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A general approach to the interpretation of pH degradation profiles

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Summary

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

Introduction

The interpretation of pH-dependent degradation profiles becomes increasingly more difficult, when the number of protolytic equilibria in which the substance investigated is involved, increases.

An example of such problems is given in the treatment of the degradation of acetylsalicylic acid by Edwards (1950). Although the phenomenon of kinetically indistinguishable reactions has been mentioned in some studies (Edwards, 1950; Hansen et al., 1983), so far, to our knowledge, no systematic treatment has been given for substances that are involved in more than one protolytic equilibrium.

Recent investigations in our laboratory (Beijnen et al., 1986a, b and c, 1988a and b) have prompted us to develop a general approach for this type of problems.

General mathematical approach

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

$$K_i = \frac{[H^+] \cdot [AH^{i-}_{n-i}]}{[AH^{1-i}_{n-i+1}]} \tag{1}$$

assuming that AH_n itself is uncharged.

The constant K_i is the equilibrium constant for the dissociation of the i^{th} proton. The concentra-

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tion 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 species are numbered from zero (AH_n) to n (A^{n-}), the concentration of the first and second species is expressed by Eqns. 2 and 3, respectively.

$$[AH^{1-}_{n-1}] = [AH_n] \cdot \frac{K_1}{[H^+]} \tag{2}$$

$$[AH^{2-}_{n-2}] = [AH_n] \cdot \frac{K_1}{[H^+]} \cdot \frac{K_2}{[H^+]} \tag{3}$$

The general equation for the concentration of the i^{th} species is then given by Eqn 4.

$$[AH^{i-}_{n-i}] = [AH_n] \cdot \frac{\prod_{j=1}^{i}(K_j)}{[H^+]^i} \tag{4}$$

By summation of Eqn. 4 from 1 to n and adding the result to $[AH_n]$ the sum of the concentration of all the species, expression 5, is obtained:

$$[AH_n] \cdot \left[1 + \sum_{i=1}^{n} \left\{ \frac{\prod_{j=1}^{i}(K_j)}{[H^+]^i} \right\} \right] \tag{5}$$

The fraction of the substance present as the i^{th} species ($i = 1, 2, \ldots n$) is then expressed by expression 6.

$$\frac{\dfrac{\prod_{j=1}^{i}(K_j)}{[H^+]^i}}{1 + \sum_{i=1}^{n} \left\{ \dfrac{\prod_{j=1}^{i}(K_j)}{[H^+]^i} \right\}} \tag{6}$$

By defining the imaginary protolytic dissociation constant K_0 equal to 1 Eqn. 7 can be substituted into expression 6 resulting in a simplified expression 8 which is valid for all species ($i = 0, 1, \ldots n$).

$$1 = \frac{K_0}{[H^+]^0} \tag{7}$$

$$\frac{\dfrac{\prod_{j=0}^{i}(K_j)}{[H^+]^i}}{\sum_{i=0}^{n} \left\{ \dfrac{\prod_{j=0}^{i}(K_j)}{[H^+]^i} \right\}} \tag{8}$$

Each of the species in solution is assumed to be involved in one single proton-, solvent- and hydroxyl-catalyzed degradation reaction. The individual reaction rate constants are denoted as a two-dimensional array $k_{i,r}$. The first index (i) indicates the species, the second one (r) the type of reaction involved. The proton-catalyzed reactions are indicated by H, the solvent catalyzed reactions by S and the hydroxyl-catalyzed reactions by OH. Using this convention, summation of the products of the fractions of the individual species in solution, the corresponding individual rate constants and the concentration of the catalyzing agent yields the overall degradation rate constant k_{obs} as expressed in Eqn. 9.

The symbol K_w indicates the autoprotolysis constant of water. The solvent concentration is regarded as constant and incorporated in the rate constant for the solvent-catalyzed reaction.

$$k_{obs} = \left(\sum_{i=0}^{n} \left\{ \frac{\prod_{j=0}^{i}(K_j)}{[H^+]^i} \left(k_{i,H} \cdot [H^+] + k_{i,S} + k_{i,OH} \cdot \frac{K_w}{[H^+]} \right) \right\} \right) \times \left(\sum_{i=0}^{n} \left\{ \frac{\prod_{j=0}^{i}(K_j)}{[H^+]^i} \right\} \right)^{-1} \tag{9}$$

The contributions of the individual reactions to k_{obs} are pH-dependent for two reasons: (1) the concentration of the individual species is pH dependent; and (2) the proton- and hydroxyl-catalyzed reactions depend on the concentrations of the protons and hydroxyl ions.

When Eqn. 9 is rearranged in such a way that all individual reactions, depending on the pH in the same way, are combined, Eqn. 10 is obtained. This equation applies to substances which are subject to at least two protolytic equilibria. When no or only one protolytic equilibrium occurs, Eqn. 10 can be adapted by eliminating the appropriate parts.

$$\begin{aligned} k_{\mathrm{obs}} = \Bigg[& k_{0,H} \cdot [\mathrm{H}^+] + k_{1,H} \cdot K_1 + k_{0,S} \\ & + \sum_{i=0}^{n-2} \left\{ \frac{\prod_{j=0}^{i} (K_j)}{[\mathrm{H}^+]^{i+1}} \cdot \left(k_{i+2,H} \cdot K_{i+1} \cdot K_{i+2} \right.\right. \\ & \left.\left. + k_{i+1,S} \cdot K_{i+1} + k_{i,OH} \cdot K_{\mathrm{w}} \right) \right\} \\ & + \frac{\prod_{j=0}^{n-1} (K_j)}{[\mathrm{H}^+]^{n}} \cdot \left(k_{n,S} \cdot K_n + k_{n-1,OH} \cdot K_{\mathrm{w}} \right) \\ & + \frac{\prod_{j=0}^{n} (K_j)}{[\mathrm{H}^+]^{n+1}} \cdot k_{n,OH} \cdot K_{\mathrm{w}} \Bigg] \\ & \Bigg/ \sum_{i=0}^{n} \left\{ \frac{\prod_{j=0}^{i} (K_j)}{[\mathrm{H}^+]^{i}} \right\} \end{aligned} \tag{10}$$

This equation can be written in the generalized form:

$$k_{\mathrm{obs}} = \frac{\sum_{i=1}^{n+3} \left(\frac{M_i}{[\mathrm{H}^+]^{i-2}} \right)}{\sum_{i=0}^{n} \left\{ \frac{\prod_{j=0}^{i} (K_j)}{[\mathrm{H}^+]^{i}} \right\}} \tag{11}$$

This Eqn. 11 is a general equation for a pH–degradation profile. We propose to call the constants M_1 to M_{n+3} "macro reaction constants". If no protolytic equilibrium occurs the physical significance of the macro reaction constants is given by the Eqns. 12, 13 and 14.

$$M_1 = k_{0,H} \tag{12}$$

$$M_2 = k_{0,S} \tag{13}$$

$$M_3 = k_{0,OH} \cdot K_{\mathrm{w}} \tag{14}$$

When a single protolytic equilibrium occurs, Eqns. 15, 16, 17 and 18 express the significance of M_1–M_4.

$$M_1 = k_{0,H} \tag{15}$$

$$M_2 = k_{1,H} \cdot K_1 + k_{0,S} \tag{16}$$

$$M_3 = k_{1,S} \cdot K_1 + k_{0,OH} \cdot K_{\mathrm{w}} \tag{17}$$

$$M_4 = k_{1,OH} \cdot K_1 \cdot K_{\mathrm{w}} \tag{18}$$

If two deprotonation steps occur the signification is given by Eqns. 19, 20, 21, 23 and 24. For more than two deprotonation steps Eqn. 22 is added to this series ($i = 1, \ldots n - 2$).

$$M_1 = k_{0,H} \tag{19}$$

$$M_2 = k_{1,H} \cdot K_1 + k_{0,S} \tag{20}$$

$$M_3 = k_{2H} \cdot K_1 \cdot K_2 + k_{1,S} \cdot K_1 + k_{0,OH} \cdot K_{\mathrm{w}} \tag{21}$$

$$M_{i+3} = \prod_{j=1}^{i} (K_j) \cdot \{k_{i+2,H} \cdot K_{i+1} \cdot K_{i+2} + k_{i+1,S} \cdot K_{i+1} + k_{i,OH} \cdot K_w\} \qquad (22)$$

$$M_{n+2} = \prod_{j=1}^{n-1} (K_j) \cdot \{k_{n,S} \cdot K_n + k_{n-1,OH} \cdot K_w\} \qquad (23)$$

$$M_{n+3} = \prod_{j=1}^{n} (K_j) \cdot k_{n,OH} \cdot K_w \qquad (24)$$

The previous mathematical approach is based upon the assumption that each species undergoes only one single proton-, solvent- and hydroxyl-catalyzed degradation reaction. If more of the reactions occur simultaneously, the sum of the rate constants has to be included in the equations instead of the original single constant.

In solvent-catalyzed degradation all reactions are included which are catalyzed by agents of which the concentration is not pH-dependent.

A hypothetical example

This generalized mathematical treatment is illustrated by the graphical representation of a hypothetical substance AH_3 which has pK_a values of 3, 7 and 11. The intervals between the pK_a values are chosen sufficiently large to avoid overlapping of the successive deprotonation steps. In Figs. 1–4 the pH values equal to the pK_a values are indicated with vertical lines.

The hypothetical individual rate constants and the resulting macro reaction constants are given in Table 1. The values of the individual rate constants are chosen in such a way that perspicuous graphs are obtained.

Fig. 1 shows the fraction of AH_3 present in the form of its different species as a function of the pH.

In Figs. 2A–D the plots of the contribution of the three hypothetical rate constants to k_{obs} are given for each individual species.

TABLE 1

Logarithms of the individual reaction constants

Species	Proton-catalyzed	Solvent-catalyzed	Hydroxyl-catalyzed
AH_3	−4.00	−7.00	2.00
AH_2^-	−3.00	−8.00	1.00
AH^{2-}	0.00	−5.00	−1.00
A^{3-}	4.00	−5.00	−2.00

Logarithms of the macro reaction constants

M_1	−4.00
M_2	−5.96
M_3	−9.95
M_4	−14.95
M_5	−24.96
M_6	−37.00

Fig. 3 shows all curves together. The kinetically indistinguishable reactions can be recognized as parallel curves, shifted to higher or lower values of k_{obs}.

Examination of the graphs in Figs. 2A–D and 3 shows that the solvent-catalyzed reaction of the species AH_2^- belongs to a kinetically indistinguishable group of 3 reactions together with the proton-catalyzed reaction of the species AH^{2-} and the hydroxyl-catalyzed reaction of the species AH_3.

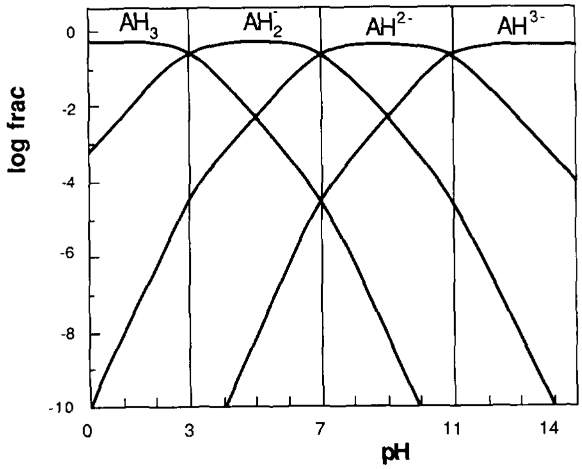

Fig. 1. Logarithms of the fraction of each of the species resulting from AH_3, present in the solution as a function of the pH.

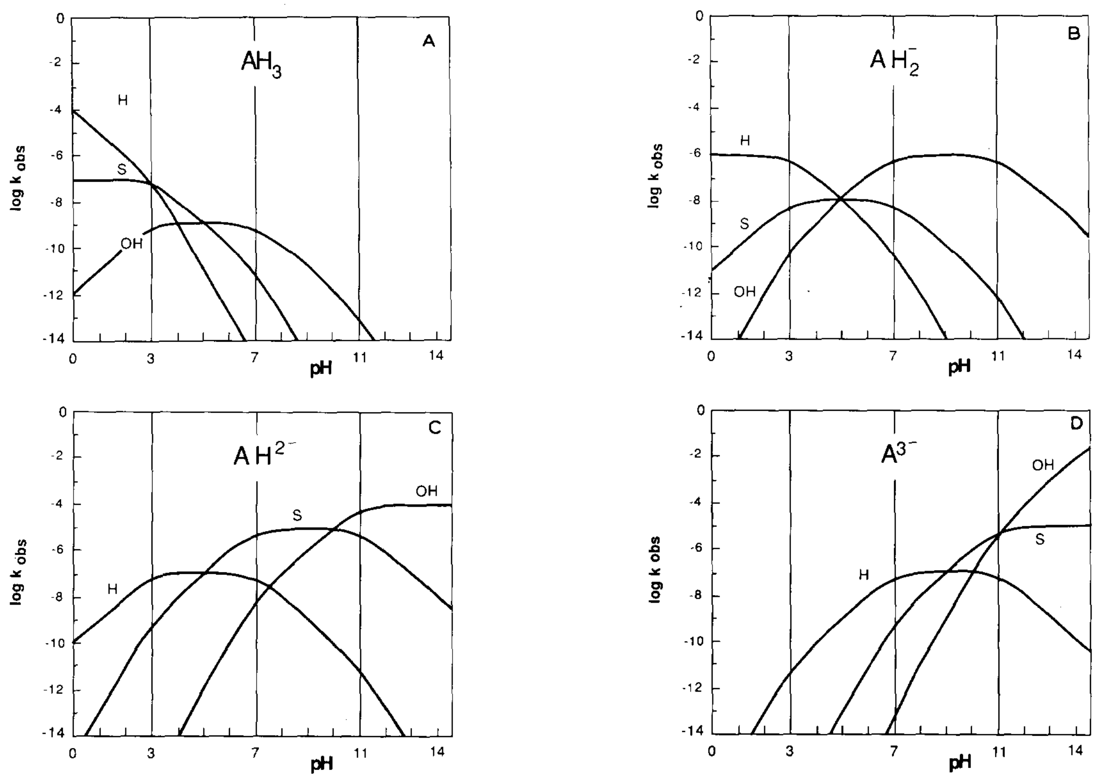

Fig. 2A–D. Logarithms of the contribution of the individual rate constants to the k_{obs} of each species resulting from AH_3, separately. The proton-, solvent- and hydroxyl-catalyzed reactions are indicated by H, S and OH, respectively.

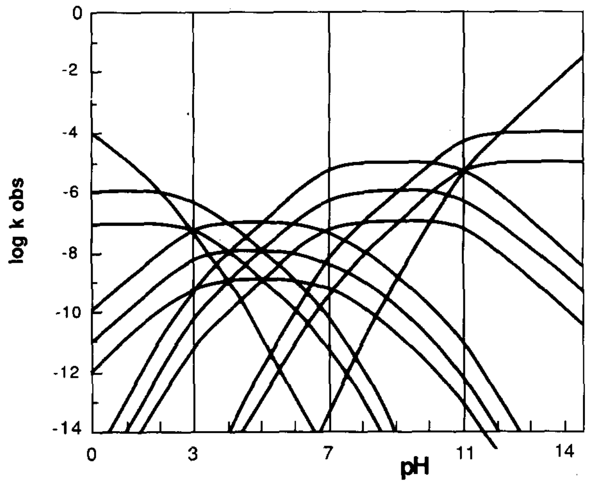

Fig. 3. Logarithm of the contribution of all reactions of the different species resulting from AH_3 to k_{obs} as a function of pH.

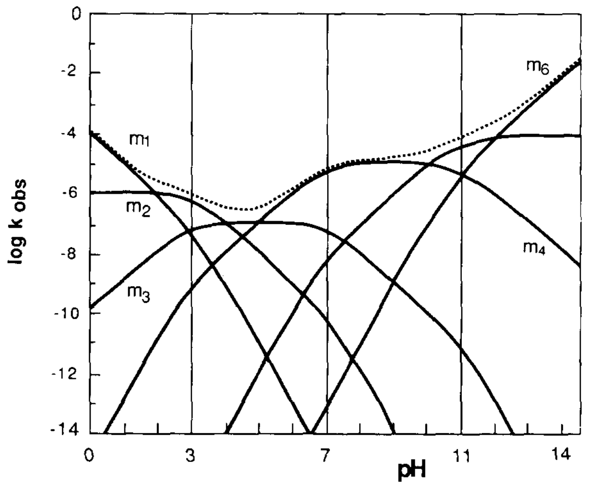

Fig. 4. Logarithm of the contribution of the macro reaction constants (M_1–M_6) to k_{obs} as a function of the pH together with the overall degradation profile of AH_3.

An analogous group is formed by the solvent-catalyzed reaction of AH^{2-}, the proton-catalyzed reaction of A^{3-}, and the hydroxyl-catalyzed reaction of AH_2^-.

The solvent-catalyzed reaction of AH_3 and the proton-catalyzed reaction of AH_2^- form a group of two kinetically indistinguishable reactions. A second group of two reactions is formed by the solvent-catalyzed reaction of A^{3-} and the hydroxyl-catalyzed reaction of AH^{2-}. These combinations of reactions into groups of kinetically indistinguishable reactions illustrate the following rule: if the sum of the charges of the catalyzing agent and the species involved are equal, the reactions are kinetically indistinguishable.

Mathematically, this rule can be derived from Eqns. 10 or 20–23. Fig. 4 shows the plots of the macro reaction constants as a function of the pH together with the overall degradation profile. The graphs in Figs. 2–4 illustrate, that apart from the first and the last macro reaction constant, it is not possible to ascribe these macro reaction constants to a single reaction nor to a single species.

The shape of the curves of the logarithms of the contribution of the individual rate constants to k_{obs} as a function of the pH can easily be understood considering that they result from adding the value of the logarithms of the fraction of the species and of the concentration of the catalyzing agent to the logarithm of the rate constant.

Examination of Figs. 1–4 shows that the logarithmic plots of the species fractions (and therefore also of those of the contributions of the individual reactions and the macro reaction constants to k_{obs}) as functions of the pH approximate straight lines in the pH intervals separated by the pH values that equal the pK_a values. The slope of these lines is equal to whole (negative or positive) numbers. The points of intersection of these straight lines occur at pH values that equal the pK_a values. Passing from a lower to a higher pH interval, the value of the slope is decremented with a value of 1. These phenomena follow directly from the fact that the nominator in the Eqns. 8–11 is a summation of separate terms. Going from low to high pH, each of the successive terms dominates the value of the nominator within the succesive pH intervals. The intersection points therefore occur for those pH values at which the successive terms are equal (Eqn. 25).

$$\frac{\prod_{j=0}^{i}(K_j)}{[H^+]^{i}} = \frac{\prod_{j=0}^{i+1}(K_j)}{[H^+]^{i+1}} \tag{25}$$

It should be clear from these considerations and from the graphs in Figs. 2–4 that the slope of (parts of) the logarithmic plot of k_{obs} as a function of the pH does not necessarily given indications about the catalyzing agent involved in the reaction. It is therefore not justified to conclude that a slope of -1 indicates that the reaction involved is proton-catalyzed, nor does a value $+1$ indicate it to be hydroxyl-catalyzed.

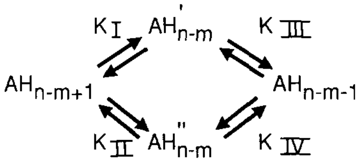

Fig. 5. Pattern of equilibria occurring on overlapping protolytic dissociations.

Influence of tautomeric equilibria

When the pK_a values are separated by less than 3 or 4 pH units, or when the substance investigated is subject to other tautomeric equilibria, the number of species in the solution increases:

The equilibria in such a case are illustrated by Fig. 5.

The distribution of the species as a function of the pH is illustrated by the diagram of Fig. 4. The macro dissociation constants K_m and K_{m+1} are related to the micro dissociation constants K_{I}, K_{II}, K_{III} and K_{IV} by Eqns. 26 and 27 (Martin, et al., 1973):

$$K_m = K_{I} + K_{II} \tag{26}$$

$$K_{m+1} = \frac{1}{\frac{1}{K_{III}} + \frac{1}{K_{IV}}} \tag{27}$$

The fraction of the substance present as the species AH_{n-m} is given by expression 28:

$$\frac{\dfrac{\prod_{j=0}^{m}(K_j)\cdot\dfrac{K_{\mathrm{I}}}{K_m}}{[H^+]^m}}{\sum_{i=0}^{n}\left\{\dfrac{\prod_{j=0}^{i}(K_j)}{[H^+]^i}\right\}} \tag{28}$$

The fraction of the substance present as the species AH_{n-m} is given by expression 29:

$$\frac{\dfrac{\prod_{j=0}^{m}(K_j)\cdot\dfrac{K_{\mathrm{II}}}{K_m}}{[H^+]^m}}{\sum_{i=0}^{n}\left\{\dfrac{\prod_{j=0}^{i}(K_j)}{[H^+]^i}\right\}} \tag{29}$$

The contribution of both species to k_{obs} can be calculated by multiplying the fraction of the substance present as each species with the corresponding individual rate constants and the concentration of the catalyzing agent.

Eqns. 12–14 are adapted to such tautomeric equilibria by replacing the rate constants involved according to Eqns. 30, 31 and 32.

$$k_{m,H}=k_{m,H}^{\mathrm{I}}\cdot\frac{K_{\mathrm{I}}}{K_m}+k_{m,H}^{\mathrm{II}}\cdot\frac{K_{\mathrm{II}}}{K_m} \tag{30}$$

$$k_{m,S}=k_{m,S}^{\mathrm{I}}\cdot\frac{K_{\mathrm{I}}}{K_m}+k_{m,S}^{\mathrm{II}}\cdot\frac{K_{\mathrm{II}}}{K_m} \tag{31}$$

$$k_{m,OH}=k_{m,OH}^{\mathrm{I}}\cdot\frac{K_{\mathrm{I}}}{K_m}+k_{m,OH}^{\mathrm{II}}\cdot\frac{K_{\mathrm{II}}}{K_m} \tag{32}$$

The rate constants of both tautomeric forms are denoted with I and II, respectively.

However, such refinements in the equation are of no practical importance, since the reactions of both tautomeric forms are kinetically indistinguishable. They only increase the number of individual reactions upon which the macro reaction constants are based. The general equation, Eqn. 10, remains valid. The only change in the equation is that K_m and K_{m+1} (belonging to the series indicated in the equation by K_j) now signify macro and not micro dissociation constants.

This mathematical treatment has practical implications for the interpretation of measured pH profiles for degradation reactions. Using adequate computer programs for non-linear regression analysis the values of the macro reaction constants and the value(s) of the protolytic dissociation constants can be calculated. The macro reaction constants can only be calculated insofar they contribute significantly to k_{obs} for at least part of the pH profile. If such is not the case, one can estimate their values to be below a certain limit.

The protolytic dissociation constants can only be calculated when the corresponding inflection points occur in the pH profile. When these inflection points are not pronounced, the calculation of the macro reaction constants will be more precise if the protolytic dissociation constants are determined by independent methods.

Calculation of the individual rate constants upon which the macro reaction constants are based is (apart from M_1 and M_{n+3}) impossible, because an infinite number of combinations of individual rate constants exists, all resulting in the same macro reaction constant. Macro reaction constants of this kind can therefore only be ascribed to a single reaction when the contribution of the other reactions (involved in the same macro reaction constant) can be neglected. Assumptions like this may be based upon the probability of the magnitude of the rate constants or upon the probability of certain reaction mechanisms in relation to the degradation products identified. For molecules involved in more than one (protolytic or tautomeric) equilibrium such assumptions will generally be rather speculative. In such cases the description of the pH profiles in terms of macro reaction constants is more appropriate in our opinion.

By interpreting of the pH profile in terms of the macro reaction constants and protolytic (macro) dissociation constants, it is simplified to its utmost mathematical simplicity. All relevant information can be derived from it provided that the number of protolytic equilibra is known. Further interpretation is then reduced to the interpretation of the reactions involved in the calculated macro reaction constants.

In our laboratory such procedures have successfully been applied to the interpretation of stability profiles of a number of pharmacological active substances such as mitomycin A, B and C (Beijnen et al., 1986a), daunorubicin (Beijnen et al., 1986b), doxorubicin (Beijnen et al., 1986c), etoposide (Beijnen et al., 1988a) 7-*N*(parahydroxyphenyl)mitomycin C (Beijnen et al., 1988b) and recently vinblastin (Vendrig et al., 1988).

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 pK_a values can be calculated.

(2) Only the first and the $n + 3^{\text{th}}$ macro reaction constants may correspond to an individual rate constant. The second and the $n + 2^{\text{th}}$ are based upon at least two individual rate constants. All other macro reaction constants are based upon at least 3 individual rate constants.

(3) In case where tautomeric equilibria occur the number of possible rate constants involved increases.

Attribution of the second to the $n + 2^{\text{th}}$ macro reaction constants to a single individual reaction cannot be based upon the measured degradation pH profile itself. For such interpretations of the profile additional information is needed.

To derive the potential information from a pH profile knowledge about tautomeric equilibria or microdissociation constants is not a prerequisite.

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