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# Simultaneous determination of the ionisation and dimerization constants and the partition and ion pair extraction coefficients for 1-8 naphtyridine derivatives

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**Abstract**

Conventional experimental studies of water/octanol partition at different pHs and concentrations were used to simultaneously evaluate (i) the true $P_{octanol/water}$ partition coefficient, (ii) acidity constants and (iii) extraction constants for ion pairs produced by the ions in the buffers used (phosphate ions). The studies also rejected the hypothesis that dimers are formed in the octanol phase. The method, applied to a few substituted 1,8-naphtyridine substances, used a *new software* whose algorithm is based on the principle of error minimisation using a simplex method. The fundamental equations used to calculate the concentration of the test species in each experimental situation (pH, concentration, phase volume ratios, counter-ions) are explained.

*Keywords:* Octanol/water partition coefficient; Ionisation constant; 1,8-Naphtyridine; Nalidixic acid; Quinolone; Zwitterion; Overlapping constant calculation

## 1. Introduction

The determination of the partition coefficients for a compound that possesses several ionisable groups necessarily requires knowledge of their acidity constants in order to calculate the true

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partition coefficient of the non-ionised form between the two phases.

The importance of these partition coefficients in evaluating the antibacterial effect of similar derivatives, such as the fluoroquinolones, has now been well established (Staroscik and Sulkowska, 1971; Bouzard et al., 1990, 1992; Ross and Riley, 1992; Takacs-Novak et al., 1992; Martorell et al., 1993).

However, determining the acidity constants of the naphtyridines is no simple matter as some derivatives form zwitterions that are globally neutral but possess two ion groups of opposing sign (Riley et al., 1989). Here, the $pK_a$ of the carboxylic acid group [loss of a proton] is lower than that of the base groups present in these compounds [gain of a proton] (Albert and Serjeant, 1971).

To reduce the considerable number of experimental measurements required to determine acidity constants and partition coefficients, we propose a method based on the evaluation of reduced experimental data obtained from the classical extraction method between two phases (shake flask method) at several pH values and initial concentrations. An algorithm that respects thermodynamic laws and the laws governing chemicals in solution is used to optimise the adaptation of the theoretical curve to the experimental points. To do this it varies, in a logical manner, the values of the physicochemical constants to be determined until values are obtained that minimise the differences between the experimental curve and the calculated curve. The statistical method used is based on the theory of maximum likelihood and the modified simplex optimisation method (Abi Khalil et al., 1986; Dubois et al., 1989).

The experimental data consist of weighing of the substance dissolved in buffered aqueous solution, the spectrophotometric determination (with or without chromatographic separation) of the concentration after partitions and the pH of these solutions.

The method proposed gives a reliable determination of the water-octanol partition coefficient, the acidity constants (taking account of the water levelling effect), the ion pair extraction constants and the dissociation constants for any dimers.

## 2. Materials and methods

### 2.1. Reagents

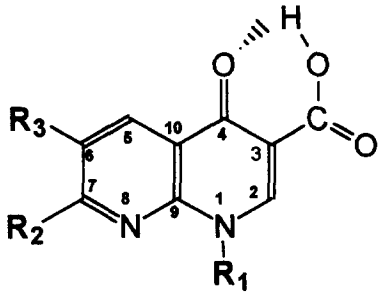
Substituted naphtyridines were analysed; their formulae are given below (Scheme 1, Scheme 2):

The compounds were synthesised and purified by Bristol-Myers Squibb. The salts used to prepare the buffer solutions were all of analytical quality ($NaH_2PO_4 \cdot H_2O$; anhydrous $Na_2HPO_4$, $H_3C_6H_5O_7 \cdot 2H_2O$, KCl, NaOH, HCl).

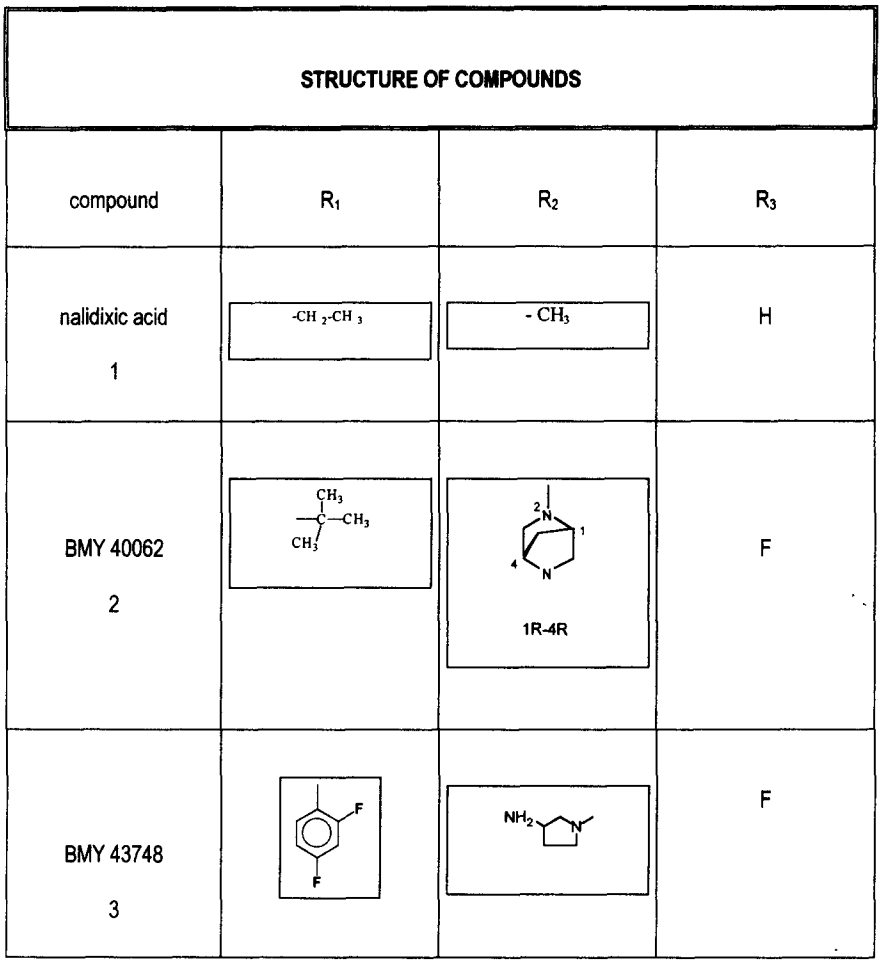
Distilled water and octanol purified by distillation. The pH 2–11 buffer solutions were prepared by adding phosphate and/or citrate ions, hydrochloric acid, 0.01 N sodium hydroxide to the sodium salts and potassium chloride to adjust the ion force to 0.15 M. The amount of potassium chloride added to the buffer solutions to obtain exactly the ion force desired was calculated using equations constructed from the true concentrations of the various salts in solution and from their thermodynamic acidity constants according to the Debye–Hückel theory (van Damme et al., 1979).

### 2.2. Experimental measurements

Three concentrations $C_i$ between 10 and 20 $\mu$g/ml were prepared by dilution of a stock solution (1.0 mg/ml) in buffer solution saturated with octanol. The buffer solution was prepared by shaking a mixture of the buffer and octanol at 20°C for 1 h before centrifuging at 4000 rev./min for 10 min (the supernatant was used as saturated organic phase). Ten solutions of each concentra-




Scheme 1. Structure of substituted naphtyridines. See Scheme 2 for composition of $R_1$, $R_2$ and $R_3$.



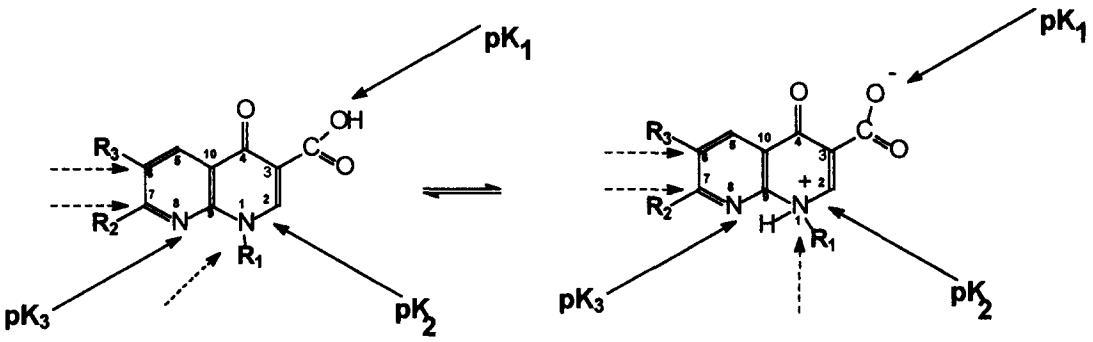

Scheme 2. Structure of substituted naphtyridines. See Scheme 1 for overall structure.

tion (but at different pHs) were prepared and their acidity determined precisely by potentiometric measurement after partition.

The concentrations in the buffer solutions at steady state $C_{Ta}$ were measured either by: (a) ultraviolet spectrophotometry using standard curves optimised by linear regression and constructed using about 20 different concentrations between 5 and 25 $\mu$g/ml in a saturated buffer solution. The measurement was made at a wavelength of 344 nm, corresponding to the isosbestic point of compound (2) [this procedure avoids sudden changes due to small modifications in the pH of the test solution], or by (b) high performance liquid chromatography.

*2.2.1. Chromatographic conditions*

**Compound 1:** 100 $\mu$l of the solution were injected into the chromatograph made up of a column 250 mm long, internal diameter 4 mm, packed with silica gel C8 of granulometry 7 $\mu$m, and a mobile phase made up of 40% v/v phosphate buffer ($NaH_2PO_4$ 0.05 M — HCl 5.82 $\times$ $10^{-3}$ M) and 60% v/v uvasol acetonitrile, at a constant flow rate of 1.5 ml/min. The detector was set at 254 nm.




Scheme 3. Partition coefficients of the two neutral species.

**Compound 3:** 200 μl of the solution were injected into the chromatograph made up of a column 250 mm long, internal diameter 4 mm, packed with Lichrosorb grafted silica RP18 of granulometry 10 μm, and a mobile phase made up of 72% v/v phosphate buffer (2 PIC A in 1000 ml of water adjusted to pH 3 with $H_3PO_4$) and 28% v/v uvasol acetonitrile, at a constant flow rate of 1.5 ml/min. The detector was set at 273 nm. Tests were also performed with nalidixic acid (compound 1) in the presence of citrate buffer in order to check the validity of the algorithm in the presence or the absence of ion pairs, as it is well known that citrate ions — as opposed to phosphate ions — do not tend to form ion pairs in the octanol phase.

### 2.3. Theoretical aspects

In very dilute solution, the partition coefficient corresponds to the ratio of the molar concentrations of the two neutral species (Scheme 3) present in the two liquid phases once the steady state has been reached:

$$P = \frac{\{BH\}_o}{\{BH\}_a} = \frac{[BH]_a f_o}{[BH]_a f_a} \approx \frac{[BH]_o}{[BH]_a} \quad (1)$$

where $P$ is the thermodynamic partition coefficient between octanol and water; $BH$, the non-ionised form of the naphtyridines; $\{X\}$, the activity of the $X$ species; $f$, the activity coefficient; $[X]$, the true concentration of species $X$ at the steady state; $o$ symbolises the octanol phase in which the species is dissolved and $a$, the buffered aqueous phase in which the species is dissolved.

The 1,8-naphtyridines may undergo different, successive dissociations as some of the derivatives possess five acid-base groups, e.g. BMY 40062 dissociates according to the six equations given below:

$$BH + H_2O \rightleftharpoons B^- + H_3O^+$$

$$K_1 = \frac{[B^-][H_3O^+]}{[BH]} \quad (2)$$

$$BH_2^+ + H_2O \rightleftharpoons BH + H_3O^+$$

$$K_2 = \frac{[BH][H_3O^+]}{[BH_2^+]} \quad (3)$$

$$BH_3^{2+} + H_2O \rightleftharpoons BH_2^+ + H_3O^+$$

$$K_3 = \frac{[BH_2^+][H_3O^+]}{[BH_3^{2+}]} \quad (4)$$

$$BH_4^{3+} + H_2O \rightleftharpoons BH_3^{2+} + H_3O^+$$

$$K_4 = \frac{[BH_3^{2+}][H_3O^+]}{[BH_4^{3+}]} \quad (5)$$

$$BH_5^{4+} + H_2O \rightleftharpoons BH_4^{3+} + H_3O^+$$

$$K_5 = \frac{[BH_4^{3+}][H_3O^+]}{[BH_5^{4+}]} \quad (6)$$

The true concentration of the non-dissociated base may be calculated in the aqueous phase using the following expression:

$$[BH]_a = \frac{C_{Ta}}{\frac{K_1}{[H^+]} + 1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_2K_3} + \frac{[H^+]^3}{K_2K_3K_4} + \frac{[H^+]^4}{K_2K_3K_4K_5}} \quad (7)$$

where: $C_{Ta}$ = the molar concentration in the aqueous phase of all the species in solution after partition; $[i]$ = the molar concentration of species $I$; pH = $-\log\{H^+\}/f\{H^+\}$ and $[H^+] = \{H^+\}/f\{H^+\} = 10^{-pH}/f\{H^+\}$

If we take account of the formation of dimers $(BH)_2$ and ion pairs not dissociated in the organic phase i.e. formed from an ionised species of the compound and monohydrogen phosphate $HPO_4^=$ or dihydrogen phosphate $H_2PO_4^-$ ions, and if we eliminate the ion pairs made up of more than three molecules, the total concentration in the organic phase may be calculated using the expression below:

$$C_{To} = [BH]_o + 2\cdot[(BH)_2]_o + [BH_2\cdot H_2PO_4] + 2\cdot[(BH_2)_2\cdot HPO_4]_o + [BH_3\cdot HPO_4]_o + [BH_3\cdot(H_2PO_4)_2]_o \quad (8)$$

where the ion pairs correspond to $BH_2\cdot H_2PO_4$, $(BH_2)_2\cdot HPO_4$, $BH_3\cdot HPO_4$ and $BH_3\cdot(H_2PO_4)_2$. The dissociation of a dimer follows the classical equilibrium law:

$$K_d = \frac{[BH]_o^2}{2[(BH)_2]_o} \quad (9)$$

The factor 2 in this relation corresponds to the transformation of the formality of the species into the corresponding concentration.

The phosphate ion concentration may be deduced with ease from the total initial concentration of the salt used $C_p$ and from the pH of the resulting buffer solution (van Damme et al., 1979)

$$[H_2PO_4^-] = \frac{C_p 10^{-2pH} K_{p_1} 10^I}{10^{-3pH} + 10^{-2pH}K_{p_1} + 10^{-pH}K_{p_1}K_{p_2} + K_{p_1}K_{p_2}K_{p_3}} \qquad \mu = \frac{1}{2}\sum c_i z_i^2 \qquad I = \frac{0.5115\sqrt{\mu}}{1+\sqrt{\mu}} \quad (10)$$

Adjusting the amount of potassium chloride to be added to the solution in such a way as to maintain ion force $\mu$ constant, avoids the necessity to perform complex iterative calculations (van Damme et al., 1979)]. The acidity constants for the phosphate ions are represented by $K_{p1}$, $K_{p2}$ and $K_{p3}$. The extraction constants $E_{m(n-1)}$ for the four possible ion pairs $PI_{(n-1)m}$ [taking account of the probability of species coexistence depending on pH and on the number of compounds involved in the formation of a neutral ion pair likely to be extracted by the organic solvent] respect classical relationships[1].

The different species possible are expressed as a function of these constants:

$$[P\,I_{11}]_o = E_{11}\cdot\frac{[BH]_a\cdot[H_3O^+]}{K_2}\cdot[H_2\,PO_4^-] \quad (11)$$

$$[P\,I_{22}]_o = E_{22}\cdot\frac{[BH]_a\cdot[H_3O^+]}{K_2\cdot K_3}\cdot[H_2PO_4^-]\cdot K_{p_2}\cdot 10^{4I} \quad (12)$$

$$[P\,I_{12}]_o = E_{12}\cdot\frac{[BH]_a^2\cdot[H_3O^+]^2}{K_2^2}\cdot[H_2PO_4^-]\cdot K_{p_2}\cdot 10^{4I} \quad (13)$$

$$[P\,I_{21}]_o = E_{21}\cdot\frac{[BH]_a\cdot[H_3O^+]^2}{K_2\cdot K_3}\cdot[H_2PO_4^-]^2 \quad (14)$$

The monomer and dimer concentrations are obtained with:

$$[BH]_o = P\cdot[BH]_a \quad [(BH)_2]_o = \frac{2[BH]_o^2}{K_d} \quad (15)$$

The relationship between these three experimental variables $C_{Ta}$, $C_{Ti}$ and pH is drawn from the equations above and can be used to optimise the $K_i$, $P$, $E_{m(n-1)}$ parameters via the simplex method (Abi Khalil et al., 1986; Dubois et al., 1989) by comparing the experimental values for aqueous concentration after partition (taking into account the initial concentration and the pH of the aqueous solution once the partition steady state has been reached) and the estimations obtained by varying the different constants over a range compatible with chemical reality (using ex-

[1] $m$ is the charge of the phosphate ion — $p$ identifies the acidity constants — $K$ indicates that this concerns one of the three dissociation constants of phosphoric acid — and $n$ is the number of ionisable protons in naphtyridine.

pressions 7 and 16 in which the terms corresponding to the constants unlikely to modify the value of the equilibria involved, are eliminated). The most probable values for the different constants are obtained by minimising total residual error:

$$C_{Ti} = C_{Ta} + \frac{v_o}{v_a}\cdot([BH]_a \times \{P + E_{11}\frac{[H_3O^+][H_2PO_4^-]}{K_2} + E_{22}\frac{[H_3O^+][H_2PO_4^-]\cdot K_{p_2}\cdot 10^{4I}}{K_2K_3} + E_{21}\frac{[H_3O^+]^2[H_2PO_4^-]^2}{K_2K_3}\} + 2[BH]^2 \times \left(\frac{P^2}{K_d} + E_{12}\frac{H_3O^+][H_2PO_4^-]\cdot K_{p_2}\cdot 10^{4I}}{K_2^2}\right)) \quad (16)$$

where, $C_{Ti}$ is the molar concentration in the aqueous phase of all the species in solution after partition.

$C_o$ being the molar concentration in the organic phase after partition, it can be replaced by $n_o/V_o$ or $(C_{Ti} - C_{Ta})\ V_a/V_o$. Actually, $n_i$ is the number of moles corresponding to $C_{ti}$; $n_a$ is the number of moles corresponding to $C_{ta}$; $n_o$ is the number of moles corresponding to $C_o$ and $V_a$ and $V_o$ the volumes respectively of the aqueous and the organic phases.

Therefore $V_a/V_o$ is the phase ratio and $n_o = (n_i - n_a) = V_a\ (C_{Ti} - C_{Ta})$.

The algorithm employed produces acidity macroconstant values for the zwitterion, not microconstant values. The latter would require spectrophotometric determination of the protonated carboxylate group (Takacs-Novak et al., 1990), as shown below:

$$\alpha_{B^-} = \frac{A_{B^-} - A_{pH}}{A_{B^-} - A_{BH}} \quad (17)$$

where $A$ is the ultraviolet absorbance of the $B^-$ form, of the BH form or of the mixture of the two forms at a given pH, $K_1K_2\ (=\beta)$ being calculated from the macroconstants or the isoelectric pH (mean pK using potentiometry $B^- \rightarrow BH_2^+$ (Akaïké, 1974; Riley et al., 1989). Here, if $k_{11}$, $k_{12}$, $k_{21}$ and $k_{22}$ correspond to the dissociation constants for the neutral BH form to the $BH_2^+$ cation form, the neutral BH form to the anion $B^-$ form, the $BH^\pm$ zwitterion to the $BH_2^+$ cation form and the $BH^\pm$ zwitterion to the $B^-$ anion form, this gives:

$$K_1 = k_{11} + k_{21}$$

$$\frac{1}{K_2} = \frac{1}{k_{12}} + \frac{1}{k_{22}}$$

$$\beta = K_1K_2 \quad (18)$$

and

$$k_{12} = \alpha_{B^-}(1 + K_1[H_3O^+] + \beta[H_3O^+]^2) - \beta[H_3O^+]^2[H_3O^+] \quad (19)$$

The proportion of zwitterion in comparison to non-ionised molecule is an important factor required to interpret the partition and determine the activity of quinolones at their receptors.

$$K_z = \frac{[BH]_a}{[BH^\pm]_a} = \frac{k_{11}}{k_{21}} = \frac{k_{22}}{k_{12}} \quad (20)$$

However, an apparent partition coefficient $P'$ calculated from the sum of the concentrations of the neutral forms in the aqueous phase is directly proportional to the true partition coefficient $P$ as,

$$P = \frac{[BH]_o}{[BH]_a} \quad P' = \frac{[BH]_o}{\langle[BH]_a + [BH_a^\pm]\rangle} \quad (21)$$

the algorithm proposed calculates $P'$ from the values for $[BH]'_a$ obtained from Eq. (7), corresponding to the sum of the neutral forms in the aqueous phase.

It can now be shown that:

$$P = P'\cdot(1 + \frac{1}{K_z}) \quad (22)$$

As $K_z$ is a constant, the value for $P'$ is a hydrophobic parameter that can be used in structure-activity relationships and constitutes a constant that is characteristic of the quinolone under study (Fig. 1).

$$P' = \frac{[BH]_o/[BH]_a}{\langle 1 + [BH_a^\pm]/[BH]_a\rangle} = \frac{P}{1 + 1/K_z} \quad (23)$$

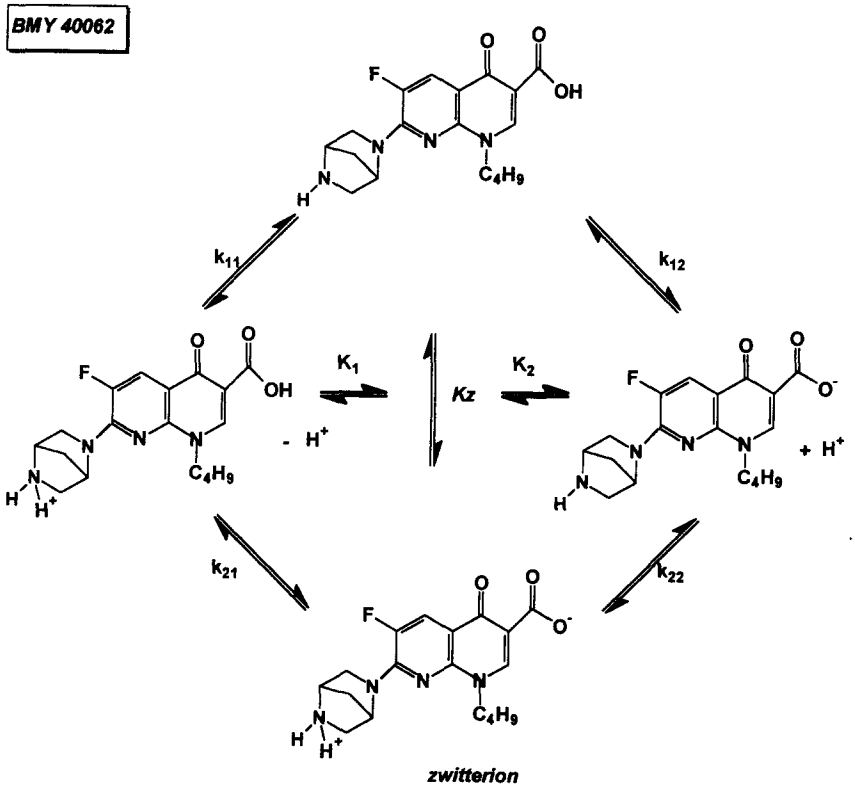



Fig. 1. BMY 40062.

## 3. Results

Applying the algorithm to the experimental values for $C_{Ti}$, $C_{Ta}$ and pH gave the constants for acidity, dimerization and ion pair extraction (Tables 1–3).

The software includes all the possible models: molecules with one or more acidity constants, ion pair formation constants, etc.

The values for the parameters evaluated may, however, be different depending on the mathematical model employed. The operator must therefore select the most appropriate model. To do this, the plausible models should be tested and the results obtained compared using an $F$-test. This checks the improvement in the residual variance after the software has chosen the best approximations for all the constants in the model. As well as evaluating the parameters under study, the software also produces values for Akaïké and Schwartz criteria (Akaïké, 1974; Schwarz, 1978). These can then be used by the experimenter to choose from models tested, the one which best fits the experimental points, even if the models do not contain the same number of parameters.

It should also be noted that, like for other methods used to determine acidity constants, the pH of the different buffers must where possible straddle the p$K$ value to be determined. If this is not the case the variations in the final concentration induced by these p$K$s may be of the same magnitude as those due to experimental error and the model thus possesses an infinite number of possible solutions.

This is shown in Table 1 which lists the different results obtained[2].

[2] The full experimental data may be obtained simply by contacting one of the authors.

Table 1
Experimental data

| Parameter | Compound 1 | | Compound 2 | Compound 3 |
|---|---|---|---|---|
| | Citrate buffer | Phosphate buffer | Phosphate buffer | Phosphate buffer |
| $P$ | 36.7 ± 0.3 | 35.0 ± 0.01 | 0.33 ± 0.01 | 8.5 ± 0.1 |
| $E_{11}$ | NS | 7.17 | 1.17 ± 0.04 | 52.7 ± 0.2 |
| $E_{12}$ | NS | NS | NS | NS |
| $E_{21}$ | NS | NS | 155.1 ± 0.1 | NS |
| $E_{22}$ | NS | NS | NS | NS |
| $K_d$ | NS | NS | NS | NS |
| $pK_I$ | 6.00 ± 0.05[a,b] | 5.97 ± 0.01[a,b] | 9.5 ± 0.06[b] | >12.5[d] |
| $pK_{II}$ | 0.85 ± 0.09[a,c] | 0.98 ± 0.09[a,c] | 6.47 ± 0.03[e] | 9.99 ± 0.06[b] |
| $pK_{III}$ | <0[a] | <0[a] | 1.56 ± 0.3[c] | 6.04 ± 0.06[f] |
| $pK_{IV}$ | | | <0 | <0 |
| $\lambda$ | | 254[h] | 344[g] | 273[h] |

Value ± standard deviation.[a]Compound 1: previous experiments 6.02, 0.94, <0 (12, 15).[b]Carboxylic group.[c]Amine N-2 of diazabicyclo ring.[d]Heterocyclic tertiary amine.[e]Heterocyclic secondary amine.[f]Primary amine.[g]Isosbestic point wavelength at which the spectral measurements were made.[h]Wavelength at which the spectral measurements were made after high performance liquid chromatography.

The pH range studied in the work performed here was deliberately restricted from 1.5 to 11 units because of the future relationship established between the physicochemical parameters and certain biological parameters. It can be seen here that when the evaluated p$K$ was close to the pH limit zone, it was rather poor, given the height of the standard deviation. This was not the case for the other p$K$ values.

## 4. Discussion

The constants obtained for nalidixic acid (compound 1) were perfectly consistent with data published in the literature (Riley et al., 1989; Takacs-Novak et al., 1990). The presence of a carbonyl group close to the carboxylic group facilitated the formation of an intramolecular hydrogen bridge which stabilised the acid group [crystallographic studies (Miyamoto et al., 1990) have shown the bridge to be 1.70 Å long]. By contrast, the fact that the two other compounds have the possibility of forming a zwitterion explains the high pK values for the carboxylic group whose dissociation is considerably influenced by the presence of the basic amine group.

Although the experimental design is rather complex, it provides values for the various parameters even when some of the constants overlap. It is then possible to estimate the octanol/water partition coefficient with very high precision.

The validity of the algorithm employed was also confirmed by analysing the results obtained in the presence of citrate buffer and phosphate buffer. The phosphate ions may form a soluble ion pair in the organic phase — expressed by the presence of the $E_{11}$ constant — whereas no ion pair extraction constant showed a statistically significant value for the tests conducted in the presence of citrate ions. Moreover, the p$K$ values for the acid group were perfectly consistent irrespective of whether these were obtained in the presence of one or the other of the counter-ions.

The algorithm developed was shown to be of unprecedented usefulness for simultaneously determining several acidity constants, ion pair ex-

Table 2
Experimental and calculated data (for compound 2)

| pH | $C_{Ti}$ (μg/ml) | $C_{Ta}$ (μg/ml) | $D_{exp}$ | $D_{calc}$ | $\Delta(D)$ % | $C_{Ti}^{calc}$ (μg/ml) |
|---|---|---|---|---|---|---|
| 2.25 | 33.39 | 30.67 | 0.0889 | 0.0663 | 25.4 | 32.70 |
| 2.25 | 43.51 | 41.01 | 0.0610 | 0.0663 | −8.7 | 43.73 |
| 2.25 | 51.97 | 48.98 | 0.0610 | 0.0663 | −8.6 | 52.23 |
| 3.05 | 25.37 | 23.59 | 0.0754 | 0.0660 | 12.4 | 25.15 |
| 3.05 | 36.56 | 34.48 | 0.0604 | 0.0660 | −9.4 | 36.75 |
| 3.05 | 60.19 | 56.36 | 0.0679 | 0.0660 | 2.7 | 60.08 |
| 4.00 | 28.19 | 26.39 | 0.0681 | 0.0603 | 11.5 | 27.98 |
| 4.00 | 38.85 | 36.71 | 0.0583 | 0.0603 | −3.5 | 38.92 |
| 4.00 | 56.41 | 53.73 | 0.0498 | 0.0603 | −21.0 | 56.97 |
| 5.00 | 28.22 | 26.00 | 0.0855 | 0.0643 | 24.8 | 27.67 |
| 5.00 | 56.88 | 53.91 | 0.0551 | 0.0643 | −16.6 | 57.38 |
| 5.00 | 56.77 | 52.92 | 0.0728 | 0.0643 | 11.6 | 56.32 |
| 6.00 | 28.05 | 25.33 | 0.1073 | 0.1050 | 2.2 | 27.99 |
| 6.00 | 29.30 | 26.32 | 0.1131 | 0.1050 | 7.2 | 29.09 |
| 6.00 | 58.10 | 52.38 | 0.1093 | 0.1050 | 3.9 | 57.88 |
| 7.00 | 27.69 | 22.41 | 0.2356 | 0.2398 | −1.8 | 27.79 |
| 7.00 | 28.93 | 23.98 | 0.2062 | 0.2398 | −16.3 | 29.73 |
| 7.00 | 58.55 | 46.90 | 0.2483 | 0.2398 | 3.4 | 58.15 |
| 8.00 | 27.58 | 20.59 | 0.3396 | 0.3101 | 8.7 | 26.97 |
| 8.00 | 28.00 | 21.89 | 0.2790 | 0.3101 | −11.2 | 28.68 |
| 8.00 | 57.99 | 45.36 | 0.2785 | 0.3101 | −11.4 | 59.43 |
| 9.00 | 27.61 | 21.11 | 0.3079 | 0.2679 | 13.0 | 26.77 |
| 9.00 | 30.88 | 23.40 | 0.3197 | 0.2679 | 16.2 | 29.67 |
| 9.00 | 57.33 | 44.30 | 0.2942 | 0.2679 | 8.9 | 56.17 |
| 10.25 | 36.71 | 35.46 | 0.0353 | 0.0655 | −85.9 | 37.78 |
| 10.25 | 46.67 | 44.56 | 0.0474 | 0.0655 | −38.3 | 47.48 |
| 10.25 | 55.55 | 53.10 | 0.0462 | 0.0655 | −42.0 | 56.58 |
| 10.8 | 45.20 | 43.87 | 0.0303 | 0.0216 | 28.8 | 44.82 |
| 10.8 | 56.83 | 54.64 | 0.0401 | 0.0216 | 46.24 | 55.81 |
| 10.8 | 56.08 | 57.03 | 0.0359 | 0.0216 | 40.02 | 58.26 |

Table 3
Main statistical parameters

| Statistic | Symbol | Value |
|---|---|---|
| Correlation coefficient | $r^2$ | 0.999759 |
| Mean of the residuals | $\mu_{res}$ | −0.0475 |
| Variance of the residuals | $s^2_{res}$ | 0.461676 |
| Schwartz criterion (Schwarz, 1978) | SC | 99.25632 |
| Akaïké criterion (Akaïké, 1974) | AIC | 90.84914 |

traction constants and the partition coefficient for a given molecule. It employs a simple technique involving distribution between a buffer and an octanol phase.

A comparison between the curves traced from experimental points and the partition calculated from the constants determined using the algorithm (distribution depending on pH) illustrates the usefulness of the algorithm for compound 2 (Fig. 2, Tables 2 and 3).

The simplified equation:

$$C_{Ti} = C_{Ta}\left[1 + \frac{v_o}{v_a}\cdot\frac{P + E_{11}\dfrac{[H_3O^+][H_2PO_4^-]}{K_2} + E_{21}\dfrac{H_3O^+]^2[H_2PO_4^-]^2}{K_2K_3}}{\dfrac{K_1}{[H^+]} + 1 + \dfrac{[H^+]}{K_2} + \dfrac{[H^+]^2}{K_2K_3}}\right] \quad (24)$$

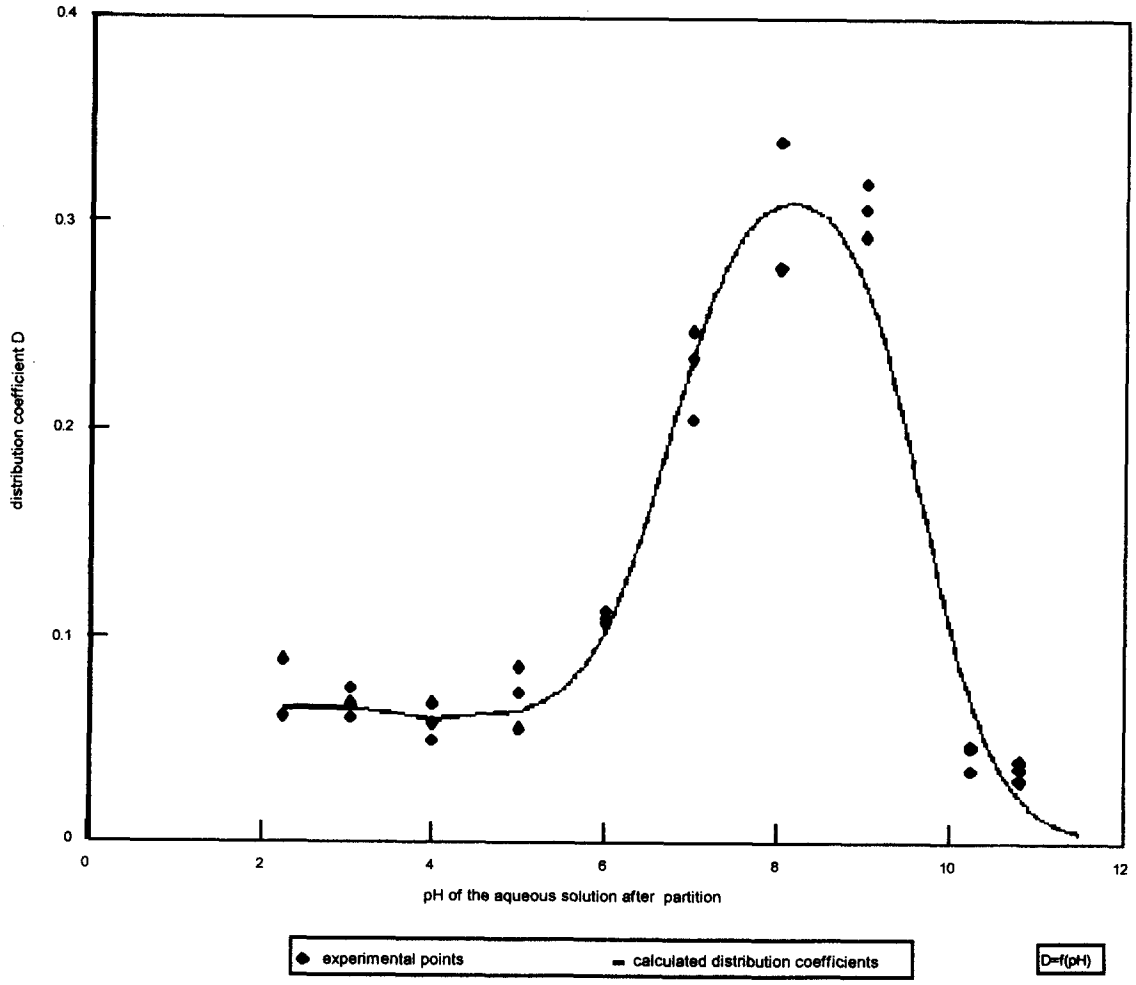



Fig. 2. Distribution curve, experimental and calculated, for compound 2.

was used to calculate the optimal concentrations $C_{Ti}$ after adjustment of the various constants. The statistical values obtained after smoothing of the curve are listed in Table 3.

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