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Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies (II) Methods to evaluate the microcalorimetric response

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Summary

In this study, a well-known reaction, the hydrolysis of acetylsalicylic acid in aqueous solutions between pH 1.9 and 7.5 at 40.0 and 50.0°C, has been investigated by a microcalorimetric technique. The heat flow produced from the reaction was monitored as a function of time, and the degradation rate constant was then calculated from the slope. The precision was evaluated by repeated measurements at 40.0 and 50.0°C, respectively. The obtained rate constant-pH profile had the same general appearance as has been reported earlier for other temperatures. At 40.0°C and in the pH region of maximum stability the value of the rate constant showed low precision, because of a small heat flow change. As alternatives, the heat flow-time curves were evaluated by other methods to characterize stability. The heat flow value for a defined time (2, 4, and 12 h) and the quantity of heat evolved during a defined time interval (2–4, 1–7, and 0–11.5 h) were used. These pH profiles had the same shape, position of the minimum and plateau, as the rate constant-pH profile, except for heat flow at 12 h at 50.0°C. The two alternatives, the heat flow at 4 h and the quantity of heat at 2–4 h, also gave improved precision and the experimental time was shortened. Thermodynamic data for the transition state were calculated for two pH values. The resulting data were found to agree with the literature. The entropy of activation has a negative value, which indicates that the activated complex has a more organized structure than do the reactants.

Introduction

In preformulation and formulation work on pharmaceuticals, accelerated isothermal studies are commonly used to predict drug stability properties. The techniques used to analyse samples include HPLC and spectroscopy, for example. Another approach is to use microcalorimetry for stability measurements (Angberg et al., 1988; Hansen et al., 1989; Pikal and Dellerman, 1989). Since microcalorimetry is a non-specific analytical technique, it has the capacity for an overall stability prediction method. Data could be collected continuously or intermittently.

In a heat-conduction microcalorimeter, the measured property is primarily presented as a heat flow signal (power, dQ/dt, in $\mu W = \mu J/s$). The heat flow change is proportional to the rate or intensity of the reaction. The time integral is proportional to the quantity of heat (Q, in mJ) evolved or absorbed during the process.

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The size of the monitored heat flow signal depends on several factors: the rate constant, the enthalpy change of the process(es) and the amount of the reacting substance(s). By increasing the temperature, the reaction rate will increase. If many reactions occur simultaneously, e.g., if the reaction products degrade further, the heat from these reactions will contribute to the heat flow and thus complicate the interpretation of the registered signal. If the enthalpy changes of the processes have different signs, the absolute heat flow level may become unexpectedly low. When the main reaction is accompanied by an ionization, the heat flow change may still give the right rate constant but the heat quantity will be affected.

In a recent paper, a microcalorimetric technique for stability testing was presented (Angberg et al., 1988). The calorimetric instrument used was a 2277 Thermal Activity Monitor (TAM) with independently working microcalorimeters of an isothermal heat-conduction type. In that study, the specific acid hydrolysis of acetylsalicylic acid (ASA) at pH 1.1 in the temperature interval 30.0–50.0 °C was investigated in order to evaluate the precision and accuracy of the method. For that simple degradation process, the technique was found to be an alternative to conventional analytical methods. The value of the activation energy and the extrapolated value for the rate constant at 25 °C were in agreement with earlier reports, although the results obtained indicated that the precision of the measurements at temperatures below 45.0 °C was relatively low as a consequence of small changes in heat flow.

The objective of the present study was to determine the rate constants and to evaluate the precision for ASA hydrolysis at different pH values (1.9–7.5), and temperatures (40.0 and 50.0 °C). The reaction paths for the hydrolytic degradation of ASA to salicylic and acetic acids in aqueous solutions have been described earlier (Edwards, 1950; Garrett, 1957). The reaction rate of the hydrolysis is affected by the ionization of the carboxylic group of ASA and is largely pH-dependent, with a maximum stability around pH 2.5 at 25 °C. The reaction shows specific acid and specific base catalysis and the rate constant-pH profile shows a sigmoid region that leads to a pH-independent plateau between about pH 5 and 8. Several degradation paths are available and one or more dominates at different pH values. However, the overall reaction can be described by a pseudo first-order kinetic equation. The proportionality constant is the rate constant, k.

Both the general appearance of the rate constant-pH profile and specific values obtained for both rate constants and for the thermodynamic parameters describing the transition state were used to evaluate the accuracy of the microcalorimetric technique. The problem of establishing reproducible and reliable data, at low degradation rates, was given special attention. Another purpose was, therefore, to evaluate the heat flow-time curves by other methods in order to gain information about the stability.

Materials and Methods

Materials

Acetylsalicylic acid was obtained from Apoteksbolaget, Sweden (ASA; sieve fraction 0.15 mm Ph.Eur., batch no 61055); acetic acid (1.00 M, Titrisol), sodium dihydrogenphosphate hydrate p.a. ($NaH_2PO_4 \cdot H_2O$), hydrochloric acid (HCl, 1.00 M, Titrisol) and sodium hydroxide (NaOH, 1.00 M, Titrisol) were purchased from Merck (F.R.G.); sample and reference vessels, glass vials (3 ml) for single use; calorimeters, type 2277-201 (two calorimeter units were available in the study); pH-meter, Metrohm 632, Switzerland.

Methods

Buffer preparation

The pH values of the buffer solutions before the addition of ASA were 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, and 6.0 when using an acetic acid buffer system, and pH 7.0 and 8.0, in the case of a phosphate buffer system. The buffers were prepared by first making a 0.10 M acetic acid and a 0.10 M phosphate solution, respectively, followed by adjustment to the desired pH by titration with 1.00 M HCl and/or 1.00 M NaOH. The influence of

differences in ionic strength on the degradation rate was neglected in this study (Edwards, 1950).

Sample and reference preparation

ASA (180 mg) was dispensed into 100.0 ml (0.010 M) of the appropriate buffer solution at room temperature (23° ± 2° C). The powder was deaggregated by sonication and then stirred until dissolved. The pH was measured directly after sample preparation. The sample, 2.40 g ASA solution, and the reference, 2.40 g buffer solution, were carefully dispensed in glass vials. The vials were immediately sealed with a teflon coated butyl rubber disc and an aluminium cap. The preparation took about 20 min.

The same solution was used for experiments in each of the two available calorimeter units.

Apparatus

The design principle of the isothermal heat-conduction calorimeter system used in the study, the 2277 Thermal Activity Monitor (TAM), Thermometric AB (formerly LKB), Sweden, has been described elsewhere (Suurkuusk and Wadsö, 1982). A functional diagram of a calorimetric unit is shown in Fig. 1. The system consists of a 25 l thermostatted water bath, which can hold up to four calorimeter units. In each calorimeter unit, the sample and reference vessels are placed between an individual pair of thermopiles, through which the heat from the reaction is quantitatively transported. The calorimeter unit registers the difference in heat flow between the sample and the reference vessels, and this corresponds to the rate of heat flow change in the sample – the twin principle.

The calorimeters are electrically calibrated with the calibration resistors shown in Fig. 1. For reactions, with rates as in this investigation, the rate is proportional to the heat flow change and a static calibration is then sufficient. However, for faster reactions, it might be necessary to calibrate the instrument dynamically in order to correct the heat flow signal (Randzio and Suurkuusk, 1980).

The precision of the temperature of the water bath is $\pm 2 \times 10^{-4}$° C, and the accuracy is $\pm 2 \times 10^{-2}$° C of the chosen experimental temperature. The calorimeter unit can measure differences in

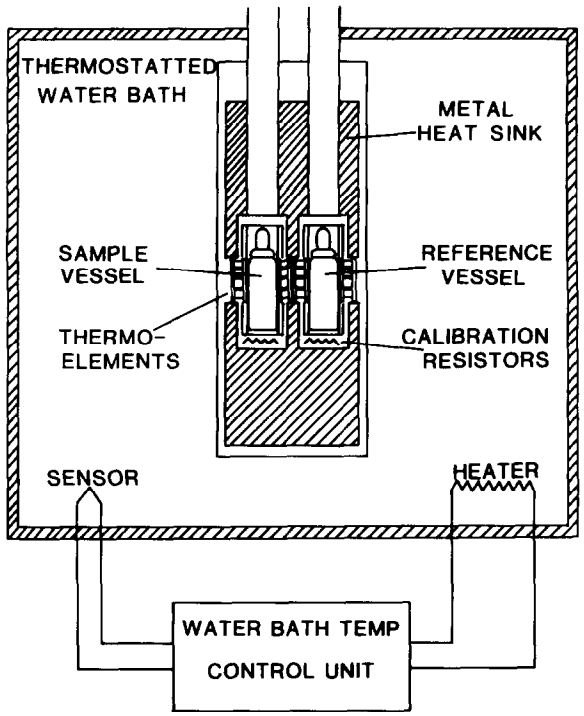

Fig. 1. Diagram of a calorimetric unit in the 2277 Thermal Activity Monitor.

temperatures of 10^{-6}° C, providing the ambient temperature does not change by more than ±1° C. In this study, room temperature did not vary more than ±1° C during a single experiment. Experiments were performed at 40.0 and 50.0° C. The experimental technique, which was as described earlier (Angberg et al., 1988), included a static electrical calibration before each experiment. The vials were then lowered to four separate temperature equilibration levels, before they reached the measurement position in the calorimeter unit. The total time allowed for the equilibration was 30 min. In all experiments the heat flow signal was recorded for at least 12 h.

Microcalorimetric disturbances

At low heat flow levels, there are some disadvantages when glass vials are used, and these can influence the experimental results (Suurkuusk and Wadsö, 1982; Angberg et al., 1988). These are mainly caused by stress relaxation in the rubber seal and temperature effects on the materials when

TABLE 1

Primary data and calculated values for the time period 7–11.5 h, for the experiments performed for the rate of the ASA hydrolysis

Temperature (°C)	pH [a]	No. of experiments n	Rate constant k ($\times 10^3$) (h^{-1})	Correlation coefficient r	Buffer system
40.0	1.1	10 [b]	32.3	−0.9991	HCl
	1.9	9	8.11	−0.7434 (1) [c]	acetate
	2.4	11	5.22	−0.7599 (4) [c]	acetate
	2.8	10	15.1	−0.9344 (1) [c]	acetate
	3.3	8	23.6	−0.9941	acetate
	3.8	6	37.8	−0.9995	acetate
	4.8	6	50.4	−0.9991	acetate
	5.4	6	48.2	−0.9986	acetate
	6.8	6	52.5	−0.9992	phosphate
	7.5	6	57.6	−0.9995	phosphate
50.0	1.1	10 [b]	81.1	−0.9997	HCl
	1.9	6	17.5	−0.9815	acetate
	2.4	6	16.9	−0.9845	acetate
	2.8	6	29.6	−0.9926	acetate
	3.3	6	56.4	−0.9986	acetate
	3.8	6	86.0	−0.9998	acetate
	4.8	6	123	−0.9998	acetate
	5.4	5	126	−0.9998	acetate
	6.8	6	135	−1.0000	phosphate
	7.5	6	137	−1.0000	phosphate

[a] The pH values refer to the measured pH after sample preparation.
[b] From Angberg et al. (1988).
[c] Number in parentheses refers to the number of experiments with positive slope values that were not included in the calculation of the mean values of the correlation coefficients.

the temperature rises. The magnitude of this effect depends on how well the sample and reference disturbances cancel each other, as it is a differential measurement. However, the use of glass vials also has advantages. Glass is a commonly used material in pharmaceutical packages, the vials are disposable and the samples can be retrieved in the vials for later microcalorimetric measurements or for further analysis.

Another factor, that can influence the measurements, especially when low heat flow levels are monitored, is baseline fluctuations (Angberg et al., 1988).

Treatment of the microcalorimetric response

The experimental data were recorded by a computer that registered the heat flow (dQ/dt, in μW) every second. The mean heat flow was calculated over a 2 min interval, every 30 min. The heat flow-time curve was also integrated each second by the trapezoidal method and then summed every 30 min, to give the heat quantity evolved (Q, in mJ). The obtained values for the heat flow and heat quantity cannot, with certainty, be regarded as absolute values, as the microcalorimeters were calibrated by the inbuilt electric calibration equipment. This is because the generation of energy is different and the position of the calibration resistors is external to the position of the vial (Fig. 1).

When the vials had reached the measurement position in the calorimeter (30 min after sample preparation had finished) the computer program was started at $t = 0$. The natural logarithms of the heat flow values ($\ln dQ/dt$) were plotted as a function of time every 30 min between 7 and 11.5 h. This time period had been found earlier to be a representative region for a 20 h ASA hydrolysis

experiment using glass vials (Angberg et al., 1988). For all experiments, the rate constant, k, for the pseudo first-order hydrolysis and the correlation coefficient, r, were calculated from the slope of the line by linear regression. The number of experiments performed at each combination of pH and temperature is in Table 1. The values at pH 1.1 are taken from the earlier study, where the buffer solution was 0.10 M HCl (Angberg et al., 1988). The logarithms of the mean rate constants were subsequently plotted as a function of pH. The shapes of the pH-rate profiles for 40.0 and 50.0 °C were then compared with the profiles reported earlier.

To evaluate the precision of the measurements, the relative standard deviation and the 95% confidence interval for the mean values were calculated (Student's t-test). As the precision was low around the pH of maximum stability, it was of interest to evaluate the heat flow-time curves also by other methods. The mean heat flow values (dQ/dt) at defined times 2, 4, and 12 h and the mean heat quantities (Q) that were evolved during defined time periods 2–4, 1–7 and 0–11.5 h were monitored. Both the precision and the general appearance of their pH profiles were then characterized as described above for the rate constants.

The transition state

In the transition state theory or the activated complex theory (Evans and Polanyi, 1935; Eyring, 1935a,b), an equilibrium is considered to exist between the reactants and an activated state (designated *). The equations relevant to this investigation are presented below (Noggle, 1985).

The equilibrium constant, K^*, can be calculated from the Gibb's free energy of activation, ΔG^*:

$$\Delta G^* = -RT \ln(K^* C) \quad (1)$$

where R denotes the gas constant, T is the absolute temperature and C is a concentration factor (1 dm^3/mol) to make K dimensionless. ΔG^* consists of contributions from the enthalpy of activation, ΔH^*, and the entropy of activation, ΔS^*, as given by the equation:

$$\Delta G^* = \Delta H^* - T\Delta S^* \quad (2)$$

For reactions in solution, the enthalpy of activation is related to the activation energy E_a by:

$$\Delta H^* = E_a - RT \quad (3)$$

The entropy of activation of the activated complex is related to the pre-exponential factor A in the Arrhenius equation by:

$$\Delta S^* = R \ln[Ah/ekT] \quad (4)$$

where h is Planck's constant and k is Boltzmann's constant.

Microcalorimetric measurements which yield mean rate constants at different temperatures permit derivation of all these parameters through use of the Arrhenius equation. In this study, data for experiments conducted at pH 1.1 and 4.8 were chosen for such analysis and the derived data was compared with thermodynamic data for the transition state in the literature (Garrett, 1957).

Results and Discussion

The rate constant-pH profiles

Primary heat flow-time curves for ASA hydrolysis at pH 2.4 and 5.4, at 40.0 and 50.0 °C, are shown in Fig. 2. The results clearly demonstrate that the reaction rate is much higher at pH 5.4 than at pH 2.4. pH 2.4 corresponds to the pH region of maximum stability and pH 5.4 to the plateau region. The heat flow change at pH 2.4 and 40.0 °C is minute.

Two buffer systems were used in this investigation, acetic acid and phosphate. The released proton from the degradation reaction is taken up by the buffer system. The heat released is larger for the phosphate buffer system than for the acetate buffer system. The heat flow will thus be slightly higher, but the rate of heat flow change should be the same.

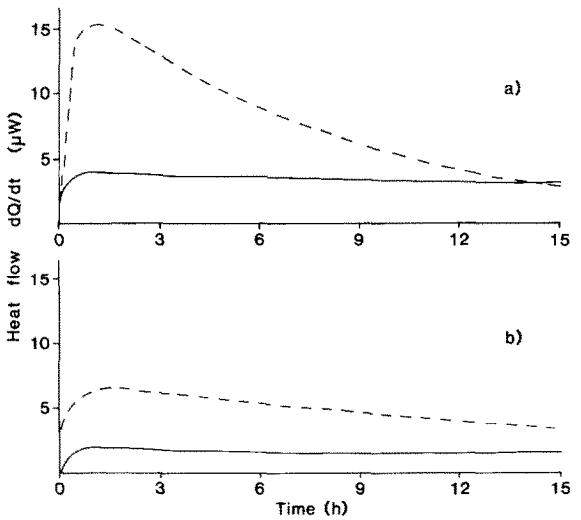

Fig. 2. Primary heat flow-time curves for ASA hydrolysis at pH 2.4 (———) and pH 5.4 (------) at (a) 50.0 °C and (b) 40.0 °C.

In Table 1, some primary data and calculated characteristics are given for the experiments performed. The pH values of the ASA solutions were measured immediately after sample preparation. These pH values are denoted in Table 1 and used in the figures. The addition of ASA decreased the pH values of the buffer solutions by 0.1–0.6 pH units. The pH was also measured after the experiments, i.e. normally 20–24 h after sample preparation. Minor decreases in pH during the experiments, normally 0.1 pH units, were observed. The actual pH during the time period, 7–11.5 h, has nevertheless been regarded as constant in this study.

The logarithms of the mean rate constants from Table 1 have been plotted as a function of pH in Fig. 3. The profiles shown in Fig. 3 are in approximate agreement with those reported earlier, for other temperatures (Edwards, 1950; Garrett, 1957). The precision and the correlation coefficients are, in general, better for the higher rate constants, due to an increase in the heat flow change.

The low precision obtained at 40.0 °C around pH 2.5 is obviously a result of the small heat flow change as can clearly be seen in Fig. 2. The heat flow signal is then much more influenced by disturbances, for instance, caused by relaxation effects in the rubber seal and fluctuations in the baseline. Some of the heat flow curves even gave rate constants with positive values over the time interval 7–11.5 h (number in parentheses, Table 1). The time interval was extended to 4–14 h, but the results did not improve to any large extent. In particular, at pH 2.4, the precision is so low that the mean value of the rate constant is uncertain, as can be seen by the pH profile shown in Fig. 3b.

For the higher temperature tested, 50.0 °C, a comparison with literature data is possible. The value of the rate constant obtained by Garrett (1957) at pH 2.5 and a temperature of 50.3 °C was

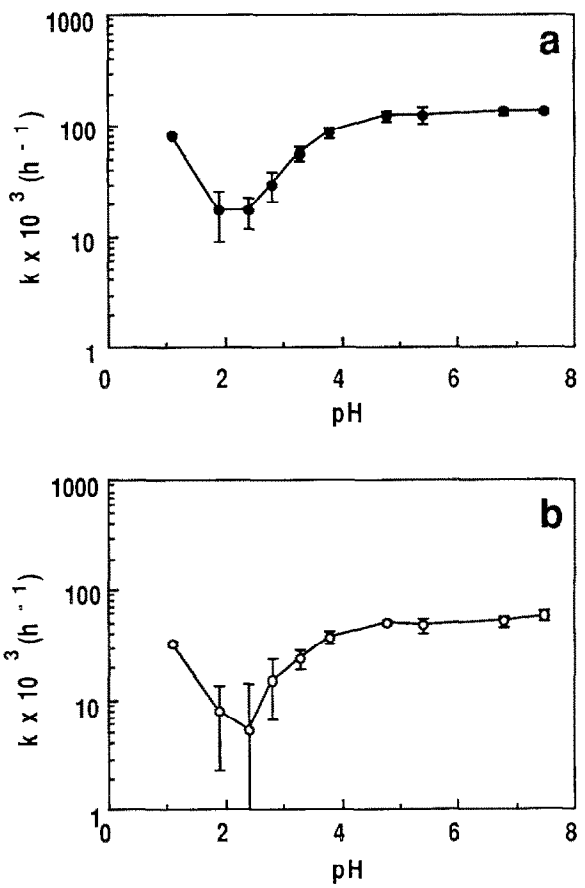

Fig. 3. Mean rate constant, k, for ASA hydrolysis for the time interval 7–11.5 h as a function of pH at (a) 50.0 °C and (b) 40.0 °C. Error bars represent the 95% confidence interval for the mean. For some values, precision is better than can be denoted in the figure.

TABLE 2

Comparison of thermodynamic quantities for the transition state for ASA hydrolysis at two pH levels and 25 °C

Method	pH	Activation energy, E_a (kJ mol^{-1})	Frequency factor, A ($\times 10^{-10}$) (h^{-1})	Entropy, ΔS^* (J mol^{-1} K^{-1})	Enthalpy, ΔH^* (kJ mol^{-1})	Gibb's free energy, ΔG^* (kJ mol^{-1})	Equilibrium constant, K^* ($\times 10^{19}$)
Micro-calorimetry	1.1 [a]	71.9 (5) [c]	3.33	−120	69.4	105	3.75
Spectro-scopy	1.1 [b]	69.5 (4) [c]	1.21	−128	67.0 [d]	105 [d]	3.70 [d]
Micro-calorimetry	4.8	75.3 (2) [c]	18.0	−106	72.8	104	5.27
Spectro-scopy	5.0 [b]	73.6 (4) [c]	10.4	−110	71.2 [d]	104 [d]	5.84 [d]

[a] From Angberg et al. (1988).
[b] From Garrett (1957).
[c] Number in parentheses defines the number of temperatures that has been used to determine the activation energy, E_a.
[d] Values calculated from the data given by Garrett (1957).

17.4×10^{-3} h^{-1}. In this study, the mean rate constant at pH 2.4 is 16.9×10^{-3} h^{-1}. Another rate constant reported by Garrett was a plateau value of 127×10^{-3} h^{-1} at pH 5.0 at 50.3 °C, and in this study the rate constant is 123×10^{-3} h^{-1} at pH 4.8 at 50.0 °C. Consequently, considering the differences in pH and temperature, the results generated in this study are in agreement with the data obtained by a conventional technique.

A further comparison of data can be made for the transition state parameters. In Table 2, values for these parameters are compared for pH 1.1 (Garrett, 1957; Angberg et al., 1988), pH 4.8 (this paper) and pH 5.0 (Garrett, 1957). The pH values 4.8 and 5.0 are both on the rate constant-pH independent plateau. The values from Garrett were converted to the units used in this study and his approximation that $\Delta H^* = E_a$ has been recalculated as $\Delta H^* = E_a - RT$.

As denoted in Table 2, the activation energy at pH 4.8 is calculated from only two temperatures. However, the use of only two data points was here regarded as acceptable for establishing the slope of the straight line. Firstly, a linear relationship is expected, since the reaction is known to follow the Arrhenius equation. Secondly, the precision of the data points used was relatively high (Fig. 7).

The values obtained in this study are also in agreement with those reported by Garrett (1957). The mean rate constants at different temperatures, i.e. the primary data obtained experimentally, are used to calculate the activation energy and subsequently the other parameters for the transition state as described above. ΔG^*, ΔH^* and ΔS^* are the respective differences between the Gibb's free energy, the enthalpy and the entropy for the transition state and for the reactants. The negative values for the entropy of activation, ΔS^*, indicate that, necessarily, the activated complex has a more organized structure than do the reactants. ΔG^* is, necessarily, positive since the equilibrium constant, K^*, is small – only a small fraction of the total number of molecules can be in the activated state.

The heat flow-pH profiles

Due to the low precision for the rate constant values at 40.0 °C and pH around 2.5, another approach was applied in order to characterize the stability of ASA. Inspection of the primary heat flow-time curves (Fig. 2) shows that for a fast reaction, the heat flow at a defined time is higher than that for a slow reaction, at least at the beginning of the experiment. Therefore, the mean heat flow values at defined times 2, 4 and 12 h were calculated and their logarithms were plotted as a function of pH (Fig. 4). For the pH interval 1.9–5.4, when acetate buffer was used, the same

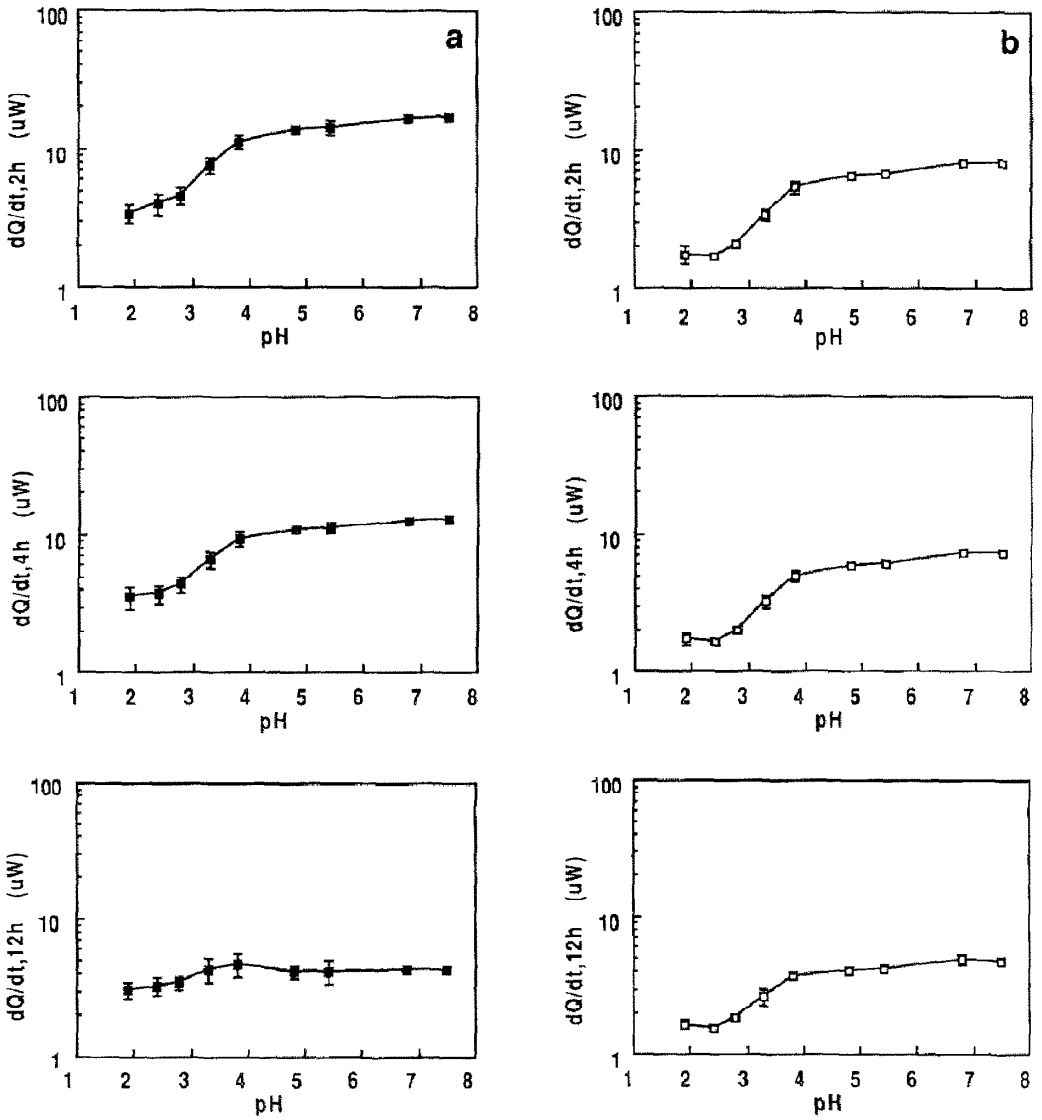

Fig. 4. Mean heat flow (dQ/dt) for ASA hydrolysis at the defined times 2, 4 and 12 h as a function of pH at (a) 50.0 °C and (b) 40.0 °C. Error bars as in Fig. 3.

type of profile is developed as for the rate constant-pH profile, except for the heat flow at 50.0 °C and 12 h. This can be understood on inspection of Fig. 2a – the heat flow after 12 h becomes very low for fast reactions; the reaction has nearly gone to completion. For pH values 6.8 and 7.5, when phosphate was used as the buffer component, the heat flow levels are higher and this, therefore, results in a limited change in the profile. The pH minimum is not exactly the same for 40.0 and 50.0 °C, but the region of maximum stability is clearly shown for the heat flow values at 2 and 4 h. The precision is approximately the same for 2 and 4 h. The experimental time is considerably reduced with this method compared to that involving rate constant calculation. It does not, of course, give the same amount of information.

The heat quantity-pH profiles

The area under the heat flow-time curve over a defined time interval represents the heat quantity (Q) evolved during that time. This area is larger for a fast reaction than that for a slow reaction, at least at the beginning of the experiment (Fig. 2). The logarithms of the mean heat quantity for the time intervals 2–4, 1–7 and 0–11.5 h plotted as a function of pH are shown in Fig. 5. All the pH-heat quantity profiles are approximately the same as the rate constant-pH curve except for the minor step in the pH-independent plateau when phosphate buffer is used (pH 6.8 and 7.5). The precision of the results for different time intervals is close in every case.

Comparisons

The heat flow level and the heat quantity pro-

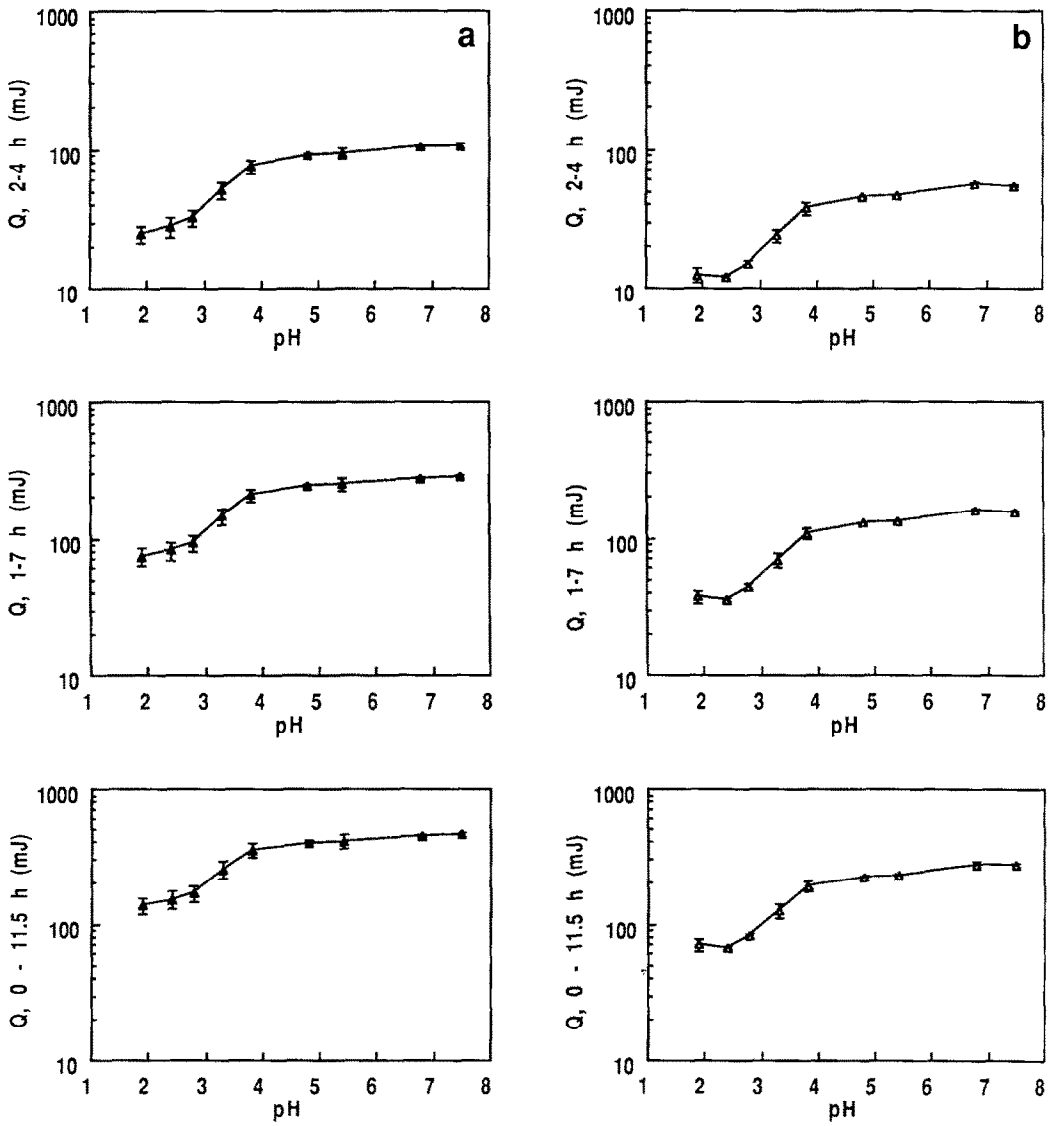

Fig. 5. Mean heat quantity (Q) for ASA hydrolysis for the time intervals 2–4, 1–7 and 0–11.5 h as a function of pH at (a) 50.0 °C and (b) 40.0 °C. Errors bars as in Fig. 3.

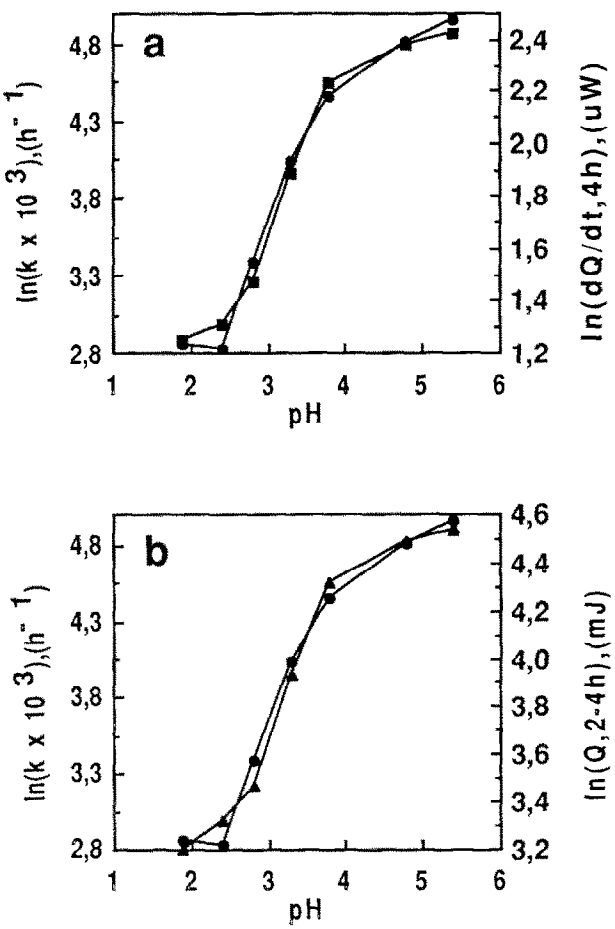

Fig. 6. Comparison between the rate constant-pH (●) and (a) the heat flow (4 h) profile (■) and (b) the heat quantity (2–4 h) profile (▲) for ASA hydrolysis at 50.0 °C.

files gave approximately the same shape as the rate constant-pH profile. It was also possible to predict the pH region with the maximum stability by both methods at 40.0 °C.

To compare the profiles in more detail, the rate constant-pH profile was normalized in Fig. 6 with the heat flow (4 h) profile and the heat quantity (2–4 h) profile at 50.0 °C for the pH interval 1.9–5.4 (acetic acid buffer). These profiles reveal the possibility of shortening the experimental time to 4 h. Using, as a measure, the heat flow at 2 h was considered to be too early in the experiment because of the risk of disturbances. The heat flow during the latter part of an experiment might, on the other hand, be impossible to use, as shown for 50.0 °C, 12 h (Fig. 4a).

In Fig. 7, the relative standard deviations for the mean are plotted vs pH for the rate constant (7–11.5 h), the heat flow (4 h), and the heat quantity (2–4 h). As can be seen, the relative standard deviations are reduced for the heat flow and the heat quantity in the pH region of maximum stability. As expected, the relative standard deviation for the rate constant is inversely related to the rate constant-pH profile (Fig. 3) – a small rate constant yields a large relative standard deviation.

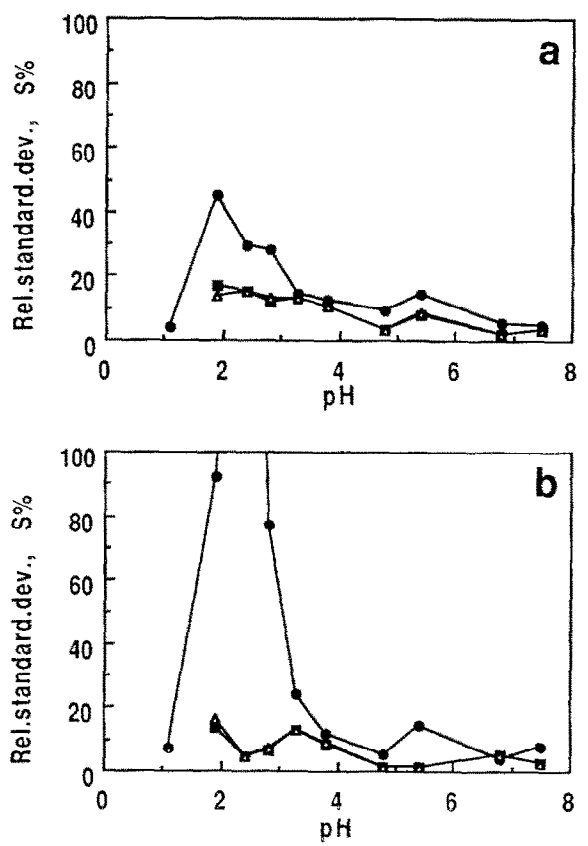

Fig. 7. Comparison between the relative standard deviations, *S*%, plotted vs pH for the mean of the rate constant (7–11.5 h) (●), the heat flow (4 h) (■) and the heat quantity (2–4 h) (△) at (a) 50.0 °C and (b) 40.0 °C. At pH 2.4, 40.0 °C, the relative standard deviation for the rate constant was 252%.

Conclusions

The possibility of using microcalorimetry for stability measurements has been shown by establishing the accuracy and precision of degradation rate constants for acetylsalicylic acid hydrolysis over a broad pH range.

To characterize stability it is possible to evaluate the microcalorimetric heat flow-time curves by several methods. The difficulties resulting from change of the buffer system in this kind of evaluation have been pointed out. Two functions, the heat flow (4 h) and the heat quantity (2–4 h), have the advantage of showing a better precision than the rate constant and experimental time is considerably reduced. For other reactions, where degradation does not result in a linear log dQ/dt vs t plot, the suggested methods of evaluating the heat flow-time curves may be an alternative to measurements of the rate constant to characterize stability.

The results of this study support the earlier results and conclusions (Angberg et al., 1988) that the microcalorimetric technique can be a complement to conventional analytical techniques for stability predictions in pharmaceutical studies.

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