Studies on the Gravimetric and Spectrophotometric Analysis of Norfloxacin using Ammonium Reineckate

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Gravimetric and spectrophotometric assay of norfloxacin was carried out using ammonium reineckate. We obtained an ion-association complex having the following formula: \([\text{C}_16\text{H}_{19}\text{FN}_3\text{O}_3]^+\text{[Cr(NCS)\(_2\)}\text{NH}_2]^\text{-}. The complex is hardly soluble in water and of a pink-purple colour. This complex was described by spectral (IR and UV-VIS) and thermal analysis (TG, DTG, DSC), by determination of its formula weight and of its water solubility.

Keywords: norfloxacin, ammonium reineckate, gravimetric and spectrophotometric assay

Norfloxacin[1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid)] is a synthetic antibiotic with a chinolonic nucleus, and it is frequently used in the treatment of urinary tract infections [1]. Due to the presence of a carboxyl group and of a 1-piperazinyl nucleus, norfloxacin is amphoteric, both functions being weak: pKa 1=6.22, pKa 2=8.51 [2]. The presence of the carboxyl and carbonyl groups in neighboring positions provides the substance with the capacity to act as a bidentate ligand. Based on this observation, spectrophotometric methods for dosing norfloxacin were developed, by including norfloxacin into a yellow-orange chelate with the Fe(III) ion [3-7]. Due to the piperazinyl nucleus norfloxacin has amine-like reactions, and forms ion-association complexes with charge transfer. A series of spectrophotometric methods were developed, in which norfloxacin is dosed as some colored complexes with: supracene violet 3B, tropeoline 000 [8], Sudan III [9], derivatives of 2,4-dinitrofluorobenzene [10], N-2,6-dimethyl-phenyl-2,3-dicloromalimide [11], the Marquis reagent [12].

In presence of ammonium diammintetraocyanato-chromate (III) (ammonium reineckate, Reinecke salt), norfloxacin forms a pink-purple microcrystalline precipitate. The resulting compound, \([\text{C}_16\text{H}_{19}\text{FN}_3\text{O}_3]^+\text{[Cr(NCS)\(_2\)}\text{NH}_2]^\text{-}\) is a 1:1 ion-association complex. To describe the complex, we used spectral analysis (IR and UV-VIS), thermal analysis (TG, DTG, DSC), determination of the formular weight and of the solubility in water at 25°C [13]. Based on the precipitation reaction and on the UV-VIS spectral behaviour of the complex dissolved in DMF, we intend to establish some simple and sensitive methods for dosage of norfloxacin.

Experimental part

Reagents

Analytical purity Merck reagents were used. For the spectrophotometric determinations the following stock solutions were prepared: stock solution for the spectrophotometric determination of norfloxacin in the visible domain, prepared by solving 0.9947 g of substance in HCl 10\(^{-1}\)M in a 100 mL volumetric flask (solution 1), stock solution for the spectrophotometric determination of norfloxacin in UV (λ=310 nm), prepared by solving 1.2084 g of substance in HCl 10\(^{-1}\)M in a 100 mL volumetric flask (solution 2), stock solution for the spectrophotometric determination of norfloxacin in UV (λ=288 nm), prepared by solving 1.3000 g of substance in HCl 10\(^{-1}\)M in a 100 mL volumetric flask (solution 3).

The synthesis of the complex

A sample of approximately 0.10 g of norfloxacin is dissolved in 80-100 mL of HCl 10\(^{-1}\)M, then the precipitation reagent is added (0.2% Reinecke salt solution in water), in small amounts, at cold (0-5°C, in ice), under continuous stirring, until complete precipitation. The pink-purple precipitate is kept cold for 15 min, then it is filtered through a G4 filter, washed with cold distilled water until the residual water no longer tests positive for the Cl\(^-\) ion, then it is dried at room temperature in a vacuum drier.

The resulting complex is hardly soluble in water, methanol, ethanol, chloroform, benzene, glacial acetic acid, soluble in acetone, highly soluble in dimethylformamide, dimethylsulfoxide, 10\(^{-1}\)M solution of sodium hydroxide.

Characterization of the complex

Apparatus

The IR spectra were recorded with a Perkin-Elmer 16 PC-FTIR spectrophotometer, using potassium bromide tablets, over the 4000-400 cm\(^{-1}\) domain.

The UV-VIS spectra were recorded with a UV-VIS Lambda 2 Perkin Elmer spectrophotometer, in 1 cm thick quartz cells.

The thermal behavior was studied with a Du Pont 2000 equipment, by recording the thermogravimetric (TG) curves and their derivate (DTG), in the 40°C-700°C temperature interval, at a heating speed of 20°C/min., and by differential scanning calorimetry (DSC), in the 40°C-300°C temperature interval, at a heating speed of 10°C/min.

Methods

Determination of the formular weight

The spectrophotometric method established by Lee Kum Tatt [14], using the maximum absorbance of the complex, dissolved in DMF was employed 15-60 mg samples of complex are brought quantitatively to 10 mL volumetric flask, dissolved in DMF, then brought up to sign with the same solvent. The absorbances of the solutions are read at 524 nm, against DMF.

Determination of the solubility in water at 25°C

A spectrophotometric method was used [14] based on the absorbance of a saturated aqueous solution of precipitate, at the wavelength of the precipitation reagent.

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 Samples of complex are brought into 25 mL volumetric flask containing distilled water, the solutions are stirred for one hour, then are completed to sign with distilled water, then are filtered through quantitative filter paper. The absorbance of the filtrate is read at $\lambda = 310$ nm, against distilled water.

**Gravimetric determination**

Samples of 0.10-0.20 g of norfloxacin are dissolved in approximately 80 mL of HCl $10^{-1}$M (or acetic acid 2M), the Berzelius beakers are put into ice and the precipitation is done with a solution of Reinecke salt. The pink-purple precipitates are kept on ice for 15 min, than filtered through G4 filter and washed with cold distilled water (0-5°C) until the residual water tests negative for the Cl ion. The resulting precipitates are dried at room temperature in dessicator to constant weight.

**Spectrophotometric determinations**

**Construction of the calibration curve for determination at the wavelenght of $\lambda = 524$ nm**

Volumes of 1, 2, 3...10 mL from stock solution 1 are brought to Berzelius beakers, approximately 80 mL of HCl $10^{-1}$M is added, then precipitation with Reinecke salt is done according to the conditions established at the gravimetric method. After filtering through G4 filter, purification, drying, the precipitates are dissolved in DMF directly on the filter. The solutions are collected in 25 mL volumetric flasks and are completed up to sign with the same solvent. The absorbances of the solutions are read at the wavelength of $\lambda = 524$ nm, against DMF.

**Construction of the calibration curve for determination at the wavelenght of $\lambda = 310$ nm**

Volumes of 1, 2, 3...10 mL from stock solution 2 are brought to Berzelius beakers, approximately 80 mL of HCl $10^{-1}$M is added, then precipitation with Reinecke salt is done according to the conditions established at the gravimetric method. After filtering through G4 filter, purification, drying, the precipitates are dissolved in DMF directly on the filter. The filtrate is collected to a 50 mL volumetric flask and is completed up to sign with the same solvent. The absorbance of solution B is read at the wavelength of $\lambda = 310$ nm, against DMF.

**Construction of the calibration curve for determination at the wavelenght of $\lambda = 288$ nm**

Volumes of 1, 2, 3...10 mL from stock solution 3 are brought to Berzelius beakers, approximately 80 mL of HCl $10^{-1}$M is added, then precipitation with Reinecke salt is done according to the conditions established at the gravimetric method. After filtering through G4 filter, purification, drying, the precipitates are dissolved in DMF directly on the filter. The filtrate is collected to a 100 mL volumetric flask and is completed up to sign with DMF (solution A). 1 mL of solution A is diluted with DMF to 100 mL in a volumetric flask (solution B). The absorbance of solution B is read at the wavelength of $\lambda = 288$ nm, against DMF.

**Results and discussion**

**Characterization of the complex**

**The IR spectrum**

In the IR spectrum of the obtained compound one can find the bands of the reagents with some modifications in position, shape and intensity. In solid state norfloxacin presents molecules that are associated by inter- and intra molecular hydrogen links. The vibrations of the –OH groups concerned in the two types of links causes some bands to appear in the 3600-2700 cm$^{-1}$ region, overlapping the bands determined by the $\nu$(N-H) vibrations of the protonated piperazinic nitrogen.

In the IR spectrum of norfloxacin reineckate (table 1) there are bands that are characteristic to norfloxacin and the most important of the are those at 1701 cm$^{-1}$ ($\nu$(CO)_{carboxilic} vibration), 1627 cm$^{-1}$ ($\nu$(CO)_{aromatic} vibration), 1271 cm$^{-1}$ ($\nu$(C-F) vibration) [15]. Bands that are characteristic to the reineckate ion are also present: at 2081 cm$^{-1}$, intense band determined by the $\nu$(CN) vibration from the Cr-NCS link, at 691 cm$^{-1}$ ($\nu$(C-S) vibration), at 489 cm$^{-1}$, corresponding to the $\delta$(NCS) deformation vibration [16, 17, 18].

**Table 1**

<table>
<thead>
<tr>
<th>$\nu$ (cm$^{-1}$)</th>
<th>assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2081</td>
<td>$\nu$(CN)</td>
</tr>
<tr>
<td>1701</td>
<td>$\nu$(CO)$_{arboxilic}$</td>
</tr>
<tr>
<td>1627</td>
<td>$\nu$(CO)$_{aromatic}$</td>
</tr>
<tr>
<td>1271</td>
<td>$\nu$(C-F)</td>
</tr>
<tr>
<td>691</td>
<td>$\nu$(C-S)</td>
</tr>
<tr>
<td>489</td>
<td>$\delta$(NCS)</td>
</tr>
</tbody>
</table>

**UV-VIS spectra**

Spectra of the obtained compound were recorded in comparison to the spectra of the reagents, both in the visible and the UV domains. To prepare the solutions we used dimethylformamide, a solvent in which both the reagents and the compound are highly soluble.

In the visible domain the norfloxacin reineckate presents a maximum of absorbion at 524 nm, corresponding to the $^{4}A_{2g} \rightarrow ^{4}T_{2g}$ electronic transitions in the Cr(III) ion. In the ultraviolet domain the norfloxacin reineckate shows two absorption peaks, at 310 nm, corresponding to the $^{4}A_{2g} \rightarrow ^{4}T_{1g}$ electronic transitions in the Cr(III) ion and at 288 nm, characteristic to norfloxacin.

**The thermal behavior**

The thermolysis curve of norfloxacin reineckate (fig. 1) and its derivate shows an almost horizontal plateau up to 190°C, followed by a breakdown in three stages: between 190°C-275°C there is a slow decrease, while between 270°C-350°C (with a peak at 300°C), and between 350°C-480°C, there is a steep decrease in the mass with residue formation. On the DSC curve (fig. 2) two endothermic peaks are present: at 11°C, with a small thermic effect and at 203°C, with a considerable thermic effect (24.7 kJ/mol), marking the start of the first stage in the breakdown of the complex. In comparison, the Reinecke salt breaks down over 190°C in two stages (190°C-320°C and 350°C-520°C), leaving a residue of Cr$_2$O$_3$, and norfloxacin breaks down over 275°C, in two sages, leaving no residue.

**The formular weight**

To calculate the formular weight we applied the following formula [15]:

$$F = \frac{a \cdot \varepsilon}{A \cdot V}$$

where:

- $F$ = the formular weight of the complex;
- $a$ = the mass of the complex used (mg);
- $\varepsilon$ = the molar absorptivity of the complex at the wavelength of $\lambda = 524$ nm;
- $A$=the experimentally determined absorbance;
- $V$ = the volume of the solution (mL)
The results were statistically interpreted and are presented in table 2.

The experimentally determined formular weight is 639.31, compared to the theoretically calculated weight of 638.84, corresponding to the nonhydrated complex in which the norfloxacin: Reinecke salt combination ratio is 1:1.

**Solubility in water**

For calculations we used the following formula [14]:

\[
S = \frac{A \cdot V \cdot F}{e}
\]

where:

- \(S\) = mg of complex dissolved in the quantity of distilled water.
- \(A\) = mg of complex disolved in 10 mL of distilled water.
- \(V\) = mL of distilled water.
- \(F\) = mg of complex.
- \(e\) = (L-mol\(^{-1}\)-cm\(^{-1}\)).

The results were statistically interpreted and are presented in table 3.

Based on the data provided by the IR and UV-VIS spectra and the results of the thermal analysis, considering the determination of the formular weight, the ion association complex obtained was assigned the following formula: \([C_{16}H_{19}FN_3O_3]^+[Cr(NCS)_4(NH_3)_2]^-\). The compound obtained has a well defined and unitary composition, high formular weight and is hardly soluble in water. The reaction of norfloxacin with the Reinecke salt proves itself to be usefull to develop some methods to dose this pharmaceutical substance.
Gravimetric determinations

For calculation we used the gravimetric factor \( f = 0.4999, \) considering the combination ratio of 1:1 between the reagents. Table 4 contains the results of the gravimetric dosage of norfloxacin as a reineckate, after statistics.

Spectrophotometric determinations

Norfloxacin reineckate dissolved in DMF shows an absorption peak, in the visible domain, at the wavelength of \( \lambda = 524 \) nm and two absorption peaks, in the UV domain, at 310 and 288 nm. Studying the variation of the absorbances in time, for all three wavelengths we proved its stability for at least 2h, time which is sufficient to perform the determinations. Table 5 contains the characteristic parameters for the spectrophotometric determination of norfloxacin at all three wavelengths. The spectrophotometric methods developed were applied for the dosage of some samples of pharmaceutical substance. Ten solutions were prepared following the procedures used to construct the calibration curves. Table 6 contains the statistically interpreted results.

### Table 3

RESULTS OF THE DETERMINATION OF THE SOLUBILITY OF NORFLOXACIN REINECKATE IN WATER (25°C)

<table>
<thead>
<tr>
<th>Nr. crt.</th>
<th>( \varepsilon ) (L·mol(^{-1})·cm(^{-1}))</th>
<th>( \nu ) (mL)</th>
<th>A</th>
<th>( S \cdot 10^7 ) (mol·L(^{-1}))</th>
<th>Error from the average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34000</td>
<td>25</td>
<td>0.931</td>
<td>2.74</td>
<td>-0.11</td>
</tr>
<tr>
<td>2</td>
<td>34000</td>
<td>25</td>
<td>0.932</td>
<td>2.74</td>
<td>-0.11</td>
</tr>
<tr>
<td>3</td>
<td>34000</td>
<td>25</td>
<td>0.965</td>
<td>2.84</td>
<td>-0.01</td>
</tr>
<tr>
<td>4</td>
<td>34000</td>
<td>25</td>
<td>0.974</td>
<td>2.87</td>
<td>+0.02</td>
</tr>
<tr>
<td>5</td>
<td>34000</td>
<td>25</td>
<td>0.985</td>
<td>2.89</td>
<td>+0.04</td>
</tr>
<tr>
<td>6</td>
<td>34000</td>
<td>25</td>
<td>1.030</td>
<td>3.03</td>
<td>+0.18</td>
</tr>
</tbody>
</table>

\( n=6 \) \( \bar{M}=2.85 \cdot 10^{-5} \) \( s=0.11 \) \( s_{\alpha}=0.04 \) \( \alpha=0.95 \) \( t_{\alpha}=2.571 \)

\( \Lambda=(2.85 \pm 0.11) \cdot 10^{-5} \)

### Table 4

RESULTS OF THE GRAVIMETRIC DOSAGE OF NORFLOXACIN AS A REINECKATE

<table>
<thead>
<tr>
<th>Nr. crt.</th>
<th>Norfloxacin used (g)</th>
<th>Complex obtained (g)</th>
<th>Norfloxacin determined (g)</th>
<th>Error from average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1043</td>
<td>0.2078</td>
<td>0.1039</td>
<td>-0.19</td>
</tr>
<tr>
<td>2</td>
<td>0.1086</td>
<td>0.2168</td>
<td>0.1084</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>0.1185</td>
<td>0.2364</td>
<td>0.1182</td>
<td>-0.07</td>
</tr>
<tr>
<td>4</td>
<td>0.1231</td>
<td>0.2456</td>
<td>0.1228</td>
<td>-0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.1425</td>
<td>0.2845</td>
<td>0.1422</td>
<td>+0.01</td>
</tr>
<tr>
<td>6</td>
<td>0.1539</td>
<td>0.3113</td>
<td>0.1556</td>
<td>-0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.1620</td>
<td>0.3237</td>
<td>0.1618</td>
<td>+0.06</td>
</tr>
<tr>
<td>8</td>
<td>0.1764</td>
<td>0.3523</td>
<td>0.1761</td>
<td>+0.03</td>
</tr>
<tr>
<td>9</td>
<td>0.1821</td>
<td>0.3639</td>
<td>0.1819</td>
<td>+0.07</td>
</tr>
<tr>
<td>10</td>
<td>0.1982</td>
<td>0.3959</td>
<td>0.1979</td>
<td>+0.08</td>
</tr>
</tbody>
</table>

\( n=10 \) \( \bar{M}=99.81 \) \( s_{\varepsilon}=0.03 \) \( \alpha=0.95 \) \( t_{\alpha}=2.262 \)

\( \Lambda=99.81 \pm 0.06 \) \( c.v.=0.08\% \)

### Table 5

CHARACTERISTIC PARAMETERS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF NORFLOXACIN AS REINECKATE

<table>
<thead>
<tr>
<th>Wavelength ( \lambda ) (nm)</th>
<th>524</th>
<th>310</th>
<th>288</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent used</td>
<td>DMF</td>
<td>DMF</td>
<td>DMF</td>
</tr>
<tr>
<td>Liniarity range</td>
<td>0.3-3.9 mg·mL(^{-1})</td>
<td>2.4-24.1 µg·mL(^{-1})</td>
<td>1.3-13 µg·mL(^{-1})</td>
</tr>
<tr>
<td>( x )</td>
<td>2.1883</td>
<td>13,2924</td>
<td>7.15</td>
</tr>
<tr>
<td>( y )</td>
<td>0.6642</td>
<td>0.7366</td>
<td>0.6705</td>
</tr>
<tr>
<td>( \sigma_x )</td>
<td>1.1428</td>
<td>6.9417</td>
<td>3.7339</td>
</tr>
<tr>
<td>( \sigma_y )</td>
<td>0.3499</td>
<td>0.3890</td>
<td>0.3596</td>
</tr>
<tr>
<td>( r )</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>( y )</td>
<td>0.3055×0.004</td>
<td>0.0359×0.0055</td>
<td>0.0957×0.0123</td>
</tr>
<tr>
<td>( \varepsilon ) (L·mol(^{-1})·cm(^{-1}))</td>
<td>97.1</td>
<td>17551</td>
<td>29906</td>
</tr>
</tbody>
</table>

### Table 6

STATISTICAL INTERPRETATION OF THE RESULTS OF SPECTROPHOTOMETRIC DOSAGE OF NORFLOXACIN AS REINECKATE

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Norfloxacin used (mg)</th>
<th>n</th>
<th>( \bar{M} )</th>
<th>( s )</th>
<th>( s_{\alpha} )</th>
<th>( \alpha )</th>
<th>( t_{\alpha} )</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>524</td>
<td>10.5-67.7</td>
<td>10</td>
<td>99.82</td>
<td>0.06</td>
<td>0.02</td>
<td>0.95</td>
<td>2.262</td>
<td>99.82±0.04</td>
</tr>
<tr>
<td>310</td>
<td>30.9-73.2</td>
<td>10</td>
<td>99.84</td>
<td>0.06</td>
<td>0.03</td>
<td>0.95</td>
<td>2.262</td>
<td>99.84±0.07</td>
</tr>
<tr>
<td>288</td>
<td>24.1-84.4</td>
<td>10</td>
<td>99.86</td>
<td>0.10</td>
<td>0.03</td>
<td>0.95</td>
<td>2.262</td>
<td>99.86±0.07</td>
</tr>
</tbody>
</table>
Conclusions

Norfloxacin forms with the Reinecke salt an ion association complex, hardly soluble in water, with the formula \( [C_{16}H_{19}FN_{3}O_{3}]^{+}[Cr(NCS)_{4}(NH_{3})_{2}]^{-} \). The precipitation reaction was used to establish a new gravimetric dosage method. The statistic interpretation of the results proves that, under the established experimental conditions, the reaction is quantitative and the methods developed are accurate and reproducible. Based on the absorption peak of the complex, three new spectrophotometric dosage methods were established, at the wavelengths of 524 nm, 310 nm and 288 nm. We found a linear dependence between the absorbance and the concentration along the following ranges: 0.3-3.9 μg . mL⁻¹, 2.4-24.1 μg . mL⁻¹ and 1.3-13 μg . mL⁻¹, respectively.

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Manuscript received: 15.07. 2008