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Determination of lipophilicity by means of reversed-phase thin-layer chromatography
III. Study of the TLC equations for a series of ionizable quinolone derivatives

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Abstract

The R_M values of a series of antibacterial quinolones were measured at pH 9.0 and 1.2 using a reversed-phase TLC system with acetone, methanol or acetonitrile as the organic modifier of the mobile phase and silicone DC 200 as the impregnating agent of the silica gel layer. The data obtained provide a further contribution to the assessment of the basic aspects of the chromatographic determination of lipophilicity for ionizable compounds. The very good correlations between experimental and extrapolated R_M values support the validity of the extrapolation technique. The overlapping of the extrapolated R_M values from three different systems shows that they are not dependent on the nature of the organic solvent. In a series of congeneric compounds there is a relationship between intercepts (a) and slopes (b) of the TLC equations. Factors affecting chromatographic congenerity are discussed. The slopes of the TLC equations and those of the equations correlating the parameters a and b are related to the solvent strength of the organic modifiers.

1. Introduction

During the last 25 years, we have been measuring the R_M values, as an expression of the lipophilic character of drugs and chemicals, by means of a reversed-phase TLC system with the silica gel layer impregnated with silicone DC 200. The chromatographic determination of lipophilicity is mainly based on the linear relationship between the R_M values and the organic solvent concentration in the mobile phase. In fact, the TLC equations describing this relationship allow the calculation of a theoretical R_M value at 0% organic solvent in the mobile phase, even for those compounds which do not migrate with an aqueous buffer alone. The chromatographic work carried out in our laboratory provided the TLC equations for about 750 drugs and chemicals. In two recent papers, the main features of the TLC equations were reviewed [1,2]. In particular, the very good correlations between experimental and extrapolated R_M values support the validity of the extrapolation technique. The overlapping of the extrapolated R_M values

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from different chromatographic systems shows that they are not dependent on the nature of the organic solvent in the mobile phase when the solvent is acetone, methanol or acetonitrile. However, as already pointed out [1], it might be questionable whether this aspect has general relevance for any chromatographic system. Grünbauer et al. [3] reached our conclusion when using acetone or methanol. On the other hand, with N,N-dimethylformamide (DMF) the extrapolated R_M values were significantly lower. They suggested that this behaviour could be due to the fact that DMF deviates the most from water as far as its liquid structure is concerned. Moreover, Smith and Burr [4] found different chromatographic parameters when analysing a series of monosubstituted aromatic compounds with an HPLC system using methanol or acetonitrile in the mobile phase. Finally, our attention was drawn to two other aspects: (a) the relationship between intercepts and slopes of the TLC equations [1] and (b) the influence of different organic modifiers on the slope of the TLC equations [2].

The aim of our previous chromatographic work was the determination of the lipophilic character of non-ionized molecules, so that the R_M values could be compared with the classical octanol–water log P values. As a consequence, the pH in reversed-phase TLC was chosen in such a way that most of the compounds were non-ionized. While the aforementioned features of the TLC equations were mostly referred to non-ionized molecules, it would be interesting to assess if the presence of ionized substituent groups is consistent with the above aspects of the TLC equations. In an attempt to investigate this point, we took advantage of some preliminary results obtained with a series of quinolones. In fact, for some time in our laboratory a research project on quinolones has been in progress. The aim is to study the lipophilic character of this important class of synthetic antibacterial drugs. The amphoteric nature of some of the investigated quinolones allowed us to study the chromatographic behaviour of compounds bearing both an acidic and a basic group. As either of these groups may be ionized depending on the pH of the chromatographic system, the reversed-phase TLC of quinolones was carried out at pH 9.0 and 1.2. At pH 9.0, the carboxyl group was ionized whereas the basic piperazine group was mostly non-ionized. In contrast, at pH 1.2 the basic moiety was fully protonated and the carboxyl group was non-ionized. In this way, by just changing the pH of the TLC system, it was possible to study the influence of both a cationic and an anionic group on the chromatographic behaviour of a single series of compounds.

2. Experimental

2.1. Chemicals

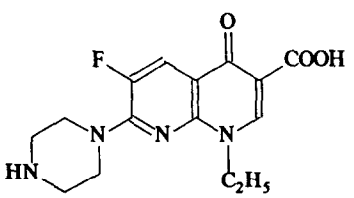
Quinolone derivatives were a generous gift from drug companies (Fig. 1). All drugs were used as received. All solvents were of analytical-reagent or HPLC grade.

2.2. Determination of R_M values by means of RP-TLC

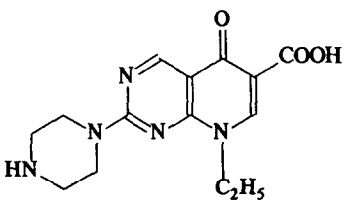
The details of the reversed-phase (RP) TLC were described previously [5]. Glass plates (20 × 20 cm) were coated with silica gel GF_{254} (Merck, Darmstadt, Germany). In order to control the pH of the stationary phase, a slurry of silica gel GF_{254} was obtained with 0.09 M hydrochloric acid or 0.36 M sodium hydroxide when the pH of the mobile phase was to be 1.2 or 9.0 respectively. A non-polar stationary phase was obtained by impregnating the silica gel layer with silicone DC 200 (350 cSt) from Applied Science Labs. (State College, PA, USA). The mobile phases, saturated with silicone, were aqueous buffers alone or mixed with various amounts of acetone, methanol or acetonitrile. Glycine buffers of pH 1.2 and 9.0 were used. The test compounds were dissolved in water or acetone (1–2 mg/ml) and 1 μl of solution was spotted randomly on the plates. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. After a few minutes at 120°C, yellow spots appeared on an intense pink background. The R_M values were calculated by means of the equation $R_M = \log[(1/R_F) - 1]$.

3. Results

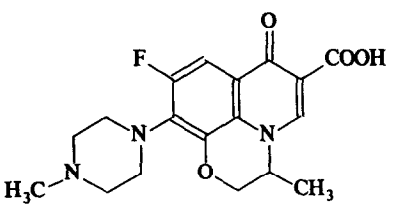
The RP-TLC of the quinolone derivatives showed that at pH 9.0 most of them did not move from the starting line when the mobile phase was aqueous buffer alone. Only with the four most hydrophilic compounds, i.e., **4**, **7**, **10** and **12**, could reliable R_M values be obtained, even with no organic modifier in the mobile phase. On the other hand, at pH 1.2 all the



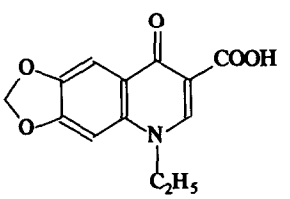
Enoxacin



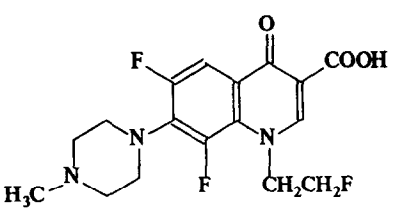
Pipemidic acid



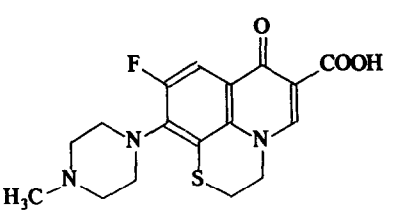
Ofloxacin



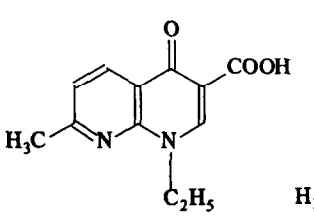
Oxolinic acid



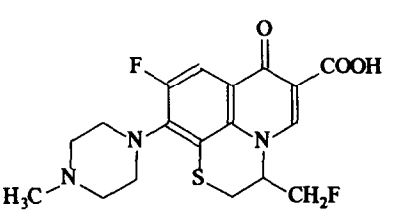
Fleroxacin



Rufloxacin



Nalidixic acid



MF 961

Fig. 1. *(Continued on p. 130)*

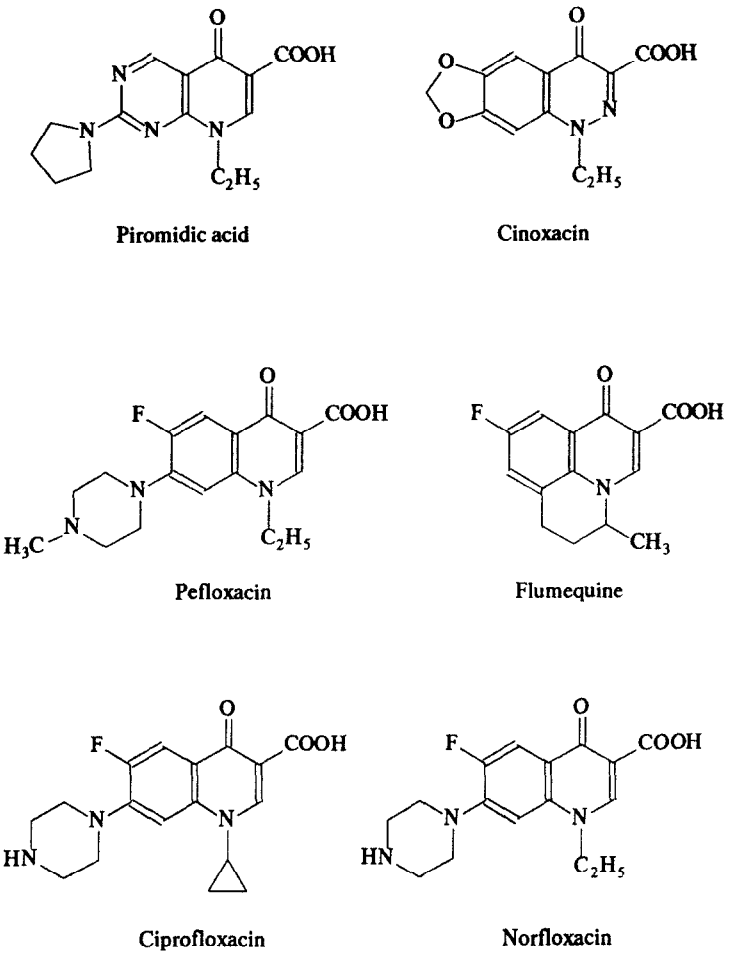


Fig. 1. Structural formulae of the compounds investigated.

derivatives except piromidic acid (**9**) moved with aqueous buffer alone. In order to obtain suitable R_M values for all the compounds at both pH values, an organic solvent was added to the mobile phase. The equations describing the linear relationship between R_M values and organic modifier concentration allowed the calculation of extrapolated R_M values also for the compounds that did not move with aqueous buffer alone. Experimental R_M values, TLC equations and ranges of organic solvent concentrations used for their calculation are reported in Tables 1 and 2.

As a first step in the analysis of the data in Tables 1 and 2, the relationship between experimental and extrapolated R_M values in each RP-TLC system was examined. In Table 3, Eqs. 1–4, with intercepts and slopes close to 0 and 1, respectively, show the overlapping of the experimental and extrapolated R_M values. As at pH 9.0 only four compounds yielded experimental R_M values with no organic solvent in the mobile phase, Eqs. 1–3 were calculated with only four data points. Nevertheless, the present results are in agreement with our recent equation correlating experimental and extrapolated R_M values for 240 compounds [1].

A second basic aspect of the TLC equations at pH 9.0 is illustrated by the intercepts and slopes of Eqs. 5–7 in Table 4. Very close extrapolated

Table 1
TLC equations of quinolones at pH 9.0 in acetone, methanol and acetonitrile systems

No.	Compound	$R_{M\ exptl}$	$R_M = a + b$(% organic modifier)											
			Acetone				Methanol				Acetonitrile			
			a	*b*	*r*	Range	*a*	*b*	*r*	Range	*a*	*b*	*r*	Range
1	Enoxacin		1.80	−0.035	0.983	16–50	1.79	−0.023	0.997	24–60	1.80	−0.030	0.990	12–55
2	Pipemidic acid		1.64	−0.031	0.975	12–45	1.68	−0.022	0.987	16–60	1.56	−0.027	0.994	20–40
3	Ofloxacin		1.92	−0.039	0.981	20–45	1.90	−0.025	0.998	24–50	1.80	−0.032	0.979	16–45
4	Oxolinic acid	1.04	1.03	−0.040	0.963	0–12	0.98	−0.027	0.992	0–40	0.95	−0.036	0.978	0–28
5	Flcroxacin		1.97	−0.041	0.941	16–50	1.93	−0.028	0.999	24–55	2.02	−0.035	0.988	16–36
6	Rufloxacin		1.98	−0.042	0.994	20–50	1.93	−0.029	0.993	20–50	1.92	−0.036	0.972	20–36
7	Nalidixic acid	1.05	1.08	−0.050	0.985	0–24	1.06	−0.029	0.992	0–40	1.01	−0.045	0.987	0–20
8	MF961		1.95	−0.045	0.985	12–50	1.97	−0.032	0.993	20–50	1.81	−0.037	0.946	20–45
9	Piromidic acid		1.21	−0.050	0.980	4–36	1.22	−0.032	0.997	4–50	1.24	−0.043	0.960	0–40
10	Cinoxacin	0.50	0.49	−0.094	0.992	0–12	0.48	−0.090	0.933	0–8	0.42	−0.077	0.998	0–16
11	Pefloxacin		1.61	−0.031	0.989	16–45	1.67	−0.020	0.992	16–50	1.63	−0.027	0.993	20–55
12	Flumequine	1.16	1.17	−0.060	0.995	0–24	1.19	−0.045	0.992	0–40	1.20	−0.057	0.986	0–20
13	Ciprofloxacin		1.49	−0.025	0.993	4–45	1.41	−0.017	0.984	12–60	1.50	−0.024	0.983	12–36
14	Norfloxacin		1.58	−0.027	0.961	16–40	1.53	−0.018	0.992	20–60	1.69	−0.026	0.991	20–45

Table 2
TLC equations of quinolones at pH 1.2 in acetone system and ΔR_M values

No.	Compound	$R_{M\ exptl}$	$R_M = a + b$(% organic modifier)				
			a	b	r	Range	ΔR_M[a]
1	Enoxacin	0.70	0.68	−0.063	0.999	0–12	1.12
2	Pipemidic acid	0.46	0.47	−0.053	0.999	0–12	1.17
3	Ofloxacin	1.02	0.99	−0.081	0.997	0–12	0.93
4	Oxolinic acid	1.25	1.18	−0.056	0.991	0–28	−0.15
5	Fleroxacin	0.88	0.88	−0.082	0.999	0–12	1.09
6	Rufloxacin	0.99	0.87	−0.075	0.944	0–12	1.11
7	Nalidixic acid	1.30	1.31	−0.059	0.998	0–28	−0.23
8	MF961	1.08	1.07	−0.084	0.999	0–12	0.88
9	Piromidic acid		1.84	−0.061	0.970	20–40	−0.63
10	Cinoxacin	1.17	1.20	−0.093	0.998	0–12	−0.71
11	Pefloxacin	1.00	0.95	−0.076	0.991	0–12	0.66
12	Flumequine	1.30	1.22	−0.047	0.992	0–40	−0.05
13	Ciprofloxacin	0.83	0.81	−0.076	0.999	0–12	0.68
14	Norfloxacin	0.83	0.81	−0.068	0.998	0–12	0.77

[a] Difference between the extrapolated R_M values at pH 9.0 and 1.2 in acetone system.

Table 3
Correlations between experimental and extrapolated R_M values at pH 9.0 and 1.2

Mobile phase		$R_{M\ exptl} = a + bR_{M\ extrap}$					
pH	Solvent	a	b	n	r	s	Eq.
9.0	Acetone	0.028	0.964	4	0.998	0.019	1
9.0	Methanol	0.058	0.948	4	0.993	0.044	2
9.0	Acetonitrile	0.151	0.979	4	0.989	0.053	3
1.2	Acetone	0.014	1.015	13	0.986	0.043	4

R_M values were obtained whether the organic modifier in the mobile phase was acetone, methanol or acetonitrile. In other words, the nature of the organic modifier does not affect the extrapolated R_M values.

Another interesting point arises from the analysis of the correlation between the intercepts ($a = R_{M\ extrap}$) and slopes (b) of the TLC equations in Tables 1 and 2. As already discussed in a previous paper [1], for series of congeneric compounds the relationship between the two parameters can be described by a straight line. In

Table 4
Correlations between extrapolated R_M values obtained with different organic modifiers at pH 9.0

Organic modifier		$R_{M\ I} = a + bR_{M\ II}$					
I	II	a	b	n	r	s	Eq.
Methanol	Acetone	−0.007	0.996	14	0.996	0.040	5
Acetonitrile	Acetone	−0.022	0.997	14	0.987	0.074	6
Acetonitrile	Methanol	0.000	0.991	14	0.981	0.090	7

Fig. 2 the intercepts of the TLC equations for the present series of quinolones are plotted against the corresponding slopes. At pH 9.0 (plots a, b and c) in all three chromatographic systems, the intercepts and slopes of nine compounds are linearly related. These derivatives are characterized by the presence of the piperazine ring. On the other hand, four derivatives, **4**, **7**, **9** and **12**, lacking the piperazine ring, are grouped below that line. Compound **10**, lacking the piperazine ring and bearing a cinnoline ring, lies even further away. At pH 1.2 (plot d, Fig. 2), the compounds are apparently divided into two groups. In fact, only compounds **4**, **7**, **9** and **12** deviate from the linear relationship, and **10** seems to be grouped with the other nine compounds. In Table 5 the equations describing these linear relationships between intercepts and slopes are reported. Compound **10** was included in the calculation of Eq. 11.

In previous reports it was shown that the slopes of the TLC equations in a given solvent system are related to the eluting power of the organic modifier, as expressed by its solvent strength parameter E_0 [2,6]. In particular, the ratios between the mean slopes in two different solvent systems are close to the ratios between the $1/E_0$ values for the corresponding solvents. The solvent strength parameter of an organic solvent in a reversed-phase chromatographic system is expressed by $1/E_0$ [7,8]. The slopes of the TLC equations of quinolones at pH 9.0 in acetone, methanol and acetonitrile systems (Table 1) were averaged and are reported in Table 6, where they can be compared with the mean slopes calculated for other series of chemical agents [2]. It can be pointed out that for all the listed chemical series the ratios between the mean slopes in different solvent systems are not far from the ratios between the corresponding $1/E_0$ values. When considering the mean ratios (x in Table 6), those referred to acetone–acetonitrile and acetonitrile–methanol, i.e., 1.09 and 1.41, are close to the ratios between the corresponding $1/E_0$ values, i.e., 1.15 and 1.47, respectively.

More recently it was shown that the same aspect could be illustrated by the b values of the equations correlating intercepts and slopes of the TLC equations [2]. For the present series of quinolones the b values of the equations in Table 5 are reported in Table 7, and their ratios are compared with those of the E_0 values for the corresponding solvents. Again, the present findings are similar to those obtained with other series of compounds [2] and reported in Table 7. The mean acetone-to-acetonitrile ratio (x in Table 7), i.e., 0.89, is particularly close to the corresponding ratio between the E_0 values, 0.86. In a previous paper [2] it was shown why in this case the E_0 values were used instead of their reciprocals ($1/E_0$ values) as in Table 6.

4. Discussion and conclusions

The present data show that the basic factors determining the chromatographic behaviour described by the TLC equations are the same when dealing with either non-ionized or ionized molecules. In fact, the four main points characterizing the TLC equations and outlined in the Introduction seem to be confirmed also for the ionized compounds. In particular, the results in Table 4 further support the finding that at least in our chromatographic system the presence of acetone, methanol or acetonitrile in the mobile phase does not change the extrapolated R_M values.

Notwithstanding, the linear relationship between slopes and intercepts of the TLC equations deserves more detailed comment. In our previous study [1], it was observed that in several instances not all the members of a chemical series fit the same straight line. Moreover, with cephalosporins, xanthines and adenosines the chromatographic data did not reveal any relationship between intercepts and slopes. Therefore, it was assumed that the linear relationship must be based on some kind of congenerity among the members of the chemical series under investigation [1]. We proposed that congenerity might be related to the shape of the hydrophobic surface area, which is available for the interaction with the non-polar stationary phase. The deviations from linearity were attributed to sev-

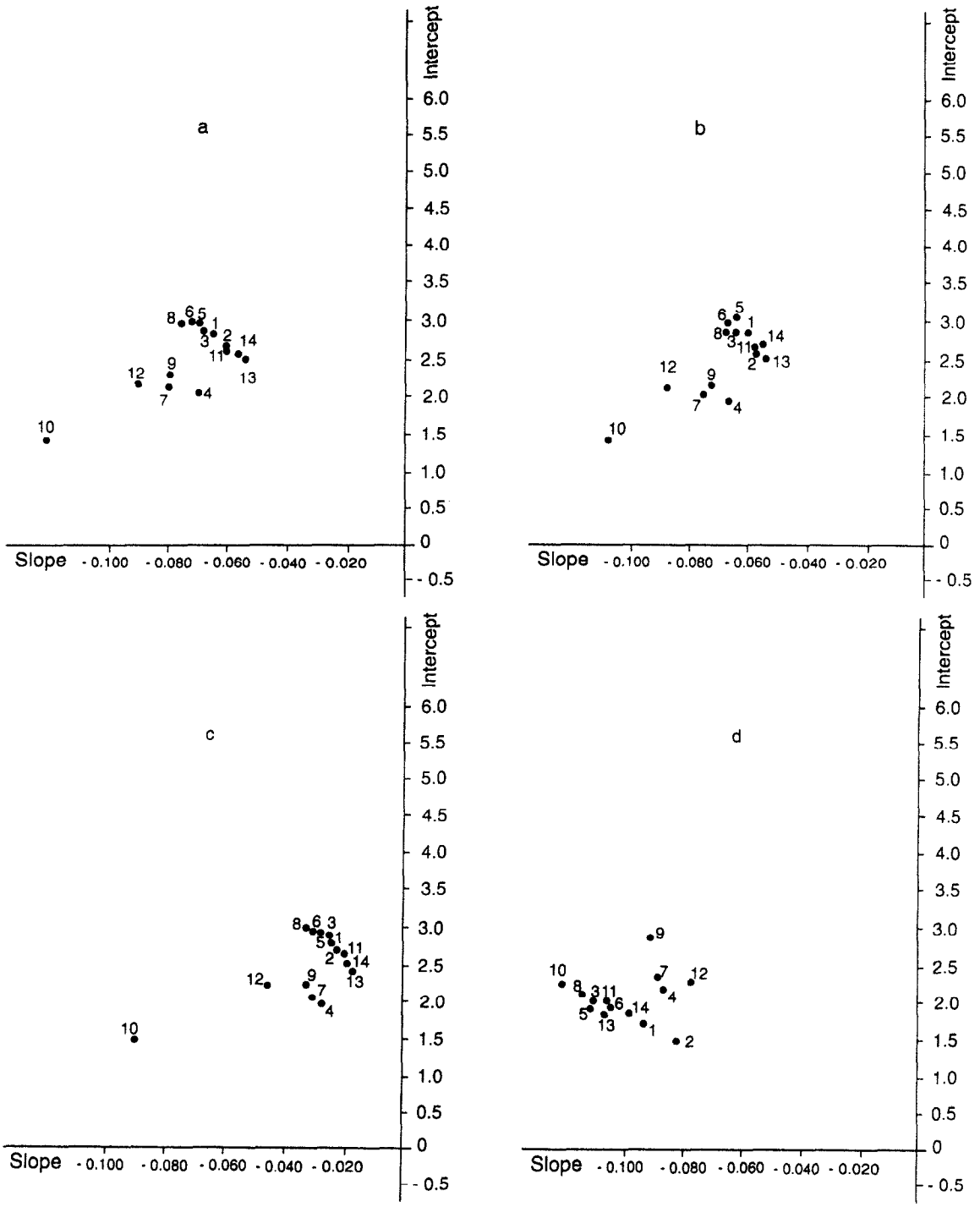


Fig. 2. Relationships between intercepts and slopes of the TLC equations at pH 9.0 (a, b and c) and 1.2 (d) in different solvent systems: (a, d) acetone, (b) acetonitrile and (c) methanol.

Table 5
Correlations between intercepts and slopes of the TLC equations at pH 9.0 and 1.2

Mobile phase		Intercept = $a + b$ (slope)					
pH	Solvent	a	b	n	r	s	Eq.
9.0	Acetone	0.838	−26.579	9	0.972	0.048	8
9.0	Methanol	0.895	−36.224	9	0.943	0.070	9
9.0	Acetonitrile	0.817	−30.561	9	0.866	0.090	10
1.2	Acetone	−0.415	−17.150	10	0.960	0.060	11

Table 6
Ratios between slopes in different TLC systems

Compound	Mean slope in solvent system			Ratio		
	Acetone	Acetonitrile	Methanol	Acetone/ acetonitrile	Acetone/ methanol	Acetonitrile/ methanol
Quinolones	−0.043(±0.005)	−0.038(±0.005)	−0.031(±0.004)	1.13	1.39	1.22
Steroids	−0.046(±0.002)	−0.041(±0.001)	−0.027(±0.001)	1.12	1.70	1.52
Triazines	−0.037(±0.001)	−0.036(±0.001)	−0.027(±0.001)	1.03	1.37	1.33
Prostaglandins	−0.072(±0.002)	−0.067(±0.001)	−0.043(±0.002)	1.07	1.67	1.56
Dermorphins	−0.064(±0.003)		−0.047(±0.003)		1.36	
Naphthalenes and quinolines	−0.046(±0.001)		−0.030(±0.001)		1.53	
$\bar{x} \pm$ S.E.[a]				1.09 ± 0.02	1.50 ± 0.06	1.41 ± 0.08
Solvent strength ($1/E_0$)	1.78	1.54	1.05	1.15	1.70	1.47

[a] Standard error of the mean.

Table 7
Ratios between the b values of the equations correlating intercepts and slopes of the TLC equations

Compound	Slope in solvent system			Ratio		
	Acetone	Acetonitrile	Methanol	Acetone/ acetonitrile	Acetone/ methanol	Acetonitrile/ methanol
Quinolones	−26.579	−30.561	−36.224	0.87	0.73	0.84
Steroids	−72.872	−80.365	−122.802	0.91	0.59	0.65
Triazines	−69.484	−74.194	−109.730	0.94	0.63	0.68
Prostaglandins	−61.014	−73.829	−86.005	0.83	0.71	0.86
Dermorphins	−56.775		−69.317		0.82	
Naphthalenes and quinolines	−62.704		−87.810		0.71	
$\bar{x} \pm$ S.E.[a]				0.89 ± 0.02	0.70 ± 0.03	0.76 ± 0.05
Solvent strength (E_0)	0.56	0.65	0.95	0.86	0.59	0.68

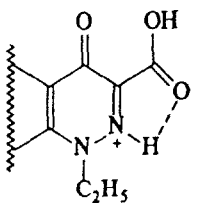
[a] Standard error of the mean.

eral structural features of the chemical agents. With benzodiazepines, penicillins and β-carbolines, the compounds deviating from the linear relationship were the only ionized members of their series, the other compounds being non-ionized at the pH of the chromatographic system. Therefore, it was suggested that at least in these series, congenerity could be broken down by the presence of ionized groups [1].

As regards the present series of quinolone drugs, Eqs. 8–11 in Table 5 show that ionized compounds can be members of a congeneric series. In this case deviations from linearity seem rather to be due to different patterns of ionization. In fact, at pH 9.0 the compounds fitting Eqs. 8–10 are characterized by the fact that their carboxyl group is fully ionized and their basic group is also at least partly ionized. On the other hand, the compounds deviating from Eqs. 8–10, i.e., **4**, **7**, **9**, **10** and **12** (plots a, b and c, Fig. 2) are lacking the partially ionized basic group. The larger deviation for **10** might be related to the presence of the N atom in position 2 of the cinnoline ring.

The ionization patterns of the quinolone drugs could also help in explaining the ranking of the R_M values at pH 9.0 in Table 1 and Fig. 2 (plots a, b and c). The higher R_M values of the compounds fitting Eqs. 8–10 compared with those of the anionic compounds **4**, **7**, **9** and **12** might result from the association of molecules in their zwitterionic form, leading to more lipophilic ion pairs. The lower R_M value of **10** could be due either to the hydrophilic character of the cinnoline N-2 atom or to the fact that it cannot form ion pairs.

At pH 1.2, whereas the carboxyl group is non-ionized, the piperazine group is ionized. Again, Eq. 11 shows a linear relationship for the compounds bearing the ionized basic group. Compounds **4**, **7**, **9** and **12** deviating from the straight line are the only non-ionized compounds in the series, and therefore the most lipophilic (plot d, Fig. 2). At this pH, **10** seems to be congeneric with the ionized subset, which should imply protonation of the cinnoline ring. However, if this were the case, the R_M value of **10** at pH 1.2 should be lower than that of **4** differing only in the lack of the N atom in position 2. This contradictory finding might be tentatively explained by assuming the formation of an intramolecular H-bond in the protonated form of **10**:



As intramolecular H-bonding is known to increase lipophilicity [9], this could to some extent counterbalance the negative contribution of the $\diagdown N^{+} =$ group.

The so far described ionization patterns also can explain the ΔR_M values between 9.0 and 1.2 in the acetone system. The quinolone derivatives with both a carboxyl and a piperazine group, i.e., **1**, **2**, **3**, **5**, **6**, **8**, **11**, **13** and **14** fitting Eqs. 8–10, have higher R_M values at pH 9.0 than at pH 1.2 (positive ΔR_M values), possibly because of the formation of ion pairs between zwitterionic forms. In contrast, **4**, **7**, **9** and **12**, bearing only the carboxyl group, are non-ionized at pH 1.2, hence their R_M values are lower at pH 9.0 than at pH 1.2 (negative ΔR_M values). The case of **10** is more complicated. This derivative, like **4**, **7**, **9** and **12**, has a negative ΔR_M value, which could be explained with the above hypothesis of an intramolecular H-bond increasing lipophilicity at pH 1.2.

However, turning back to the point at issue here, i.e., the relationship between intercepts and slopes of the TLC equations, the conclusion one can draw from the above discussion is that the definition of congenerity in chromatographic terms is still far from being clearly established. In fact, many unpredictable factors can affect chromatographic congenerity, and thereby one cannot state a priori that for a given series of structural analogues it is possible to find a linear correlation between the two parameters. This casts further doubts on the reliability of the slope of the TLC equation as a chromatographic lipophilicity parameter alternative to R_M. In any

event, the findings arising from this paper and the two earlier parts [1,2] make a contribution to a more detailed knowledge of the interrelationships between slopes and intercepts, with a view to the final assessment of the best suited alternative to the octanol–water log P values.

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