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Fast drug stability determination by LC variable-parameter kinetic experiments

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Abstract

Variable-parameter kinetic experiments were carried out using HPLC as analytical instrument. The hydrolysis of aspirin was followed both at variable-temperature and at variable-pH conditions. The peak areas relative to salicylic acid were processed by direct fit to a mathematical model and/or by differential method obtaining, by single experiments, the values of the apparent rate constant in the whole range of temperature and pH studied. The results, although the discontinuity of this kind of analysis, are in agreement with those obtained by constant-parameter kinetics but saving experimental time.

Keywords: Variable-parameter kinetics; Variable-temperature kinetics; Variable-pH kinetics; HPLC; Aspirin; Hydrolysis

1. Introduction

The effect of environmental parameters on the stability of drugs in solution is a vitally important information in pharmaceutical industry. It often requires a detailed kinetic investigation on new molecules of interest before its release on the market, to have quantitative data on the dependence of the reaction rate constant on temperature, pH, ionic strength, metal ions concentration, etc [1]. This gives technical useful data on the time the species maintains its chemical identity in specified conditions and, more deeply, a panoramic picture of the reactive behaviour useful for mechanistic studies. The usual way consists of following many times the reaction, by a suitable analytical instrument, in pseudo-first order conditions, for different values of a parameter and for each parameter enquired. Variable-parameter ki-

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netics [2] is a new, more economic way of collecting kinetic data. It consists on carrying out kinetic experiments while varying the value of a physical parameter. The mathematical model describing the process is given by Eq. (1), where C is the concentration of the monitored reacting species.

$$-\frac{dC}{dt} = \{k_{obs}[Par_i(t)]\}C \quad (1)$$

In this equation k_{obs} is not a constant but depends on a parameter Par_i varying with time. $k_{obs}[Par_i(t)]$ is then a function of function: $k_{obs}(Par_i)$ is the dependence function (D), describing the dependence of the rate constant on the parameter i, and $Par_i(t)$ is the modulating function (M), showing the way the parameter changes with time. Kinetic profiles obtained in these experiments have, obviously, shapes different from the exponential first order curve and different from each other in dependence of D and M functions. A visualisation can be easily obtained by computer simulation [3]. The experimental profile contains in each point information about the value of the rate constant at that time ($-(dC/dt)/C = k_{obs}[Par_i(t)]$) and, knowing the mathematical form of the dependence function, a fitting to Eq. (1) gives the terms regulating such dependence. For example, in variable-temperature kinetic experiments the dependence function is the Eyring equation and the terms are the activation parameters $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$; in generic variable-concentration kinetic experiments the dependence function is the rate law and the terms to optimise are the rate constants of rate determining elementary steps. We studied variable-temperature kinetics carrying out experiments on various reacting systems [4–8] but several examples are reported in the literature as non-isothermal kinetic analysis [9–12]. A variable-concentration kinetic experiment was a subject of a communication [2] and a variable-pH kinetic experiment appeared recently in literature [13].

During our studies we followed the reacting species using various analytical instruments: UV/vis spectrophotometer [4–7], fluorometer [4], conductometer [2], polarimeter [8]. We know of examples of non-isothermal kinetic experiments followed by IR spectrophotometer [14] and liquid chromatography [15]. The spectrophotometric is by far the most powerful method used for kinetic monitoring. The UV/vis regions are particularly useful. However, when it is not possible to use a spectrophotometer or some problems can be avoided by the separation of the signals, alternative methods are necessary. In this paper we carried out both variable-temperature and variable-concentration kinetic experiments following them by HPLC to directly compare the results with those we obtained spectrophotometrically and applying the same methodology we have developed in the last few years. HPLC is a largely employed separation method in pharmaceutical field, for analytical and preparative purposes but even for kinetic investigations. Moreover, the use of chiral stationary phases allowed the direct separation of chiral analytes without any preliminary derivatisation making it a powerful alternative to polarimeter for racemisation kinetics.

The reaction model is the hydrolysis of aspirin which we studied in various conditions [4,13] and of which we have direct comparative data. Acetylsalicylic acid and its reaction product, salicylic acid, can be easily separated and monitored by HPLC.

2. Experimental

2.1. Material

Acetylsalicylic acid was obtained from J.T. Baker (Phillipsburg, NJ, USA) and recrystallised from ethanol.

2.2. Solutions

The hydrogen ion concentrations were obtained by adding a 0.5 M solution of NaOH to a solution containing H_3PO_4 (0.01 M) and H_3BO_3 (0.01 M).

2.3. Kinetic measurements

The kinetics of the hydrolysis of aspirin were carried out chromatographically by both variable-

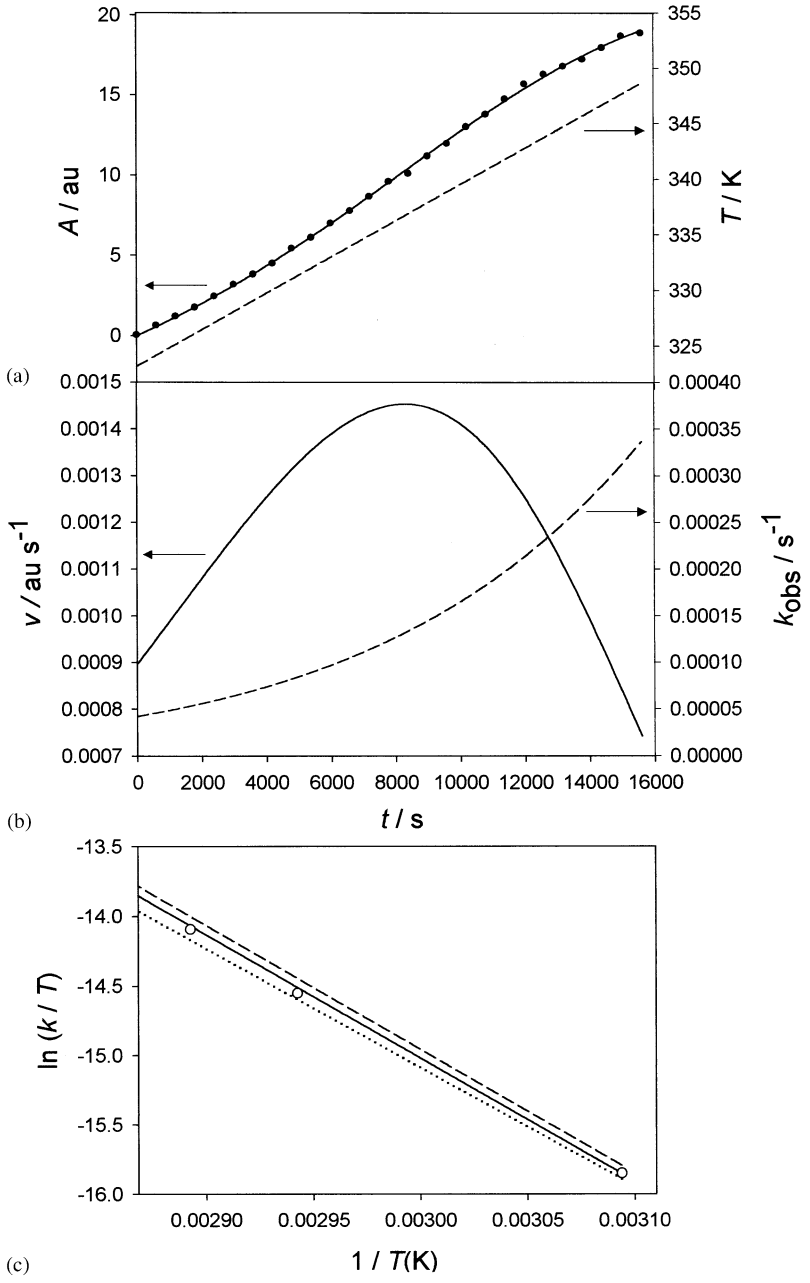

Fig. 1. (a) Change in HPLC peak area of salicylic acid (solid circles) during the hydrolysis of aspirin at pH 7.00 at the variable temperature T (K) = 323.2 + 1.631 × $10^{-3}t$ (s) (dashed line). The solid line is the theoretical curve obtained by the best fit to Eq. (4). (b) Trend of the reaction rate as obtained by differentiation of the kinetic curve (solid line) and $k_{obs}(T)$ profile obtained by dividing the derivative to $-(A_0-A_f)$ (dashed line). (c) Eyring plot of the results obtained by various methods: HPLC-VTK (fitting; solid line), HPLC-VTK (differ. meth.; dashed line); UV/vis-VTK (pointed line), CTK (open circles).

temperature kinetic (VTK) runs and variable-pH kinetic (VpHK) runs.

2.3.1. VTK HPLC method

The experiment was carried out in a 250 ml reaction vessel, equipped with a stirrer to ensure chemical and thermal homogeneity, immersed in a thermostated bath HAAKE C 25 which allows a controlled change of the temperature with time with an accuracy of ± 0.05 K. The temperature was checked by a platinum resistor connected to the computer (readout resolution 0.01 °C). At suitable times, samples were withdrawn, stored at low temperature and analysed by HPLC.

2.3.2. VpHK HPLC method

The experiment was carried out in a 250 ml reaction vessel immersed in a thermostated bath (± 0.01 K) equipped with a stirrer to ensure chemical and thermal homogeneity, a glass electrode to measure the actual pH and a platinum resistor to control the reaction temperature. An autoburette was used to vary the pH by adding 1.19 μl s^{-1} of a 0.5 M solution of NaOH into the reaction vessel containing H_3PO_4 (0.01 M), H_3BO_3 (0.01 M) and NaOH (to adjust the pH at about 7.00) in 200 ml of distilled water. Under these conditions the H^+ concentration varies not linearly with time but in a more appropriate way to ensure a sufficient standing of the reaction in the different zones of pH inside the range 7–10 and it is not altered by the interaction with the substrate. The actual values of pH and temperature were automatically acquired by a computer connected to the experimental apparatus. At suitable times samples were withdrawn, stored at low temperature and analysed by HPLC.

HPLC system used was a Hitachi chromatograph equipped with a D-7000 interface, a solvent pump L-7100 and a spectrophotometric detector L-7400. A Varian Omnispher 5 C18 150 × 4.6 mm column was used with a mobile phase methanol–water–acetic acid (65:33:2, v/v/v). Measurements were carried out at a flow rate of 0.450 ml min^{-1} and at room temperature.

2.4. Data processing

Processing [16] of stored data was done using the Jandel Scientific Table Curve 2D.

3. Results and discussion

Fig. 1 summarises the results obtained in the HPLC-VTK experiment. Fig. 1a shows the kinetic profile obtained by hydrolysing aspirin at pH 7.00 at a rising temperature $T = T_0 + \alpha t$ with $T_0 = 323.2$ K and $\alpha = 1.631 \times 10^{-3}$ K s^{-1}. Data points were obtained by integration of HPLC peak areas relative to salicylic acid. The direct fitting of this data was carried out using Eq. (4) as a model:

$$A = (A_0 - A_f)\exp\left\{-\frac{k}{h}\exp\left[\frac{\Delta S^{\ddagger}}{R}\right] \times \int_0^t (T_0 + \alpha t)\exp\left[-\frac{\Delta H^{\ddagger}}{R(T_0 + \alpha t)}\right]dt\right\} + A_{\infty} \quad (4)$$

where A is the peak area at the time t and A_0, A_f (respectively, areas at time zero and at the end of the reaction), $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the parameters to be optimised. Twenty-seven points were analysed, very few compared with the hundreds or even thousands of points possible to acquire by a computer aided instrument [2,4–8]; nevertheless, the obtained values of $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are quite similar to those obtained spectrophotometrically, apart from a greater statistical error (HPLC: $\Delta S^{\ddagger} = -102 \pm 8$ J K^{-1} mol^{-1}, $\Delta H^{\ddagger} = 73 \pm 2$ kJ mol^{-1}, $R^2 = 0.9997$, 27 points analysed; UV/vis: $\Delta S^{\ddagger} = -110 \pm 1$ J K^{-1} mol^{-1}, $\Delta H^{\ddagger} = 71 \pm 1$ kJ mol^{-1}, $R^2 = 0.9999$, 335 points analysed). Indeed, correlation coefficient is lower and standard deviation is higher. Results are comparable to those obtained by traditional constant-temperature kinetic experiments (CTK: $\Delta S^{\ddagger} = -115 \pm 7$ J K^{-1} mol^{-1}, $\Delta H^{\ddagger} = 69 \pm 3$ kJ mol^{-1}, $R^2 = 0.9982$, five k_{obs} values). Interpolation, mild smoothing and application of the Savitsky–Golay [17] method allowed the calculation of the derivative of the kinetic profile (Fig. 1b). It shows well the trend of

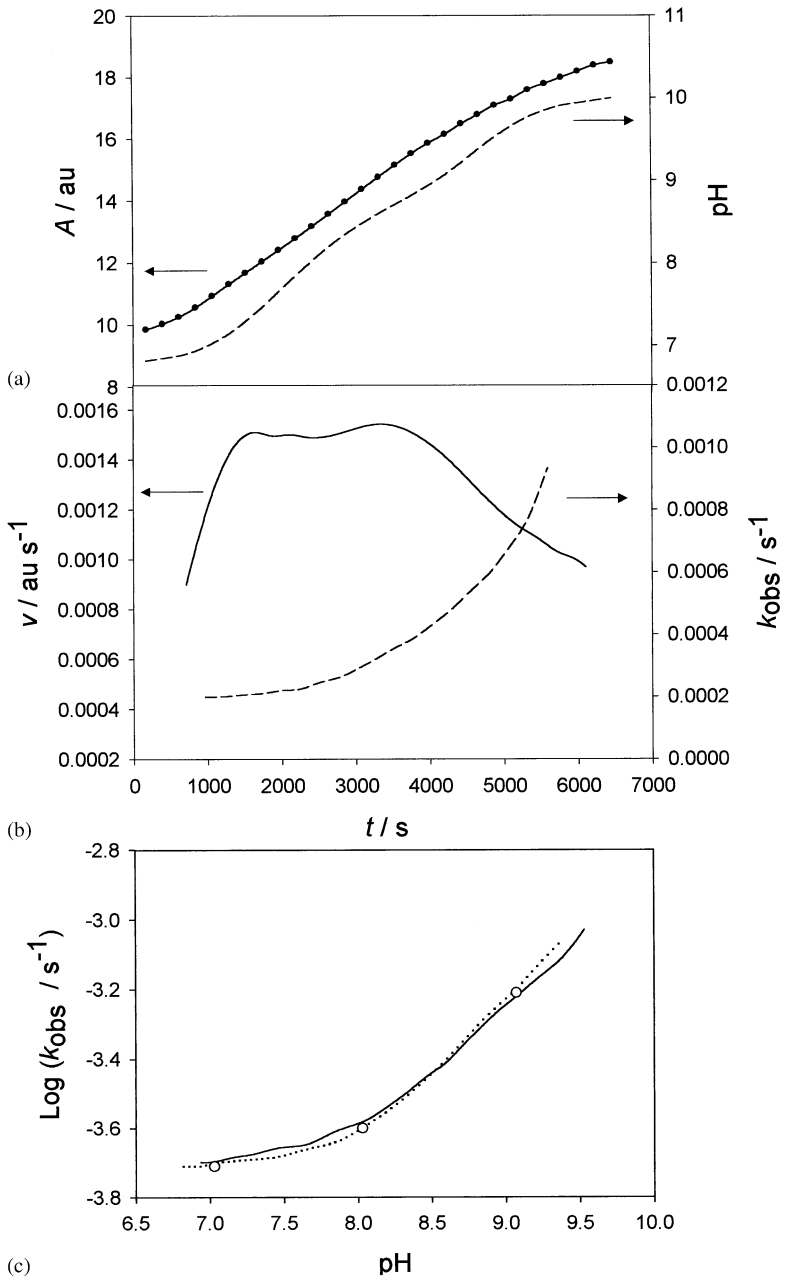

Fig. 2. (a) Change in HPLC peak area of salicylic acid (solid circles) during the hydrolysis of aspirin at $T = 342.3$ K at variable pH (dashed line). The solid line is an interpolated curve. (b) Trend of the reaction rate as obtained by differentiation of the kinetic curve (solid line) and k_{obs} (pH) profile obtained by dividing the derivative to $-(A_0-A_f)$ (dashed line). (c) pH-rate profile obtained by various methods: HPLC-VpHK (differ. meth.; solid line), UV/vis-VpHK (pointed line), CpHK (open circles).

the reaction rate during the process. The ratio of the derivative to $-(A_0-A_f)$, according to Eq. (5):

$$-\frac{1}{A-A_f}\frac{dA}{dt}=k_{obs}[T(t)] \quad (5)$$

gave the entire $k(T)$ profile (Fig. 1b). Fig. 1c shows a logarithmic plot of the results obtained either by the direct fit and by the differential method together with those obtained by VTK spectrophotometric experiments [4]. They are all on the same straight line confirming the goodness of the method and the reproducibility of the results. They are all coherent confirming that both the two processing methods are useful. Some kinetic data obtained by traditional CTK experiments are also shown in Fig. 1c. They are, between the experimental error, also in agreement.

Fig. 2 summarises the results obtained in the HPLC-VpHK experiment. Fig. 2a shows the kinetic profile obtained by hydrolysing aspirin at the temperature $T=342.3$ K at the variable pH shown in the figure with a dashed line. A direct fit of the profile to a mathematical model, although possible, was not attempted because of some difficulty to describe analytically both the modulating function pH(t) and the dependence function k_{obs}(pH). Having obtained good results in VTK experiment, we used the differential method. Fig. 2b shows the derivative of the kinetic profile as obtained by interpolation, mild smoothing and Savitsky–Golay algorithm. The trend of the reaction rate looks irregular but the curve contains in each point information about the rate constant. Indeed, dividing the derivative by $-(A_0-A_f)$, according to Eq. (6), the k_{obs}(pH) profile was obtained (Fig. 2b, dashed line).

$$-\frac{1}{A-A_f}\frac{dA}{dt}=k_{obs}[pH(t)] \quad (6)$$

Fig. 2c shows the results in logarithmic form, i.e. the pH-rate profile, together with results obtained in the same range in a spectrophotometric VpHK experiment and some points obtained by the traditional, and so far universally used, constant-parameter kinetic method. The pH-rate profile of aspirin in this range of pH shows an upper bend caused by a change in the reaction mechanism [18,19]. It would require at least ten kinetic experiments to be obtained. The result obtained by a single VpHK kinetic experiment describes the curve in a very good manner. HPLC-VpHK, UV/vis-VpHK, UV/vis-CpHK data are almost coincidental confirming the goodness of the method even when a discontinuous analytical monitoring is used.

4. Conclusions

The choice of the analytical instrument to be used in VPaK experiments requires a compromise between the response of the instrument to the method and the analytical signal arising from the physical properties of the system studied. HPLC is surely not the best choice because of the difficulties in automating the analysis for acquiring hundreds of points. Nevertheless, when it is necessary to use it, every kind of variable-parameter kinetic experiment can be carried out. The results, although with some limitation, are good and, particularly in the case of numerous routine measurements, very frequent in the tuning process of pharmaceutical molecules, can bring about a great saving of experimental time.

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References

[1] K.A. Connors, G.L. Amidon, V.J. Stella, Chemical Stability of Pharmaceuticals. A Handbook for Pharmacists, second ed., Wiley-Interscience, New York, 1986.
[2] G. Alibrandi, J. Chem. Soc. Chem. Commun. (1994) 2709–2710.
[3] G. Alibrandi, S. D'Aliberti, R. Pedicini, Chem. Educator 6 (2001) 185–191.
[4] G. Alibrandi, N. Micali, S. Trusso, A. Villari, J. Pharm. Sci. 10 (85) (1996) 1105–1108.
[5] R. Ficarra, A. Villari, N. Micali, S. Tommasini, M.L. Calabrò, M.R. Di Bella, S. Melardi, M.F. Agresta, S. Coppolino, R. Stancanelli, J. Pharm. Biomed. Anal. 20 (1999) 283–288.

[6] G. Alibrandi, Inorg. Chim. Acta 221 (1994) 31–34.
[7] R. Romeo, G. Alibrandi, Inorg. Chem. 36 (1997) 4822–4830.
[8] G. Alibrandi, S. Coppolino, S. D'Aliberti, P. Ficarra, N. Micali, A. Villari, J. Pharm. Biomed. Anal. 29 (2002) 1025–1029.
[9] E. Koch, Non-isothermal Reaction Analysis, Academic Press, London, 1977.
[10] B.W. Madsen, R.A. Anderson, D. Herbison-Evans, W. Sneddon, J. Pharm. Sci. 63 (1974) 777–781.
[11] J.M. Hempenstall, W.J. Irwin, A. Li Wan Po, A.H. Andrews, J. Pharm. Sci. 72 (1983) 668–673.
[12] S. Yoshioka, Y. Aso, M. Uchiyama, J. Pharm. Sci. 76 (1987) 794–798.
[13] G. Alibrandi, S. Coppolino, N. Micali, A. Villari, J. Pharm. Sci. 3 (90) (2001) 270–274.
[14] S. Zhang, T.L. Brown, Inorg. Chim. Acta 240 (1995) 427–432.
[15] G.H. Jiunnarkar, S. Stavchansky, Pharm. Res. 12 (1995) 599–604.
[16] W.H. Press, B.P. Flannery, S.A. Teukolsky, W.T. Vetterling, Numerical Recipes, Cambridge University Press, Cambridge, 1986.
[17] A. Savitzky, M.J.E. Golay, Anal. Chem. 36 (1964) 1627–1639.
[18] W.P. Jenks, Catalysis in Chemistry and Enzymology, McGraw Hill, New York, 1969.
[19] G.M. Loudon, J. Chem. Educ. 68 (1991) 973–984.