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Improved kinetic parameter estimation in pH-profile data treatment

Issa T. Some [a], Philippe Bogaerts [b], Raymond Hanus [b], Michel Hanocq [a], Jacques Dubois [a,*]

[a] *Laboratoire de Chimie bioanalytique, de Toxicologie et de Chimie physique appliquée, Institut de Pharmacie, Université Libre de Bruxelles, Campus de la Plaine, CP 205/1 Boulevard du Triomphe, B-1050 Brussels, Belgium*
[b] *Service d'Automatique et d'Analyse des Systèmes, Faculté des Sciences Appliquées, Université Libre de Bruxelles, CP 165 /55 Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium*

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Abstract

Statistical problems in temperature stability parameter estimation have been the subject of many papers whereas statistics in, pH-profile parameter estimation have focused little attention. However, the conventional two step method used in data treatment in both cases leads to identical statistical problems. The aim of this study is then to introduce a method that improves statistics in pH-profile parameter estimation. A one step non-linear method that takes into account the errors in drug content determination is proposed. A mathematical relationship between drug content C, pH and time t is tested. The proposed method allows the estimation of the specific kinetic constants and the dissociation constant (pK_a) in a single run. The most likely experimental initial drug contents C_{0j},. where j is the index of a given experiment, are also determined. This approach that takes into account all relevant experimental information for the estimation of kinetic parameters is more rigorous from a statistical viewpoint than the classical two step methods. Kinetic data from acetylsalicylic acid (ASA) hydrolysis was used for the tests.

Keywords: Acetylsalicylic acid; Stability; pH-profile; Non-linear fitting; Simplex algorithm

1. Introduction

Pharmaceutical stability studies include performances of drug in different forms and its pharmaceutical properties. Accelerated testing and pH-profile kinetics are the most important parts of chemical stability performances (Connors, 1981; Carstensen, 1990).

The purpose of accelerated testing is to determine the stability parameters (activation energy and shelf life). The aim of pH-profile studies is to understand the drug degradation mechanisms. It also provides useful information for drug formu-

* Corresponding author. Tel.: +32-2-6505286; fax: +32-2-6505187.

E-mail addresses: pbogaert@labauto.ulb.ac.be (P. Bogaerts), jdubois@ulb.ac.be (J. Dubois)

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lation and storage conditions. The kinetic parameters are the specific rate constants k_i, the dissociation constant pK_a and the pH of maximum stability.

In both cases, good practical decisions have to be taken on the basis of statistically correct determined parameters.

There exist various statistical treatments of data from accelerated testing, dealing with isothermal and non-isothermal approaches (King et al., 1984; Connors et al., 1986; Junnarkar and Stavchansky, 1995). They include linear and non-linear models. The statistical problems of both approaches are discussed by many authors (Bentley, 1970; Davies and Hudson, 1982; King et al., 1984). Recently, a non linear method which takes into account all relevant experimental information as it considers batch effect in the parameter estimation has been introduced (Some et al., 1999).

In spite of the similarity of the data treatment in temperature stability and pH-profile studies (a two step approach), little attention is given to the statistical problem of pH-profile parameter statistics.

The treatment of pH-profile data also includes linear and non-linear methods. Linear approaches (Connors, 1981) in pH-profile data treatment, that consist in making hypothesis depending on the pH domains, lead to false kinetic parameters. The assumptions underlying these methods are not correct (the pH-profile in that domain is supposed to be linear and the variances on estimated kinetic constants for each pH are supposed to be constant). In addition, it does not consider all the experimental data points describing the pH-profile.

Non-linear identification approaches consider all the experimental data points in parameter estimation. In these methods, the mean prior identified kinetic constants $k_{(T,\mathrm{pH})}$ (for each pH at constant temperature) are used to estimate the specific kinetic constants. This approach leads to a better parameter estimation, but still suffers from some statistical problems. First the prior estimation of the observed kinetics constants $k_{(T,\mathrm{pH})}$ is always based on assumptions on the drug content measurements. It then yields a given parametric error distribution on these kinetic constants. These parametric errors should be taken into account in the second identification step, which leads to the determination of specific kinetic constants. This is not the case in published pH-profile data treatment (Powell, 1987; Skwierczynski and Connors, 1993). Secondly, the initial drug contents are supposed to be equaled to their measurements although they should be considered as an unknown parameter (due to measurement errors). As demonstrated for the temperature stability studies (Some et al., 1999), initial concentrations affect the estimated parameters.

For these reasons, a non-linear approach which avoids the above mentioned problems is proposed. It uses a mathematical relationship between drug content C, pH and time t for the estimation of pH-profile stability parameters (k_i, pK_a) and all experimental drug contents C_{0j}. This approach is more rigorous from statistical viewpoint. The model is tested on real kinetic data from acetylsalicylic acid (ASA).

It should be noticed that the aim of this study is not to establish equations describing the degradation of acetylsalicylic acid as function of pH. Many authors (Edwards, 1950; Kelly 1970) have already discussed the matter and a general approach to the interpretation of pH degradation is proposed by Van Der Houwen et al. (1997). This paper deals specifically with how to estimate pH-profile specific kinetic parameters with better statistical considerations.

2. Materials and methods

2.1. Materials

ASA, salicylic acid and 2,4 dihydroxybenzoïc acid (internal standard) are bought from Sigma–Aldrich (Belgium). HPLC methanol is supplied by Merck Belgolabo (Belgium). $NaH_2PO_4 \cdot H_2O$, $Na_2HPO_4 \cdot 2H_2O$, H_3PO_4, HCl and NaOH are analytical grade from Merck Belgolabo (Belgium). High purified water from Milli-Q filters system (Millipore, USA) is used for the preparation of all solutions.

2.2. HPLC analysis

The HPLC system consisted of a solvent delivery pump (Gilson, France), an injection system with 20 μl loop (Rheodyne, USA), a 300×4.6 mm column (μBondapak) packed with 10 μm irregular particles from Waters (USA) with a temperature control device operating at 25°C, a UV detector (Gilson Holochrome, USA) and a computing system equipped with a data acquisition software program (Brown Chromatographic software, J.M.B.S., France). The mobile phase (methanol 40% (v/v)-phosphate buffer (0.05M; pH 2.5) 60% (v/v)) is filtered through 0.45 pm pore nylon membrane (Milipore, USA) and deaerated under reduced pressure. The flow rate is maintained at 1.5 ml min^{-1}. The detection wavelength is set at 230 nm (isobestic point).

2.3. Kinetic method

A stock solution of ASA containing about 2 mg ml^{-1} in pure ethanol is prepared. Five milliliters are taken and transferred into a 50-ml volumetric flask and brought up to volume with buffer solutions previously maintained at 25°C. The flask is then shaken to mix and the solution is distributed into sealed vials and stored in an oven (Bekso, Belgium). Samples of 1 ml are withdrawn at appropriate time intervals and immediately diluted with 18 ml of 0.18 M phosphate buffer (pH $2.5 \approx$ maximum stability) and 1 ml of internal standard from a stock solution (26 μM) to quench the reaction. The samples are immediately analysed or frozen and kept at -20°C until the analysis was performed. The time scales for each pH is selected to achieve at least 70% of drug degradation. The initial drug concentration is about 5.55×10^{-8} M or half of this concentration. The pH range is from 0.93 to 12.55 and up to 18 pH are monitored. Phosphate buffers are used from pH 2 to 8. NaOH (0.023–0.01 M) and HCl (0.2–0.1 M) are used for other pH. All buffer solutions are adjusted to the same ionic strength (0.5 M) with potassium chloride. The amount of potassium chloride added is calculated as suggested by Van Damme et al. (1979).

3. Basics of classical approach

In most papers, the determination of the specific kinetic constants k_i is made in two steps. In the first step, the mean observed kinetic constant $\bar{k}_{(T,\mathrm{pH})}$ is identified with the model linking the drug content C variation to time t. For a first order kinetic model, the relation is

$$C = C_0 \exp(-k_{(T,\,\mathrm{pH})}t) \tag{1}$$

Where C_0 is the initial drug content.

In a second step, the specific kinetic constants k_i are obtained either by a linear model or a non-linear model (generally in a logarithmic form) through a function relating the observed rate constants to the pH. For ASA, this equation is (Connors, 1981)

$$\bar{k}_{(T,\mathrm{pH})} = [K_1 \times 10^{(-2\mathrm{pH}+pK_a)} + k_2 \times 10^{(-\mathrm{pH}+pK_a)} + k_4 + k_3 \times 10^{(\mathrm{pH}-pK_w)}]/[10^{(-\mathrm{pH}+pK_a)} + 1] \tag{2}$$

where $\bar{k}_{(T,\mathrm{pH})}$ is the observed rate constant at a given pH, k_1 is the specific second order acid catalysed constant, k_3 the specific second order base catalysed constant, k_2 and k_4 the first order non-catalysed constants of unionised and ionised forms of ASA. K_a is the dissociation constant of the drug, K_w is the water ionisation constant at the temperature of the study. The pH-profile of ASA is an interesting one in hydrolysis studies. It shows a V form followed by a sigmoïd shape and differences between kinetic constants at different pH are such that the logarithmic form of Eq. (2) is used for the graphical representation (Fig. 1) or the fitting process.

In the framework of the validation of pH profile data treatment, Eq. (1) and its logarithmic form were identified using classical approaches on kinetic data from the hydrolysis of ASA. The observed kinetic constants $\bar{k}_{(T,\mathrm{pH})}$ obtained on the basis of Eq. (1) linking the concentration with time at different pH through a first order kinetic model, are presented in Table 1.

The least-square cost function for the identification of Eq. (2) is

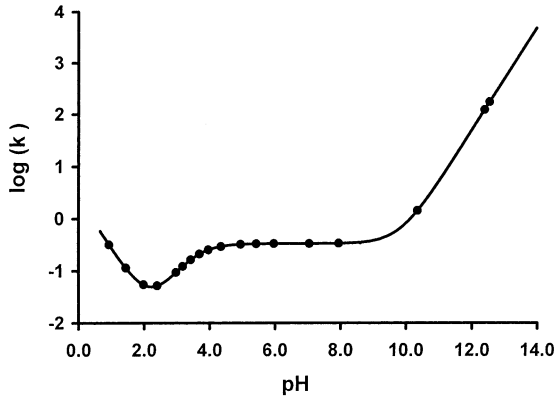

Fig. 1. pH-profile of ASA at 25°C and 0.5 M ionic strength. ●, experimental points represent the logarithim of the mean kinetic constants values at each pH.

Table 1
Kinetic constants from the hydrolysis of ASA as a function of pH at 25°C and 0.5 M ionic strength[a]

PH	$k_{(298.16,pH)}$	pH	$k_{(298.16,pH)}$
0.93	0.346	4.96	0.317
	0.335		0.330
	0.337		0.334
1.45	0.106	5.42	0.330
	0.111		0.331
	0.108		0.352
1.95	0.054	5.96	0.337
	0.051		0.333
	0.052		0.347
2.4	0.055	7.04	0.372
	0.055		0.370
	0.053		0.370
2.95	0.096	7.96	0.389
	0.094		0.381
	0.095		0.392
3.45	0.167	10.35	1.780
	0.156		1.770
	0.163		1.780
3.95	0.241	12.40	134.60
	0.225		141.04
	0.238		140.01
4.25	0.272	12.55	148.16
	0.262		149.17
	0 278		147.52

[a] The kinetic constants are obtained on the basis of the model (l) and a least square function. The units are day^{-1}. All experiments are in triplicate.

Table 2
Specific kinetic constants obtained from the identification of Eq. (2)

Parameters	Initial values θ_0	Estimated values $\hat{\theta}$	Standard errors
k_1 (day^{-1} M^{-1})	2.0	2.0	5.0
k_2 (day^{-1})	0.2	2.0×10^{-8}	0.0001
k_3 (day^{-1} M^{-1})	5000	2.14×10^{-5}	3
k_4 (day^{-1})	0.3	188.2	0.6
pK_a	3.5	11.96	0.02
$J(\theta)$	15.45	56.23	

Table 3
Specific kinetic constants obtained from the identification of Eq. (2) (logarithmic form)

Parameters	Initial values θ_0	Estimated values $\hat{\theta}$	Standard errors
$k_1(day^{-1}M^{-1})$	2.0	2.56	0.14
k_2 (day^{-1})	0.2	0.020	0.004
k_3 (day^{-1} M^{-1})	5000	5087	174
k_4 (day^{-1})	0.3	0.348	0.008
pK_a	3.5	3.54	0.75
$J(\theta)$	15.45	0.39	

$$j(\theta)=\sum_{j=1}^{N}(\bar{k}_{j(T,\mathrm{pH})}(\mathrm{pH}_j) - f(\bar{k}_{j(T,\mathrm{pH})},\mathrm{pH}_j;k_1,k_2,k_3,k_4,pK_a))^2 \quad (3)$$

where $\theta=[k_1, k_2, k_3, k_4, pK_a]$ is the parameter vector, N is the number of experiments, $\bar{k}_{(T,\mathrm{pH})}$ is the estimated kinetic constant for the pH_j in experiment j and $f(\bar{k}_{T,\mathrm{pH}},\mathrm{pH}_j;k_1,k_2,k_3,k_4,pK_a)$ is the model given by Eq. (2). A similar cost function is defined for the logarithmic form of this equation.

The optimisation of these cost functions by the simplex algorithm (Nelder and Mead, 1965) implemented in Matlab (version 5.1) leads to the results presented in Table 2 for Eq. (2) and Table 3 for its logarithmic form.

The analysis of results in Table 2 shows that the estimated values of specific kinetic constants are

meaningless. The standard errors are greater than the estimated values. This is confirmed by the estimated value of the dissociation constant of ASA (11.96) which is incorrect compared to published values (≈ 3.5). It should be notice that the good initialisation values were chosen for the optimisation process. On the contrary, Table 3 gives results in accordance with values from literature (Garrett, 1957; Connors, 1981).

The discrepancies between the estimated values using Eq. (2) and its logarithmic form has justified further tests on literature published data. The question is: are the differences due to our data used or are they the consequences of statistical considerations?

Tests on data from Garrett (1957) lead to the same discrepancies (results not shown). To verify if our difficulties in optimising the cost function (3) are unique, tests were performed on the pH-profile data of L-phenylalanine, which presents the same equation as ASA (Skwierczynski and Connors, 1993). The identified parameters show the same discrepancies with the logarithmic form always giving better results.

But, whatever the chosen method (Eq. (2) or its logarithmic form), statistical problems arise from the use of the two step identification procedure. The parametric error distribution on the observed kinetic constants $k_{(T,\mathrm{pH})}$ at different pH, resulting from the error distribution on the measured concentrations C are not taken into account in the second step of the identification process. This leads to biased estimation of specific kinetic constants k_i. As stated by Perrella (1988), to eliminate the biasing of data, regression analysis should be performed on the untransformed data by non-linear methods.

4. The proposed method

In this paper, a global pH-profile data treatment by eliminating the global kinetic constant $\bar{k}_{(T,\mathrm{pH})}$ from Eq. (1) and Eq. (2) is proposed. The equation used here is obtained as follows. Substituting of $\bar{k}_{(T,\mathrm{pH})}$ in Eq. (1) by its expression from Eq. (2) leads to an equation relating the drug content C, the time t and the pH.

$$C = C_0 \exp[(k_1 \times 10^{(-2\mathrm{pH}+\mathrm{p}K_a)}) + (k_2 \times 10^{(-\mathrm{pH}+pK_a)}) + (k_4) + (k_3 \times 10^{(\mathrm{pH}-\mathrm{p}K_w)})]t/(10^{(-\mathrm{pH}+\mathrm{p}K_a)}+1) \quad (4)$$

The determination of the unknown parameters is then reduced to the minimisation of a least-square function based on (Eq. (4)) and on an a priori distribution of the measurement errors on the drug content determination.

This procedure then takes into account the a priori knowledge of the measurement error distribution in drug content C determinations. This model is statistically better as it takes into account all relevant experimental information for the specific kinetic parameter estimation.

4.1. Theoretical considerations and cost functions definition

Let us consider the non-linear model (for one experiment):

$$C(t_k) = f(\theta, y\,(t_k), t_k) \quad (5)$$

where $C(t_k) \in \Re$ is a measured signal (in our case the concentration) at time t_k; $y(t_k) \in \Re^m$ is a measured vector of signals (in our case [pH, t]) at time t_k; $\theta \in \Re^n$ is the parameter vector (in our case. [k_1, k_2, k_3, k_4, pK_a,C_0]). The Markov estimate is given by

$$\hat{\theta}_M = \underset{\theta}{\mathrm{ArgMin}} \sum_{k=1}^{N} \frac{1}{\sigma^2(t_k)} (C(t_k) - f(\theta, y(t_k), t_k))^2 \quad (6)$$

This expression is statistically valid (hence corresponding to the maximum likelihood estimate) if and only if:

— there are additive measurement errors only on the signal $C(t_k)$ (*and* not on the measured signals $y(t_k)$):

$$C(t_k) = \bar{C}(t_k) + \varepsilon(t_k) \quad (7)$$

where $\bar{C}\,(t_k)$ are the real values and $\varepsilon(t_k)$ are the measurement errors;

— these errors $\varepsilon(t_k)$are supposed to be white noises (i.e. with no correlation between samples: $E[\varepsilon(t_k)\varepsilon(t_l)]=0 \quad \forall t_k \neq t_l$) with zero mean and variance $\sigma^2(t_k)$

$E[\varepsilon(t_k)]=0 \ \forall t_k$

$E[\varepsilon(t_k)\varepsilon(\mathrm{t_l})]=\delta_{k,l}\ \sigma^2(t_k)$ (*where* $\delta_{k,l}$ is the Kronecker's symbol $\forall t_k,\ t_l$)

— the distribution of these errors $\varepsilon(\mathrm{t_k})$ is supposed to be Gaussian. If moreover $\sigma^2\ (t_k)$ is supposed to be constant ($\sigma^2\ (t_k)=\sigma^2 \quad \forall t_k$) then the Markov estimate reduces to the least squares estimate:

$$\hat{\theta}_{\mathrm{LS}}=\underset{\theta}{\mathrm{ArgMin}}\sum_{k=1}^{N}(C(t_k)-f(\theta,y(t_k),t_k))^2 \qquad (8)$$

Two cases must be considered:

1. All the variances $\sigma^2(t_k)$ are known (up to a constant factor). Then the Markov estimate $\hat{\theta}_M$ can be used directly. However, it must be noticed that this estimate consists of the solution of a non-linear optimisation problem. Hence, a numerical optimisation algorithm must be used (simplex, Gauss–Newton, Levenberg–Marquardt…). The initial estimate of the parameters is very important. It determines the speed of convergence, the convergence itself and the problem of obtaining local minima or the minimum minimorum. Therefore, if a non-linear transformation (such as a logarithmic one) allows to linearise the model with respect to time, it can be very useful to use it in order to find a first estimate as a unique solution of a least squares problem.
2. The variances $\sigma^2(t_k)$ are unknown. Then a question must be answered: may people accept the above mentioned assumptions for the least squares estimate or are these assumptions more acceptable if we apply a transformation on the model and hence on the measured signals? This question often reduces to the following one: concerning the measurement errors on $C(t_k)$, what are the 'more constant' ones: the absolute errors or the relative errors?

If the answer is 'the absolute errors' (i.e. $\sigma\ (t_k) \approx$ constant) then the least squares estimate $\hat{\theta}_{\mathrm{LS}}$ may be used. If the answer is 'the relative errors' (i.e. $\sigma(t_k)/C(t_k) \approx$ constant), then a least squares estimate may be used with a logarithmic transformation of the model (because the absolute errors on $\log \sigma(t_k)$ correspond to the relative errors on $C(t_k)$).

4.2. Application to the parameter identification in pH profile

In this study, the development of the analytical method for measurement of the drug content $C(t_k)$ does not lead to the quantification of the variances $\sigma^2\ (t_k)$ (i.e. for each sample measurement) but it can reasonably be assumed that, within the range of measurement, the absolute errors on $C(t_k)$ are 'more constant' than the relative ones. Hence, even if a non-linear transformation can be used for finding an initial guess, the least squares estimate $\hat{\theta}_{\mathrm{LS}}$ based on the original non-linear model is the best choice.

In order to define the cost function we consider the time values t and the pH measurements as precise enough to neglect the errors in their measurements. On the contrary, the errors in drug content measurements C cannot be considered as negligible. We make the a priori assumption that the measurements are stationary white noises with a Gaussian distribution with zero mean and unknown variance σ^2.This assumption leads to a least-square cost function:

$$j(\theta) = \sum_{j=1}^{M}\sum_{i=1}^{N_j}(C_{ij}\ (t_{ij},\mathrm{pH}_j) - f\ (C_{ij},t_{ij},\mathrm{pH}_j;\ k_1,\ k_2,\ k_3,\ k_4,\ \mathrm{p}K_a,\ C_{0j}))^2 \qquad (9)$$

$\theta=[k_1,\ k_2,\ k_3,\ k_4,\ \mathrm{p}K_a, C_{01,}\ldots C_{0M}]$is the known parameter vector, M is the number of experiments, N_j is the number of measurement samples in experiment j, C_{ij} is the measured concentration and $f(C_{ij},t_{ij},\mathrm{pH}_j;k_1,\ k_2,\ k_3,\ k_4,\ \mathrm{p}K_{\mathrm{a}}\ \ \mathrm{C}_{01},\ldots C_{0M})$ is the model given by Eq. (4).

The simplex algorithm allows solving the non-linear optimisation problem that consists in minimising the least squares cost function with respect to the parameter vector θ.

Based on the least-squares cost function J and the identified parameters $\hat{\theta}$ where

$$\hat{\theta}=\underset{\vartheta}{ArgMin}\ J\ (\vartheta)$$

the variance-covariance matrix can be approximated by

$$\hat{E}[\tilde{\theta}\,\tilde{\theta}^T] \cong \hat{\sigma}^2 P\,(\hat{\theta}) \tag{10}$$

where $\tilde{\theta} = \theta - \tilde{\theta}$ is the parametric error,

$$\hat{\sigma}^2 = \frac{J(\hat{\theta})}{\sum_{j=1}^{M} N_j - \dim\,(\theta)} \tag{11}$$

is an estimate of the measurement noise variance, which is nothing but the minimised least-squares cost function divided by the difference between the number of measurements and the number of parameters and

$$p^{-1}\,(\hat{\theta}) = \sum_{j=1}^{M} \sum_{i=1}^{N_j} (J_{ij})\,(J_{ij})^T \tag{12}$$

is the sensitivity matrix of the model with respect to the parameters where

$$J_{ij} = \frac{df(C_{ij},t_{ij},\mathrm{pH}_j;[k_1,k_2,k_3,k_4,\mathrm{p}K_a,C_{0j}])}{d\theta}\,|\theta = \hat{\theta} \tag{13}$$

5. Results and discussion

All the kinetic parameters, including the pK_a and all initial drug contents are estimated in a single run (Table 4). The initial drug contents and the errors in their estimation are given in Appendix A.

Because of the non-linearity of the proposed model, the optimisation procedure lead to final estimates dependent of initial values (i.e initial estimates). This is checked by running the optimisation procedure several times again using different initial values. The simplex algorithm converges to the same final estimates (Table 4) in a large domain of initial values. On the contrary, the classical methods (Eq. (2) and its logarithmic form) showed more sensitivity with respect to starting values.

As different initial drug contents are used to gather kinetic constants at different pH, there are as many initial drug contents as experiments. The classical identification procedure does not lead to the estimation of initial drug contents. In a previous paper (Some et al., 1999), it was showed how initial experimental conditions could affect the correctness of temperature stability parameters. The identification procedure used in this study also allows dealing with significantly different initial experimental conditions. Table 5 shows results of the identification of Eq. (4) from data of statistically different experimental initial drug contents. The comparison with those on Table 4 shows that the specific kinetic constants are not significantly different although the initial experimental concentrations are quite different. When different, some initial drug concentrations are half of those presented in Appendix A.

The analysis of the correlation coefficient matrix from the variance-covariance matrix shows different relations between parameters. For exam-

Table 4
Estimated parameters using the proposed identification method and influence of initialisation on final values using data from similar initial conditions[a]

Parameters	Initial estimates			Final estimates	Standard errors
	Test 1	Test 2	Test 3		
K_I	2.50	25	0.50	2.54	0.06
k_2	0.20	10	20	0.02	0.002
k_3	5000	1000	100	4869	95
k_4	0.34	0.30	30	0.35	0.004
pK_a	3.50	5	2	3.60	0.30
$J(\theta)$	41.60	43.80	32.50	5.73	

[a] The experimental initial concentrations are similar and are given in Appendix A.

Table 5
Estimated parameters using the proposed identification method and influence of initialisation on final values using data from different initial conditions[a]

Parameters	Initial estimates			Final estimates	Standard errors
	Test 1	Test 2	Test 3		
k_1	2.50	25	0.50	2.34	0.08
k_2	0.20	10	20	0.025	0.002
k_3	5000	1000	100	4965	93
k_4	0.34	0.30	30	0.348	0.004
pK_a	3.50	5	2	3.60	0.22
$J(\theta)$	45.60	25.90	31.80	3.96	

[a] In this case, some initial drug contents are about 5.55×10^{-5} M and the others are half of these concentrations ($\approx 2.75 \times 10^{-5}$ M).

ple, it shows correlations between k_1 and k_2 (-0.67), between k_2 and pK_a (-0.77) and between k_4 and pK_a (-0.69). The correlation between other parameters is less than 0.35. All the correlations are explained by the nature of the reactions in solution. (Some et al., 1999).

The analysis of the residuals (Appendix B) does not exhibit any systematic errors neither for each pH nor for the data as a whole.

6. Conclusion

The proposed equation and the identification method used in this study are able (on the contrary of the classical method) to provide all the pH-profile kinetic parameters and all the initial drug contents C_{0j} in one step. It avoids the statistical problems of the usual approaches and uncertainties on final estimates are directly the consequences of errors in drug content determination. The kinetic parameters can also be estimated directly from statistically different initial estimates. No further transformation is needed and the results are largely independent from initialisation values.

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Appendix A. Experimental and estimated initial drug contents corresponding to the results on Table 4

Number of experiments	Experimental initial drug contents (10^{-5} M)	Estimated initial drug contents (10^{-5} M)	Standard errors
1	5.56	5.47	0.08
2	5.56	5.42	0.08
3	5.56	5.47	0.08
4	5.57	5.58	0.09
5	5.58	5.51	0.09
6	5.58	5.53	0.09
7	5.55	5.41	0.08
8	5.57	5.64	0.08
9	5.58	5.54	0.08
10	5.55	5.31	0.08

Appendix A. ***(Continued)***

Number of experiments	Experimental initial drug contents (10^{-5} M)	Estimated initial drug contents (10^{-5} M)	Standard errors
11	5.55	5.37	0.08
12	5.58	5.39	0.08
13	5.55	5.30	0.08
14	5.57	5.34	0.08
15	5.58	5.34	0.08
16	5.55	5.54	0.10
17	5.55	5.52	0.10
18	5.55	5.57	0.10
19	5.55	5.43	0.10
20	5.57	5.56	0.10
21	5.58	5.50	0.11
22	5.55	5.63	0.11
23	5.55	5.62	0.11
24	5.55	5.64	0.11
25	5.55	5.58	0.11
26	5.57	5 70	0.11
27	5.58	5.63	0.11
28	5.55	5.60	0.11
29	5.57	5.68	0.11
30	5.58	5.55	0.10
31	5.57	5.65	0.10
32	5.55	5.58	0.10
33	5.58	5.52	0.10
34	5.55	5.70	0.10
35	5.57	5.64	0.10
36	5.58	5.48	0.10
37	5 55	5 53	0.10
38	5.57	5.60	0.10
39	5.58	5.53	0.10
40	5.57	5.48	0.10
41	5.58	5.47	0.10
42	5.58	5.42	0.10
43	5.57	5.39	0.10
44	5.58	5.42	0.10
45	5.58	5.39	0.10
46	5.57	5.24	0.08
47	5.57	5.28	0.08
48	5.62	5.52	0.08
49	5.62	5.34	0.08
50	5.62	5.39	0.08
51	5.57	5.72	0.12
52	5.57	5.70	0.12
53	5.57	5.71	0.12

Appendix B. Residuals: differences between all the experimental and the estimated drug contents for the 18 pH used in this study

These are 326 experimental data points.

0.090	0.107	−0.097	−0.079	−0.082	−0.155	−0.093	0.140	−0.052
0.010	−0.108	−0.035	−0.095	−0.029	0.090	0.035	−0.022	−0.121
−0.069	−0.064	−0.027	−0.015	−0.051	−0.058	0.132	0.208	0.056
0.065	−0.076	−0.069	−0.005	0.112	0.062	0.050	0.051	−0.118
−0.048	0.073	0.131	0.137	0.080	0.136	−0.046	−0.267	0.020
0.119	0.196	0.062	−0.072	−0.091	−0.077	−0.088	0.561	0.091
0.051	0.040	−0.105	−0.078	−0.012	0.164	0.242	0.239	0.005
−0.220	0.008	−0.109	−0.026	−0.085	0.184	0.022	−0.123	−0.139
0.062	−0.104	−0.081	0.192	−0.050	−0.075	−0.123	0.001	−0.001
0.250	0.066	−0.242	−0.152	−0.163	−0.033	−0.037	0.229	−0.005
−0.172	−0.070	−0.100	−0.121	−0.078	0.239	−0.036	−0.107	−0.141
−0.090	−0.122	−0.088	0.015	−0.098	0.034	0.061	0.041	−0.013
0.019	0.029	−0.092	0.015	0.100	−0.054	−0.024	0.092	−0.022
−0.090	0.075	0.067	0.024	0.068	−0.010	0.125	−0.038	−0.177
−0.034	0.038	0.005	0.016	0.007	−0.083	0.040	−0.094	0.441
0.080	−0.098	−0.021	0.042	−0.003	−0.077	−0.039	0.107	0.160
0.076	0.086	0.037	−0.072	−0.027	0.150	0.063	0.099	0.041
0.018	−0.086	−0.087	0.191	0.159	0.130	0.113	0.101	−0.032
0.009	0.003	−0.027	0.171	0.097	−0.132	0.064	0.096	0.214
0.131	−0.049	−0.025	0.086	0.095	0.072	0.024	−0.053	0.021
−0.034	0.084	0.290	0.152	−0.111	0.065	0.141	0.181	0.136
0.026	−0.105	0.007	0.117	0.097	0.071	−0.075	0.073	−0.006
0.088	0.105	−0.028	0.049	−0.011	−0.039	0.039	0.062	0.054
−0.099	−0.008	0.054	0.037	0.055	−0.151	0.205	−0.009	−0.029
0.051	0.048	−0.065	0.067	0.006	0.035	0.066	0.096	−0.009
−0.204	0.055	0.096	0.022	−0.075	0.029	0.038	0.068	0.084
−0.034	−0.021	0.023	0.068	0.101	0.047	−0.078	−0.010	0.034
0.039	0.085	0.026	−0.063	−0.136	−0.117	−0.043	0.111	−0.013
−0.104	−0.068	−0.071	−0.065	0.159	−0.084	−0.105	−0.101	−0.046
−0.021	0.173	−0.060	−0.055	−0.243	−0.086	−0.030	0.154	−0.056
−0.102	−0.122	−0.079	−0.055	0.188	−0.034	−0.135	−0.179	−0.119
−0.064	0.329	−0.003	−0.110	−0.349	−0.144	0.290	0.033	−0.057
−0.362	−0.195	0.099	0.004	−0.063	−0.014	0.015	−0.159	0.126
0.281	0.039	−0.094	−0.125	−0.063	−0.189	−0.114	0.225	0.106
0.036	−0.226	−0.159	−0.097	−0.122	−0.152	0.069	0.256	0.287
0.176	−0.127	0.025	0.278	0.284	0.165	−0.135	0.021	0.306
0.295	0.180							

For each pH, the errors follow a Gaussian distribution and no systematic bias was noticed. The units are 10^{-5} M.

References

Bentley, D.L., 1970. Statistical techniques in predicting thermal stability. J. Pharm. Sci. 59, 464–468.

Carstensen, J.T., 1990. Drug Stability Principles and Practices. In: Drug and Pharmaceutical Science, vol. 43. Marcel Dekker, USA.

Connors, K.A., 1981. The Study of reaction kinetics. J. Parenter. Sci. Technol. 35, 187–211.

Connors, K.A., Amidon, G.L., Valentino, J.S., 1986. Chemical Stability of Pharmaceutical. A Handbook for Pharmacist, 2nd Edn. John Wiley, New York.

Davies, O.L., Hudson, H.E., 1982. Stability of drugs: accelerated storage tests. In: In Statistics in Pharmaceutical Industry, 2nd edn. New York, Marcel Dekker, pp. 445–480.

Edwards, L.J., 1950. The hydrolysis of aspirin: a determination of the thermodynamic dissociation constant and a study of the reaction kinetics by UV spectrophotometry. Trans. Faraday Soc. 46, 723–735.

Garrett, E.R., 1957. The kinetics of solvolysis of acyl esters of salicylic acid. J. Am. Chem. Soc. 79, 3401–3408.

Junnarkar, G.H., Stavchansky, S., 1995. Isothermal and non-isothermal decomposition of famotidine in aqueous solution. Pharm. Res. 12, 599–604.

Kelly, A.C., 1970. Determination of the decomposition of Aspirin. J. Pharm. Sci. 59, 1053.

King, P.S., Kung, M., Fung, H., 1984. Statistical prediction of drug stability based on non-linear parameter estimation. J. Pharm. Sci. 73, 657–662.

Nelder, J.A., Mead, R., 1965. A simplex method for function optimization. Comput. J. 7, 308–313.

Perrella, F.W., 1988. EZ-FIT: a practical curve-fitting microcomputer program for the analysis of enzyme kinetic data on IBM-PC compatible computers. Anal. Biochem. 174, 437–447.

Powell, M.F., 1987. Stability of lidocaine in aqueous solution: effect of temperature pH buffer and metals ions on amide hydrolysis. Pharm. Res. 4, 42–45.

Skwierczynski, R.D., Connors, K.A., 1993. Demethylation kinetics of Aspartame and L-Phenylalanine methyl ester in aqueous solution. Pharm. Res. 10, 1174–1180.

Some, I.T., Bogaerts, P., Dubois, J., Hanus, R., Hanocq, M., 1999. Incorporating batch effects in the estimation of drug stability parameters using Arrhenius model. Int. J. Pharm. 184, 165–172.

Van Damme, M., Hanocq, M., Molle, L., 1979. Méthodes de calcul de la force ionique de solutions tampons. J. Pharm. Belg. 34, 5–12.

Van Der Houwen, A.O.G.J., De Loos, M.R., Beijnen, J.H., Bult, A., Underberg, W.J.M., 1997. Systematic interpretation of pH-degradation profiles: a critical review. Int. J. Pharm. 155, 137–152.