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Stability study of piroxicam and cinnoxicam in solid pharmaceuticals☆

R. Ficarra [a,*], A. Villari [a], N. Micali [b], S. Tommasini [a], M.L. Calabrò [c], M.R. Di Bella [a], S. Melardi [a], M.F. Agresta [a], S. Coppolino [a], R. Stancanelli [a]

[a] *Dipartimento Farmaco-Chimico, Università di Messina, Facoltà di Farmacia, Vill. Annunziata 98168 Messina, Italy*
[b] *Istituto di Tecniche Spettroscopiche, CNR, Sperone 31, Vill. S. Agata 98166 Messina, Italy*
[c] *Facoltà di Farmacia, Università di Catanzaro, compl. Barbieri, 88021 Roccelletta di Borgia (CZ), Italy*

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Abstract

The pseudo-first order rate constant for the hydrolysis of cinnoxicam as a function of temperature was obtained by variable-temperature kinetic experiments. The method used is on a generalization of non-isothermal analysis, and takes advantage of the capabilities of modern data collection and processing systems. A spectrophotometric method under non isothermal conditions was carried out. The results obtained are identical to those obtained under the same conditions by using traditional constant-temperature kinetic runs. This provides the possibility of reducing the amount of time spent and chemicals usually used in collecting kinetic data in mechanistic studies in solution by an order of magnitude. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The mechanistic investigation of a chemical reaction is a very important step in the effort to understand the submicroscopic world and it is often very useful for practical applications [1,2]. Unfortunately, in the kinetic world, a great deal of work is often necessary in order to clarify in detail the behavior of the systems studied [3].

The aim of this work is to apply a new recently proposed method [4,5], based on a generalization of non-isothermal analyses [6], for an in vitro investigation of piroxicam and cinnoxicam stability in two pharmaceutical formulations.

Variable-temperature kinetic (VTK) experiments are not new and have all stemmed from 'non-isothermal reaction analysis', extensively used in thermochemistry. Some interesting examples of applications have been reported in the pharmaceutical field [7–9].

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* Corresponding author. Fax: +39-90-6766407.
E-mail address: pficarra@pharma.unime.it (R. Ficarra)

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The method consists of carrying out kinetic experiments while varying, in a known way, a parameter (T, P, $[H^+]$, etc.) and in obtaining, with a single kinetic run, instead of a single rate constant, the entire dependence of the rate constant on that parameter [10].

Spectrophotometry represents one of the most powerful and utilized method for monitoring the progress of a chemical reaction [1] for several reasons: good sensitivity, simple apparatus and good control of temperature. These, together with the easy connection to a computer for real time collection of the experimental data, makes it the best choice for VTK experiments.

2. Experimental

2.1. Materials

Piroxicam and cinnoxicam were separately extracted with methanol from the pharmaceutical formulations Feldene® (Pfizer Italiana, Latina) and Sinartrol® (Società Prodotti Antibiotici, Milano).

2.2. Extractions

Ten capsules of Feldene® (120 mg) and ten tablets of Sinartrol® (30 mg) were separately extracted with 30 ml of methanol (three times for 30 min) under magnetic stirring at 37°C.

The extractive solutions were collected and evaporated under vacuum. Pureness control was carried out by TLC and HPLC.

2.3. Solutions

Solutions at different pH values (0.5, 1.5, 3.0, 7.0, 8.0) were prepared. The hydrogen ion concentration was obtained at 25°C, according to F.U., by using solutions of HCl 0.1 M and KCl 0.1 M (pH 0.5, 1.5), disodium phosphate 0.2 M and citric acid 0.1 M (pH 3.0, 7.0, 8.0), pH values refer to a temperature of 25°C. The water used for solutions was distilled, deionized and filtered through a 0.22 μm Millipore. Solvents of analytical grade were used.

2.4. Instruments: drug stability

Kinetic runs were carried out using a Perkin-Elmer Lambda 3 spectrophotometer connected to a 486/DX2 microcomputer and equipped with a cell compartment thermostated by a Perkin-Elmer PTP (Peltier temperature programmer) which allows a controlled change of the temperature with an accuracy of ± 0.05°C. The temperature was checked by a platinum resistor inserted into the spectrophotometric cell and connected to the computer (readout resolution 0.01°C). The absorbance–time data were automatically acquired by using the Perkin-Elmer PECSS program.

Processing of data for the fitting was done using the Jandel Scientific PEAKFIT program with the Marquardt algorithm [11] and every experiment was repeated three times.

3. Results and discussion

3.1. Stability test

Cinnoxicam, piroxicam and cinnamic acid standard solutions were prepared by dissolving the substances (4 mg) in 100 ml of four different buffers (pH 0.5, 3.0, 7.0 and 8.0). Every solution was analyzed spectrophotometrically in a range of 200–400 nm at 25°C (Fig. 1).

In order to verify substances stability, all solutions were analyzed at 65°C. The studies on cinnoxicam showed hydrolysis of the molecule; at pH 0.5, the substance was transformed into piroxicam and cinnamic acid and this hydrolysis occurred within 5 h. At pH 3.0, under the same temperature conditions, this process proved to be slower, taking place, in fact, within 8 h.

Analyses of the solutions at other pH values (7.0 and 8.0) indicated that hydrolysis occurred, but in an insignificant percentage, within 24 h. In the experimental conditions piroxicam kept an unchanged structure.

Cinnoxicam kinetics measurements. The kinetics of cinnoxicam hydrolysis were monitored spectrophotometrically either by variable-temperature or by constant-temperature kinetic runs in order to have some direct comparative data.

Cinnoxicam hydrolysis can be represented by the expression

$$A \rightarrow B$$

where the concentration change as function of time is explained by equation:

$$[B] = [A_0](1\ e^{-(kt)}) \quad (1)$$

which describes the exponential increase of concentration as a function of time.

Since the hydrolysis is monitored by the UV absorbance value (D), by replacing the spectrophotometric term in Eq. (1) it will become:

$$D_t = (D_0 - D_\infty)\exp(-kt) + D_\infty \quad (2)$$

where D_t, D_0 and D_∞ are the absorbances at time t, at the beginning and at the end of the reaction, respectively.

In a traditional way, reaction profile, k/T, in a range of temperature, is calculated by various experiments at constant temperatures according to the Eyring equation. On the contrary, in this work, during the hydrolysis reaction the temperature was varied ($T = T_0 + \alpha t$) and the following equation is applied:

$$[B] = ([A_t] - [A_0]) \exp\left\{ -\frac{k_B}{h}\exp\left[\frac{\Delta S^{\mp}}{R}\right]\int_0^t (T_0 + \alpha t)\exp\left[-\frac{\Delta H^{\mp}}{R(T_0 + \alpha t)}\right]dt\right\} \quad (3)$$

where k_B is Boltzman constant; h is Plank constant; R is the universal gas constant.

It was possible, by one experiment at variable temperature, to calculate $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ parameters and obtain the kinetic profile in the range of the temperature considered. Reaction parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) correspond to an hypothetical of the first order reaction, that shares only the hydrolysis speed with the 'true' reaction of the pseudo-first order.

Fig. 2 shows spectrophotometric variable-temperature kinetic profile obtained for cinnoxicam

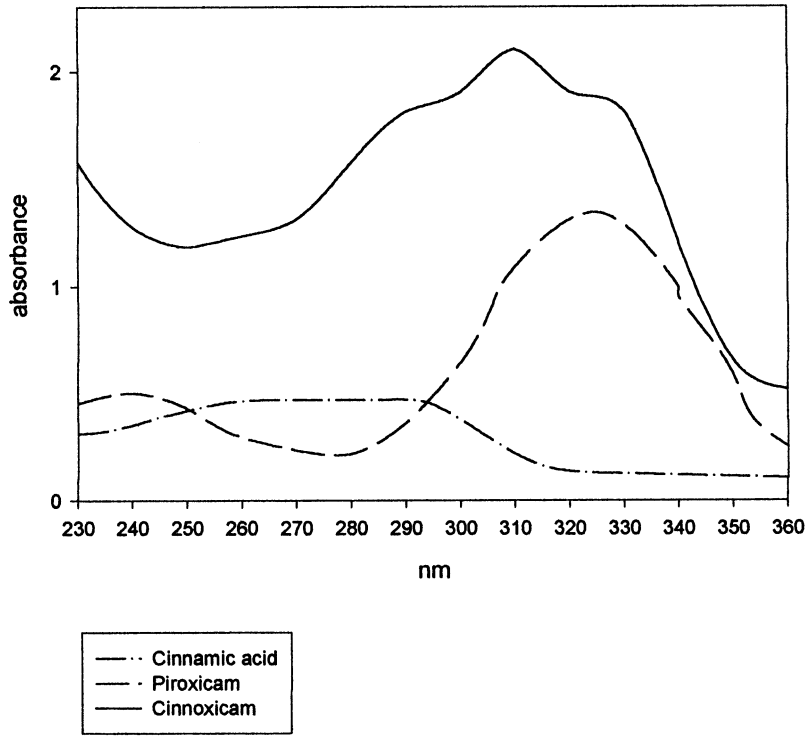

Fig. 1. UV spectra of piroxicam, cinnoxicam and cinnamic acid.

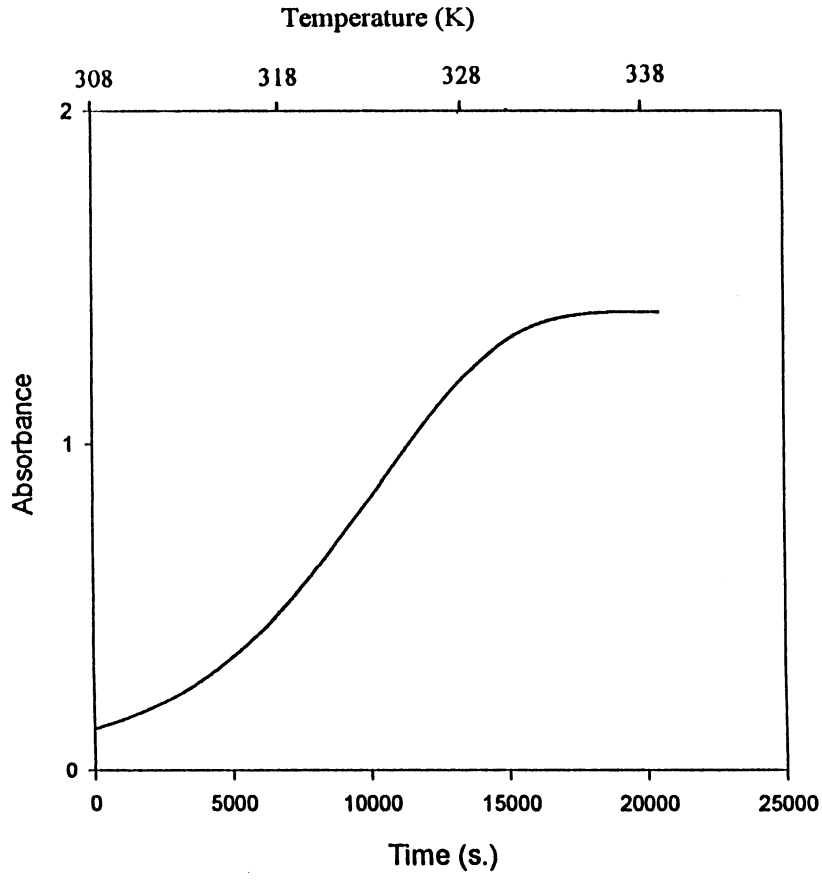

Fig. 2. Spectrophotometric variable-temperature kinetic profile obtained for the cinnoxicam hydrolysis at pH 0.5, at $\lambda = 325$ nm.

hydrolysis at pH 0.5 at a linearly increasing temperature ($T = T_0 + \alpha t$), $\lambda = 325$ nm. It has the typical sigmoid shape, which is due to the acceleration of the reaction in its first part caused by the increase of the temperature. After the inflection point, the acceleration cuts down because the substrate concentration decreases. The 'plateau' of the curve is indicative of completion of the hydrolysis.

By using the mathematical expression (Eq. (3)), it was possible, by fitting absorbance values as a function of temperature, to obtain $\Delta H^{\ddagger}$ ($1.2323 \times 10^5 \pm 1$ kJ mol^{-1}), $\Delta S^{\ddagger}$ (64.8244 ± 1 J K^{-1} mol^{-1}) values with residue ± 0.02, residue% ± 4. ΔG (1.0132×10^5 kJ mol^{-1}) and subsequently ΔE (128.84 kJ mol^{-1}) values were calculated at 338 K. The temperature increasing, α, was $1.566 \pm 0.001 \times 10^{-3}$ K s^{-1}. The replacing of these values in the Eyring's equation, made it possible to trace out the hydrolysis constant profile (k).

In order to confirm the previous data, some experiments were performed at constant temperature (35, 45, 55, 65°C) (Fig. 3). In this test four exponential curves were obtained, that evidenced, in the beginning, an acceleration of the reaction, then a 'plateau'. This is indicative of the completion of cinnoxicam hydrolysis. The data obtained, reported in Table 1, are in agreement with the results of conventional kinetic studies.

The velocity of reaction (k), measured at different constant temperatures, coincides with that calculated from the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ obtained by the experiment at variable temperature, from 35 to 65°C (Fig. 4), with an experimental error of 1%.

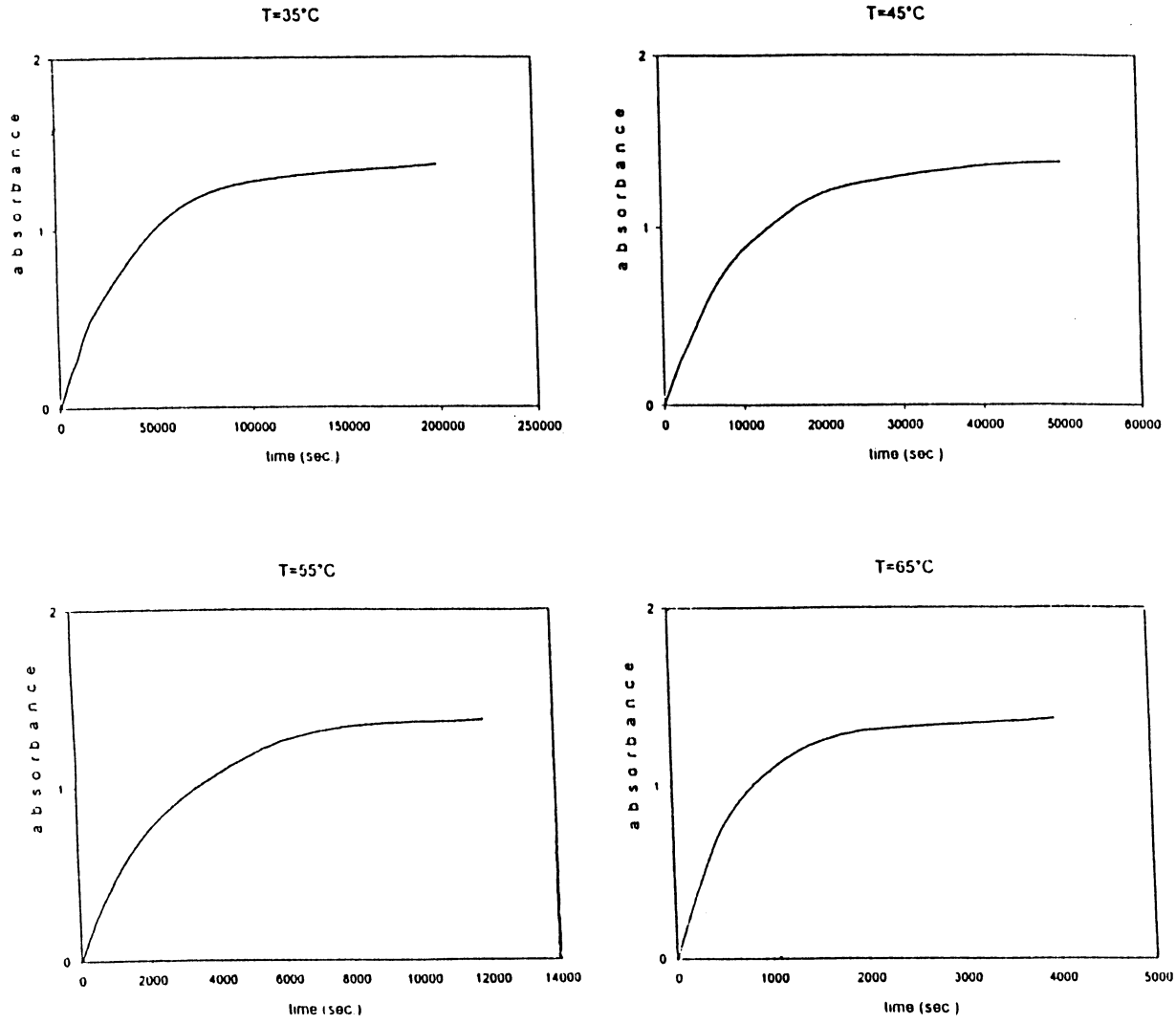

Fig. 3. Spectrophotometric constant-temperature kinetic profiles obtained at pH 0.5 for the cinnoxicam hydrolysis at 35, 45, 55, 65°C, respectively.

4. Conclusion

The results pointed out that piroxicam maintained an unchanged structure, despite of the pH and temperature variations, thus indicating great stability. On the contrary stability studies on cinnoxicam, established hydrolysis of the molecule. The use of variable-parameter kinetics adds a new dimension to the experimental data describing the reactivity of a system studied.

The experimental apparatus was made up of routine instruments. The kinetic data were collected automatically and processed by microcom-

Table 1
Reaction velocity (k) measured at constant temperatures

T°C	k (s^{-1})
35	2.449×10^{-5}
45	9.716×10^{-5}
55	3.448×10^{-4}
65	1.528×10^{-3}

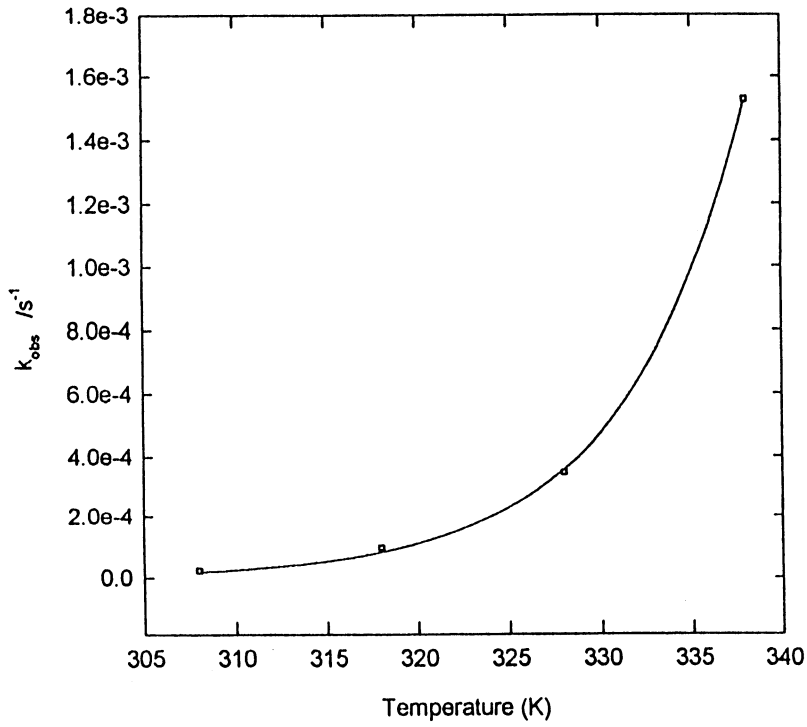

Fig. 4. Dependence on the temperature of the pseudo-first-order rate constant ($k_{obs}(T)$ profile) of the cinnoxicam hydrolysis in water at pH 0.5 as obtained by a single variable-temperature kinetic run (solid line). Squares refer to traditional constant-temperature kinetic runs carried out under the same conditions.

puters using commercially available software. The results, obtained quickly by using a simple mathematical process, are subjected to a minor statistical uncertainty compared to those obtained with a traditional isothermal analysis.

This method can be applied as well to obtain from a single kinetic run the dependence on other parameters as, for example, the concentration of a reactant and the role of other parameters such as pH, ionic strength.

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