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QUANTITATIVE FLUORESCENCE INTENSITY-STRUCTURE RELATIONSHIPS OF CERTAIN QUINOLONE-METAL CHELATES

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يتضمن هذا البحث دراسة العلاقة الكميه بين شدة الطيف اللصفى للمتراكبات الناتجة من تفاعل ثمانية مركبات من مشتقات الكينولون المضادة للبكتيريا وهم: الاميفلوكساسين ، السيبروفلوكساسين ، الدايفلوكساسين ، اللوميفلوكساسين ، حمض الناليديكسيك ، النورفلوكساسين ، الاوفلوكساسين مع محاليل أملاح الزركونيوم أو الموليبدينيوم أو الفانيديوم أو التنجستين فى وسط حمضى عند أس هيدروجينى ٣,٥ والتركيب الكيميائى لمشتقات الكينولون محل الدراسة.

وقد تم استنباط عشرين معادلة تصف العلاقة الكميه بين شدة اللصف للمتراكبات الناتجة من التفاعل المذكور والتركيب الكيميائى للمركبات الصيدلية موضوع الدراسة ممثلا فى بعض الثوابت الفراغية والالكترونية الخاصة بهذه المركبات. كما تم استخدام المعادلات المستنبطة لحساب بعض الثوابت الالكترونية لمركب الاوفلوكساسين والتى لم يتم حسابها من قبل.

A quantitative relationship was found between the relative fluorescence intensities of certain metal chelates of some quinolone antibacterials and their physicochemical parameters namely: the pKa_1 (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 ($^2\chi^v$), Hammett constant (σ_m), polar constants ($\mathcal{F}$) and resonance constants ($\mathcal{R}$) 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 σ_m, $\mathcal{F}$ and $\mathcal{R}$ 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.

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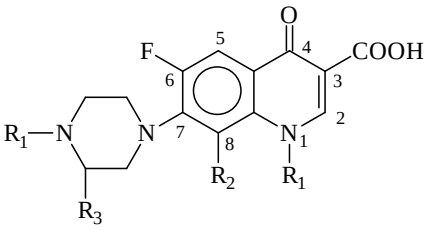
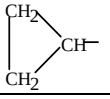
INTRODUCTION

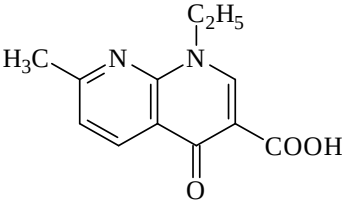
In a previous communication, simple, rapid, reliable, and sensitive spectrofluorometric methods for the determination of eight quinolone antibacterials namely ciprofloxacin, norfloxacin, lomefloxacin, difloxacin, amifloxacin, pefloxacin, ofloxacin, and nalidixic acid (Figure I) based on their chelation with zirconium, molybdenum, vanadium or tungsten to produce intensely fluorescent chelates were developed.[1]

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.



Compound	R_1	R_2	R_3	R_4
1- Amifloxacin	$NHCH_3$	H	H	CH_3
2- Ciprofloxacin		H	H	H
3- Difloxacin	C_6H_4-F	H	H	CH_3
4- Lomefloxacin	C_2H_5	F	CH_3	H
5- Norfloxacin	C_2H_5	H	H	H
6- Ofloxacin	$-CH_2CHCH_3)-$	-O-	H	CH_3
7- Pefloxacin	C_2H_5	H	H	CH_3



Nalidixic acid

There are two general aspects of structure which can be identified and expressed in the form of numerical parameters:[2]

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 pKa_1 of the studied drug (ionization of 3-carboxylic group) as well as Hammett constant (σ_m), polar constant ($\mathcal{F}$) and resonance constant ($\mathcal{R}$) for the substituent at position 1 of the quinolone nucleus. These electronic parameters were obtained from literature[3-5] and included as the electronic contribution variables.

The molecular topography was expressed using the molecular connectivity (χ) adapted by Kier *et al.*[6] 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 $(\delta_i\ \delta_j)^{-0.5}$. The molecular connectivity index is the simple sum of the computed bind values over the entire molecule according to the equation:[2]

$$^1\chi = \Sigma\ (\delta_i\ \delta_j)^{-0.5}$$

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.[2]

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]

$$^2\chi = \Sigma\ (\delta_i\ \delta_j\ \delta_k)^{-0.5}$$

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 (δ^v) and the computed connectivity index is called the valence chi (χ^v).

Another progress in this field was the treatment of heteroatoms introduced by Kier and Hall,[7] 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 δ^v values of different heteroatoms in various hybrid states.[2]

In this work, we tried to correlate the experimentally measured RFI of the studied chelates with the pKa_1 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 ($^2\chi^v$), Hammett constant (σ_m), polar constant ($\mathcal{F}$) and resonance constant ($\mathcal{R}$) for the substituent at position 1 of the quinolone nucleus.

Similar correlations were previously reported for phenothiazines,[8] catecholamines[9] and thiols,[10] where highly significant relationships were obtained between molar absorptivities of certain derivatives and some physicochemical parameters of the parent compound.

COMPUTATION

Instrument

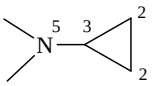
Intel Pentium IV PC (USA) equipped with Microsoft Excel 2000® data analysis tool pack.

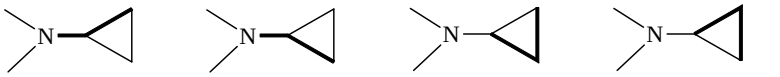
Mathematical and statistical treatment of data

a- The correlation between the experimentally measured RFI of the formed chelates obtained in the previously published work[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).

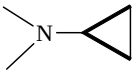
b- The correlation between the experimentally measured RFI of the studied chelates and the studied parameters obtained from literature,[3-5] was carried out using simple or multiple regression analysis models in the Microsoft Excel 2000® data analysis tool pack.

c- The second order connectivity index ($^2\chi^v$) for the substituent at postion 1 of the quinolone nucleus was calculated according to Kier and Hall's rules.[2] An illustrative example for calculating $^2\chi^v$ for the substituent at position 1 in ciprofloxacin is given in scheme 1.





$^{2}\chi^{v} = (5 \times 3 \times 2)^{-0.5} + (5 \times 3 \times 2)^{-0.5} + (3 \times 2 \times 2)^{-0.5} + (2 \times 2 \times 3)^{-0.5} +$



$(2 \times 3 \times 2)^{-0.5} = 1.2312$

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:

RFI = a + b K_s ……(equations 1-4)

RFI= a + b log K_s……(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.[11] 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:

$$RFI = a + b\ pKa_1 + c \log K_s$$

(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*.

Drug	Zirconium		Molybdenum		Vanadium		Tungsten	
	Ks x 10^{-7}	Log Ks	Ks x 10^{-7}	Log Ks	Ks x 10^{-7}	Log Ks	Ks x 10^{-7}	Log Ks
CIP	101.3047	9.0056	105.7894	9.0184	108.4924	9.0353	103.3569	9.0143
NOR	128.0265	9.1073	164.9301	9.2173	185.1399	9.2675	130.8579	9.1168
DIF	131.4519	9.1187	147.9200	9.1700	190.0561	9.3076	133.9248	9.1268
AMI	121.3845	9.0841	146.9355	9.1671	184.8486	9.2668	124.4474	9.0949
LOM	106.0566	9.0255	125.4870	9.0986	135.7062	9.1326	110.5605	9.0436
OFL	140.2168	9.1468	156.1550	9.2749	293.9681	9.4683	142.0365	9.1524
PEF	110.9430	9.0451	127.9484	9.1070	150.3009	9.2049	117.9248	9.0668
NAL	87.9781	8.9443	99.4947	8.9978	102.0469	9.0088	95.8956	8.9817

* Average of 3 determinations.

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

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

Parameter	Chelated metal	Eq. no.	a	b	r	F^+	SE
K_s	Zirconium**	1	-33.710	0.668	0.9314	41.056	4.809
	Molybdenum**	2	-24.136	0.569	0.8111	10.663	10.886
	Vanadium**	3	9.388	0.183	0.8735	19.332	6.723
	Tungsten**	4	-49.792	0.725	0.9477	52.897	4.8922
Log K_s	Zirconium**	5	-1524.230	173.076	0.9319	39.639	4.883
	Molybdenum**	6	-1407.330	159.847	0.9230	26.509	7.794
	Vanadium**	7	-689.072	79.174	0.9352	41.849	4.892
	Tungsten**	8	-1740.471	195.886	0.9483	53.532	4.169

*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.

Table 3: Some electronic parameters of the studied drugs.

Drug	pKa_1*	Hammett constant (σ_m)**	Polar constant F **	Resonance constant R**	$^2\chi^{v\ \$}$
Amifloxacin	6.15	-0.3	-0.11	-0.74	0.224
Ciprofloxacin	6.13	-0.07	-0.03	-0.19	1.231
Difloxacin	5.86	0.4	0.51	-0.42	1.476
Lomefloxacin	5.82	-0.07	-0.05	-0.10	0.316
Norfloxacin	6.26	-0.07	-0.05	-0.10	0.316
Ofloxacin	6.21	$1.27^{\$}$	$0.90^{\$}$	$1.43^{\$}$	1.016
Pefloxacin	6.02	-0.07	-0.05	-0.10	0.316

*References 3 and 4. **Reference 5.

$^{\$}$Calculated values.

The constants σ_m, F, R and $^2\chi^v$ 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 pKa_1 and log K_s *.

$$RFI = a + b\ pKa_1 + c\ \log K_s$$

Chelated metal	Eq. no.	a	b	c	r	F^+	$SE^{\$}$
Zirconium**	9	-1527.221	0.163	173.505	0.9322	16.601	5.337
Molybdenum**	10	-1168.500	2.3855	132.278	0.9373	15.348	7.438
Vanadium**	11	-907.798	4.431	103.294	0.9741	46.369	5.851
Tungsten**	12	-1466.410	1.516	164.779	0.9645	32.976	3.819

*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.

$^{\$}$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 ($\mathcal{F}$) and resonance constant ($\mathcal{R}$) 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 postion 1 of the quinolone nucleus (Table 5) according to the general equation:

$$RFI = a + b\,\mathcal{F} + c\,\mathcal{R} + d\,\sigma_m$$

(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 ($^2\chi^v$) calculated according to Kier and Hall's rules.[2] On using simple linear regression model to correlate the RFI of the studied chelates to $^2\chi^v$ of the substituent the substituent at postion 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 $^2\chi^v$ of the substituent at position 1 and its electronic parameters F and σ_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*.

$$RFI = a + b\,\mathcal{F} + c\,\mathcal{R} + d\,\sigma_m$$

Chelated metal	Eq. no.	a	b	C	d	r	F^+	$SE^\$$
Zirconium**	13	52.888	1406.443	373.653	-1402.852	0.9376	4.851	2.696
Molybdenum**	14	72.562	3174.061	843.942	-3183.130	0.9966	6.587	1.001
Vanadium**	15	55.027	2416.935	630.059	-2415.641	0.9236	2.967	6.334
Tungsten**	16	48.589	1794.789	472.546	-1793.411	0.9211	3.732	3.803

*Probability = 95 %.

**Number of compounds included in analysis = 6 compounds.

$^+$F= F-ratio between the variances of the observed and calculated values at the given probability.

$^\$$ 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*.

$$RFI = a + b\,{}^2\chi^v + c\,\mathcal{F} + d\,\sigma_m$$

Chelated metal	Eq. No.	A	b	C	d	r	F^+	$SE^\$$
Zirconium**	17	46.938	-6.681	30.657	-1.525	0.9426	5.927	2.982
Molybdenum**	18	59.124	-15.089	67.031	-18.077	0.9986	11.852	1.0216
Vanadium**	19	44.995	-11.265	97.069	-52.671	0.9203	3.891	7.782
Tungsten**	20	41.064	-8.449	54.886	-21.206	0.9299	5.328	4.762

*Probability = 95 %.

**Number of compounds included in analysis = 6 compounds.

$^+$F= F-ratio between the variances of the observed and calculated values at the given probability.

$^\$$Overall SE of the correlation.

between the RFI of the studied chelates with both the electronic and steric parameters of the substituent at postion 1 of the quinolone nucleus according to the general equation:

$$RFI = a + b\,{}^{2}\chi^{v} + c\,\mathcal{F} + d\,\sigma_m$$

(equations 17-20)

The regression analysis studied were further utilised to predict the unreported electronic parameters R, F and σ_m of the substituent ($-OCH_2CH(CH_3)-$) in ofloxacin. The known values of RFI and ${}^{2}\chi^{v}$ for ofloxacin were substituted in equations (17) and (18) to give the F and σ_m constants of this group. The deduced F and σ_m values were then substituted in equation (14) to obtain the R value (Table 3).

The electronic parameters of ($-OCH_2CH(CH_3)-$) 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.

Compound	Relative fluorescence intensities				
	Calculated		Observed	Error (%)	
	(14)*	(18)*		(14)*	(18)*
Amifloxacin	53.84	53.79	53.80	0.069	0.012
Ciprofloxacin	39.81	39.80	39.81	0.026	0.010
Difloxacin	63.63	63.81	63.83	0.274	0.012
Lomefloxacin	52.28	52.27	51.55	1.522	1.495
Norfloxacin	52.28	52.27	53.47	2.090	2.117
Ofloxacin	82.83	81.17	81.10	2.134	0.088
Pefloxacin	52.28	52.27	51.92	0.740	0.712

*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 ($^2\chi^v$), Hammett constant (σ_m), polar constant ($\mathcal{F}$) and resonance constant ($\mathcal{R}$) for the substituent at position 1 of the qinolone nucleus. Twenty highly significant regression equations were obtained and used to calculate the values of σ_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.

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