

Review paper

Systematic interpretation of pH-degradation profiles. A critical review

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Abstract

In this study we discuss the application of the general models for pH degradation profiles for specific acid, solvent and base catalysis, both in the absence and presence of ligands, and for the general acid and base catalysis, that we have published recently, we also present a systematic step by step procedure for the interpretation of pH profiles, which we apply to a number of recent publications. To facilitate the comparison of the mathematical treatment of the data the model equations reported in these studies have been transformed analogous to our equations. Many of these studies raise minor to serious objections. These objectives vary from unjustified conclusions regarding the content of specific reactions to the degradation, mathematical errors in the model equations, unjustified neglect of pK_a values close to or within the pH range investigated, unjustified linearization of non linear relationships to the application of model equations with non integer exponents without any theoretical foundation. Application of our model equations explains discrepancies in some of the original publications and offers acceptable alternatives to some rather stretched hypotheses. © 1997 Elsevier Science B.V.

Keywords: pH-degradation profiles; Specific acid; Solvent and base catalysis

1. Introduction

pH-Degradation profiles are an essential part of degradation studies of drugs. From a theoretical point of view they provide indications for degradation mechanisms of pharmacologically active substances. From a practical perspective they may

provide useful information for the optimal formulation and storage conditions of pharmaceutical products containing these active substances.

The majority of drugs are involved in protolytic equilibria when dissolved in aqueous solutions. These protolytic equilibria complicate the pH-degradation profiles of the drugs involved. In 1988 we reported a general approach to the interpretation of pH-degradation profiles, dealing sys-

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tematically with the influences of protolytic equilibria (Van der Houwen et al., 1988). Since then this mathematical methodology has been extended to pH-degradation profiles applying to drugs degrading in the presence of ligands (Van der Houwen et al., 1991) and to the influence of buffer catalyzed degradation processes (Van der Houwen et al., 1994). After a brief description of a step-by-step procedure for the interpretation of pH-degradation profiles an overview is presented of the most common misinterpretations in this respect, illustrated with literature examples.

Hopefully this review contributes to a more systematic approach for the interpretation of pH-degradation profiles.

2. Model equations describing the relationship between the pH and the observed degradation rate

As described earlier (Van der Houwen et al., 1988) the general equations containing all individual degradation reactions for substances involved in 0, 1, 2, 3 or 4 protolytic equilibria, are given by Eqs. (1)–(5), respectively. The combinations of kinetically indistinguishable reactions, which contribute to the same macro reaction constant, are included in parentheses ().

$$k_{\text{obs}} = (k_0^{\text{H}} \cdot [\text{H}^+]) + (k_0^{\text{S}} + \left(\frac{k_0^{\text{OH}} \cdot K_{\text{w}}}{[\text{H}^+]} \right)) \quad (1)$$

$$\begin{aligned} k_{\text{obs}} = & \left[(k_0^{\text{H}} \cdot [\text{H}^+]) + (k_0^{\text{S}} + k_1^{\text{H}} \cdot K_{\text{a}}) \right. \\ & + \frac{(k_0^{\text{OH}} \cdot K_{\text{w}} + k_1^{\text{S}} \cdot K_{\text{a}})}{[\text{H}^+]} \\ & \left. + \frac{(k_1^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}})}{[\text{H}^+]^2} \right] / \left[1 + \frac{K_{\text{a}}}{[\text{H}^+]} \right] \quad (2) \end{aligned}$$

$$\begin{aligned} k_{\text{obs}} = & \left[(k_0^{\text{H}} \cdot [\text{H}^+]) + (k_0^{\text{S}} + k_1^{\text{H}} \cdot K_{\text{a}1}) \right. \\ & + \frac{(k_0^{\text{OH}} \cdot K_{\text{w}} + k_1^{\text{S}} + k_2^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2})}{[\text{H}^+]} \\ & + \frac{(k_1^{\text{OH}} \cdot K_{\text{a}1} \cdot K_{\text{w}} + k_2^{\text{S}} K_{\text{a}1} \cdot K_{\text{a}2})}{[\text{H}^+]^2} \\ & \left. + \frac{(k_2^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2})}{[\text{H}^+]^3} \right] / \left[1 + \frac{K_{\text{a}1}}{[\text{H}^+]} + \frac{K_{\text{a}1} \cdot K_{\text{a}2}}{[\text{H}^+]^2} \right] \quad (3) \end{aligned}$$

$$\begin{aligned} k_{\text{obs}} = & \left[(k_0^{\text{H}} \cdot [\text{H}^+]) + (k_0^{\text{S}} + k_1^{\text{H}} \cdot K_{\text{a}1}) \right. \\ & + \frac{(k_0^{\text{OH}} \cdot K_{\text{w}} + k_1^{\text{S}} \cdot K_{\text{a}1} + k_2^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2})}{[\text{H}^+]} \\ & + \frac{(k_1^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} + k_2^{\text{S}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} + k_3^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3})}{[\text{H}^+]^2} \\ & + \frac{(k_2^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} + k_3^{\text{S}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3})}{[\text{H}^+]^3} \\ & \left. + \frac{(k_3^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3})}{[\text{H}^+]^4} + \right] \\ & / \left[1 + \frac{K_{\text{a}1}}{[\text{H}^+]} + \frac{K_{\text{a}1} \cdot K_{\text{a}2}}{[\text{H}^+]^2} + \frac{K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3}}{[\text{H}^+]^3} \right] \quad (4) \end{aligned}$$

$$\begin{aligned} k_{\text{obs}} = & \left[(k_0^{\text{H}} \cdot [\text{H}^+]) + (k_0^{\text{S}} + k_1^{\text{H}} \cdot K_{\text{a}1}) \right. \\ & + \frac{(k_0^{\text{OH}} \cdot K_{\text{w}} + k_1^{\text{S}} \cdot K_{\text{a}1} + k_2^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2})}{[\text{H}^+]} \\ & + \frac{(k_1^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} + k_2^{\text{S}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} + k_3^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3})}{[\text{H}^+]^2} \\ & + \frac{(k_2^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} + k_3^{\text{S}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} + k_4^{\text{H}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} \cdot K_{\text{a}4})}{[\text{H}^+]^3} \\ & + \frac{(k_3^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} + k_4^{\text{S}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} \cdot K_{\text{a}4})}{[\text{H}^+]^4} \\ & \left. + \frac{(k_4^{\text{OH}} \cdot K_{\text{w}} \cdot K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} \cdot K_{\text{a}4})}{[\text{H}^+]^5} \right] \\ & / \left[1 + \frac{K_{\text{a}1}}{[\text{H}^+]} + \frac{K_{\text{a}1} \cdot K_{\text{a}2}}{[\text{H}^+]^2} + \frac{K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3}}{[\text{H}^+]^3} + \frac{K_{\text{a}1} \cdot K_{\text{a}2} \cdot K_{\text{a}3} \cdot K_{\text{a}4}}{[\text{H}^+]^4} \right] \quad (5) \end{aligned}$$

The subscript of a micro reaction constant in Eq. (1), Eq. (2), Eq. (3), Eq. (4), Eq. (5) indicates the successive deprotonation steps and a superscript indicates the type of catalysis. The fully protonated species is indicated with the subscript 0. The proton, solvent and hydroxyl catalysis are indicated with the superscripts H, S and OH, respectively. The constants for the successive protolytic equilibria are indicated with K_{a} with the number corresponding to the equilibrium added to the subscript a. K_{w} is the autoprotolysis con-

stant of water. Eqs. (2)–(5), however, cannot be applied for regression analysis, because each value of the sum of the combined kinetically indistinguishable reactions yields an infinite number of values for the contributing kinetically indistinguishable micro reaction constants. For regression analysis it is therefore necessary to replace each group of kinetically indistinguishable reaction constants by a single constant: the macro reaction constant (M_i). By doing so Eqs. (1)–(5) are transformed into Eqs. (6)–(10).

$$k_{\text{obs}} = M_0 \cdot [\text{H}^+] + M_1 + \frac{M_2}{[\text{H}^+]} \quad (6)$$

$$k_{\text{obs}} = \frac{M_0 \cdot [\text{H}^+] + M_1 + \frac{M_2}{[\text{H}^+]} + \frac{M_3}{[\text{H}^+]^2}}{1 + \frac{K_a}{[\text{H}^+]}} \quad (7)$$

$$k_{\text{obs}} = \frac{M_0 \cdot [\text{H}^+] + M_1 + \frac{M_2}{[\text{H}^+]} + \frac{M_3}{[\text{H}^+]^2} + \frac{M_4}{[\text{H}^+]^3}}{1 + \frac{K_{a1}}{[\text{H}^+]} + \frac{K_{a1} \cdot K_{a2}}{[\text{H}^+]^2}} \quad (8)$$

$$k_{\text{obs}} = \frac{M_0 \cdot [\text{H}^+] + M_1 + \frac{M_2}{[\text{H}^+]} + \frac{M_3}{[\text{H}^+]^2} + \frac{M_4}{[\text{H}^+]^3} + \frac{M_5}{[\text{H}^+]^4}}{1 + \frac{K_{a1}}{[\text{H}^+]} + \frac{K_{a1} \cdot K_{a2}}{[\text{H}^+]^2} + \frac{K_{a1} \cdot K_{a2} \cdot K_{a3}}{[\text{H}^+]^3}} \quad (9)$$

$$k_{\text{obs}} = \frac{M_0 \cdot [\text{H}^+] + M_1 + \frac{M_2}{[\text{H}^+]} + \frac{M_3}{[\text{H}^+]^2} + \frac{M_4}{[\text{H}^+]^3} + \frac{M_5}{[\text{H}^+]^4} + \frac{M_6}{[\text{H}^+]^5}}{1 + \frac{K_{a1}}{[\text{H}^+]} + \frac{K_{a1} \cdot K_{a2}}{[\text{H}^+]^2} + \frac{K_{a1} \cdot K_{a2} \cdot K_{a3}}{[\text{H}^+]^3} + \frac{K_{a1} \cdot K_{a2} \cdot K_{a3} \cdot K_{a4}}{[\text{H}^+]^4}} \quad (10)$$

Comparison of the corresponding pairs of equations (Eq. (1) and Eq. (6), Eq. (2) and Eq. (7), Eq. (3) and Eq. (8), Eq. (4) and Eq. (9), Eq. (5) and Eq. (10)) shows which of the individual reaction constants are combined to a particular macro reaction constant.

3. A step-by-step procedure for the interpretation of a pH-degradation profile

After the determination of the overall degradation rate constants (k_{obs}) over a range of pH values the first step in the interpretation of the profile is the choice of the equation corresponding to the number of protolytic equilibria in which the degrading substance is involved. It is pivotal that this model equation is selected in accordance to the number of protolytic equilibria and not to the shape of the profile. Profiles of drugs involved in different numbers of protolytic equilibria may nevertheless have similar shapes. The interpretation of the shape of the profile, however, is entirely different, if different numbers of species are involved in the degradation process. The ultimate objective of the interpretation of pH-degradation profiles is not only to obtain a theoretical model, which corresponds closely to the measured values for k_{obs} , but also to correlate each part of the profile to the actually contributing reaction(s). A classification of pH-degradation profiles on their shape, as described by Carstensen (1990), is therefore of limited use. The shape of the pH-degradation profile is only important as far as it enables the investigator to recognize the contributing macro reaction constants. If a $\text{p}K_a$ value of the degrading substance lies more than 2 pH units above the pH range investigated, the profile can very often adequately be described with a reduced model equation based solely upon the number of protolytic equilibria relevant within the pH range of interest. The reduction is obtained by omitting the last term of the numerator and that of the nominator, resulting in a model equation equivalent to the model equation for a degrading substance that is involved in one protolytic equilibrium less. The new equation, however, still contains contributions of the proton catalyzed degradation and the solvent catalyzed degradation of the fully deprotonated species in the last but one and the last macro reaction constant, respectively. If the last macro reaction constant from the numerator of the original equation con-

tributes significantly to the overall degradation at the end of the pH range investigated the reduction of the numerator is not allowed. Such cases (which are rare) can be recognized from the fact that the $\log k_{\text{obs}}\text{-pH}$ plot exhibits a slope higher than +1 in the last part of the pH profile.

If the neglected $\text{p}K_{\text{a}}$ lies more than 2 pH units below the pH range investigated, the model equation can be adapted by omitting the first term of the numerator ($k_0^{\text{H}} \cdot [\text{H}^+]$) and that of the nominator (1) and subsequently dividing all remaining terms of the numerator and the nominator by the second term of the nominator ($K_{\text{a1}}/[\text{H}^+]$). This results in a model equation which is mathematically equivalent to a model equation for a degrading substance that is involved in one protolytic equilibrium less. The new equation, however, still contains the contributions of the solvent catalyzed degradation and the hydroxyl catalyzed degradation of the fully protonated species in M_0 and M_1 , respectively. If the first macro reaction constant of the numerator of the original equation contributes significantly to the first part of the pH-degradation profile, it may be necessary to maintain the first term of the original numerator, divided by $K_{\text{a1}}/[\text{H}^+]$, in the numerator of the reduced model equation. Such cases can be recognized from the fact that the $\log k_{\text{obs}}\text{-pH}$ plot exhibits a slope lower than -1 in the first part of the pH profile.

The second step is the calculation of the macro reaction constants and the $\text{p}K_{\text{a}}$ values by regression analysis. A reliable calculation of the $\text{p}K_{\text{a}}$ values, however, requires that the corresponding inflection points in the profile are pronounced, which means that minimally two measurements (at least 1 pH unit apart) at both sides of the inflection point of the $\text{p}K_{\text{a}}$ should be available and that these four measurements are (mainly) determined by the same macro reaction constant. If that is not the case the precision in the estimated values of the macro reaction constants may decrease dramatically. In such cases the $\text{p}K_{\text{a}}$ values should be determined by an independent method and used as constants in the regression analysis.

The third step in the interpretation of the pH-profile consists of the analysis of the potential contributions of the kinetically indistinguishable

micro reactions to the macro reaction constants. Such interpretations can be based upon the nature of the degradation product(s), the expected reaction mechanisms, the required order of magnitude to contribute significantly, the influence of ionic strength on the observed degradation rate and/or solvent isotope effects (Isaacs, 1987). One should, however, keep in mind that every macro reaction constant is essentially the sum of the contributions of all corresponding micro reactions constants and that it is possible that more than one individual reaction contributes significantly to a macro reaction constant.

If the degrading substance is involved in more than one protolytic equilibrium and if the protolytic equilibria overlap (partially), it may be impossible to determine which of the specific micro reaction constants contributes to the macro reaction constant. In this case the use of macro reaction constants is then to be preferred for the description of the pH-degradation profiles.

To illustrate the usefulness of our approach in comparison with those in the literature we evaluated a number of recent publications in the field of drug stability studies.

4. Interpretations of pH-degradation profiles from recent literature

4.1. Recognition of kinetically indistinguishable micro reactions

Almost all investigators by-pass the problem of kinetically indistinguishable reactions in their model equations by reducing each group of kinetically indistinguishable reactions to a single reaction while neglecting the others. The model equation thus obtained is based on only one of the many potential combinations of kinetically indistinguishable micro reactions. The number of these combinations increases with the number of protolytic equilibria: if the substance is involved in a single protolytic equilibrium the investigator has to choose one from four possible combinations. For each additional protolytic equilibrium

the number of combinations increases three-fold: twelve combinations for two protolytic equilibria, thirty six combinations for three protolytic equilibria etc. One should justify selection by proving that the reactions chosen are the determinants of the observed degradation. Although some authors recognize the existence of kinetically indistinguishable reactions (Al-Razzak and Stella, 1990; Bundgaard et al., 1986; Buur et al., 1988; Jensen and Bundgaard, 1991; Kahns and Bundgaard, 1990, 1991; Kearney and Stella, 1993; Oliyai and Borchardt, 1993; Safadi et al., 1993), a complete account for the choice of each of the micro reactions is very rare. Consequently the reactions included in the model equations are not necessarily the reactions that lead to the degradation, even when the plot of the model equation and the measured values of the degradation rate fit perfectly. To avoid this type of misunderstanding the use of macro reaction constants is to be preferred in model equations. The existence of kinetically indistinguishable reactions may easily be overlooked when the protolytic equilibria of the drug involved lie outside the pH range of interest and the model equation is reduced to an equivalent of Eq. (6). An example of such studies is the interpretation of the pH-degradation profile of diltiazem (Won and Iula, 1992) with a pK_a value above the pH range investigated.

In some studies the micro reactions proposed by the authors do not correspond with the macro reaction constants in their model equation. In the study of the decomposition of sodium diethyldithiocarbamate Martens et al. (1993) conclude that the degradation is acid catalyzed and that the degrading species is protonated. Their model equation, however, is equivalent with Eq. (7) from which all macro reaction constants except M_1 have been omitted. M_1 combines the kinetically indistinguishable proton catalyzed degradation of the deprotonated species with the solvent catalyzed degradation of the protonated species. The shape of the pH-degradation profile is in agreement with that model. Proton catalyzed degradation of the protonated species corresponds with M_o and as a consequence the investigators conclusions about the degradation mechanism are not correct.

4.2. Neglect of pK_a -values outside the investigated pH range

pK_a values within 2 pH units from the pH range investigated will influence the shape of the pH-degradation profile. The corresponding protolytic equilibrium should, in these cases, be taken into consideration in the model equation. This has not been done in the degradation studies of ASP hexapeptide by Oliyai and Borchardt (1993), batanopride ([4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-[(1-methylacetyl)oxy]benzamide) by Nassar et al. (1992) and 3-phosphoryloxymethyl-5,5-diphenylhydantoin by Kearney and Stella (1993). The ASP hexapeptide Val-Tyr-Pro-Asp-Gly-Ala, studied by Oliyai and Borchardt (1993), contains four protolytic functions associated with the terminal carboxylic group of the peptide chain, the carboxylic group of aspartic acid, the phenolic moiety of tyrosine and the terminal amino function of the peptide chain. Degradation products originate from hydrolysis at the Asp-Gly bond and the formation of a cyclic imide. The authors presented two partial pH-rate profiles, each of which corresponded to the degradation into one of these degradation products. Such partial pH-rate profiles are described by the same equations as the pH profiles of the overall degradation rate. These equations are derived analogous to Eq. (1), Eq. (2), Eq. (3), Eq. (4), Eq. (5) (Van der Houwen et al., 1988). The main difference is that in the model equation for the partial pH-rate profiles the individual rate constants refer to reactions resulting in a particular degradation product, while in that for overall pH-degradation profile these constants refer to all degradation reactions. For the hydrolysis reactions the authors provided a partial pH-rate profile from pH 0.3–4.0 (Fig. 1A). The authors calculated a pK_a of 3.5 ± 0.2 by regression analysis. For the formation of the cyclic imide the authors presented a partial pH-rate profile for the pH range 0.3–5.0. The model equation for this profile was based on an equation for two protolytic equilibria equivalent to Eq. (8) from which the macro reaction constants M_3 and M_4 were deleted. From this equation the authors calculated pK_a values of 3.1 ± 0.05 and 5.2 ± 0.05 , respec-

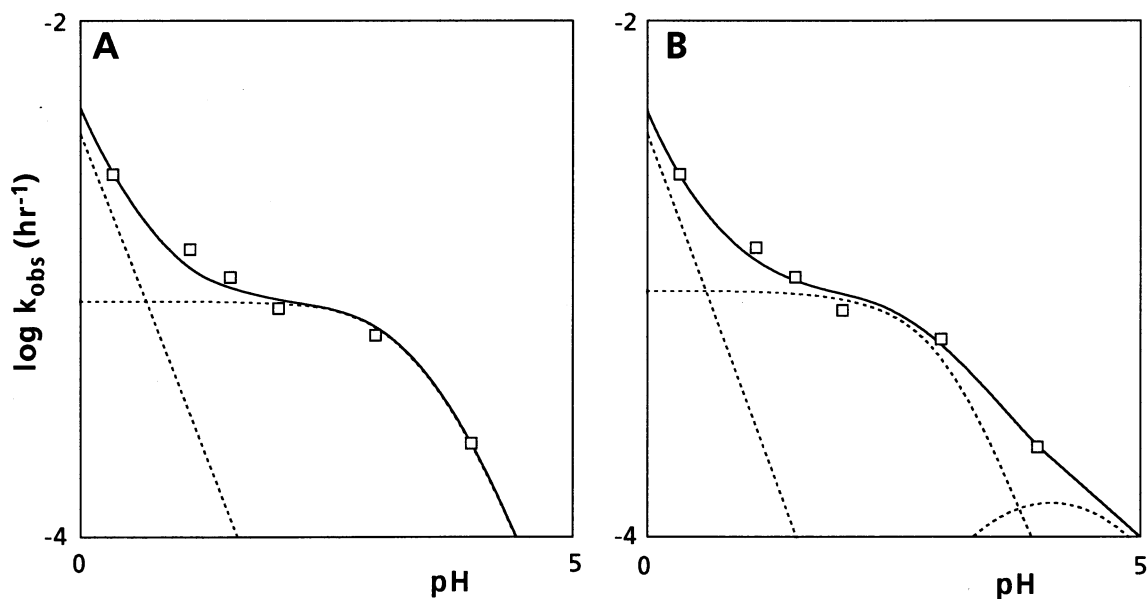


Fig. 1. Partial pH rate profiles for the hydrolysis of Val-Tyr-Pro-Asp-Gly-Ala hexapeptide. The contributions of the macro reactions are indicated with dotted lines. A: calculated with the model equation with pK_a 3.5 and the two rate constants as reported by Oliyai and Borchardt (1993). B: calculated with a model equation for two protolytic equilibria with pK_a values of 3.1 and 5.2 and values of $3.4 \times 10^3 \text{ h}^{-1} \text{ M}^{-1}$, $8.8 \times 10 \text{ h}^{-1}$ and $1.3 \times 10^{-8} \text{ h}^{-1} \text{ M}^{-1}$ for M_0 , M_1 and M_2 , respectively.

tively, by regression analysis. The authors noticed that a pK_a value of 5.2 is significantly higher than the value expected for a C-terminus carboxylic acid (3.0–4.7), which they explained as: ‘that the inflection in the pH-rate profile at a pH value of approximately 5.2 is, in reality a kinetic pK_a and corresponds to a change in the rate-determining step in the reaction sequence rather than the second dissociation of the reactant’. Regarding the use of the model equations in this study, it is inconsistent that the authors used two model equations based on different numbers of protolytic equilibria for two nearly identical pH ranges. The neglect of the second protolytic equilibrium (pK_a 5.2) in the model equation for the hydrolysis is not allowed if the degradation is studied at pH 4. It would be even more unjustifiable, if the second pH would have had the expected value between 3.0 and 4.7. The difference between the values obtained for the pK_a of the first protolytic equilibrium seems to be somewhat large (0.4). This difference increases to 0.5 units if the second protolytic equilibrium (with the

assumed pK_a of 5.2) is taken into account. A lower value for the second pK_a would increase this difference even more. The influence of the second pK_a on the value obtained for the first pK_a is pronounced because the degradation rate at pH 4.0 is the only one measured value above the first pK_a and pH 4.0 is rather close to the value of the second pK_a .

If the macro reaction constant M_2 (which was omitted from the equation for the partial pH-rate profile for the hydrolysis) is included in the model equation the pK_a obtained by regression analysis decreases to 3.2. The contribution of M_2 would be in agreement with the hydrolysis between pH 4 and 5 reported by the authors in their summary. Fig. 1B shows the partial pH-rate profile for the hydrolysis obtained with regression analysis using Eq. (8) from which M_4 and M_5 have been omitted and in which 3.1 and 5.2 are used as constants for the pK_a values.

Comparison with the original profile (Fig. 1A) illustrates that by omitting M_2 from the equation the discrepancy between the pK_a values as calcu-

lated from the two partial pH-rate profiles emerges. The unexpected high value for the pK_a of the second protolytic equilibrium can be explained by a contribution of the macro reaction constant M_3 to the degradation measured at pH 5 (again the only degradation measurement above the pK_a). This may explain its value rather than a 'kinetic' pK_a , which is different from the pK_a responsible for the protolytic equilibrium. The existence of such a 'kinetic' pK_a would add one more pK_a to the model equation for the actual pK_a would still exert its influence on the pH profile by changing the ratio of reacting species on changing pH. The authors did not present an overall pH-degradation profile. However, if the data presented by the authors for k_{obs} are analyzed with regression analysis a pK_a value of 4.5 ± 0.1 is obtained for the second protolytic equilibrium.

In the study of the degradation of batanopride (Nassar et al., 1992) the pK_a of the aromatic amino group is expected to be close to or even within the pH range investigated (2–10). Its influence, however, is not taken into account and the potential contribution of the fully protonated species to the degradation is not considered. The correct model equation would have been Eq. (8).

In the study of the degradation of 3-phosphoryloxymethyl-5,5-diphenylhydantoin in the pH range 1–7 Kearney and Stella (1993) used a model equation based upon two protolytic equilibria with the solvent catalyzed contribution to M_2 as the only degradation reaction. By doing so they neglected the acidic character of 5,5-diphenylhydantoin (expected pK_a value of approximately 8) which may contribute significantly to the shape of the pH-degradation profile at pH 7 and higher. Above pH 6 their calculated pH-degradation profile deviates from the observed degradation rate in such a manner which strongly suggests that it is due to the neglected macro reaction constant M_3 . Thus in this case the macro reaction constant M_3 is wrongly omitted from the model equation. The authors calculated the first protolytic dissociation constant of 3-phosphoryloxymethyl-5,5-diphenylhydantoin from the de-

crease in k_{obs} between pH 1 and 2. In this pH range only one measurement was performed. The calculation of a pK_a value is here not justified since it is impossible to conclude from these data whether M_1 and M_0 contribute or not.

4.3. Neglect of pK_a values within the investigated pH range

In the following examples we discuss studies in which pK_a values situated within the pH range investigated were not taken into consideration in the model equations. The pH-degradation profile of morphine dipropionyl ester (Drustrup et al., 1991) was interpreted according to Eq. (6). The authors drew attention to the fact that no inflection point was observed in the profile at the pH value that corresponds with the pK_a of the substance. They explained this by assuming that the reactivities of the protonated and the deprotonated species towards hydroxide ions are equal. The implication of this assumption is that Eq. (6) cannot be utilized. The correct description is given by Eq. (7). The interpretation of the profile by proton, hydroxyl and solvent catalysis ignores the existence of kinetically equivalent reactions, which are included in Eq. (7).

Brandl et al. (1993) used a model equation, that is mathematically equivalent to Eq. (6), for the degradation of 4'-azidothymidine in the pH range 0–10. They determined a pK_a of 9.33 at 50°C by potentiometric titration. They interpreted 'the absence of curvature at high pH' as the result of 'equal reactivity of the protonated and deprotonated species' although the pH profile clearly suggests that such a curvature exists above pH 9. The recognition of the existence of a protolytic equilibrium in combination with the use of Eq. (6) as a model equation is inconsistent.

Lee and Lee (1989) ignored the protolytic equilibrium of the carboxylic acid function of tolrestat (*N*-[[6-methoxy-5-(trifluoromethyl)-1-naphthalenyl]thioxomethyl]-*N*-methylglycine) by using a model equation equivalent to Eq. (6) from which M_2 has been omitted. The pH-degradation profile was determined over the pH range 0–10. The

profile exhibits a slope of -1 between pH 0 and 2 and almost a plateau between pH 4 and pH 10. The pK_a value of tolrestat can be expected to be between 3 and 5. If the profile is explained on the basis of Eq. (7), which is the appropriate model equation, M_0 is the main contributing macro reaction between pH 0 and 2, and a combination of M_1 and M_2 is responsible for the pH-degradation profile above pH 3. This combination can account for the slight minimum between pH 3 and 5 in the pH profile measured at 75°C , presented by the authors (Lee and Lee, 1989, Fig. 4). M_3 does not contribute within the pH range studied. For a correct interpretation of the pH profile the pK_a value of tolrestat should have been determined by an independent method since the profile does not exhibit a sufficiently marked inflection point to enable a reliable calculation of the pK_a value.

Maniar et al. (1992) described the pH-degradation profiles of the oligomers of tartrate esters with Eq. (6), although they recognized the existence of two protolytic equilibria in their study. The pK_a values of the carboxylic groups involved are expected to be approximately 3–4. For two protolytic equilibria the correct model equation is Eq. (8). If the pH-degradation profile is interpreted with this equation it becomes clear that M_0 and M_4 contribute significantly in the pH ranges of 1–3 (slope -1) and 5–9 (slope $+1$), respectively. Between pH 3 and 5 the macro reaction constants (M_1 , M_2 and M_3) contribute. The interpretation of the authors that between pH 3 and 5 there is no proton nor hydroxyl catalysis, is therefore not correct.

Longhi and Bertorello (1990) observed an inflection point at about pH 1 in the pH-degradation profile of *N*-(3,4-dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone, which they attributed to a protolytic equilibrium with a pK_a value of 1.10 (quoted from the literature). The authors, however, used Eq. (6) as a model equation while omitting the constant M_2 . The use of their model equation, meant for compounds not subject to protolytic equilibria and thus conflicting with their observation, makes the interpretation of the inflection point thus irrational. Eq. (7) is the correct model. The shape of the pH profile indicates that M_1 , and M_2 are the main contributing

macro reaction constants. These constants contain two kinetically equivalent, and thus indistinguishable, degradation reactions. It is therefore not justified to interpret the pH-degradation profile as the result of hydrogen catalyzed and solvent catalyzed degradation only.

4.4. Mathematical errors in model equations

Kahns and Bundgaard used a model equation for substances involved in a single protolytic equilibrium which differed from Eq. (7) in that M_0 and M_1 are not divided by the nominator, in opposition to M_2 and M_3 . They applied this equation both in a study of the degradation of esters of *N*-acetylcysteine (Kahns and Bundgaard, 1990) and in a study of the degradation of various *N*-acylderivatives (Kahns and Bundgaard, 1991). The implication of their equation is that the contribution of proton and solvent catalyzed degradation of the protonated species would occur independently of the degree of protonation of the substance (in opposition to the hydroxyl catalyzed degradation of the protonated and deprotonated species). This implication shows that the equation is fundamentally wrong. For the substances investigated this error fortunately has no consequences for the regression analysis because the contributions of M_2 and M_3 dominate the pH profile starting from more than 2 pH units below the pK_a value.

The studies of Jordan, Quigley and Timoney on the hydrolysis of oxprenolol esters (Jordan et al., 1992) and propranolol esters (Quigley et al., 1994) deserve some comment. In both studies the authors used a model equation in which the proton, the solvent and the hydroxyl catalyzed degradation of the protonated species were combined with the hydroxyl catalyzed degradation of the deprotonated species. This model should have been an equation equivalent to Eq. (7). However, the authors multiplied the rate constant of the solvent catalyzed degradation reaction with the proton activity. As a result the solvent catalyzed degradation constant was combined with the proton catalyzed degradation constant in the term corresponding with M_0 by which the model was missing the term corresponding with M_1 .

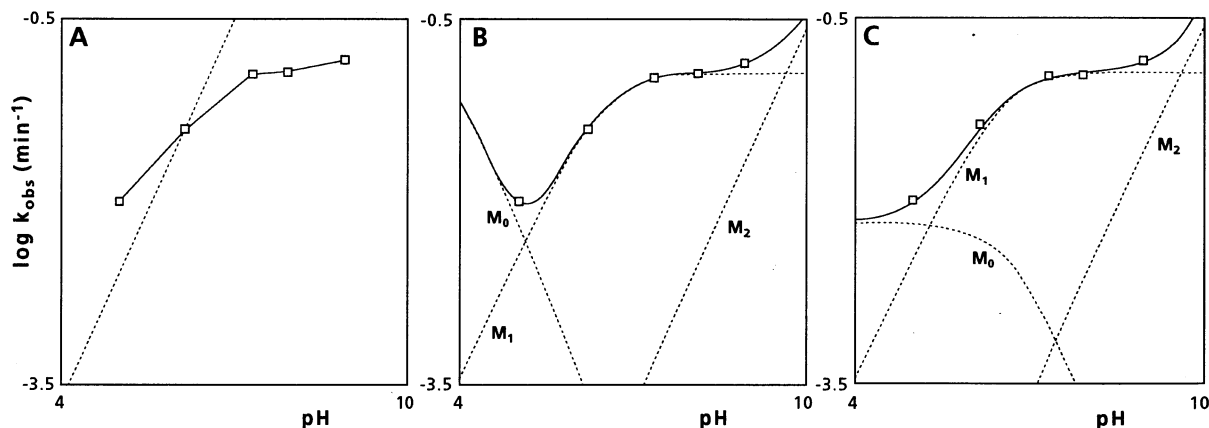


Fig. 2. pH-Degradation profiles of propranolol acetate. A: The pH-degradation profile as presented by Quigley et al. (1994). The dotted line represents the calculated model based upon a pK_a value of 8.39, a reaction constant of 1.09×10^5 for the hydroxyl catalyzed degradation of the protonated propranolol acetate and a pK_w value of 13.62 as reported by Quigley et al. B: The pH-degradation profile obtained by recalculation with Eq. (7) from which M_1 has been omitted (model equation as described by Quigley et al.). The contribution of the macro reactions is indicated with dotted lines and the symbols M_0 , M_2 and M_3 . C: The pH-degradation profile obtained by recalculation with Eq. (7) from which M_0 has been omitted. The contribution of the macro reactions is indicated with dotted lines and the symbols M_1 , M_2 and M_3 .

The authors used their model equation in the discussion about the potential contributions of the three terms in the numerator and not for regression analysis. They concluded that the hydroxyl catalyzed degradation of the protonated species is the main degradation reaction in the pH range investigated. When the pH profile of propranolol acetate, based on the reported values for this rate constant and the pK_a , is plotted in combination with the observed degradation rates Fig. 2A is obtained. The pH profile presented by the authors is included in this figure. If the authors had used regression analysis with their own (incomplete) equation, they would have obtained the pH profile in Fig. 2B.

Regression analysis with the complete equation (Eq. (7)) is impossible because the calculation of the two macro reaction constants M_0 and M_1 , depends on only a single measurement of the degradation rate (pH 5.0). It is therefore impossible to discriminate between the contribution of these two macro reaction constants. Each of these two constants, however, can be calculated if the other constant is arbitrarily set to zero. The pH profile obtained using the macro reaction constant M_1 and neglecting the macro reaction constant

M_0 is depicted in Fig. 2C. Comparison of Fig. 2B and Fig. 2C shows that a single measurement at pH 4 would have given sufficient additional information to calculate both macro reaction constants.

The difference between the calculated pH profiles (Fig. 2B and 2C) and the pH profile presented by the authors (Fig. 2A) is caused by the fact that the inflection point in the pH profile does not correspond with the pK_a values reported by the authors. Table 1 shows the differences between the pK_a values obtained by titration and those obtained with our regression analysis for a number of propranolol esters. The discrepancy be-

Table 1

Comparison of the pK_a values of Quigley et al. (1994), determined by titration and the pK_a values determined by recalculation from the pH-degradation profile

Propranolol ester	pK_a From titration	pK_a From pH-degradation profile
<i>O</i> -acetyl	8.39	6.56
<i>O</i> -isobutyryl	7.63	6.37
<i>O</i> -cyclopropionyl	7.66	6.76
<i>O</i> -crotonyl	8.44	6.79

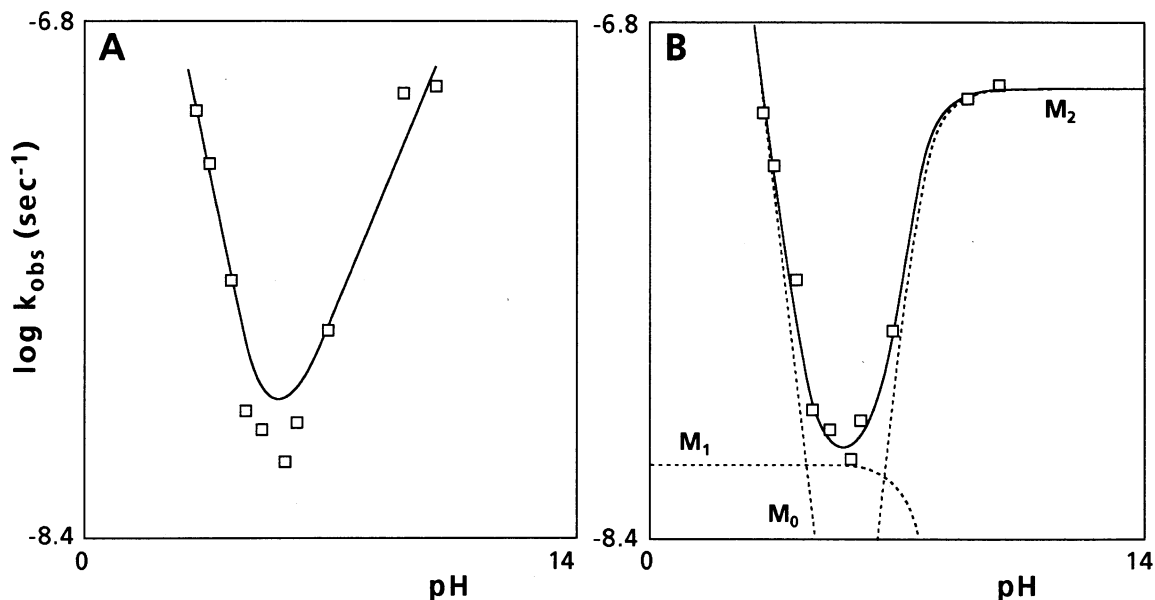


Fig. 3. pH-Degradation profiles of nefopam hydrochloride. A: as presented by Tu et al. (1990) B: as obtained by recalculation with Eq. (7). The contribution of the macro reactions is indicated with dotted lines and the symbols M_0 – M_2 .

tween the calculated profile and the measured degradation rates explains why the authors calculated different values for the rate constant for hydroxyl catalyzed degradation of the protonated species at pH 6.2 ($1.09 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$) and at pH 7.4 ($0.203 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$).

The differences between the reported pK_a values and those from our regression analysis are unacceptably large. The occurrence of buffer catalysis could explain these differences. The authors used phosphate and citrate buffers in the pH range 2–8. Buur et al. (1988), however, reported that there is no catalysis by phosphate buffers for the decomposition of propranolol esters. Another explanation might be that the values for the pH or the observed degradation rate constants were not correct. An indication for this hypothesis are the significantly different values for the pK_a (8.3) and the rate constant for hydroxyl catalyzed degradation ($2.6 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$) of the protonated species calculated by Buur et al. (1988) from the degradation pH-profile of the acetyl ester of propranolol. Adequate mathematical treatment of their data should have informed the authors about the discrepancies between their model and experimental data.

4.5. Application of a model equation with non-integer exponents

A number of authors describe the pH-degradation profile with the equation

$$k_{\text{obs}} = k^{\text{H}} \cdot [\text{H}^+]^m + k^{\text{S}} + k^{\text{OH}} \cdot [\text{OH}]^n \quad (11)$$

in which the exponents m and n may be non-integer values. If the exponents have the value -1 and 1 , respectively, Eq. (11) equals Eq. (6). There is no theoretical basis for Eq. (11) with non-integer values for m and n . It may originate from attempts to apply Eq. (6) to pH-degradation profiles in which protolytic equilibria play a role but in which inflection points are not very pronounced. Parts of such profiles may, at a superficial glance, appear to be linear although in reality they are a combination of non-linear contributions of macro reactions. This is the case with the following four studies.

Although the existence of a protolytic equilibrium within the pH range investigated for nefopam is recognized by Tu et al. (1990) they did not use Eq. (7) but Eq. (11) instead as a model equation (Fig. 3A). This equation gave a rather unsatisfactory fit between the model and the ob-

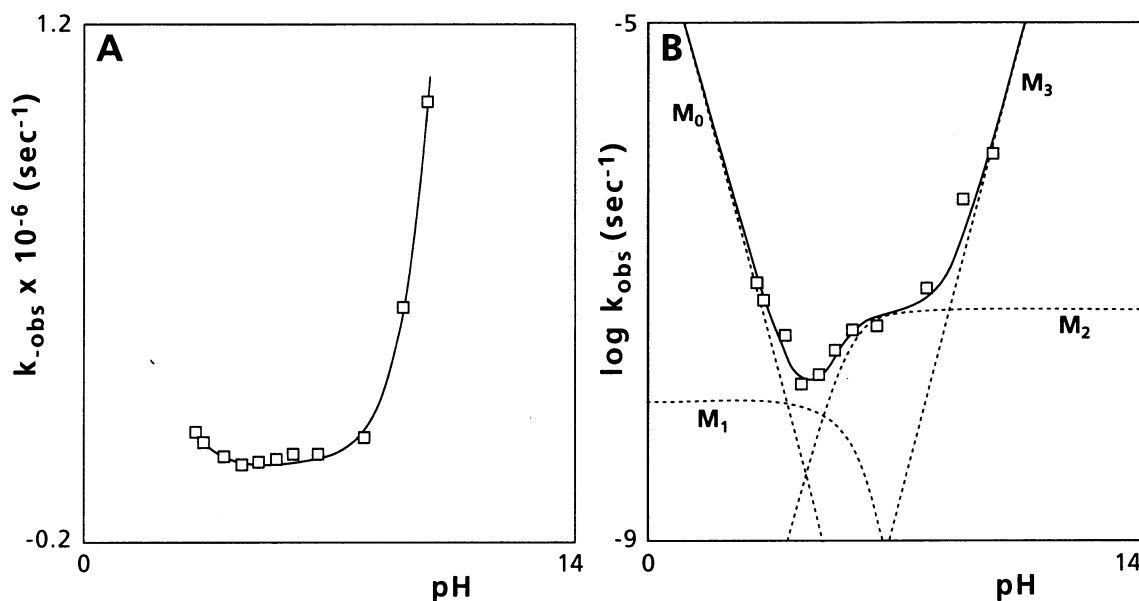


Fig. 4. pH Rate profiles of metronidazole. A: as presented by Wang and Yeh (1993) B: as obtained by recalculation with Eq. (7). The contribution of the macro reactions is indicated with dotted lines and the symbols M_0 – M_3 .

served degradation rate. Their explanation that the non-integer orders of m and n might imply the existence of some intermediates in the acid base catalytic degradation sequences is not convincing. Reanalyzing the pH-degradation profile using Eq. (7) results in a much better fit (Fig. 3B).

In the investigation of the degradation of metronidazole (Wang and Yeh, 1993) the authors found values of $m = -0.57$ and $n = 0.61$ for the exponents in Eq. (11). The authors interpret these non-integer exponents by 'the existence of some intermediate formations during the acid/base catalytic degradation sequences of metronidazole' which is inconsistent with the nature of a first order reaction. The inflection point due to the pK_a is obscured in the pH-profile presented by the authors as result of the use of a non-logarithmic scale for the observed degradation rate. Non-linear regression analysis of the observed degradation with Eq. (7) results in an acceptable fit as is illustrated by comparison of the original plot in Fig. 4A with the logarithmic plot in Fig. 4B.

The cyclic heptapeptide MT-II, studied by Ugwu et al. (1994), has prototropic functions due to a histidine and arginine residue with estimated

pK_a values of, 6 and 13, respectively (Perrin, 1965a,b). For the interpretation of the pH-degradation profile Eq. (8) is the appropriate model equation. As the degradation is investigated between pH 2 and 10, the pK_a of the arginine residue may be neglected whereby Eq. (8) is replaced by Eq. (7) for this limited pH range. Ugwu et al. (1994) however, used Eq. (11). For the exponents m and n they found values of -0.102 and 0.127 , respectively. They explained this result by assuming that 'MT-II degrades by several different pathways, each with its own pH dependence and true catalytic coefficients'. Non-linear regression analysis of the observed degradation with Eq. (7) as the model and a pK_a value of 6.0 for the histidine moiety yields an acceptable fit as is illustrated by Fig. 5. As no values for the observed rate constants have been reported in this study the calculation has been performed with estimations from one of the figures (Ugwu et al., 1994, Fig. 5).

A particular case is the study of the degradation of moricizine (King et al., 1992). The authors described a model equation equivalent to Eq. (7) from which the term containing M_3 was omitted and in which M_0 , M_1 and M_2 were replaced by the

proton, solvent and hydroxyl catalyzed degradation reactions of the protonated moricizine, respectively. They claimed to have used this model equation for non-linear regression analysis and report values for the three reaction constants.

The plot of this model equation should exhibit an inflection point around the pK_a value of the morpholinyl group. However, this inflection point is absent in the plot presented and the authors do not report a calculated value for the pK_a . Moreover the authors mentioned a value of -0.92 for the slope between pH 0 and pH 2 and a slope of 0.79 between pH 4 and 6 which suggests linear regression on parts of the pH profile rather than the reported non linear regression. The authors claim that the fractional values of the descending and ascending slopes are indications for the complexity of the reactions involved. To our opinion these are artifacts resulting from an unjustified attempt to apply linear regression to a non-linear system. Comparison of the plot presented by the authors (Fig. 6A) with the fit obtained with Eq. (7) (Fig. 6B) illustrates that there is a good correlation between the model equation and the values for k_{obs} which implies that there is no need for

slopes with non-integer values. The slope of 0.79 clearly appears to be the result of extending the linear part of the profile in the area of the inflection point caused by the pK_a . Regression analysis indicates that this pK_a has a value of approximately 6. This value is roughly in accordance with literature values for the pK_a of some substituted morpholines (Perrin, 1965c). For a more precise calculation of the pK_a a few measurements of k_{obs} at pH values above pH 6 would be necessary.

5. Buffer-catalysis

The occurrence of buffer-catalysis is investigated in a number of studies. Such effects are generally demonstrated by a linear relationship between the degradation rate and the buffer concentration at fixed pH and fixed ionic strength. Usually this investigation is performed at a single pH. The following studies are exceptions in this respect.

In the study of the degradation of moricizine (King et al., 1992) the catalytic effects of phosphate and acetate have each been measured at two different pH values. The authors interpreted the increase in degradation rate with increase of pH as a catalytic effect of acetate and secondary phosphate ions on the protonated form of moricizine. Kearney and Stella (1993) observed in their study on the hydrolysis of phosphate esters a catalytic effect by phosphate buffer. As the influence decreased with higher pH values they interpreted this as an 'apparent general acid catalyzed reaction pathway'. An analogous remark was made by Naringrekar and Stella (1990) regarding the catalytic effect of phosphate buffers on the hydrolysis of enaminones. Won and Iula (1992) observed catalytic effects from acetate and phosphate on the degradation of diltiazem. Fort and Mitra (1990) investigated the influence of acetate, phosphate and borate on the degradation of methotrexate dialkyl esters. Reubsæet et al. (1995) described phosphate catalyzed degradation of Antagonist [Arg⁶,D-Trp^{7,9},MePhe⁸]-Substance P{6-11}. None of these studies, except that of Reubsæet et al. (1995), offered a pH-degradation profile of the observed buffer catalyzed degrada-

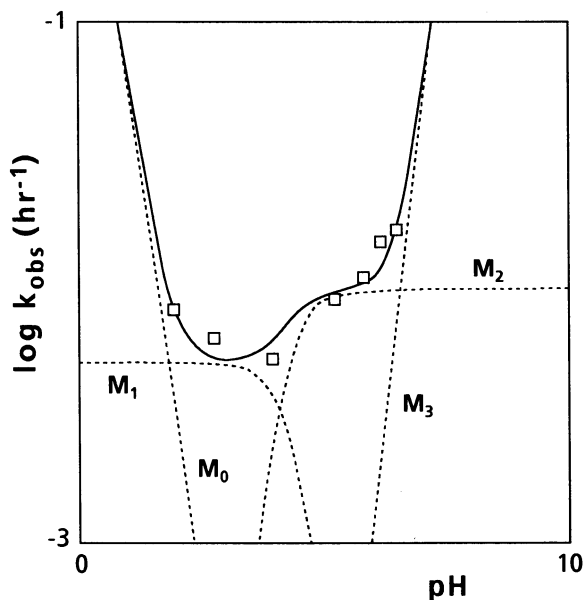


Fig. 5. pH-Degradation profile of MT-II recalculated with Eq. (7). The contribution of the macro reactions is indicated with dotted lines and the symbols M_0 – M_4 .

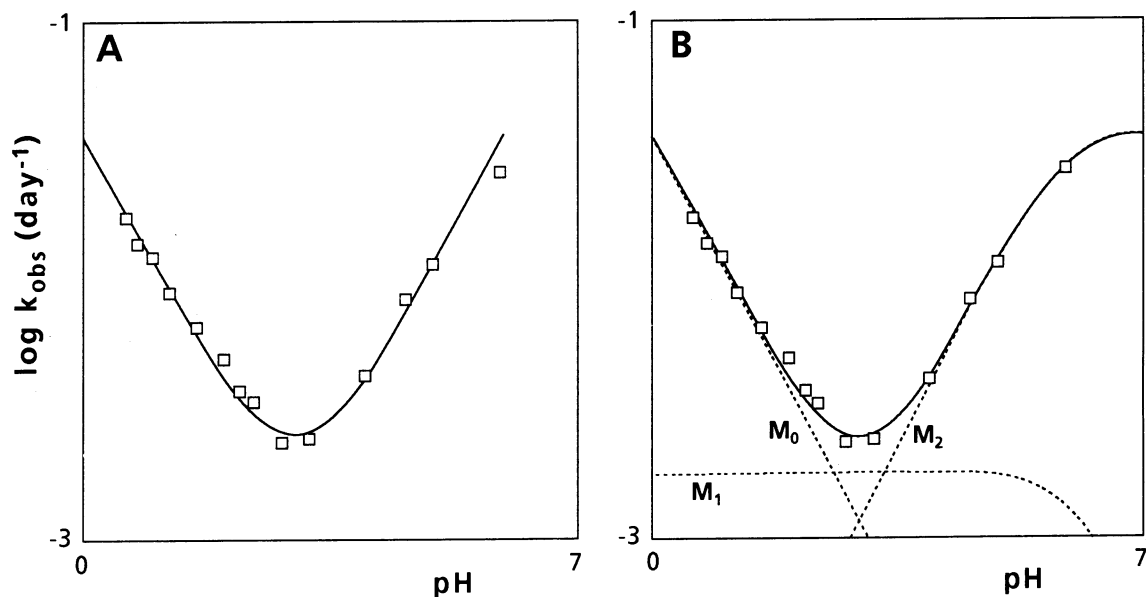


Fig. 6. pH-Degradation profiles of moricizine. A: As presented by King et al., 1992. B: Calculated with Eq. (7) as model equation. The contributions of the macro reaction constants are indicated by dotted lines and the symbols.

tion, nor did they refer to the existence of kinetically indistinguishable buffer catalyzed reactions. All attributions of catalytic buffer effects to specific combinations of buffer species and species of the degrading substance investigated are limited selections from combinations of kinetically indistinguishable micro constants, characterized by a constant sum of the charges of the reacting and catalyzing species (Van der Houwen et al., 1994).

6. pH-Degradation profiles in the presence of ligands

The degradation of drugs in the presence of ligands has been described in a number of studies. Examples of such studies are the degradation of mitomycin C in the presence of cyclodextrins (Bekers et al., 1989), chlorambucil and melphalan in the presence of 2-hydroxypropyl- β -cyclodextrin (Loftsson et al., 1989), lomustine in the presence of various cyclodextrins (Loftsson and Fridriksdottir, 1990), nitrosourea derivatives in the presence of Tris (Loftsson and Fridriksdottir, 1992),

estramustine in the presence of various cyclodextrins (Loftsson et al., 1992), tauromustine in the presence of cyclodextrins (Loftsson and Baldvinsdottir, 1992), medroxyprogesterone and megestrol acetate (Loftsson et al., 1993) or acetylsalicylic acid in the presence of 2-hydroxypropyl- β -cyclodextrin (Choudhury and Mitra, 1993).

In these studies the degradation rate is measured as a function of the ligand concentration at a constant pH. The relation between the observed rate constant k_{obs} , the complexation constant K^L , the degradation rate constant at ligand concentration zero k_0 , the degradation rate constant at infinite ligand concentration k_c and the ligand concentration $[L]$ is given by Eq. (12).

$$k_{\text{obs}} = \frac{k_0 + k_c \cdot K^L \cdot [L]}{1 + K^L \cdot [L]} \quad (12)$$

The equation can be transformed into Lineweaver–Burk plots to calculate K^L and k_c . If the degrading drug is involved in one or more protolytic equilibria the complexation constant, as calculated with this method, is an ‘apparent constant’. Its value depends on the true complexation

constants of each of the species of the degrading drug and the pK_a values and it varies with pH.

It is noted that almost no attempt has been made to calculate the true complexation constants. An exception in this respect is the study of mitomycin C (Bekers et al., 1989) to which a sequel has been added in which the true complexation constant is calculated (Van der Houwen et al., 1993).

For a drug involved in a single protolytic equilibrium such as acetylsalicylic acid the relation between the apparent complexation constant K^L and the respective complexation constants of the protonated and deprotonated acetylsalicylic acid (K_{HA}^L and K_{A-}^L), the pH and the pK_a value is given by Eq. (13)

$$K^L = \frac{K_{HA}^L + \frac{K_a}{[H^+]} \cdot K_{A-}^L}{1 + \frac{K_a}{[H^+]}} \quad (13)$$

This equation can be used for the calculation of the true complexation constants.

The degradation rate constant k_c depends on the magnitude of the proton catalyzed, solvent catalyzed and hydroxyl catalyzed degradation reactions of all species of the degrading drug, their complexation constants, the protolytic dissociation constants and the pH.

A series of values of k_c , obtained at a range of pH values, represents the pH-degradation profile of the complexed drug in the same way as a series of values of k_0 represents the pH-degradation profile of the non-complexed, free drug. It is regrettable that in none of the studies mentioned, an attempt has been made to interpret the values obtained for k_c in this way.

For acetylsalicylic acid, serving here as an example, the pH-degradation profile of the complexed drug is described with Eq. (14).

$$k_c = \frac{K_{HA}^L \cdot k_{IHA}^H \cdot [H^+] + (K_{HA}^L \cdot k_{IHA}^S + K_a \cdot K_{A-}^L \cdot k_{IA-}^H) + \frac{(K_{HA}^L \cdot K_W \cdot k_{IHA}^{OH} + K_a \cdot k_{IA-}^S)}{[H^+]} + \frac{K_a \cdot K_W \cdot K_{A-}^L \cdot k_{IA-}^{OH}}{[H^+]^2}}{K_{HA}^L + \frac{K_a}{[H^+]} \cdot K_{A-}^L} \quad (14)$$

in which the rate constants of the proton, solvent and hydroxyl catalyzed degradation reactions of the complexed protonated and deprotonated acetylsalicylic acid are indicated by k_{IHA}^H , k_{IHA}^S , k_{IHA}^{OH} and k_{IA-}^H , k_{IA-}^S , k_{IA-}^{OH} , respectively.

The kinetically indistinguishable reactions have been combined between parentheses (). Using the concept of macro reactions Eq. (14) can be transformed into Eq. (15). This equation can be used to calculate the contributions of the groups of kinetically indistinguishable reactions by regression analysis, provided the stability constants (K_{HA}^L , K_{A-}^L) are known.

$$k_c = \frac{M_0 \cdot [H^+] + M_1 + \frac{M_2}{[H^+]} + \frac{M_3}{[H^+]^2}}{K_{HA}^L + \frac{K_a}{[H^+]} \cdot K_{A-}^L} \quad (15)$$

Comparison of this equation with Eq. (7) shows the mathematical resemblance of the equations for the description of pH-degradation profiles of non-complexed and complexed drugs.

The derivation of Eq. (13) and Eq. (14) and the corresponding equations for more than one protolytic equilibrium have been described by us (Van der Houwen et al., 1991).

7. Conclusions

Most studies published in the literature treat degrading substances as if they are involved in a single protolytic equilibrium. If the substance is involved in more than one protolytic equilibrium, the study is often limited to such a small pH range that the other protolytic equilibria can be neglected. The systematic approach, as described in this study, has shown how to improve the mathematical description and the interpretation of the

pH-degradation profile. This is particularly important for drugs involved in more than one protolytic equilibrium.

The analysis of a number of recent studies in the field of stability research indicates that emphasis should be placed on the choice of the right model equation in accordance with the number of protolytic equilibria involved. Too often incorrect model equations and assumptions are made during the interpretation of experimental data. This can lead to incorrect conclusions of the underlying reaction mechanisms and calculated data such as pK_a values. The model equations presented by us show clearly that in almost all cases a straightforward treatment of the experimental data is possible.

Use of the concept of macro reaction constants should make investigators aware of the obligation to motivate their choice of a particular reaction from a series of kinetically indistinguishable ones. If no evidence for such a choice can be offered the use of macro reactions is the only acceptable alternative.

Knowledge of the shape of the contributions of the various macro reactions to the pH-degradation profile of buffer catalyzed degradation may enable investigators to recognize buffer-catalysis in complex degradation profiles.

The mathematical description of the degradation profile of complexed drugs leads to a more fundamental interpretation of the reactivity of complexed drugs.

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