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STUDY ON THE RATE OF DECOMPOSITION OF AMOXICILLIN IN SOLID STATE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Amoxicillin sodium salt degradation in solid state relies on a sequential reaction consisting of two pseudo-first-order processes. Amoxicillin trihydrate, now used in pharmaceutical formulations, is significantly more stable than sodium amoxicillin. It degrades according to Prout-Tompkins model. We studied the stability of amoxicillin at temperatures of 37°, 50°, 80°, 90°, 100° y 110°C. HPLC was chosen as the analytical method. Amoxicillin and its decomposition products are separated by reversed-phase (C18) HPLC with gradient elution.

INTRODUCTION

The study of the stability of penicillins in solid state is of great economic and sanitary importance, for it is in this state that they are stored, transported, distributed and administered ; furthermore, the period between the manufacture of a medicine and its use varies greatly, and in many cases may be several years.

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Several papers exist concerning the decomposition kinetics of amoxicillin in aqueous solution [1, 2]. The purpose of the present paper is to study the stability of amoxicillin in the solid state, both as sodium salt and as free acid (trihydrate). It was determined the effect of temperature on the stability of these antibiotics.

MATERIALS AND METHODS

Samples.

Sodium amoxicillin (purity greater than 95 %) with a water content of 2.3 % and amoxicillin trihydrate (purity greater than 98 %) with a 12.4 % water content (Karl Fischer's method) were used. These compounds were supplied by Antibióticos S.A. (León, Spain).

Apparatus.

HPLC chromatograms were obtained on a Konik KNK-500 liquid chromatograph. UV spectral measurements were performed with a Spectronic 2000 spectrophotometer (Bausch Lomb) and IR spectra were obtained on a Shimadzu IR-435 spectrophotometer. Heraeus T 5060E and Heraeus RVT 220 ovens were used.

Chromatographic method.

For the HPLC quantitative analyses of the antibiotics, both isocratic and gradient elutions were performed, using a 25 cm x 0.46 cm I.D. column, packed with Microbondapak C18 (10 μm), and a 3 cm x 0.46 cm I.D. pre-column packed with Microbondapak C18 (40-60 μm).

Isocratic elution was performed with a mobile phase of 0.05 M KH_2PO_4 (pH 4.4)-methanol (95:5,v/v), detection being carried out at 229 nm, where the injection volume was 20 μl and the flow rate 2 ml/min. Gradient elution was performed with mobile phases A, methanol-0.2 M phosphate buffer (pH 7.0)-water (5:5:90, v), and B, methanol-0.2 M phosphate buffer (pH 7.0)-water (50:5:45, v). The gradient elution programme was as follows : 5 % of mobile phase B for 5 min, increased at a rate of 2 % of B per min to 65 % of B, held at 65 % of B for 30 min, decreased at a rate of 8 % of B per min until the original concentration was reached. Detection was at 279 nm.

Kinetic method.

20 mg lots of the substance investigated were weighed out in solid state and put into small glass vials which were hermetically sealed and placed in sand baths. These were put into ovens at a predetermined temperature, which it was important to keep even through-out each oven. At regular intervals, vials were removed from the ovens and the sample dissolved in 25 ml of 0.05 M phosphate buffer, pH 6.0, in the case of sodium amoxicillin, and in 25 ml of acetonitryl-0.05 M phosphate buffer, pH 6.0, (10:90, v/v) for amoxicillin trihydrate. The solution thus prepared was used to determine the quantity of intact antibiotic by HPLC (isocratic method).

To analyse the products originated by the degradation of solid state amoxicillin using gradient elution HPLC, 40 mg of the sample was dissolved in 5 ml of mobile phase A, for sodium amoxicillin, and in 3 ml of 0.2 M phosphate buffer, pH 11.0, and 2 ml of mobile phase A, for amoxicillin trihydrate. The sample was injected into the column one minute after its preparation.

Preparation of piperazine-2,5-dione (2-(6'-hydroxyphenylpiperazin-2',5'-dion-3'-yl)-5,5-dimethylthiazolidine-4-carboxylic acid).

A method was used analogous to that described by Bundgaard and Larsen [3] for ampicillin : 5 g of sodium amoxicillin was dissoved in 20 ml of a 10 % w/v aqueous solution of glucose, the pH being adjusted to 9.2, which solution was kept at room temperature (22°C) for 24 hours. The pH was maintained at 9.2 ± 0.1 by periodical adjustments with 2 M sodium hydroxide, adjustments being necessary only during the first 5 or 6 hours. After the whole reaction the solution was cooled to 4°C and the pH was brought down to 2.0 by means of 5 M hydrochloric acid. The resultant yellow precipitate was left undisturbed at 4°C for 24 hours, filtered, washed with water and vacuum-dried at 40°C, there finally being obtained 3.8 g of 2-(6'-hydroxyphenylpiperazin-2',5'-dion-3'-yl)-5,5-dimethyl thiazolidine-4-carboxylic acid; IR (KBr) ν_{max} 2739, 2359, 1666, 1298, 1099 and 924 cm^{-1}; UV max. 274 nm.

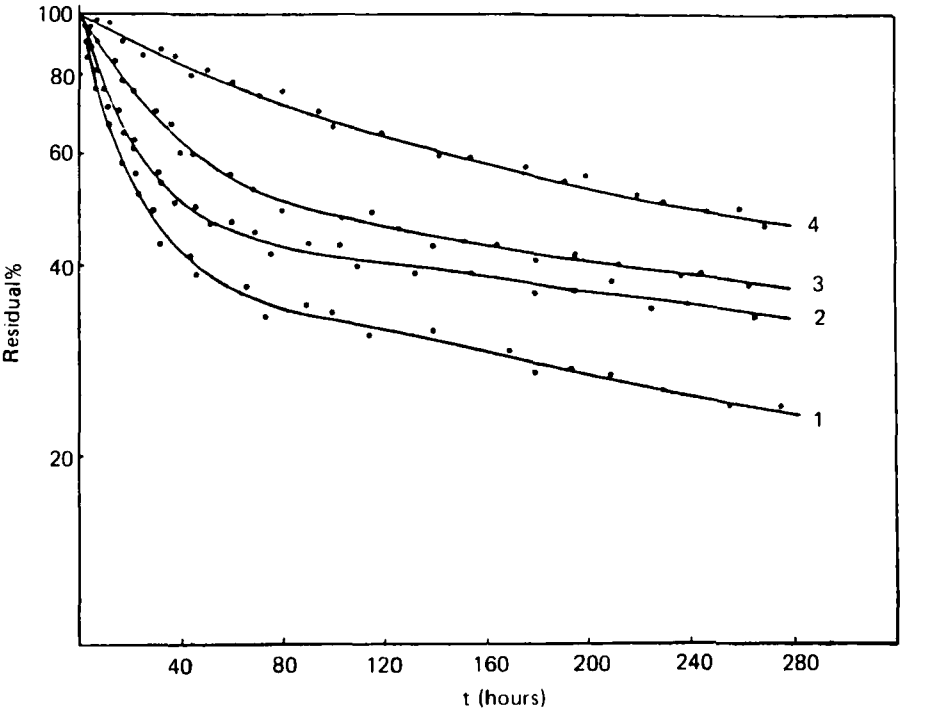

FIGURE 1

Plot residual percentage versus time for a thermal degradation of amoxicillin sodium salt at different temperatures : 1 = 110°C, 2 = 100°C, 3 = 90°C, 4 = 80°C.

Preparation of amoxicilloic acid.

This was carried out using Munro and workers'procedure [4].

RESULTS AND DISCUSSION

Thermal degradation of sodium amoxicillin.

Figure 1 shows the residual percentage of sodium amoxicillin against time at temperatures of 80°, 90°, 100° and 110°C. By applying the "residuals method" or "subtraction technique" [5,6] to the decomposition curves, straight lines were obtained as a consequence of the first subtraction, which allows us to postulate that the

TABLE 1

Partial Pseudo-First Order Rate Constants (k_1, k_2 in s^{-1}) for Amoxicillin Sodium Salt Thermal Degradation in Solid State.

Temperature (°C)	$k_1.10^6$	$k_2.10^8$
37	0.0249[a]	0.995[a]
80	2.32	12.98
90	9.95	20.41
100	14.17	40.66
110	18.23	51.70
110	18.18[b]	51.17[b]

[a]Calculated from Arrhenius representation.

[b]Calculated from "subtraction technique".

degradation of sodium amoxicillin takes place via a mechanism of successive reactions in two steps, as in the following model :

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad \text{(model 1)}$$

where k_1 and k_2 are the first-order partial rate constants, and A, B and C are the substrate and the products formed sequentially during the process of degradation.

The curves obtained may be described by a biexponential equation:

$$A = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}$$

The kinetic constants (Table 1) were obtained by adjusting these curves by means of a programme for the analysis of non-linear regression by the least squares method. For a temperature of 110°C, these constants were also obtained graphically by applying the so-called "subtraction technique", the same results being arrived at by both methods. As may be observed, the values for the constants

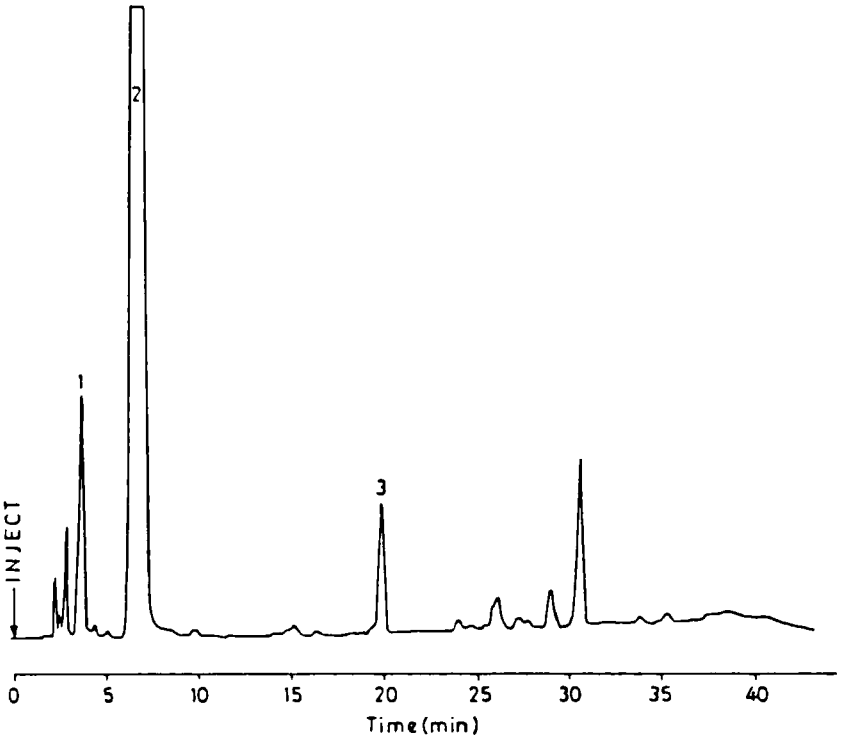

FIGURE 2

HPLC chromatogram of sodium amoxicillin. Amount injected : 20 μl. See material and methods for chromatographic conditions. Peaks : 1 = amoxicilloic acid, 2 = amoxicillin, 3 = piperazine-2,5-dione.

k_1 are much higher than those of k_2 whence it is deduced that product B (model 1) will at first accumulate and then change into C.

Partially degraded amoxicillin produces chromatograms like the one in figure 2, where we have identified the peaks due to amoxicillioc acid, amoxicillin and piperazine-2,5-dione. With time, the piperazine-2,5-dione peak increase, while the amoxicilloic acid peak tends to keep its level until it finally decrease.

When degradation of the sample is advanced, many small peaks appear which may be due to dimeroates, trimeroates, tetrameroates, trimers and tetramers.

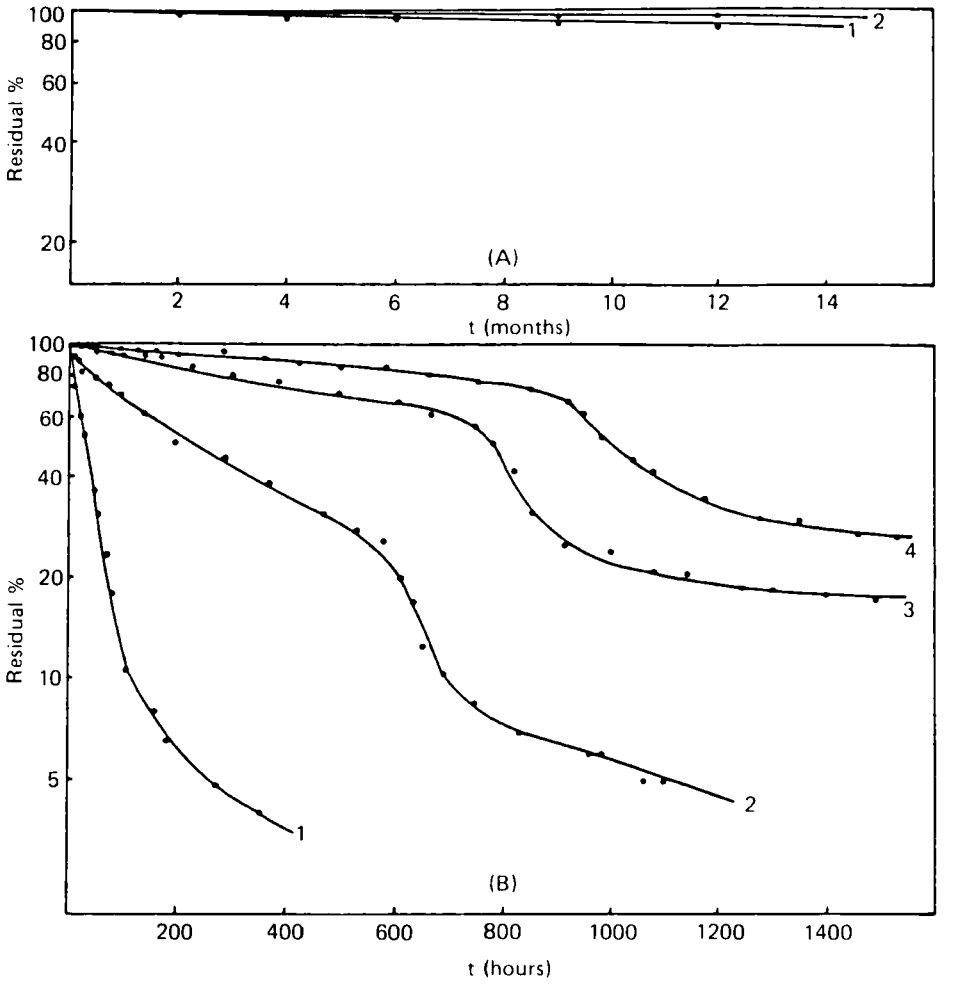

FIGURE 3

Typical S-shaped curves for a thermal degradation of amoxicillin trihydrate at different temperatures : 1 = 110°C, 2 = 100°C, 3 = 90°C, 4 = 80°C.

Thermal degradation of amoxicillin trihydrate.

The degradation of amoxicillin trihydrate was investigated at 37°, 50°, 80°, 90°, 100° and 110°C. The plot of the residual percentage against time for the different temperatures is shown in figure 3, (A) and (B).

The sigmoidal shape of these curves, fig. 3 (B), would suggest that the degradation of amoxicillin trihydrate may correspond to the following type of reaction [7] :

$$A_{sol.} \xrightarrow{k} B_{sol.} + C_{gas} \qquad \text{(model 2)}$$

where k is a first-order rate constant.

In a curve of this type, three periods are to be distinguished: an initial induction period, the time taken for 10 % of the decomposition to take place; an acceleratory period, when from 10 % to 50 % degradation occurs, and a decay period, which is less reproducible than the other two.

Prout-Tompkins's model is the one most generally used for explaining decomposition curves of this type [8, 9], its interpretation being based on this equation :

$$\log \frac{C_t}{C_o - C_t} = -\frac{k}{2.303} t + b$$

where C_o and C_t represent substrate concentrations at t = 0 and t, respectively, and k is the decomposition reaction rate constant.

The plot of $\log C_t/(C_o - C_t)$ against time is a straight line after the end of the induction period (figure 4). The rate constants for the acceleration period were calculated from the slopes of these lines (Table 2).

It may be observed that these constants have much higher values than those found at 37° and 50°C, for which the curves obtained are not sigmoidal, probably because at low temperatures the crystalline form of trihydrate is stable, whereas at higher temperatures it decomposes following Prout-Tompkins kinetic.

HPLC chromatographic analysis of a partially decomposed sample of amoxicillin trihydrate gives, under the conditions indicated above, a chromatogram like the one in figure 5. It is to observed that in this case too amoxicilloic acid and piperazine-2,5-dione

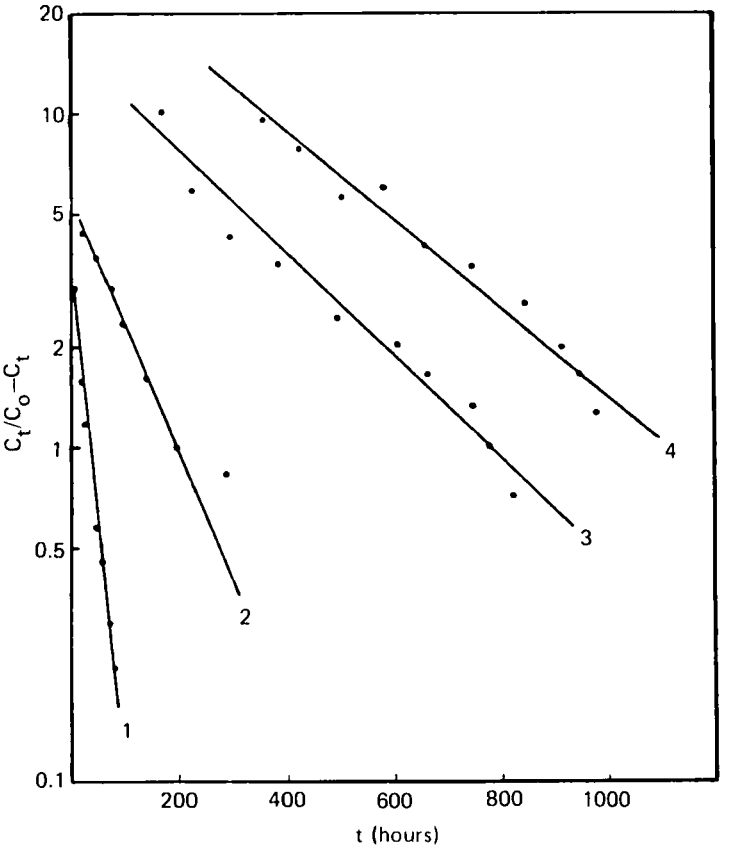

FIGURE 4

Linear plots log $C_t/(C_o - C_t)$ versus time constructed according to Prout-Tompkin's concept for thermal degradation of amoxicillin trihydrate at different temperatures : 1 = 110°C, 2 = 100°C, 3 = 90°C, 4 = 80°C.
C_t = concentration (%) of amoxicillin trihydrate at time t.
C_o = initial concentration (%) of amoxicillin trihydrate.

are formed as degradation products of the antibiotic, as are other compounds which may be polymers and polymeroates.

We also determined, by Karl Fischer's method, the water content of the samples on their removal from the oven, which content becomes less and less with degradation time, indicating that amoxicillin trihydrate loses its water of hydration as thermal degradation advances.

TABLE 2

Rate Constants (k in s^{-1}) and Induction Periods for Amoxicillin Trihydrate Thermal Degradation in Solid State.

Temperature (°C)	Induction Period (h)	$k.10^7$
37	---	0.0098
50	---	0.0306
80	396	8.40
90	131	9.37
100	8	23.10
110	5	94.11

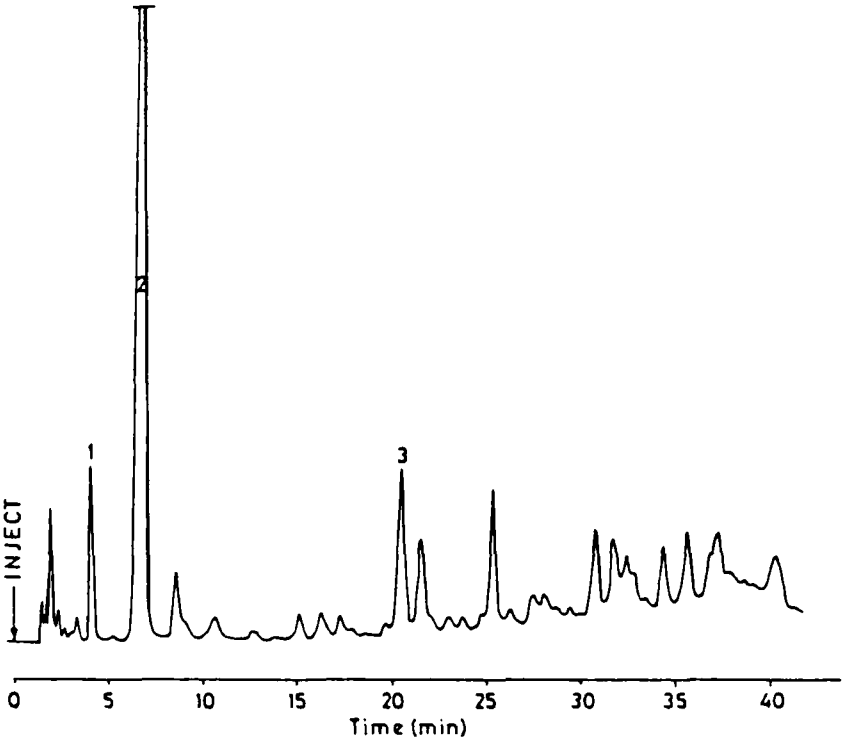

FIGURE 5

HPLC chromatogram of amoxicillin trihydrate. Amount injected : 20 µl. See material and methods for chromatographic conditions. Peak : 1 = amoxicilloic acid, 2 = amoxicillin, 3 = piperazine-2,5-dione.

TABLE 3

Arrhenius Parameters for Amoxicillin Sodium Salt as well as for Amoxicillin Trihydrate Degradation Reaction in Solid State.

Arrhenius Parameters	Sodium Amox.	Amox. Trihydrate
ΔH_a ($J.mol^{-1}$)	99502.09	90754.39
log A	9.1568	7.1959
$10^9.k_1$ (s^{-1}) at 20°C	2.665	1.041
$t_{0.1}$ (years) at 20°C	1.25	3.20

Table 3 shows the activation enthalpy and frecuency factor logarithm values for the degradation reaction of sodium amoxicillin and amoxicillin trihydrate, respectively, together with the rate constant at 20°C for each antibiotic and their expiry period, that is the time taken for 10 % degradation to take place ($t_{0.1}$).

In order to calculate the Arrhenius parameters of sodium amoxicillin, were used the rate constants k_1, which represent the first step in the degradation of substrate A (model 1).

A comparison of the results obtained demonstrates that the zwitterion form (amoxicillin trihydrate) is more stable than the anionic one (sodium amoxicillin), the latter's degradation rate constant being, for example, at a temperature of 100ºC, about six times greater than the former's. These differences are much greater at room temperature.

As may be seen in table 3, the two antibiotics have very different expiry periods: sodium amoxicillin takes 1.25 years to degrade 10% at room temperature, whereas amoxicillin trihydrate takes 3 years.

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