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Study of accelerated inactivation of cefadroxil

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Summary

A study was made of the accelerated inactivation rate of cefadroxil, a cephalosporin intended for oral use, as a function of the following pH values of the solution: 2.5, 3.2, 4.0, 5.0, 6.0, 7.0 and 8.0, after adjusting their ionic strength to $\mu = 0.5$.

Once the inactivation rate constants of the cephalosporin had been obtained, for the temperatures studied, the values of these constants were calculated for temperatures of −4, 20 and 37°C, using the Arrhenius equation. Also calculated as a function of pH and temperatures were the values of activation energy and the frequency factor of the inactivation reaction of cefadroxil.

Introduction

Cefadroxil is a semi-synthetic cephalosporin whose chemical structure is 7-D-(−)-α-amino-(4-hydroxyphenyl)-acetamido-3-methyl-3-cephem-4-carboxylic acid. This antibiotic has proved effective in the treatment of both Gram-positive and Gram-negative organisms (Buck and Price, 1977) and is of special use in the treatment of infections of the respiratory (Mercado et al., 1978) and urino-genital tracts (Hennes and Richards, 1978).

Intended for oral use, it shows good bioavailability when administered by this route. Once in the systemic circulation, its binding to the plasma proteins is poor and it is excreted in an unaltered state principally by the kidney (Pfefer et al., 1978). Previous studies carried out in volunteers with normal renal function have shown that at usual clinical doses cefadroxil shows dose-independent behaviour (Mariño and Dominguez-Gil, 1980).

Studies on accelerated instability are principally based on subjecting the drug in question to extremes in temperature, pH, etc., where it undergoes a process of degradation, generally measured in physical terms, in such a way that the results

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obtained may easily be extrapolated to the kinds of conditions in which the drug is normally found, in particular, during storage.

This kind of study has the disadvantage that if the functional group or system used for the quantifying physical measurement is not responsible for the therapeutic effect of the drug, it is possible that the calculation of the instability of the drug might not be a true index of its loss of activity. Thus on undertaking this work, an attempt was made not to determine the instability of cefadroxil but rather, to determine the inactivation of this antibiotic; in order to this a microbiological method was used which permitted the measurement of the antibiotic.

The reasons behind such a study are two-fold; on the one hand, to determine the inactivation of cefadroxil during its path through the body, in particular through the GI tract, and on the other hand, to ensure that the solutions of this drug, essential for use in multiple experiments, do maintain their expected activity.

Analytical technique

The determination of the antibiotic in aqueous solutions at different pH values was carried out by a microbiological plate diffusion method using *Bacillus subtilis* (ATCC no. 6633) as the test organism.

Standard curves of the antibiotic in aqueous solutions buffered at the same pH values as the test solutions were constructed using known concentrations. All determinations were carried out a minimum of 4 times. Repeated analysis of the reference standards indicates that the accuracy of the method expressed as relative standard deviation was 5%. The lower sensitivity limit of the method was 1.60 μg/ml.

Materials and methods

The accelerated inactivation of cefadroxil was studied in aqueous solutions buffered at different pH values (2.5, 3.2, 4.0, 5.0, 6.0, 7.0 and 8.0) using citrate-phosphate-borate buffer of Teorell and Stenhagen, adjusted to an ionic strength of $\mu = 0.5$ in each case. An initial solution of cefadroxil was prepared at a pH value corresponding to the study which was to be carried out. From this solution aliquots of 5 ml were taken and sealed in glass vials under a stream of N_2. The batch of vials in turn was divided into 3 groups, each of which was later subjected to a particular temperature in a thermostatted water bath at different temperatures. At previously programmed times, the samples were removed from the baths, one by one, and immediately plunged into crushed ice. After labelling, the vials were stored at $-20°C$ until assay.

Theoretical

Experiments in studies on accelerated instability usually consist in determining a series of concentrations of the undegraded drug as a function of time, at a given pH

and temperature. With the resulting data a determination is first made of the order of the degradation reaction, after which it is possible to determine the instability constant in such a way that as a function of the pH value, we will obtain a value of the instability constant for each temperature (Lachmam et al., 1976).

By means of the Arrhenius equation, it is possible to relate constants and temperatures.

$$K = A \cdot e^{-E_a/RT}$$

where K = rate constants of the process, A = frequency factor, E_a = activation energy, R = gas constant (1.987 cal/deg · mol) and T = temperature in absolute degrees.
That is:

$$\log K = \log A - \frac{E_a}{2.303\,R} \cdot \frac{1}{T}$$

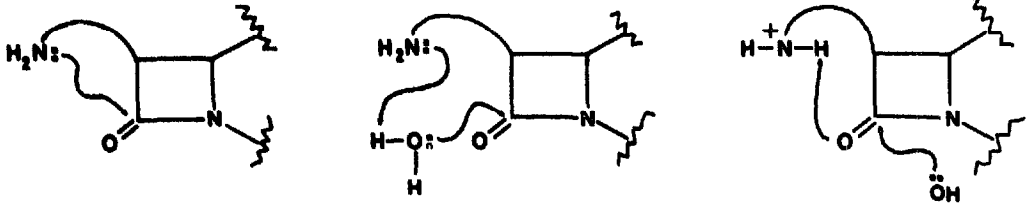
With the relationships established between the constants and the temperatures, it is possible to extrapolate the values of the inactivation constants at any other temperature, such as body temperature (37°C), normal storage temperature (20°C) and freezer storage temperature (−4°C) for the same pH value. From these constants it is possible to determine the inactivation half-life; i.e. the time it takes to inactivate 50% of the initial drug. It is also possible to determine the expiry date if the drug is formulated in solution at that pH; i.e. the time 10% of the initial drug would take to degrade. Similarly, all determinations were repeated for the complete range of pH values studied.

It is classically recognized that the possible degradation mechanisms for cefalosporin antibiotics are: hydrolytic breakdown of the β-lactam ring by direct attack by water, and degradation caused by intramolecular participation of the nearby amide group on the C = 0 bond of the β-lactam ring.

Where a side-chain amide participates in the degradation of cefalosporins by an intramolecular reaction, the introduction of an electron-donating substituent into the side-chain would facilitate the breaking down of the ring, while an electron-withdrawing substituent would retard it. This theory necessitates the existence of an intermediate degradation product, highly reactive, which could be formed under physiological conditions of temperature and pH and which might play an important part in allergies to cephalosporins (Rattie et al., 1979).

Yamana et al. (1976) propose that for the α-amino cephalosporins there are a series of possible degradation mechanisms in which the α-amino group participates; these are shown in Scheme I: if these mechanisms may coexist, from the kinetic point of view they are identical.

SCHEME 1



Results and discussion

Fig. 1 shows the initial results of non-inactivated cefadroxil concentrations for each of the temperatures and pH values as a function of time.

Using an HP-97 minicomputer, it was determined from these values that the inactivation of cefadroxil in solution, as a function of pH, follows first-order kinetics (Ray-Bellet and Etter, 1980).

Table 1 shows the values of the inactivation rate constants obtained for the different temperature and pH values studied in this work together with the corresponding degradation half-lives and expiry periods.

With the results obtained for the inactivation constants for each temperature as a function of pH, the Arrhenius equation was applied, fitting, by means of the least-squares method with a HP-97 programmable computer, the linear relationships between the logs of the inactivation constants and the reciprocals of the temperatures expressed on the Kelvin scale; the equations obtained are shown in Table 2 together with the correlation coefficients and statistic probability levels. The same table shows the values obtained with these equations for the activation energy and the frequency factor, which in turn are shown in Fig. 2.

By extrapolating the regression lines for each pH, we obtain the theoretical values of the degradation constants, half-lives and expiry periods at temperatures of 37, 20 and −4°C, as shown in Table 3.

It may be seen from these results that cefadroxil is stable in solution at body temperature (37°C) for pH values between 2–8, though the values of the activity half-life, obtained for pH values between 6–8, are lower than those reported by Buck and Price (1977), who in a similar study report that the activity half-life of cefadroxil remains greater than 24 h at a temperature of 37°C and throughout the physiological pH range.

On comparing the values of the activity half-life of cefadroxil at 37°C with the results reported in the literature of this same parameter in other antibiotics, it may be seen that cefadroxil is acceptable in terms of effect. Table 4 shows the values obtained by us for the activity half-life of cefadroxil at the pH values of maximum and minimum stability, together with those appearing in the literature for other oral cephalosporins. At pH values of maximum stability, cefadroxil has an active half-life similar to that described for cephalexin and cephradine (Yamana and Tsuji, 1976), and much greater than cephaloglycine (Yamana and Tsuji, 1976), cephroxadine (Perotas van Herckenrode and Cadorniga) and cefaclor (Fogleson et al., 1978). For pH values of minimum stability, it may be seen that in terms of degradation, cefadroxil is similar to cephradine (Yamana and Tsuji, 1976) and cephroxadine (Perotas van Herckenrode and Cadorniga) and is considerably more stable than cephaloglycine, cephalexin (Yamana and Tsuji, 1976) and cefaclor (Fogleson et al., 1978).

The plot shown in Fig. 3 may be obtained by representing the logs of the inactivation constants theoretically derived by extrapolating for cefadroxil at 37°C as a function of the pH value. Fig. 3 shows 3 clearly differentiated zones; one for pH values lower than 3.2, which would be the acid inactivation zone; another between

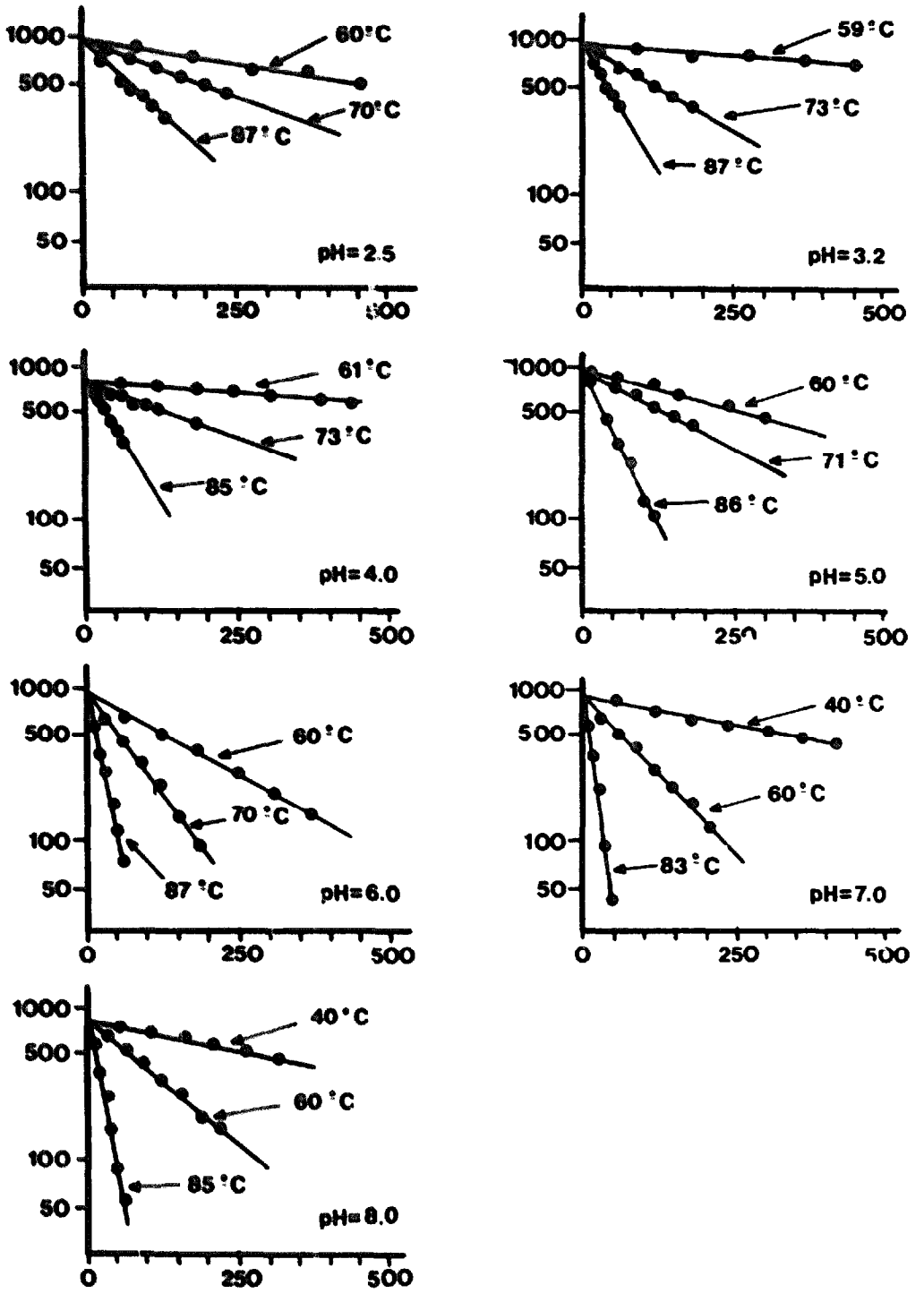


Fig. 1. Non-inactivated cefadroxil concentrations (μg/ml) as a function of the pH and temperature values studied versus the time (min).

pH values of 3.2–4.0 in which degradation seems to be independent of pH, and a third zone of alkaline degradation for pH values greater than 4.0 in which after a certain pH a loss of linearity may be noted.

TABLE I

INACTIVATION CONSTANTS, HALF-LIFE AND $t_{90\%}$ OBTAINED AS A FUNCTION OF THE EXPERIMENTAL pH AND TEMPERATURE VALUES

pH	T (°C)	$K \times 10^{-3}$ (min^{-1})	$t_{1/2}$ (h)	$t_{90\%}$ (h)
2.5	60	1.5337	7.531	1.141
2.5	70	3.3566	3.441	0.521
2.5	87	8.1827	1.412	0.206
3.2	59	0.5659	20.411	3.122
3.2	73	5.0384	2.222	0.351
3.2	87	15.4222	0.749	0.115
4.0	61	0.7620	15.157	2.297
4.0	73	3.7772	3.058	0.463
4.0	85	17.7667	0.654	0.099
5.0	60	2.5373	4.552	0.690
5.0	71	4.7233	2.445	0.371
5.0	86	19.7889	0.584	0.088
6.0	60	5.0782	2.274	0.348
6.0	70	12.7050	0.904	0.139
6.0	87	43.3870	0.266	0.041
7.0	40	1.8470	6.261	0.949
7.0	60	9.4667	1.220	0.185
7.0	83	61.6490	0.187	0.028
8.0	40	1.4797	7.806	1.183
8.0	60	7.5900	1.522	0.231
8.0	85	43.8640	0.263	0.040

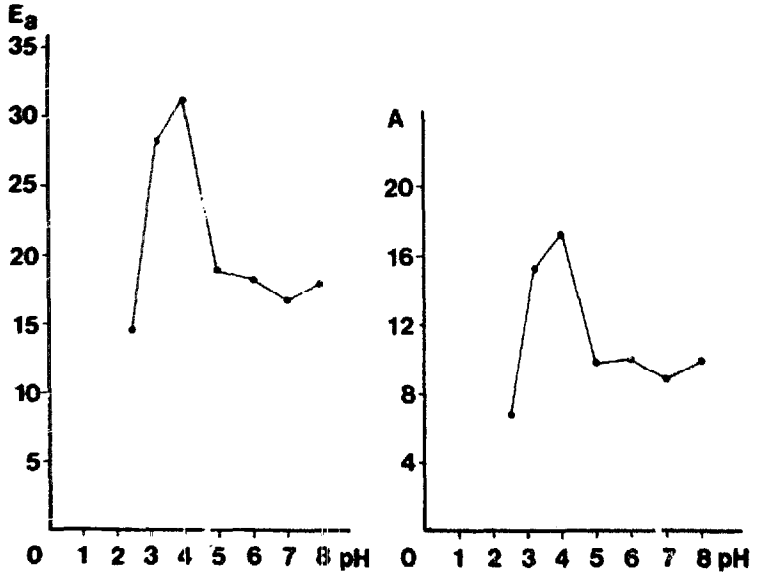


Fig. 2. Plot of inactivation energy (E_a) and frequency factor (A) as a function of pH values.

TABLE 2

RESULTS OBTAINED AFTER TREATMENT OF ARRHENIUS

y = logs of the inactivation constants (min^{-1}).
x = reciprocals of the temperatures expressed on the Kelvin scale.

pH	Regression equation	*P*	Statistical level	Activation energy	log of frequency factor
2.5	y= 6.7935−3.1917x	0.9959	<0.001	14.6050	6.7935
3.2	y=15.3411−6.1479x	0.9871	<0.001	28.1415	15.3411
4.0	y=17.2406−6.8010x	0.9999	<0.001	31.1220	17.2406
5.0	y= 9.8433−4.1538x	0.9937	<0.001	19.0080	9.8433
6.0	y=10.0744−4.1140x	0.9990	<0.001	18.2860	10.0744
7.0	y= 9.8687−3.9497x	0.9993	<0.001	16.7690	8.8805
8.0	y= 8.8805−3.6646x	1.0000	<0.001	18.0740	9.8687

TABLE 3

RESULTS OBTAINED AFTER EXTRAPOLATION TREATMENT FOR THE INACTIVATION CONSTANT, HALF-LIFE AND $t_{90\%}$ AT −4, 20 AND 37°C.

pH	T (°C)	$K \times 10^{-3}$ (min^{-1})	$t_{1/2}$ (h)	$t_{90\%}$ (h)
2.5	−4	0.01867	618.640	93.733
2.5	20	0.07947	145.322	22.020
2.5	37	0.35023	32.978	4.977
3.2	−4	0.00014	83673.727	12798.579
3.2	20	0.00225	5134.289	777.922
3.2	37	0.03187	362.514	55.440
4.0	−4	0.00005	236570.630	35859.190
4.0	20	0.00107	10806.512	1637.350
4.0	37	0.02005	576.146	87.295
5.0	−4	0.00704	1640.578	248.572
5.0	20	0.04638	249.046	37.734
5.0	37	0.27790	41.562	6.297
6.0	−4	0.01669	692.018	105.850
6.0	20	0.10798	106.956	16.361
6.0	37	0.63603	18.159	2.778
7.0	−4	0.04074	283.447	42.951
7.0	20	0.24465	47.210	7.153
7.0	37	1.34250	8.603	1.304
8.0	−4	0.04477	257.991	33.090
8.0	20	0.23620	48.899	7.409
8.0	37	1.14630	10.076	1.527

TABLE 4

VALUES OF ACTIVITY HALF-LIVES (h) AT pH VALUES OF MAXIMUM AND MINIMUM STABILITY AT 37°C FOR SOME ORAL CEPHALOSPORINS

	Maximum stability		Minimum stability	
	pH	$t_{1/2}$ (h)	pH	$t_{1/2}$ (h)
Cephaloglycine	2.5	126.00	10.00	1.00
Cefalexin	2.5	630.00	10.00	4.78
Cefradine	2.5	577.50	10.00	10.34
Cefroxadine	2.5	157.92	8.00	9.60
Cefaclor	2.5	223.57	7.00	4.74
Cefadroxil	4.0	576.15	7.00	8.60

This kind of plot is similar to that obtained for other cephalosporins; cephaloglycine also shows these 3 clearly differentiated zones (Yamana and Tsuji, 1976). In contrast to this, with cephalexin and cephradine (Yamana and Tsuji, 1976) only two zones appear; one which is independent of pH and the other corresponding to alkaline degradation.

As has been reported above, on representing the logs of the constants against the pH of cefadroxil a loss of linearity occurs in the zone of alkaline degradation. This is attributable to the fact that as from a pH value of 6.0, as a consequence of the zwitterionic nature of cefadroxil (Mariño and Dominguez-Gil, 1981), different ionic species exist which show different degradation rates according to the pH value. This

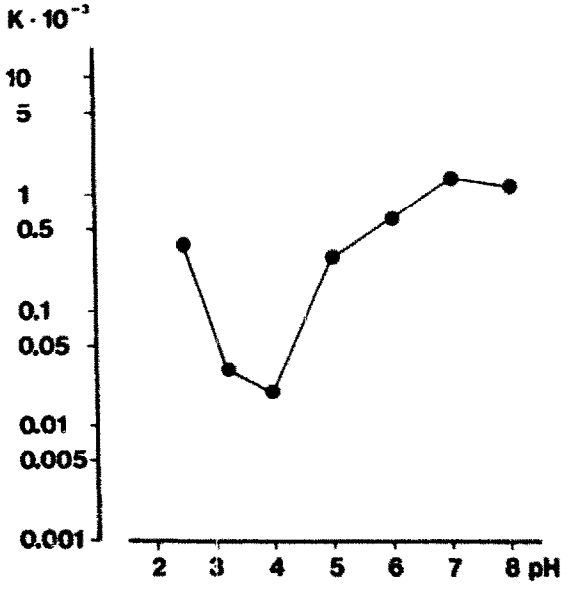


Fig. 3. Plot of the inactivation constants of cefadroxil calculated by extrapolation at 37°C as a function of pH values.

same feature has already been described in the literature for the penicillin antibiotics (Schwartz and Buefwacter, 1962).

Acknowledgement

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