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A general approach to the interpretation of pH degradation profiles in the presence of ligands

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Summary

A general equation for pH-dependent degradation profiles of polybasic weak electrolytes in the presence of a ligand is presented. The potential information which can be deduced from such profiles is discussed. The method is graphically illustrated with a hypothetical example.

Introduction

The interpretation of pH-dependent degradation profiles which has been treated in general terms in a previous study (Van der Houwen et al., 1988), becomes even more complicated if the substance investigated is involved in equilibria with a ligand. The addition of ligands may be pharmaceutically important to increase the solubility of the substance involved or the stability of its preparations, or to obtain preparations with more desirable biopharmaceutical properties (Frank, 1975; Uekama and Otagiri, 1987).

General Mathematical Approach

The symbols used in this mathematical approach are summarized in Table 1. The weak electrolyte AH_n exists in solution in different species depending on the degree of deprotonation (and therefore of the pH). The stepwise dissociation of the substance is described by a series of equations with the general form of Eqn 1:

$$K_i = [H^+] \cdot \left[AH_{n-i}^{i-}\right] / \left[AH_{n-i+1}^{1-i}\right] \qquad (1)$$

assuming that AH_n itself is uncharged. The constant K_i is the equilibrium constant for the dissociation of the i-th proton.

The concentration of each species can be expressed as a function of the concentration of the totally protonated species by applying Eqn 1 to the successive deprotonation steps. When the

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species are numbered from zero (AH_n) to n (A^{n-}), then the concentration of the i-th species is given by Eqn 2.

To simplify the equations the non-existent dissociation constant K_0 is defined to be equal to 1, the symbol j is used to indicate the subscript of the successive protolytic dissociation constants (Van der Houwen et al., 1988).

$$[AH^{i-}_{n-i}] = [AH_n] \cdot \prod_{j=0}^{i} K_j/[H^+]^i \qquad (2)$$

TABLE 1

Symbols used in the mathematical approach

K_i	protolytic dissociation constant of the i-th deprotonation step
K_0	constant without physical meaning, defined equal to unity, used for simplification of the equations
$\prod_{i=1}^{n} K_i$	product $K_1 \cdot K_2 \cdot K_3 \cdots K_n$
K_i^L	ligand binding constant of the i-th species of the solute
[L]	ligand concentration
K_w	autoprotolysis constant of water
k_{obs}	observed degradation rate constant
k_i^H	rate constant for the proton catalyzed degradation reaction of the i-th free species
k_i^{OH}	rate constant for the hydroxyl catalyzed degradation reaction of the i-th free species
k_i^S	rate constant for the solvent catalyzed degradation reaction of the i-th free species
k_{1i}^H	rate constant for the proton catalyzed degradation reaction of the i-th ligand bound species
k_{1i}^{OH}	rate constant of the hydroxyl catalyzed degradation reaction of the i-th ligand bound species
k_{1i}^S	rate constant of the solvent catalyzed degradation reaction of the i-th ligand bound species
M_i	'macro reaction constant' resulting from a combination of degradation rate constants, ligand binding constants and protolytic dissociation constants relevant for the i-th group of the kinetically indistinguishable degradation reactions and the ligand concentration
K_i^c	constant resulting from a combination of the protolytic dissociation constants for the species 1 to i, the ligand binding constant of the i-th species and the ligand concentration
M_i^f	contribution of the degradation of the free species to the i-th macroreaction constant M_i
M_i^1	contribution of the degradation of the ligand bound species to the i-th macroreaction constant M_i

If each of the successive deprotonation products of AH_n can bind to the ligand L in a 1 : 1 complex, this results in $n + 1$ equilibria which are described by the general form of Eqn 3.

$$K_i^L = [AH_{n-i}L^{i-}]/([AH^{i-}_{n-i}] \cdot [L]) \qquad (3)$$

The binding constant of the complex is given by K_i^L.

The concentration of each ligand bound species can be expressed as a function of the corresponding binding constant, the protolytic equilibrium constants and the ligand concentration and $[AH_n]$ by combining Eqns 2 and 3 to Eqn 4.

$$[AH_{n-i}L^{i-}] = [AH_n] \cdot \prod_{j=0}^{i} K_j \cdot K_i^L \cdot [L]/[H^+]^i \qquad (4)$$

The fraction of the substance AH_n which is present as one of the free or ligand bound species can be calculated by dividing the concentration of the corresponding species by the sum of the concentration of all species (Eqn 5).

$$[AH_n] \cdot \left[\sum_{i=0}^{n} \left\{ \prod_{j=0}^{i} K_j \cdot (1 + [L] \cdot K_i^L)/[H^+]^i \right\} \right] \qquad (5)$$

Each of the species present in solution can be involved in proton, solvent or hydroxyl catalyzed degradation reactions. The observed degradation rate constant is equal to the sum of the products of the individual rate constants and the fraction of the substance present in the form of the corresponding species. When the substance is involved in n protolytic equilibria and in complex formation with a ligand the total number of species present in solution is $2n + 2$. The number of individual reaction constants is then $6n + 6$.

The proton catalyzed solvent catalyzed and hydroxyl catalyzed contributions of the ligand bound i-th species are given by Eqn 6. The corresponding individual degradation rate constants of the ligand bound species are indicated by k_{1i}^{H}, k_{1i}^{S} and k_{1i}^{OH}. K_{w} is the autoprotolysis constant of water.

$$\left[\left(\prod_{j=0}^{i} K_j \cdot [\mathrm{L}] \cdot K_i^{\mathrm{L}}/[\mathrm{H}^+]^i\right) \cdot \left(k_{1i}^{\mathrm{H}} \cdot [\mathrm{H}^+] + k_{1i}^{\mathrm{S}} + k_{1i}^{\mathrm{OH}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+]\right)\right] \cdot \left[\sum_{i=0}^{n} \left\{\prod_{j=0}^{i} K_j \cdot \left(1 + [\mathrm{L}] \cdot K_i^{\mathrm{L}}\right)/[\mathrm{H}^+]^i\right\}\right]^{-1} \tag{6}$$

The proton catalyzed, solvent catalyzed and hydroxyl catalyzed degradation reactions of the non-ligand-bound *i*-th species are given by Eqn 7. The individual reaction constants are given by k_i^{H}, k_i^{S} and k_i^{OH}.

$$\frac{\left(\prod_{j=0}^{i} K_j/[\mathrm{H}^+]^i\right) \cdot \left(k_i^{\mathrm{H}} \cdot [\mathrm{H}^+] + k_i^{\mathrm{S}} + k_i^{\mathrm{OH}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+]\right)}{\left[\sum_{i=0}^{n} \left\{\prod_{j=0}^{i} K_j \cdot \left(1 + [\mathrm{L}] \cdot K_i^{\mathrm{L}}\right)/[\mathrm{H}^+]^i\right\}\right]} \tag{7}$$

By summation of Eqns 6 and 7 for all species the equation for the overall degradation rate constant (k_{obs}) is obtained (Eqn 8)

$$k_{\mathrm{obs}} = \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j \cdot [\mathrm{L}] \cdot K_i^{\mathrm{L}}/[\mathrm{H}^+]^i\right) \cdot \left(k_{1i}^{\mathrm{H}} \cdot [\mathrm{H}^+] + k_{1i}^{\mathrm{S}} + k_{1i}^{\mathrm{OH}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+]\right) + \left(\prod_{j=0}^{i} K_j/[\mathrm{H}^+]^i\right) \cdot \left(k_i^{\mathrm{H}} \cdot [\mathrm{H}^+] + k_i^{\mathrm{S}} + k_i^{\mathrm{OH}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+]\right)\right\} / \sum_{i=0}^{n} \left\{\prod_{j=0}^{i} K_j \cdot \left(1 + [\mathrm{L}] \cdot K_i^{\mathrm{L}}\right)/[\mathrm{H}^+]^i\right\} \tag{8}$$

The numerator of Eqn 8 can be rearranged in such a way that all individual degradation reaction constants that are kinetically indistinguishable (i.e. that depend on the pH in the same way) are combined in a single constant M.

Eqns 9–13 indicate how this is done.

$$M_0 = k_0^{\mathrm{H}} + [\mathrm{L}] \cdot K_0^{\mathrm{L}} \cdot k_{10}^{\mathrm{H}} \tag{9}$$

$$M_1 = \left(k_1^{\mathrm{H}} + [\mathrm{L}] \cdot K_1^{\mathrm{L}} \cdot k_{11}^{\mathrm{H}}\right) \cdot K_1 + k_0^{\mathrm{S}} + [\mathrm{L}] \cdot K_0^{\mathrm{L}} \cdot k_{10}^{\mathrm{S}} \tag{10}$$

$$M_i = \left(k_i^{\mathrm{H}} + [\mathrm{L}] \cdot K_i^{\mathrm{L}} \cdot k_{1i}^{\mathrm{H}}\right) \cdot \prod_{j=0}^{i} K_j + \left(k_{i-1}^{\mathrm{S}} + [\mathrm{L}] \cdot K_{i-1}^{\mathrm{L}} \cdot k_{1\,i-1}^{\mathrm{S}}\right) \cdot \prod_{j=0}^{i-1} K_j + \left(k_{i-2}^{\mathrm{OH}} + [\mathrm{L}] \cdot K_{i-2}^{\mathrm{L}} \cdot k_{1\,i-2}^{\mathrm{OH}}\right) \cdot K_{\mathrm{w}} \cdot \prod_{j=0}^{i-2} K_j \tag{11}$$

$$M_{n+1} = \left(k_n^{\mathrm{S}} + [\mathrm{L}] \cdot K_n^{\mathrm{L}} \cdot k_{1n}^{\mathrm{S}}\right) \cdot \prod_{j=0}^{n} K_j + \left(k_{n-1}^{\mathrm{OH}} + [\mathrm{L}] \cdot K_{n-1}^{\mathrm{L}} \cdot k_{1\,n-1}^{\mathrm{OH}}\right) \cdot K_{\mathrm{w}} \cdot \prod_{j=0}^{n-1} K_j \tag{12}$$

$$M_{n+2} = \left(k_n^{\mathrm{OH}} + [\mathrm{L}] \cdot K_n^{\mathrm{L}} \cdot k_{1n}^{\mathrm{OH}}\right) \cdot K_{\mathrm{w}} \cdot \prod_{j=0}^{n} K_j \tag{13}$$

The subscript i in Eqn 11 has a range from 2 to n.

According to Eqn 14 a number of constants can be combined in a 'combination constant' K_i^{c}

$$K_i^{\mathrm{c}} = \prod_{j=0}^{i} K_j \cdot \left(1 + [\mathrm{L}] \cdot K_i^{\mathrm{L}}\right) \tag{14}$$

The equation for the overall degradation constant is given by Eqn 15.

$$k_{\text{obs}} = \frac{\sum_{i=0}^{n+2} \left(M_i / [\text{H}^+]^{i-1} \right)}{\sum_{i=0}^{n} \left(K_i^{\text{c}} / [\text{H}^+]^{i} \right)}. \tag{15}$$

This equation bears a strong resemblance to that for the degradation profile in the absence of ligands (Van der Houwen et al., 1988).

The constants M_i and K_i^{c} in Eqn 15 can be calculated from measurements of the observed overall rate constants at different pH values using non-linear regression analysis. The constants M_i are linear functions of the ligand concentration. When measurements are repeated for a range of ligand concentrations, the contribution of the ligand bound species to the overall degradation rate constant can be calculated separately. The constants K_i^{c} are also linearly dependent on the ligand concentration. This makes it in principle possible to measure the ligand binding constant from a range of values of K_i^{c} determined at different ligand concentrations. However, such determinations are only possible when $[\text{L}] \cdot K_i^{\text{L}} \gg 1$.

For graphical representation of pH degradation profiles the logarithm of the observed rate constant is generally plotted against the pH. Analogous to what has been described for the pH profile in the absence of ligands (Van der Houwen et al., 1988) the separate profiles corresponding with the macro reaction constants can be approached by a combination of straight lines. The i-th intersection point of these lines lies at that pH value for which Eqn 16 is valid

$$K_i^{\text{c}} / [\text{H}^+]^{i} = K_{i-1}^{\text{c}} / [\text{H}^+]^{i-1} \tag{16}$$

Combination of Eqns 14 and 16 gives

$$[\text{H}^+] = K_i \cdot \left(1 + [\text{L}] \cdot K_i^{\text{L}}\right) / \left(1 + [\text{L}] \cdot K_{i-1}^{\text{L}}\right). \tag{17}$$

Eqn 17 shows that the inflexion points in the presence of ligands do not necessarily correspond with the pK_{a} values, in contrast to the pH profile in the absence of ligands. This is in agreement with the observation of apparent dissociation constants of acidic solutes in the presence of cyclodextrins (Connors and Lipari, 1976; Connors et al., 1982). The use of Eqn 15 for the study of the degradation rate of a substance in the presence of ligands requires measurements to be carried out at a sufficient number of pH values and ligand concentrations.

Measurements of the observed rate constant for different ligand concentrations at a single pH value or a very limited range of pH values cannot be interpreted in this way. In these cases, the following approach may be adopted: Eqns 9–14 indicate that the constants M_i and K_i^{c} are the sum of a term which is dependent on the ligand concentration and one that is not. If these terms are separated, Eqn 15 can be written as

$$k_{\text{obs}} = \frac{\sum_{i=0}^{n+2} \left\{ \left(M_i^{\text{f}} + [\text{L}] \cdot M_i^{1} \right) / [\text{H}^+]^{i-1} \right\}}{\sum_{i=0}^{n} \left\{ \left(\prod_{j=0}^{i} K_j + [\text{L}] \cdot \prod_{j=0}^{i} K_j \cdot K_i^{\text{L}} \right) \right\} / [\text{H}^+]^{i}} \tag{18}$$

The meaning of M_i^{f} is given in Eqns 19–23, and that of M_i^{1} in Eqns 24–28.

$$M_0^{\text{f}} = k_0^{\text{H}} \tag{19}$$

$$M_1^{\text{f}} = k_1^{\text{H}} \cdot K_1 + k_0^{\text{S}} \tag{20}$$

$$M_i^{\text{f}} = k_i^{\text{H}} \cdot \prod_{j=0}^{i} K_j + k_{i-1}^{\text{S}} \cdot \prod_{j=0}^{i-1} K_j + k_{i-2}^{\text{OH}} \cdot K_{\text{w}} \cdot \prod_{j=0}^{i-2} K_j \tag{21}$$

$$M_{n+1}^{\text{f}} = k_n^{\text{S}} \cdot \prod_{j=0}^{n} K_j + \left(k_{n-1}^{\text{OH}}\right) \cdot K_{\text{w}} \cdot \prod_{j=0}^{n-1} K_j \tag{22}$$

$$M_{n+2}^{\text{f}} = k_n^{\text{OH}} \cdot K_{\text{w}} \cdot \prod_{j=0}^{n} K_j \tag{23}$$

$$M_0^1 = K_0^L \cdot k_{10}^H \tag{24}$$

$$M_1^1 = K_1^L \cdot k_{11}^H \cdot K_1 + K_0^L \cdot k_{10}^S \tag{25}$$

$$M_i^1 = K_i^L \cdot k_{1i}^H \cdot \prod_{j=0}^{i} K_j + K_{i-1}^L \cdot k_{1i-1}^S \cdot \prod_{j=0}^{i-1} K_j + K_{i-2}^L \cdot k_{1i-2}^{OH} \cdot K_w \cdot \prod_{j=0}^{i-2} K_j \tag{26}$$

$$M_{n+1}^1 = K_n^L \cdot k_{1n}^S \cdot \prod_{j=0}^{n} K_j + K_{n-1}^L \cdot k_{1n-1}^{OH} \cdot K_w \cdot \prod_{j=0}^{n-1} K_j \tag{27}$$

$$M_{n+2}^1 = K_n^L \cdot k_{1n}^{OH} \cdot K_w \cdot \prod_{j=0}^{n} K_j. \tag{28}$$

The subscript i in Eqn 26 has a range from 2 to n. Eqn 18 can be rearranged to

$$k_{obs} = \left[\sum_{i=0}^{n+2} \left(M_i^f/[H^+]^{i-1}\right) + [L] \cdot \sum_{i=0}^{n+2} \left(M_i^1/[H^+]^{i-1}\right)\right] \times \left[\sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\} + [L] \cdot \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right) \cdot K_i^L/[H^+]^i\right\}\right]^{-1}. \tag{29}$$

If the numerator and denominator of this equation are divided by the first part of the denominator, Eqn 30 is obtained.

$$k_{obs} = \left[\sum_{i=0}^{n+2} \left(M_i^f/[H^+]^{i-1}\right) \Big/ \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\} + \sum_{i=0}^{n+2} \left(M_i^1/[H^+]^{i-1}\right)[L] \Big/ \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\}\right] \times \left[1 + \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right) \cdot K_i^L/[H^+]^i\right\}[L] \Big/ \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\}\right]^{-1} \tag{30}$$

The observed rate constant in the absence of ligand (k_{obs}^0) is given by Eqn 31.

$$k_{obs}^0 = \frac{\sum_{i=0}^{n+2} \left(M_i^f/[H^+]^{i-1}\right)}{\sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\}}. \tag{31}$$

Eqn 30 can be simplified to Eqn 32.

$$k_{obs} = \left[k_{obs}^0 + [L] \cdot \sum_{i=0}^{n+2} \left(M_i^1/[H^+]^{i-1}\right) \Big/ \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\}\right] \times \left[1 + [L] \cdot \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right) \cdot K_i^L/[H^+]^i\right\} \Big/ \sum_{i=0}^{n} \left\{\left(\prod_{j=0}^{i} K_j\right)/[H^+]^i\right\}\right]^{-1}. \tag{32}$$

This equation can be used for non-linear regression analysis for a series of measurements of the observed rate constant at varying ligand concentrations and at fixed pH.

Eqn 32 can be written in an abbreviated form

$$k_{\mathrm{obs}} = \frac{k_{\mathrm{obs}}^{0} + A \cdot [\mathrm{L}]}{1 + B \cdot [\mathrm{L}]}. \tag{33}$$

This equation can be rearranged to

$$\frac{[\mathrm{L}]}{k_{\mathrm{obs}}^{0} - k_{\mathrm{obs}}} = \frac{1}{k_{\mathrm{obs}}^{0} - A/B} \cdot [\mathrm{L}] + \frac{1}{B \cdot \left(k_{\mathrm{obs}}^{0} - A/B\right)}. \tag{34}$$

If the substance involved is not subject to protolytic equilibria the meaning of A and B is given by

$$A = k_{10}^{\mathrm{H}} \cdot K_0^{\mathrm{L}} \cdot [\mathrm{H}^+] + k_{10}^{\mathrm{S}} \cdot K_0^{\mathrm{L}} + k_{10}^{\mathrm{OH}} \cdot K_0^{\mathrm{L}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+] \tag{35}$$

$$B = K_0^{\mathrm{L}} \tag{36}$$

Combination of Eqns 35 and 36 gives Eqn 37:

$$A/B = k_{10}^{\mathrm{H}} \cdot [\mathrm{H}^+] + k_1^{\mathrm{S}} + k_{10}^{\mathrm{OH}} \cdot K_{\mathrm{w}}/[\mathrm{H}^+]. \tag{37}$$

Substitution of Eqns 36 and 37 into Eqn 34 gives the Lineweaver-Burk equation (Lineweaver and Burk, 1934; Bender and Komiyama, 1978). If the substance investigated is involved in one or more protolytic equilibria Eqn 32 can still be applied. The meaning of A and B is then given by equations more complex than Eqns 35–37.

A Hypothetical Example

The generalized mathematical treatment is illustrated by the graphical representation of the degradation reactions of a hypothetical substance AH_2, with pK_a values of 5 and 10, in the presence of a ligand. When not indicated otherwise the ligand concentration is supposed to be equal to unity. The ligand binding constants of the three species AH_2, AH^- and A^{2-} are defined to be 100, 10 and 1, respectively.

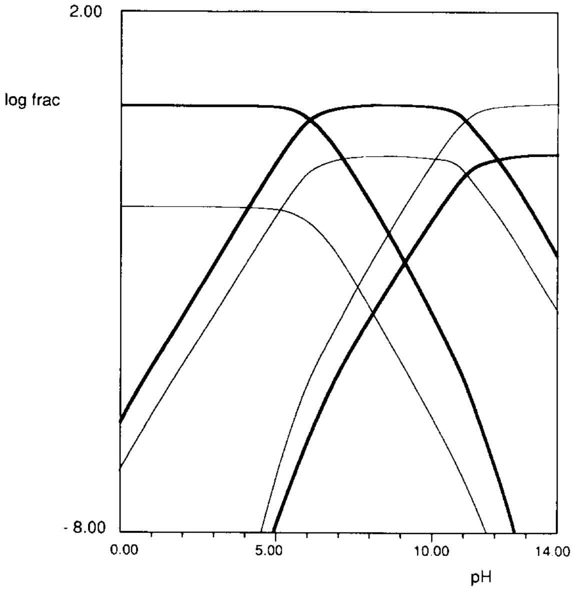

Fig. 1. Logarithms of the fractions of each of the species resulting from AH_2, present in the solution as a function of the pH. The graphs of the ligand bound species are indicated with emphasized lines.

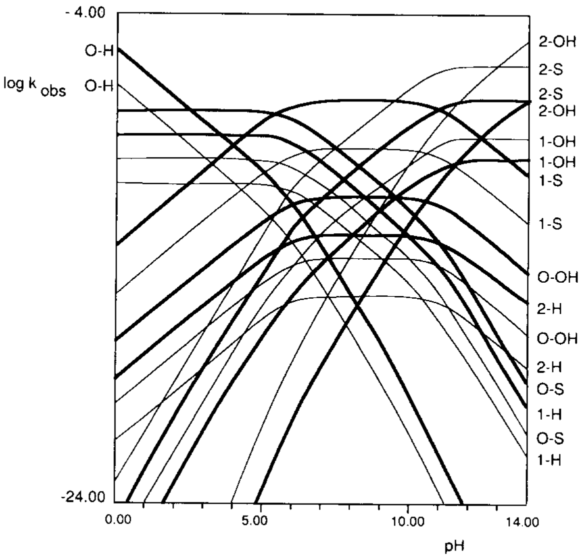

Fig. 2. Contributions of the individual rate constants to the log k_{obs}. The individual reactions are identified by the combination of a number (the number of protons dissociated) and the symbols H, S and OH (for the proton-, solvent- and hydroxyl-catalyzed reactions, respectively). The graphs of the ligand bound reactions are indicated by bold lines.

TABLE 2

Logarithms of the individual reaction constants

	Proton catalyzed	Solvent catalyzed	Hydroxyl catalyzed
Free species			
AH_2	-5.00	-8.00	-4.00
AH^-	-4.00	-8.50	-5.00
AH^{2-}	-4.50	-6.00	-5.00
Bound species			
AH_2	-5.50	-8.00	-3.50
AH^-	-3.00	-7.50	-7.00
AH^{2-}	-1.00	-6.50	-6.50

Logarithms of the macroreaction constants (ligand concentration = 1)

M_1	-3.49
M_2	-5.95
M_3	-11.50
M_4	-20.99
M_5	-34.00

The fractions of the bound and the free species as functions of the pH are given in Fig. 1. The graphs of the corresponding bound and free species are parallel. The inflexion points are shifted from the pK_a values for approx. 1 pH unit (0.96 and 1.0 pH units according to Eqn 17). The hypothetical individual reaction constants of the bound and free species are given in Table 2. The values of the individual reaction constants have been chosen in such way that the graphs of the kinetically indistinguishable reactions (parallel graphs) are easily recognized (Fig. 2). The reactions can be divided in five groups containing, respectively, 2, 4, 6, 4 and 2 kinetically indistinguishable reactions. Each of these groups results in a single macro reaction. The number of reactions in each group corresponds with the number of terms in Eqns 9–13.

The graphs of the individual reactions and the macro reactions have approximately the shape of a combination of straight lines. The pH of the inflection points of the graphs correspond with those of Fig. 1. The overall pH-profile and the contribution of the separate macro reaction constants are given in Fig. 3. The dependence of the overall pH-profile on the ligand concentration is illustrated by Fig. 4. This figure clearly demonstrates the dependence of the pH of the inflection

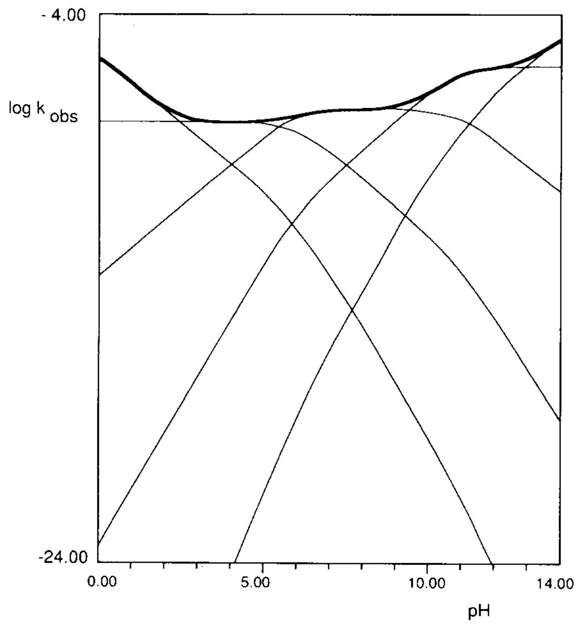

Fig. 3. Contributions of the macro reaction constants to the log k_{obs} as a function of the pH together with the overall degradation profile of AH_2 (bold line).

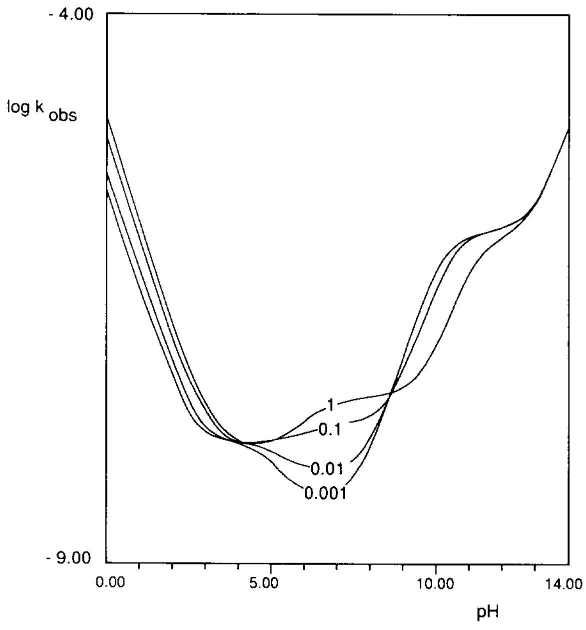

Fig. 4. Log k_{obs} at ligand concentrations of 1, 0.1, 0.01 and 0.001.

points on the ligand concentration as predicted by Eqn 17.

Conclusions

From these considerations the following conclusions can be drawn.

(1) From a pH degradation profile of a weak electrolyte from which n protons can dissociate, a maximum of $n + 3$ macro reaction constants and n inflection pH values can be calculated.

(2) The first and the $n + 3$rd macro reaction constant correspond to the sum of a single rate constant of both a free and a ligand bound rate constant. The second and the $n + 2$nd macro reaction constant are based on a combination of two rate constants of free species and two rate constants of ligand bound species.

All other macro reaction constants are based on combinations of three rate constants of free species and three rate constants of ligand bound species.

(3) The contribution of the ligand bound species can be calculated from the dependence of the macro reaction constants on the ligand concentration.

(4) The ligand binding constants can be calculated from the profiles if the product of the complex stability constant and the ligand concentration is sufficiently high.

(5) Except for those contributing to the first and $n + 3$rd macroreaction constants the calculation of the individual rate constants cannot be based upon the measured degradation profile itself.

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