International Journal of Pharmaceutics, 26 (1985) 259–266
Elsevier

IJP 00888

A kinetic study of tetracycline decomposition in acid solution

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(Received March 27th, 1985)
(Accepted June 5th, 1985)

Key words: tretracycline decomposition kinetics – dehydration reactions of tetracyclines – reversible epimerizations of tetracyclines – anhydrotetracycline – epitetracycline

Summary

The individual rate constants of the reactions involved in the reversible epimerizations of tetracycline and anhydrotetracycline are reported together with those for the dehydration reactions of tetracycline and epitetracycline. These values, obtained by the initial rate method, are compared with literature values obtained by curve fitting procedures. At pH 1.5 dehydration is the faster process. The initial rate method is compared with the conventional integrated rate equation method for the decomposition of the parent tetracycline at different temperatures between 30 and 75°C and over a wider range of pH than has previously been reported. The advantages and limitations of the initial rate method are discussed.

Introduction

In the study of the stability of a drug, the most usual purpose is to determine loss of potency with time under specified storage conditions. This is generally accomplished by measuring, directly or indirectly, the concentration variation of intact drug with time during decomposition. It has been suggested, however, that monitoring the decomposition product concentration with time may provide an alternative and possibly better approach (Carstensen et al., 1968). This approach has been used

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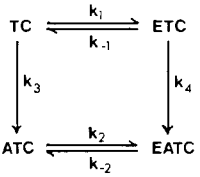

Fig. 1. Representation of the accepted decomposition pathways of tetracycline in acid solution.

in the study of some drug decompositions (Deeks et al., 1983). The conventional or integrated method using reactant concentration has been compared quantitatively with the initial rate method utilizing product concentration for the simple case of a drug decomposing irreversibly to a single product (Taylor et al., 1983). As part of an ongoing investigation into the relative merits of these two approaches to drug stability testing, the decomposition of tetracycline (TC) constitutes a very useful test system for several reasons.

Firstly, its instability is important in that, as is well known, one of its products of decomposition is the toxic but antibiotically inactive epianhydrotetracycline (EATC). Thus the rate of production of this product is arguably more relevant medically than the loss in potency of TC. The clinical importance of this particular decomposition has been reported recently (Thomson et al., 1984).

Also, this decomposition has been shown to be complex, involving both reversible epimerization of TC to epitetracycline (ETC) as well as dehydration of these epimers to anhydrotetracycline (ATC) and EATC, respectively. The widely accepted reaction scheme is shown in Fig. 1. The merits and limitations of the initial rate method have not been evaluated for such a reaction. In addition, while many stability investigations have been made of this reaction, most have assumed that, by adjusting reaction conditions, one or other of the decomposition pathways can be minimized and rate constants obtained for the predominant route. Only one report can be located in the literature where the various rate constants, defined in Fig. 1 have been individually evaluated (Yuen and Sokoloski, 1977). In that case the reaction was allowed to proceed to completion at a single pH and rate constants were obtained by curve fitting of the data according to the kinetic equations derived from Fig. 1.

The present work reports rate constants obtained by treating individual compounds in the decomposition scheme as reactants and measuring the rate of production of the resulting decomposition products over a small extent of reaction. Under such conditions the reaction can be assumed zero-order with respect to the reactant. Conventional first-order rate constants are calculated using the relationship (Carstensen and Su, 1971):

$$k_0 = k_1[R]_0$$

where k_0 is the apparent zero-order slope of the product concentration–time graph, k_1 is the rate constant assuming that the reaction is first-order if allowed to proceed to large extents of decomposition and $[R]_0$ is the initial concentration of the species chosen as reactant.

In addition, the log k–pH profiles for the decomposition reactions involving TC are reported over a wider range than appears in the literature. These are obtained by measuring the increase of ETC and ATC over small extents of reaction and also by the conventional means of monitoring TC concentration directly. The temperature dependence of these reactions is also reported.

In order to carry out the required analyses, previously reported separation systems using HPLC were found to be inadequate and the development of a separating system involving hydrophobic pairing ion is described (Hung and Taylor, 1980, 1981).

Materials and Methods

Chromatographic measurements were carried out using a Waters Associates M6000A pump and M441 fixed wavelength (254 nm) detector. Columns were 100 or 200×4.6 mm, slurry packed using 5 μm ODS Hypersil (Shandon Laboratories). Injection was by a Rheodyne 7125 valve fitted with a 20–μl loop.

Tetracycline and its decomposition products were supplied by Lederle Laboratories, cetrimide was obtained from Thornton and Ross and sodium lauryl sulphate from Fisons. Acetonitrile was obtained from Rathburn Chemicals and water was purified using a Millipore MilliQ system. All other chemicals used were of AnalaR or equivalent grade.

Results and Discussion

Chromatography

Previously reported reverse-phase solvent systems involving buffer/organic modifier alone (Dihuidi et al., 1982) or incorporating ethylenediaminetetraacetic acid (Knox and Jurand, 1979) were found to give inadequate resolution, in particular between TC and ETC, in times short enough to provide adequate sensitivity for the longer retained ATC.

The four compounds of interest are amphoteric and should, therefore, be amenable to control of retention by the addition of anionic hydrophobic pairing ions at low pH or cationic hydrophobic pairing ions at high pH (Taylor and Reid, 1984). Fig. 2 shows the variation of column capacity factor (k′) with both sodium lauryl sulphate (SLS) and cetrimide (CTAB) concentrations. Optimum chromatograms obtained using these pairing ions are shown in Fig. 3. The SLS pairing ion produced the better overall resolution. If, however, quantitation of EATC only were required, say for limit testing, the CTAB system produces adequate resolution of this compound in a much shorter overall analysis time. The solvent system indicated in Fig. 3a was used for subsequent stability analyses.

The calibration lines for the four compounds were determined to be linear ($r^2 > 0.99$) over the different concentration ranges used. The precision of the peak height quantitation method was determined by repeated injection of a partially

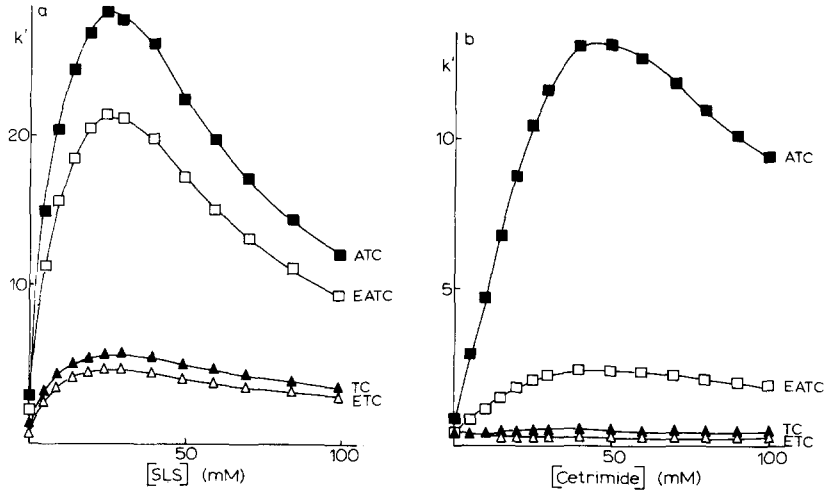

Fig. 2. Plots showing the variation in capacity factor k' with anionic and cationic pairing ion. a: sodium lauryl sulphate in $CH_3CN:H_2O$ (40/60)/0.05 M phosphate buffer at pH 2.0. b: cetrimide in $CH_3CN:H_2O$ (40/60)/0.05 M phosphate buffer at pH 7.0.

decomposed TC solution containing all species. For 8 replicates, the following relative standard deviations were obtained: TC (1.3%), ETC (0.91%), EATC (1.6%), ATC (1.3%). During decomposition runs, standards of concentration appropriate to the absorbance range of the detector were used.

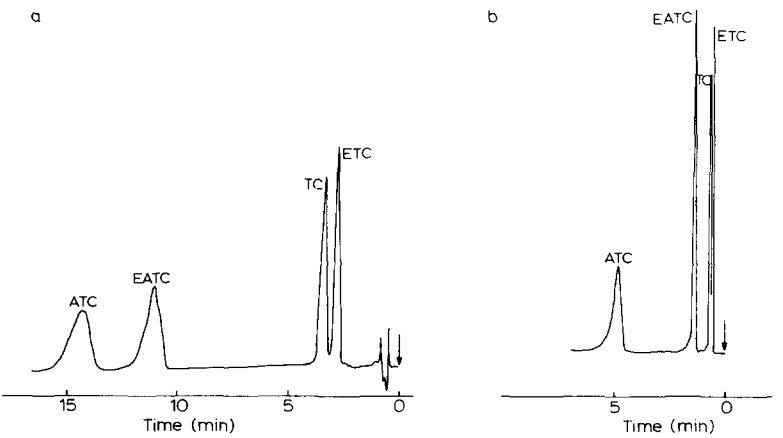

Fig. 3. Specimen chromatograms showing separation of TC, ETC, ATC and EATC in a partially decomposed TC sample. Chromatographic conditions using a 5 μm ODS Hypersil column 200 × 4.6 mm. a: $CH_3CN:H_2O$ (40/60)/0.05 M phosphate buffer at pH 2.0 containing 50 mM sodium lauryl sulphate. b: $CH_3CN:H_2O$ (40/60)/0.05 M phosphate buffer at pH 7.0 containing 2.5 mM cetrimide.

TABLE 1

RATE CONSTANTS FOR THE DECOMPOSITION OF TC ACCORDING TO FIG. 1 OBTAINED OVER LOW EXTENTS OF DECOMPOSITON BY THE INITIAL RATE METHOD AT 30°C IN pH 1.5 BUFFER

Rate constant	Present work			Literature (Yuen 1977) ($min^{-1} \times 10^4$)
	k ($min^{-1} \times 10^4$)	RSD (%)	r^2	
k_1	0.65	2.3	0.999	3.51
k_{-1}	1.98	by difference *		4.63
k_2	1.80	2.5	0.998	15.0
k_{-2}	0.85	3.6	0.996	12.7
k_3	4.40	1.7	0.999	2.83
k_4	4.31	2.4	0.997	0.71

* The value for k_{-1} quoted was obtained indirectly as indicated in the text.

Stability measurements

Determination of individual rate constants

In order to evaluate the individual rate constants defined in Fig. 1, the appropriate compound was used as the reactant at an accurately prepared concentration of approximately 5×10^{-3} M in pH 1.5 phosphoric acid/sodium dihydrogen phosphate buffer. These solutions were stored in a constant temperature bath at 30°C. The solutions were analyzed for decomposition products over time scales such that the extent of reaction did not exceed 5% of the initial reactant concentration. Under these conditions the slope of the product concentration–time lines were found to be linear ($r^2 > 0.99$) and first-order rate constants were determined using the above equation. The calculated values of the various rate constants so obtained are shown in Table 1 together with their relative standard deviations. The decomposition of TC to ETC and ATC allowed determination of k_1 and k_3, respectively. ATC and EATC as reactants enabled k_2 and k_{-2} to be determined. When ETC was used as the reactant, however, k_4 could be calculated but the resolution between TC and ETC was inadequate in the situation where TC was present as the minor component and direct determination of k_{-1} was not possible. The value of k_{-1} shown in Table 1 was calculated indirectly after measuring the conventional first order rate constant for ETC decomposition, i.e. $(k_4 + k_{-1})$. The values shown in Table 1, with the exception of k_{-1} could readily be determined in an overall time of 2 h at 30°C. Also shown, for comparison, in Table 1 are values of rate constants calculated from literature data obtained at higher temperatures (Yuen and Sokoloski, 1977).

Comparison of corresponding values show that in the present work consistently lower rate constants are obtained for epimerization process but the values for the dehydration reactions are higher. The present work is considered more reliable due to the direct measurements involved and also since other data (Hoener et al., 1974) show that at this pH dehydration is faster than epimerization.

TABLE 2

KINETIC DATA FOR THE DECOMPOSITION OF TC OBTAINED BY THE INTEGRATED AND INITIAL RATE METHODS AT DIFFERENT TEMPERATURES AND IN SOLUTIONS OF DIFFERENT pH. k_{TC} IS THE OVERALL RATE CONSTANT FOR TC DECOMPOSITION AND k'_{TC} THE SUM OF k_1 AND k_3 AS DEFINED IN FIG. 1

Temp. (°C)	pH	Integral method		Initial rate method				
		$k_{TC} \times 10^3$ (min^{-1})	RSD (%)	$k_1 \times 10^3$ (min^{-1})	RSD (%)	$k_3 \times 10^3$ (min^{-1})	RSD (%)	$k'_{TC} \times 10^3$ (min^{-1})
75	2.3	7.60	3.4	11.5	1.9	22.2	4.0	13.7
	2.6	9.32	4.9	10.3	4.2	9.75	2.7	11.3
	3.1	11.2	1.1	12.1	4.4	5.46	1.5	12.6
	3.3	8.72	1.3	8.65	2.0	4.74	1.6	9.12
	3.6	9.82	4.4	10.0	5.0	2.90	1.6	10.3
	3.9	7.21	3.4	10.2	2.8	2.32	1.2	10.4
	4.2	7.70	5.5	8.11	1.7	3.30	2.4	8.44
	5.0	6.86	7.2	6.64	2.7	1.59	2.7	6.80
	5.5	6.50	4.6	5.89	3.8	1.28	3.8	6.02
	6.0	5.49	9.3	4.59	0.7	0.61	1.9	4.65
	6.2	5.20	4.8	3.40	5.0	0.95	5.9	3.50
	7.3	5.24	5.9	2.19	1.1	0.62	5.1	2.25
	8.0	4.52	3.3	1.14	2.1	0.15	7.0	1.29
40	7.0	0.22	6.6	0.33	2.0	0.02	5.2	0.33
50		0.43	12	0.67	1.6	0.03	2.8	0.67
60		0.93	15	1.65	2.7	0.40	2.7	1.66
70		2.10	2.0	3.01	14	0.61	1.6	3.07
Act. energy ($kJ \cdot mol^{-1}$)		66.3 RSD = 4.3		66.6 RSD = 2.1		98.0 RSD = 19		69.0 RSD = 2.6

Temperature and pH dependence of TC decomposition

The decomposition of TC has hitherto been studied over very limited pH ranges. To provide more complete log k–pH profiles and allow activation energies for the individual reactions involving TC as reactant, the initial rate method was applied to TC at 40, 50, 60 and 70°C at pH 7 and at a single temperature of 75°C over a pH range of 2.3–8.0. McIlvaine's buffer was used in this work to maintain constant ionic strength. Under the same conditions the TC decompositions were allowed to proceed to higher extents of reaction (30–70%) in order to obtain first-order rate constants by conventional means.

The rate constants so obtained namely k_1, k_3 and k_{TC} the overall rate constant obtained by following TC concentration are shown in Table 2 together with their associated activation energies. Also shown in Table 2 are values of $k'_{TC} = k_1 + k_3$. At all temperatures the dehydration reaction is slow at pH 7 and large errors are associated with the determination of k_3 which are reflected in the uncertainty in the activation energy quoted for this rate constant.

The log k–pH profiles are shown in Fig. 4 for the various rate constants. In all cases there is an increase with decreasing pH. This is most marked with k_3 and is

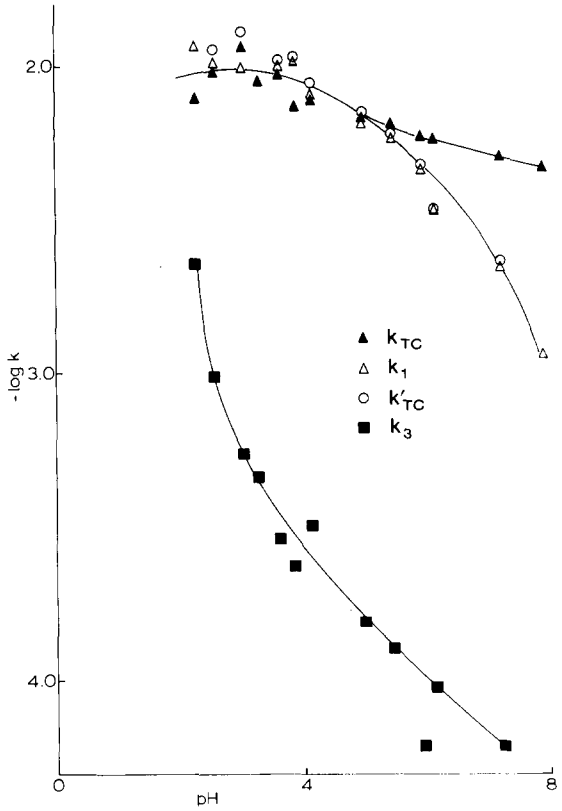

Fig. 4. Log k–pH profile obtained from data contained in Table 2. ▲, $-k_{TC}$; △, $-k_1$; ■, $-k_3$; ○, $-k'_{TC}$.

consistent with the higher rate of dehydration compared with epimerization at pH 1.5 shown in Table 1. Good correspondence is obtained between k_{TC} and k'_{TC} over the pH range 2.6–5.5. At pH values below 2.6 the TC reaction is studied to longer extents of reaction than at higher pH values and, as a result the rate constant k_{TC} measured by the integral method will be reduced by the reverse reaction. At pH values above 5.5 other reactions than those shown in Fig. 1 occur. This is evident by the appearance of two additional peaks in the chromatograms obtained at higher pH values and results in k'_{TC} values lower than k_{TC}. These additional decomposition product peaks are inadequately resolved for quantitation and do not interfere with any of the compounds in Fig. 1.

Conclusions

The reverse-phase ion pairing system suggested for TC degradation studies is adequate to follow the kinetics of decomposition by the initial rate method except in the situation where ETC is reactant. It also demonstrates the existence of other

reactions at high pH. The additional compounds produced do not interfere with the quantitation of any of the compounds in Fig. 1. The initial rate method has been shown to be capable of producing realistic rate constants for this complex reaction scheme. While it is required that the reaction pathway be known, this method allows evaluation of rate constants at realistic extents of decomposition of the drug, that is, below that normally accepted as limiting shelf-life. The limitations of the initial rate method are also demonstrated by the different values of k_{TC} and k'_{TC} shown in Table 2. This points out the dangers of applying the initial rate method to obtain rate constants for use in the determination of shelf-life. Measurement of decomposition products, however, which is a prerequisite for applying the initial rate method has the obvious advantage of establishing concentration levels of toxic impurities as a function of time.

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