A quantitative relationship was found between the relative fluorescence intensities of certain metal chelates of some quinolone antibacterials and their physicochemical parameters namely: the $pK_{a1}$ (corresponding to the ionisation of the 3-carboxylic group) and the calculated stability constants of the formed chelates as well as the second order connectivity indexes ($\chi^2$), Hammett constant ($\sigma_m$), polar constants ($F$) and resonance constants ($R$) for the substituent at position 1 of the quinolone nucleus. The studied quinolone antibacterials are amifloxacin, difloxacin, ciprofloxacin, nalidixic acid, norfloxacin, lomefloxacin, ofloxacin and pefloxacin and the metals involved are zirconium, molybdenum, vanadium and tungsten.

Twenty highly significant regression equations were obtained and used to calculate the unreported values of $\sigma_m$, $F$ and $R$ for the substituent at position 1 of ofloxacin. Two correlation equations were used also to predict the fluorescence intensity of molybdenum chelates of the studied quinolones.
INTRODUCTION

In a previous communication, simple, rapid, reliable, and sensitive spectrofluorometric methods for the determination of eight quinolone antibacterials namely ciprofloxacin, norfloxacin, lomefloxacin, difloxacin, amifloxacin, pefloxacin, ofloxacin, and nalidixic acid (Figure I) based on their chelation with zirconium, molybdenum, vanadium or tungsten to produce intensely fluorescent chelates were developed. It is well known that the relative fluorescence intensity (RFI) is dependent on the molecular structure and linking pattern or bonding scheme of the atoms in the fluorescent species. The conversion of structural formula into numerical values or indices, which encode structural information, will be extremely helpful. Thus, a numerical description of a molecule derived from knowledge of the molecular structure itself can be developed.

Figure I: Chemical structures of the investigated quinolones.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
<th>( R_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Amifloxacin</td>
<td>NHCH(_3)</td>
<td>H</td>
<td>H</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>2- Ciprofloxacin</td>
<td>C(_2)H(_5)</td>
<td>F</td>
<td>CH(_3)</td>
<td>H</td>
</tr>
<tr>
<td>3- Difloxacin</td>
<td>C(_2)H(_4)-F</td>
<td>H</td>
<td>H</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>4- Lomefloxacin</td>
<td>C(_2)H(_5)</td>
<td>F</td>
<td>CH(_3)</td>
<td>H</td>
</tr>
<tr>
<td>5- Norfloxacin</td>
<td>C(_2)H(_5)</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>6- Ofloxacin</td>
<td>-CH(_2)CHCH(_3)-</td>
<td>-O-</td>
<td>H</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>7- Pefloxacin</td>
<td>C(_2)H(_5)</td>
<td>H</td>
<td>H</td>
<td>CH(_3)</td>
</tr>
</tbody>
</table>

Nalidixic acid
There are two general aspects of structure which can be identified and expressed in the form of numerical parameters:

1- The topology of the molecule: information about the identities of atoms and their electronic properties and connections.

2- The molecular topography: various three-dimensional aspects e.g. size, shape, branching, volume and surface area of the molecule.

In the present work, the topology of the molecule was expressed using several electronic parameters namely: the pKa1 of the studied drug (ionization of 3-carboxylic group) as well as Hammett constant (σm), polar constant (f) and resonance constant (r) for the substituent at position 1 of the quinolone nucleus. These electronic parameters were obtained from literature and included as the electronic contribution variables.

The molecular topography was expressed using the molecular connectivity (χ) adapted by Kier et al. as a descriptive title for the general method leading to indexes derived from the molecular structure. The structural formula of the compound is written down as a molecular skeleton without hydrogens. Each carbon atom is designated by a cardinal number, which is a count of the number of adjacent carbon atoms. This count of adjacent or formally bonded carbon atoms is called the delta value (δ). The molecular skeleton is then dissected into all constituent bonds, each designated by the two carbons, i and j, forming the bond. A value for each bond is computed from the equation (δ_i, δ_j)^0.5. The molecular connectivity index is the simple sum of the computed bind values over the entire molecule according to the equation:

\[ \chi = \sum (\delta_i \delta_j)^{0.5} \]

Where the prefix 1 indicates that the index is for a one bond dissection of the molecule.

However, physico-chemical properties measured experimentally such as fluorescence intensity have a complex dependence on three-dimensional structural features. Further dissection of the molecule into two-bond fragments (three contiguous atoms) may provide this dimensionality.

To calculate the second order molecular connectivity index (\( ^2\chi \)), a term for each two bond fragment (three contiguous atoms i, j and k) is computed using the following general equation:

\[ ^2\chi = \sum (\delta_i \delta_j \delta_k)^{0.5} \]

To include the effect of unsaturation in the calculated connectivity indexes, a double bond would be counted twice when calculating the δ value for adjacent atoms e.g.

\[ ^2C = ^3C - ^3C = ^2C \]

This procedure takes explicit account of the valence and the hybrid state of each carbon atom. The modified delta value calculated according to this procedure is called
the valence delta ($\delta^v$) and the computed connectivity index is called the valence chi ($\chi^v$).

Another progress in this field was the treatment of heteroatoms introduced by Kier and Hall, where the delta value for each heteroatom is calculated according to the equation:

$$\delta^v = z^v - h$$

Where $z^v$ is the number of valence electrons and $h$ is the number of hydrogen atoms attached to the heteroatom. Kier and Hall also reported the $\delta^v$ values of different heteroatoms in various hybrid states.

In this work, we tried to correlate the experimentally measured RFI of the studied chelates with the $pK_a$ values (ionisation of the 3-carboxylic group) of the studied drugs, the calculated stability constants of the formed chelates (Table 1) as well as the second order connectivity indexes ($\chi^v$), Hammett constant ($\sigma_m$), polar constant ($\sigma_f$) and resonance constant ($\sigma_R$) for the substituent at position 1 of the quinolone nucleus.

Similar correlations were previously reported for phenothiazines, catecholamines and thiols, where highly significant relationships were obtained between molar absorbivities of certain derivatives and some physico-chemical parameters of the parent compound.

**COMPUTATION**

**Instrument**

Intel Pentium IV PC (USA) equipped with Microsoft Excel 2000\textsuperscript{6} data analysis tool pack.

**Mathematical and statistical treatment of data**

a- The correlation between the experimentally measured RFI of the formed chelates obtained in the previously published work\textsuperscript{1} and the corresponding drug concentrations was carried out using linear regression model to obtain the intercept (a), the slope (b), the correlation coefficient (r) and the determination coefficient ($r^2$).

b- The correlation between the experimentally measured RFI of the studied chelates and the studied parameters obtained from literature,\textsuperscript{3-5} was carried out using simple or multiple regression analysis models in the Microsoft Excel 2000\textsuperscript{6} data analysis tool pack.

c- The second order connectivity index ($\chi^v$) for the substituent at position 1 of the quinolone nucleus was calculated according to Kier and Hall’s rules.\textsuperscript{2} An illustrative example for calculating $\chi^v$ for the substituent at position 1 in ciprofloxacin is given in scheme 1.
Scheme 1: calculation of $^{2}\chi^v$ for the substituent at position 1 in ciprofloxacin.

**RESULTS AND DISCUSSION**

Usually, the RFI of chelates are directly proportional to their stability constants. To find the quantitative relationship between the experimentally measured RFI of the formed chelates and the calculated stability constants $K_s$ for these chelates (Table 1), simple linear regression model was used. Good results (Table 2) were obtained upon correlating the RFI with either $K_s$ or log $K_s$ according to the general equations:

\[
\text{RFI} = a + b \, K_s \quad \text{(equations 1-4)}
\]

\[
\text{RFI} = a + b \, \log K_s \quad \text{(equations 5-8)}
\]

However, the correlation between the RFI of the formed chelates and log $K_s$ was statistically more significant than the correlation between RFI and $K_s$ values in all cases (Table 2).

For further understanding of the differences in the RFI of the studied chelates resulting from changes in the structure of the investigated drugs, multiple linear regression analysis was performed including several parameters representing the structural features that differ from one drug to another. In addition to log $K_s$, the pKa$_1$ of the studied drugs (Table 3) was used in the multiple linear regression as an indicator of the electron density of the carboxylic acid moiety of quinolones. Excellent correlation of RFI versus pKa$_1$ and log $K_s$ was obtained for all the studied chelates (Table 4) according to the general equation:

\[
\text{RFI} = a + b \, \text{pKa}_1 + c \, \log K_s \quad \text{(equations 9-12)}
\]

The regression models obtained in equations 9-12 (Table 4) demonstrated that an increase in the pKa$_1$ value resulted in an increase in the measured RFI. This can be easily explained by the fact that the increase
Table 1: Calculated stability constants of the formed chelates*.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Zirconium</th>
<th>Molybdenum</th>
<th>Vanadium</th>
<th>Tungsten</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_s \times 10^7$</td>
<td>$\log K_s$</td>
<td>$K_s \times 10^7$</td>
<td>$\log K_s$</td>
</tr>
<tr>
<td>DIF</td>
<td>131.4519</td>
<td>9.1187</td>
<td>147.9200</td>
<td>9.1700</td>
</tr>
<tr>
<td>AMI</td>
<td>121.3845</td>
<td>9.0841</td>
<td>146.9355</td>
<td>9.1671</td>
</tr>
<tr>
<td>OFL</td>
<td>140.2168</td>
<td>9.1468</td>
<td>156.1550</td>
<td>9.2749</td>
</tr>
<tr>
<td>NAL</td>
<td>87.9781</td>
<td>8.9443</td>
<td>99.4947</td>
<td>8.9978</td>
</tr>
</tbody>
</table>

* Average of 3 determinations.

Table 2: Regression analysis of RFI of the quinolone chelates versus their stability constants $K_s$ or $\log K_s$*.

RFI = $a + b K_s$
RFI = $a + b \log K_s$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chelated metal</th>
<th>Eq. no.</th>
<th>$a$</th>
<th>$b$</th>
<th>$r$</th>
<th>$F^*$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_s$</td>
<td>Zirconium**</td>
<td>1</td>
<td>-33.710</td>
<td>0.668</td>
<td>0.9314</td>
<td>41.056</td>
<td>4.809</td>
</tr>
<tr>
<td></td>
<td>Molybdenum**</td>
<td>2</td>
<td>-24.136</td>
<td>0.569</td>
<td>0.8111</td>
<td>10.663</td>
<td>10.886</td>
</tr>
<tr>
<td></td>
<td>Vanadium**</td>
<td>3</td>
<td>9.388</td>
<td>0.183</td>
<td>0.8735</td>
<td>19.332</td>
<td>6.723</td>
</tr>
<tr>
<td></td>
<td>Tungsten**</td>
<td>4</td>
<td>-49.792</td>
<td>0.725</td>
<td>0.9477</td>
<td>52.897</td>
<td>4.892</td>
</tr>
<tr>
<td>Log $K_s$</td>
<td>Zirconium**</td>
<td>5</td>
<td>-1524.230</td>
<td>173.076</td>
<td>0.9319</td>
<td>39.639</td>
<td>4.883</td>
</tr>
<tr>
<td></td>
<td>Molybdenum**</td>
<td>6</td>
<td>-1407.330</td>
<td>159.847</td>
<td>0.9230</td>
<td>26.509</td>
<td>7.794</td>
</tr>
<tr>
<td></td>
<td>Vanadium**</td>
<td>7</td>
<td>-689.072</td>
<td>79.174</td>
<td>0.9352</td>
<td>41.849</td>
<td>4.892</td>
</tr>
<tr>
<td></td>
<td>Tungsten**</td>
<td>8</td>
<td>-1740.471</td>
<td>195.886</td>
<td>0.9483</td>
<td>53.532</td>
<td>4.169</td>
</tr>
</tbody>
</table>

*Probability = 95 %.
**Number of compounds included in analysis = 8 compounds.
$F^*$= F-ratio between the variances of the observed and calculated values at the given probability.

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Table 3: Some electronic parameters of the studied drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>pKa₁*</th>
<th>Hammett constant (σₑ)**</th>
<th>Polar constant F **</th>
<th>Resonance constant R**</th>
<th>2χ⁻²χ'**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amifloxacin</td>
<td>6.15</td>
<td>-0.3</td>
<td>-0.11</td>
<td>-0.74</td>
<td>0.224</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.13</td>
<td>-0.07</td>
<td>-0.03</td>
<td>-0.19</td>
<td>1.231</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>5.86</td>
<td>0.4</td>
<td>0.51</td>
<td>-0.42</td>
<td>1.476</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>5.82</td>
<td>-0.07</td>
<td>-0.05</td>
<td>-0.10</td>
<td>0.316</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>6.26</td>
<td>-0.07</td>
<td>-0.05</td>
<td>-0.10</td>
<td>0.316</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>6.21</td>
<td>1.27</td>
<td>0.90</td>
<td>1.43</td>
<td>1.016</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>6.02</td>
<td>-0.07</td>
<td>-0.05</td>
<td>-0.10</td>
<td>0.316</td>
</tr>
</tbody>
</table>

*References 3 and 4. **Reference 5.

The constants σₑ, F, R and 2χ⁻²χ' were determined for the substituent at position 1 of the quinolone nucleus.

Table 4: Multiple linear regression analysis of RFI of the studied chelates versus pKa₁ and log Kₑ.*

RFI = a + b pKa₁ + c log Kₑ

<table>
<thead>
<tr>
<th>Chelated metal</th>
<th>Eq. no.</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>r</th>
<th>F⁺</th>
<th>SE $^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zirconium**</td>
<td>9</td>
<td>-1527.221</td>
<td>0.163</td>
<td>173.505</td>
<td>0.9322</td>
<td>16.601</td>
<td>5.337</td>
</tr>
<tr>
<td>Molybdenum**</td>
<td>10</td>
<td>-1168.500</td>
<td>2.3855</td>
<td>132.278</td>
<td>0.9373</td>
<td>15.348</td>
<td>7.438</td>
</tr>
<tr>
<td>Vanadium**</td>
<td>11</td>
<td>-907.798</td>
<td>4.431</td>
<td>103.294</td>
<td>0.9741</td>
<td>46.369</td>
<td>5.851</td>
</tr>
<tr>
<td>Tungsten**</td>
<td>12</td>
<td>-1466.410</td>
<td>1.516</td>
<td>164.779</td>
<td>0.9645</td>
<td>32.976</td>
<td>3.819</td>
</tr>
</tbody>
</table>

*Probability = 95 %. **Number of compounds included in analysis = 8 compounds. +F= F-ratio between the variances of the observed and calculated values at the given probability. $^5$Overall SE of the correlation.
in electron density at the carboxylate ion would result in increasing the attraction of the metal ion for the drug molecule. This increased attraction will cause an increase in the stability constant and consequently the RFI of the formed chelate.

It is obvious that the studied fluoroquinolones differ mainly in the substituent at position 1 of the quinolone nucleus. Thus, the quantitative relationship between the measured RFI of the studied chelates and certain physico-chemical parameters of the substituent at position 1 of the quinolone nucleus was also investigated (nalidixic acid was excluded from this study because it has a somewhat different nucleus).

Several electronic parameters namely, Hammett constant (σ_m), polar constant (F) and resonance constant (R) were included in this study using simple linear regression analysis but none of them gave satisfactory results, indicating a complex relationship between these parameters and the RFI of the studied chelates. Upon applying multiple linear regression analysis to this study, excellent results were obtained showing a strong relationship between the RFI of the formed chelates and the electronic parameters of the substituent at position 1 of the quinolone nucleus (Table 5) according to the general equation:

\[ \text{RFI} = a + b \sigma_m + c R + d F \]

(equations 13-16)

It is apparent that the three electronic parameters of this substituent contribute to the RFI of the formed chelates to different degrees. A logic explanation for that correlation is the stabilizing effect of this substituent on the formed anionic form of the drug before chelation or its stabilizing effect on the formed chelate itself.

Another factor was also studied in this work, which is the effect of the bulkness and branching of the substituent at position 1 of quinolone nucleus on the RFI of the formed chelates. The bulkness and branching of the substituent \( R_1 \) was expressed as the second order connectivity index of this substituent (\( \chi' \)) calculated according to Kier and Hall’s rules. On using simple linear regression model to correlate the RFI of the studied chelates to \( \chi' \) of the substituent the substituent at position 1 of the quinolone nucleus, unsatisfactory results were obtained. This can be explained by the fact that the relationship between the RFI of the chelates and the substituent is complex and depending not only on its steric parameters but also on its electronic parameters as was proven by the equations 13-16 (Table 5). Hence, multiple linear regression model was again used to correlate the RFI of the formed chelates with \( \chi' \) of the substituent at position 1 and its electronic parameters F and \( \sigma_m \). Excellent results were obtained (Table 6) showing a good correlation.
Table 5: Multiple linear regression analysis of RFI of the studied chelates versus some electronic parameters of the studied fluoroquinolones*.

\[ RFI = a + b F + c R + d \sigma_m \]

<table>
<thead>
<tr>
<th>Chelated metal</th>
<th>Eq. no.</th>
<th>a</th>
<th>b</th>
<th>C</th>
<th>d</th>
<th>r</th>
<th>F*</th>
<th>SE(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zirconium**</td>
<td>13</td>
<td>52.888</td>
<td>1406.443</td>
<td>373.653</td>
<td>-1402.852</td>
<td>0.9376</td>
<td>4.851</td>
<td>2.696</td>
</tr>
<tr>
<td>Molybdenum**</td>
<td>14</td>
<td>72.562</td>
<td>3174.061</td>
<td>843.942</td>
<td>-3183.130</td>
<td>0.9966</td>
<td>6.587</td>
<td>1.001</td>
</tr>
<tr>
<td>Vanadium**</td>
<td>15</td>
<td>55.027</td>
<td>2416.935</td>
<td>630.059</td>
<td>-2415.641</td>
<td>0.9236</td>
<td>2.967</td>
<td>6.334</td>
</tr>
<tr>
<td>Tungsten**</td>
<td>16</td>
<td>48.589</td>
<td>1794.789</td>
<td>472.546</td>
<td>-1793.411</td>
<td>0.9211</td>
<td>3.732</td>
<td>3.803</td>
</tr>
</tbody>
</table>

*Probability = 95 %.
**Number of compounds included in analysis = 6 compounds.
\(^1\)F= F-ratio between the variances of the observed and calculated values at the given probability.
\(^5\) Overall SE of the correlation.

Table 6: Multiple linear regression analysis of RFI of the studied chelates versus some electronic and steric parameters of the studied fluoroquinolones*.

\[ RFI = a + b \chi_v + c F + d \sigma_m \]

<table>
<thead>
<tr>
<th>Chelated metal</th>
<th>Eq. No.</th>
<th>A</th>
<th>b</th>
<th>C</th>
<th>d</th>
<th>r</th>
<th>F*</th>
<th>SE(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zirconium**</td>
<td>17</td>
<td>46.938</td>
<td>-6.681</td>
<td>30.657</td>
<td>-1.525</td>
<td>0.9426</td>
<td>5.927</td>
<td>2.982</td>
</tr>
<tr>
<td>Molybdenum**</td>
<td>18</td>
<td>59.124</td>
<td>-15.089</td>
<td>67.031</td>
<td>-18.077</td>
<td>0.9986</td>
<td>11.852</td>
<td>1.0216</td>
</tr>
<tr>
<td>Vanadium**</td>
<td>19</td>
<td>44.995</td>
<td>-11.265</td>
<td>97.069</td>
<td>-52.671</td>
<td>0.9203</td>
<td>3.891</td>
<td>7.782</td>
</tr>
<tr>
<td>Tungsten**</td>
<td>20</td>
<td>41.064</td>
<td>-8.449</td>
<td>54.886</td>
<td>-21.206</td>
<td>0.9299</td>
<td>5.328</td>
<td>4.762</td>
</tr>
</tbody>
</table>

*Probability = 95 %.
**Number of compounds included in analysis = 6 compounds.
\(^1\)F= F-ratio between the variances of the observed and calculated values at the given probability.
\(^5\) Overall SE of the correlation.
between the RFI of the studied chelates with both the electronic and steric parameters of the substituent at position 1 of the quinolone nucleus according to the general equation:

\[
\text{RFI} = a + b \cdot x^v + c \cdot F + d \cdot \sigma_m
\] (equations 17-20)

The regression analysis studied were further utilised to predict the unreported electronic parameters \( R, F \) and \( \sigma_m \) of the substituent (-OCH\(_2\)CH(CH\(_3\))-) in ofloxacin. The known values of RFI and \( x^v \) for ofloxacin were substituted in equations (17) and (18) to give the \( F \) and \( \sigma_m \) constants of this group. The deduced \( F \) and \( \sigma_m \) values were then substituted in equation (14) to obtain the \( R \) value (Table 3).

The electronic parameters of (-OCH\(_2\)CH(CH\(_3\))-) group, like those already reported for other groups are of important value in chemical and biochemical correlations.

It is apparent from Tables 5 and 6 that molybdenum chelates of fluoroquinolones give the highest statistical significance for correlation between their RFI and the electronic and steric parameters of their 1-substituent. Therefore, equations 14 and 18 were used to predict the RFI of fluoroquinolone-molybdenum chelates (Table 7).

**Table 7: Prediction of the RFI of fluoroquinolone-molybdenum chelates.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative fluorescence intensities</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated</td>
<td>Observed</td>
</tr>
<tr>
<td>Amifloxacin</td>
<td>53.84</td>
<td>53.79</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>39.81</td>
<td>39.80</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>63.63</td>
<td>63.81</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>52.28</td>
<td>52.27</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>52.28</td>
<td>52.27</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>82.83</td>
<td>81.17</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>52.28</td>
<td>52.27</td>
</tr>
</tbody>
</table>

*Number of equation.
Conclusion

A quantitative relationship was found between the RFI of the studied chelates and certain physicochemical parameters of the studied drugs namely: the pKa$_1$ values (ionisation of the 3-carboxylic group) of the studied drugs, the calculated stability constants of the formed chelates as well as the second order connectivity indices (\(\tilde{\chi}''\)), Hammett constant (\(\sigma_m\)), polar constant (\(\varphi\)) and resonance constant (\(\tilde{\rho}\)) for the substituent at position 1 of the qinolone nucleus. Twenty highly significant regression equations were obtained and used to calculate the values of \(\sigma_m\), F and R for the substituent at position 1 of ofloxacin which were not previously reported. In addition, two of these equations were exploited for the prediction of RFI of fluoroquinolone-molybdenum chelates.

REFERENCES