 25±1.0°C**

<table>
<thead>
<tr>
<th>Add of pure (mg/25ml)</th>
<th>Found (mg/25ml)</th>
<th>Recovery (%)</th>
<th>SD (n=5)</th>
<th>Confidence (n=5)</th>
<th>α=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.232</td>
<td>0.222 (0.221)</td>
<td>95.5 (95.1)</td>
<td>0.45 (0.52)</td>
<td>0.39 (0.46)</td>
<td></td>
</tr>
<tr>
<td>0.697</td>
<td>0.671 (0.668)</td>
<td>96.34 (95.8)</td>
<td>0.38 (0.46)</td>
<td>0.33 (0.40)</td>
<td></td>
</tr>
<tr>
<td>1.161</td>
<td>1.142 (1.135)</td>
<td>98.4 (97.8)</td>
<td>0.35 (0.42)</td>
<td>0.31 (0.37)</td>
<td></td>
</tr>
<tr>
<td>1.625</td>
<td>1.628 (1.615)</td>
<td>100.2 (99.4)</td>
<td>0.28 (0.38)</td>
<td>0.25 (0.33)</td>
<td></td>
</tr>
<tr>
<td>2.09</td>
<td>2.142 (2.115)</td>
<td>102.5 (101.2)</td>
<td>0.23 (0.31)</td>
<td>0.20 (0.27)</td>
<td></td>
</tr>
<tr>
<td>2.55</td>
<td>2.657 (2.64)</td>
<td>104.2 (103.5)</td>
<td>0.22 (0.33)</td>
<td>0.19 (0.29)</td>
<td></td>
</tr>
</tbody>
</table>
The data in Table (4) shows that the NAL 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 (95.8-98.68) %, with standard deviations (SD) were within (0.18–0.4) (%). 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 Nalidixic Acid in Pharmaceutical Preparations

The data between brackets were from conductivity method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Manufacturer</th>
<th>Label of content (mg)</th>
<th>Proposed methods</th>
<th>Found (mg)</th>
<th>Recovery (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAL (Tablet)</td>
<td>(Life Pharmatfarm aco-group Italy)</td>
<td>500</td>
<td></td>
<td>493.4 (489)</td>
<td>98.68 (97.8)</td>
<td>0.32 (0.4)</td>
</tr>
<tr>
<td>Dar Al-daaw - Jordan</td>
<td>Uroneg (Tablet)</td>
<td>500</td>
<td></td>
<td>493.2 (484)</td>
<td>98.64 (96.8)</td>
<td>0.18 (0.2)</td>
</tr>
</tbody>
</table>

Conclusion:-

Potentiometric and Conductometric methods are excellent methods for calculation of stability constant of metal ligand complexes. NAL has one dissociation constant log$K_{H}^{1}$=5.0.

However, NAL forms complexes with metal ions of types 1:1, 1:2 and/or 1:3 metals to ligand complexes. The stoichiometric ratio obtained from potentiometric method was consistent to the results of conductometric method. Finally, the species distribution of ligands and its metal complexes under investigation are variables during the pH ranges.

Compared with many of already existing methods for the determination of nalidixic acid, 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 nalidixic acid in pharmaceutical formulation. Recovery of nalidixic acid for various tablet dosage formulation from 95.8 to 98.68 %, and no interferences observed.
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The study aims to evaluate the equilibrium of the metal complexes of NAL with the metal ion Na+ at different NAL to metal ratios (1:1, 2:1, 3:1). The Complexes were characterized by their infra-red

References:

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