ELSEVIER

International Journal of Pharmaceutics 173 (1998) 221–232

international journal of pharmaceutics

Hydrolysis kinetics and stability predictions for a mixture of R and S temocillin isomers

S. Craig Dyar, Robert E. Notari *

Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425-2303, USA

Received 1 May 1998; received in revised form 10 July 1998; accepted 13 July 1998

Abstract

The hydrolysis kinetics of a temocillin isomer mixture (~68% R and ~32% S) were studied as a function of pH, temperature and buffers. Temocillin concentrations were determined from HPLC analyses of the individual isomers where [TEM] = [R] + [S]. Rate constants (k_R, k_S and k_{SUM}) were determined from linear first-order plots for [R], [S] and [TEM], respectively. The true time for 10% loss of temocillin (T_{true}) was determined by examining the time-course for the sum, [R] + [S]. Although the k_R and k_S values were generally similar, they differed by 30–40% under some conditions. Nevertheless, the calculated time for 10% loss ($T_{90} = -\ln 0.9/k_{SUM}$) agreed with T_{true} for all experimental conditions. This was explained using computer simulations that indicated epimerization was faster than hydrolysis. Under these circumstances, reliable predictions of temocillin stability were achieved with $k_{SUM} = k_{H1}(a_{H+})f_1 + k_{H2}(a_{H+})f_2 + k_{H3}(a_{H+})f_3 + k_{H_2O} + k_{OH}(a_{OH-}) + k_{B_4O_7}[B_4O_7^{2-}]$, where the catalytic constants were k_{H1}, k_{H2} and k_{H3} for hydrogen ion activity, k_{OH} for hydroxyl ion activity, $k_{B_4O_7}$ for $B_4O_7^{2-}$ (in borate buffer only); k_{H_2O} was the first-order rate constant for spontaneous hydrolysis and f_1, f_2, f_3 were the fractions of temocillin in three stages of dissociation. The Arrhenius expression, $k = Ae^{-E/RT}$, and the experimentally determined A and E values were substituted for k_{H1}, k_{H2} k_{H3}, k_{OH} and $k_{B_4O_7}$. This equation successfully calculated 92% of the observed k_{SUM} values with $< 10\%$ error. A shelf-life of ~1.8 days was predicted and experimentally verified at 30°C in the region of maximum stability, pH 6.5–7.5.

Keywords: Temocillin isomers; Temocillin stability; Temocillin hydrolysis; Diastereoisomers; Hydrolysis kinetics; Isomeric mixtures

1. Introduction

Ariens et al. (1988) indicated that ~25% of drugs were marketed as racemates or mixtures of diastereoisomers. It has been recognized for more than 25 years that stereoisomers can differ signifi-

* Corresponding author. Tel.: +1 843 7928427; fax: +1 843 7920759; e-mail notariro@musc.edu

0378-5173/98/$ - see front matter
PII S0378-5173(98)00239-7

cantly with respect to pharmacokinetic characteristics such as binding, metabolism, clearance, biological half-life and volume of distribution (Shand and Rango, 1972; Ariens, 1983; Simonyi, 1984; Hutt and O'Grady, 1996). A pharmacokinetic description of an isomeric mixture requires that each isomer be independently assessed. This necessitates specific analytical methodology for each isomer. If their pharmacokinetics are sufficiently different, failure to analytically resolve the two isomers provides meaningless pharmacokinetic data composed of the sum of two superimposed individual time-courses.

In contrast, the potential impact of the degradation kinetics of two isomers on stability predictions for an isomeric mixture has not been widely studied. Moore and Pearson (1981) presented a method for obtaining the individual rate constants for an isomeric mixture when epimerization was negligible relative to hydrolysis. The semilogarithmic plot of total concentration versus time was biphasic. The kinetic analysis was illustrated using a mixture of diethyl-*t*-butylcarbinyl chloride isomers where the isomer present as 35% hydrolyzed approximately four times faster than the dominant isomer.

Nguyen (1991) studied the hydrolysis kinetics of a 50:50 mixture of cefuroxime axetal diastereoisomers, A and B. Epimerization was negligible relative to hydrolysis. First-order plots for [A], [B], or [A + B], were linear with negative slopes k_A, k_B and k_{SUM}, respectively. Although k_A and k_B differed by as much as 27%, the rate of cefuroxime axetal loss was described by $-d[A+B]/dt = k_A[A] + k_B[B] \approx k_{SUM}[A+B]$. Shelf-life estimates based on k_{SUM} were equivalent to those based on $k_A[A] + k_B[B]$.

Neither of those studies provide examples wherein an unrecognized error would be introduced by calculating the shelf-life from a first-order plot of total concentration. The diethyl-*t*-butylcarbinyl chloride isomer mixture plot was so clearly biphasic as to be obvious that first-order analysis was not valid. Although cefuroxime axetal provided a first-order plot for [A + B], the differences in isomer reactivities were not sufficient to result in an error by calculating the shelf-life with k_{SUM}.

However, a significant error can result if a semilogarithmic plot for the sum of the isomer concentrations is a shallow biexponential curve that appears to be first order with negative slope k_{SUM}. The shelf-life determined from k_{SUM} overestimates the true shelf-life (T_{true}) defined as the time for 10% loss of drug. For the hypothetical example in Fig. 1, the shelf-life calculated from a first-order plot of total concentration was 40% longer than T_{true}.

Temocillin is a bactericidal penicillin with relatively high stability to β-lactamases (Scheme 1). The disodium salt of temocillin is available for parenteral use as a mixture of ~68% R isomer and ~32% S isomer for reconstitution before injection (Walker, 1994). Although both isomers are active, they are not equivalent in potency or disposition in man (Guest et al., 1985). The R and S isomers

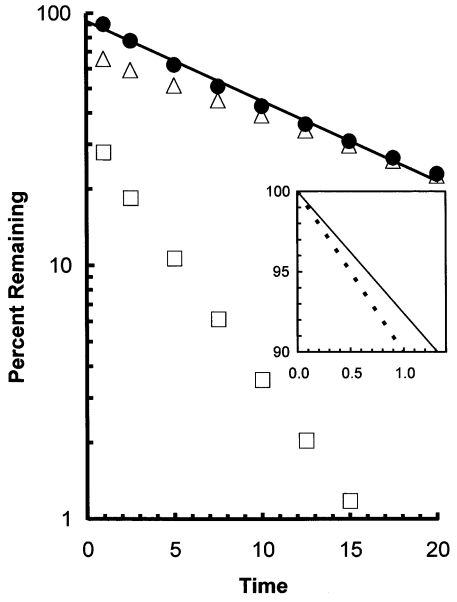
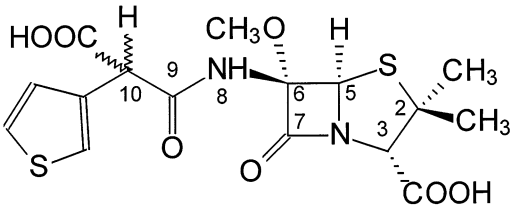


Fig. 1. First-order plots of simulated data for total drug (●), its R isomer (△), and its S isomer (□) when hydrolysis rate constants ($k = 0.0560^{-t}$ for the R and $k = 0.224^{-t}$ for the S isomer) produce a shallow biexponential curve for total drug. The regression line on the total drug curve resulted from treating these data (●) as first order. The insert demonstrates how the time for 10% loss, erroneously calculated with a first-order rate constant (solid line), overestimated the true time (T_{true}) required for a biexponential curve to lose 10% of the initial concentration (dashed line). The initial mixture was 68% R and 32% S.



Scheme 1. The structure for temocillin (6β-[2-carboxy-2-thien-3-ylacetamido]-6α-methoxypenicillanic acid) showing the unresolved C-10 asymmetric center that is responsible for the diastereoisomeric mixture.

differ significantly in clearance, half-life, volume of distribution and plasma protein binding.

Just as the R and S isomers differ in pharmacokinetic behavior, they may also differ in hydrolysis rates. Burton et al. (1986) reported hydrolysis half-lives and degradation products for loss of temocillin at pH 3, 4 and 12, 20°C. Bird et al. (1984) reported epimerization half-lives in acetate (pH 5) and phosphate (pH 7) buffers at 25 and 37°C. However, a literature search did not disclose any kinetic studies for hydrolysis of temocillin or its R and S isomers.

The specific aims of this investigation were (1) to study the hydrolysis kinetics of R and S temocillin as a function of pH, temperature and buffer, (2) to determine whether or not the individual R and S temocillin isomer time-courses are required to predict temocillin shelf-life, (3) to employ computer simulations to develop the kinetic theory required to interpret these results, (4) to develop equations that predict temocillin shelf-life at any pH and temperature in the presence or absence of buffers and (5) to predict and experimentally verify the shelf-life of a solution buffered to the pH of maximum stability and stored at 30°C.

2. Materials and methods

2.1. Materials

Temocillin sodium was used as received from SmithKline Beecham (Worthing, West Sussex, UK). The sample was a mixture of ~68% R isomer and ~32% S isomer (by HPLC analysis, Section 2.2). All other chemicals were analytical or HPLC grade.

2.2. HPLC assays

An HPLC system (Waters, Bedford, MA) consisting of a M-600E gradient control system, a M-484 variable wavelength detector, a M-745B data module and a 3.9×300 mm μBondapak C_{18} reverse phase column with a μBondapak C_{18} guard column was used.

The analytical method was an adaptation of an isocratic HPLC assay that resolved the R and S isomers and separated them from the degradation products that formed during hydrolysis (Bird et al., 1984). Typical chromatograms with the R isomer eluting before the S isomer are shown in that paper. The assay used a mobile phase consisting of phosphate buffer and methanol at a flow rate of 1.5 ml/min, a 20-μl injection volume and ambient column temperature. Monobasic sodium phosphate monohydrate (13.8 g) was dissolved in 900 ml of distilled water, the pH was adjusted to 7.0 with 1 N NaOH and distilled water added to make 1 l. A detection wavelength of 230 nm was selected because it is an isosbestic point for the R and S isomers. This allowed the proportion of each isomer to be calculated from the percentage contribution of its peak area to the total area of both peaks.

In the current study, methanol and phosphate buffer were deaerated under vacuum with sonication. During analysis, the mobile phase was purged with helium at 45 ml/min. The R and S isomers were analyzed using the areas under their respective HPLC peaks and total temocillin was determined from the sum of these two areas. When reactions at $pH > 5.5$ were analyzed with the mobile phase employed for studies at $pH \leq 5.5$, degradation products coeluted with the R and S isomers. Therefore, two different ratios of methanol to phosphate buffer were required to quantitatively separate the R and S isomers from the degradation products formed during kinetic studies at $pH \leq 5.5$ and those formed at $pH > 5.5$.

At $pH \leq 5.5$, the mobile phase consisted of 10% v/v methanol and 90% v/v phosphate buffer. The retention time was 9 min for the R isomer and 11

Table 1
First-order hydrolysis rate constants (10^3 k in min^{-1}) determined from R isomer (k_R), S isomer (k_S) and temocillin (k_{SUM}) in HCl

Temperature (°C)	HCl concentration (M)	pH	10^3 k_R	10^3 k_S	10^3 k_{SUM}
25	0.50	0.42	45.5	45.8	45.6
	0.20	0.83	19.2	18.2	18.9
	0.10	1.13	10.3	9.85	10.1
	0.05	1.44	6.31	6.08	6.22
30	0.50	0.42	67.8	82.7	72.0
	0.20	0.83	31.4	30.0	30.4
	0.10	1.14	17.0	16.7	16.9
	0.05	1.44	11.5	10.2	10.8
40	0.50	0.43	202	181	185
	0.20	0.84	83.2	81.4	82.6
	0.10	1.14	49.7	46.6	48.6
	0.05	1.44	34.1	29.1	32.7
50	0.05	1.44	195	197	195
60	0.05	1.44	353	464	384

min for the S isomer. The capacity factor was 4.36 and replication (CV) was 1.20% for the R isomer; 5.63 and 1.80, respectively, for the S isomer. For temocillin, the CV was 0.80% and the linear detection range was $1–6 \times 10^{-4}$ M.

At pH values > 5.5, the mobile phase was 4% v/v methanol and 96% v/v phosphate buffer. The retention time was 14 min for the R isomer and 18 min for the S isomer. The capacity factor was 4.59 and the CV was 1.01% for the R isomer; 6.08 and 1.31%, respectively, for the S isomer. For temocillin, the CV was 0.67% and the linear detection range was $1–6 \times 10^{-4}$ M.

Several methods were used to insure that the assays were specific for the R isomer and the S isomer in the presence of their degradation products. Following ~50 and 75% hydrolysis, the UV spectra at the leading edge, maximum and trailing edge of the R and S isomer peaks were superimposable with those of the original temocillin sample (diode array detector, Model 996, Waters, Bedford, MA). No residual peaks were found beneath their peaks when reactions were allowed to proceed to completion. No deviations were observed in first-order plots. Representative reactions in acidic and buffered solutions were analyzed in duplicate with the appropriate mobile phase described above and a second mobile phase which provided retention times of 7 min for the R isomer and 8 min for the S isomer. This mobile phase consisted of 95% v/v phosphate buffer and 5% v/v acetonitrile. The concentrations and first-order plots were similar for the duplicate assays.

2.3. Kinetics

The temperatures (stable to ± 0.1°C), compositions of the reaction solutions, and the pH values are given in Tables 1 and 2. The ionic strength was adjusted to 0.5 by addition of NaCl. The pH values for the HCl solutions were calculated as a function of temperature with hydrogen ion activity coefficients determined from published values (Harned and Owen, 1958). The pH values for the buffered solutions were measured at the temperatures of the studies before and after completion of the reactions.

Reactions were initiated by adding 1 ml of a temocillin solution to 20 ml of HCl or buffered solution at the temperature of the study to provide a 10^{-3} M temocillin reaction solution. Reactions were quenched prior to analysis in the following manner. Samples were removed as a function of time, rapidly cooled with an ice and water mixture and a 1.0 ml aliquot was immediately added to 1 or 2 ml of water or buffer to give

Table 2
First-order hydrolysis rate constants (10^3 k in min^{-1}) determined from R isomer (k_R), S isomer (k_S) and temocillin (k_{SUM}) in buffered solutions

Temperature (°C)	pH	Buffer concentration (M)	10^3 k_R	10^3 k_S	10^3 k_{SUM}
Formate					
40	2.49	0.91	15.3	15.6	15.4
		0.45	15.3	15.6	15.4
		0.23	14.5	16.0	15.0
	3.52	0.82	7.01	7.18	7.07
		0.41	6.57	7.98	7.03
		0.21	6.57	6.65	6.65
	4.55	0.45	0.968	1.19	1.03
		0.11	1.14	1.33	1.19
50	2.50	0.23	39.5	38.3	39.1
	3.52	0.21	16.8	18.0	17.2
	4.58	0.11	2.65	3.04	2.76
60	2.51	0.23	86.7	87.3	86.9
	3.50	0.41	37.2	44.4	36.9
	4.59	0.23	6.34	7.17	6.58
Phosphate					
40	5.41	0.09	0.232	0.294	0.252
	6.52	0.16	0.122	0.140	0.128
	7.52	0.09	0.111	0.114	0.112
50	5.40	0.09	0.638	0.662	0.647
	6.52	0.16	0.336	0.385	0.353
	7.53	0.09	0.355	0.352	0.358
60	5.41	0.09	1.49	2.09	1.67
	6.51	0.16	0.950	1.23	0.984
	6.56	0.04	0.971	1.26	1.04
	7.54	0.09	1.02	1.08	1.01
Borate					
40	8.69	0.08	0.453	0.497	0.469
		0.04	0.353	0.348	0.351
		0.02	0.266	0.320	0.284
50	8.59	0.08	1.25	1.45	1.32
		0.02	0.761	0.876	0.801
		0.01	0.717	0.721	0.719
60	8.55	0.04	2.65	2.76	2.69
		0.02	2.06	2.01	2.04
		0.01	2.10	1.72	2.00
	8.72	0.04	4.20	4.12	4.17
		0.02	2.91	2.99	2.94
		0.01	2.31	2.38	2.34
Carbonate					
40	9.50	0.10	0.846	0.987	0.896
		0.06	0.764	0.852	0.793
		0.02	0.713	0.827	0.752
50	9.41	0.10	2.32	2.43	2.36
		0.06	2.16	2.06	2.13
		0.02	1.60	1.91	1.70
60	9.34	0.10	6.47	6.48	6.48
		0.06	5.99	6.14	6.05
		0.02	4.75	5.04	4.86

Table 2 (Continued)

Temperature (°C)	pH	Buffer concentration (M)	$10^3\ k_R$	$10^3\ k_S$	$10^3\ k_{SUM}$
40	10.4	0.11	4.28	4.49	4.35
		0.09	3.53	3.90	3.64
		0.07	3.51	3.90	3.64
50	10.4	0.11	12.8	13.0	12.9
		0.09	10.6	11.3	10.9
		0.07	10.3	10.6	10.4
60	10.2	0.11	32.7	32.5	32.6
		0.09	28.1	28.2	28.1
		0.07	26.6	26.2	26.5

a pH of 6.5–7.5. Most diluted samples were stored in an ice bath and analyzed within 12 h. Control studies showed that temocillin concentrations were unchanged throughout the quenching and storage procedure. Dilutions from longer reactions were stored under refrigeration prior to analysis. No loss of temocillin was detected by HPLC when these solutions were stored for 24 h in the refrigerator.

3. Results

3.1. Determination of first-order rate constants

The rate of temocillin hydrolysis was first order in aqueous HCl or buffers at constant pH, temperature, and ionic strength. Pseudo-first-order rate constants (k) were calculated from linear plots based on Eq. (1),

$$\ln[C_t] = \ln[C_0] - kt \quad (1)$$

where t is time, $[C_0]$ is initial concentration and $[C_t]$ is the time-dependent concentration for the R isomer, the S isomer, or for temocillin defined as the sum of the two isomers; k is k_R when determined from the concentration of the R isomer, k_S from the S isomer, and k_{SUM} from the sum of the isomers. Each study was comprised of eight or more assays spaced to provide changes of ~0.1$[C_0]$ per sampling interval. Rate constants (Tables 1 and 2) were obtained by linear regression ($r^2 > 0.99$) for data in the range, $[C_0]$ to ~0.2$[C_0]$. Differences between k values for duplicate studies were $\leq 2\%$. There were no significant differences between k values obtained for hydrolysis of 10^{-3} M temocillin and those obtained for a clinically employed concentration of 50 mg/ml (0.11 M) at pH 7.5, 60°C.

The rate constants obtained from the R isomer and the S isomer were generally similar. More than 91% of the k_R and k_S pairs varied by $< 10\%$. The values for k_{SUM} were within 5–10% of the means of the k_R and k_S values. The largest differences were observed at 60°C in phosphate buffers, pH 5.4–6.6, where k_S was 29–40% larger than k_R (Table 2 and Fig. 2). Despite this difference, the time to achieve 10% loss of temocillin was similar when calculated with k_{SUM} or observed from the time-course of the sum of the R and S isomers. The reason for this agreement is presented in Section 4. The goal of the following kinetic analy-

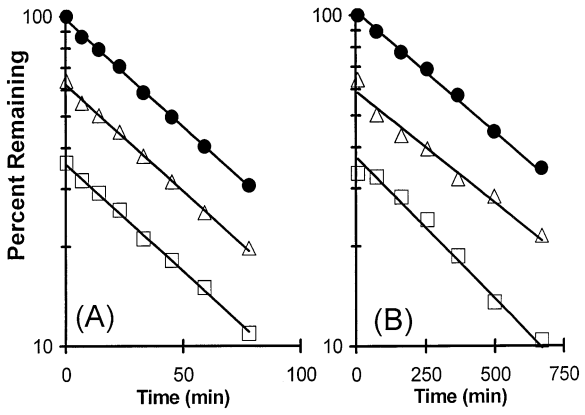


Fig. 2. First-order plots of the percent of temocillin (●), its R isomer (△), and its S isomer (□) remaining as a function of time in: (a) 0.45 M formate buffer at pH 2.54, 40°C and (b) 0.09 M phosphate buffer at pH 5.41, 60°C.

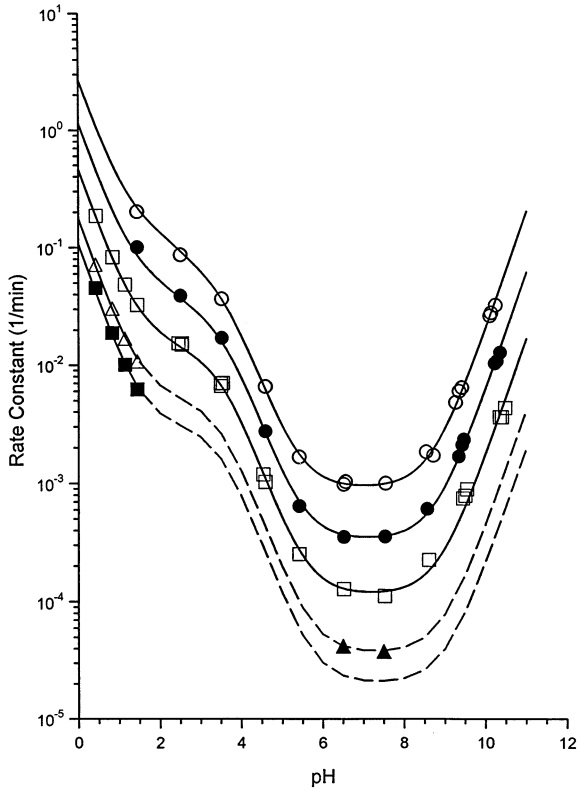


Fig. 3. The solid curves were obtained by simultaneous nonlinear regression using Eq. (11) to fit $k_{SUM} = k_{(pH)}$ at 60°C (○), 50°C (●), 40°C (□), 30°C (△), and 25°C (■) using the observed temocillin hydrolysis rate constants in Tables 1 and 2 and the intercepts of k vs borate buffer concentration. The dashed curves were predicted with Eq. (11) and the A and E values in Table 3. The two rate constants on the dashed curve for 30°C (▲) were experimentally determined to verify their predicted values.

sis was to develop equations to calculate k_{SUM} as a function of pH, temperature and buffer in order to predict the time for 10% loss.

3.2. pH-rate profiles

Fig. 3 shows pH-rate profiles for temocillin hydrolysis rate constants (k_{SUM}). Each of these curves exhibited an inflection at pH 2–4. Inflections in a pH-rate profile are frequently observed near the pK_a of a substrate when the reactivity of the protonated and unpronated forms are sufficiently different.

The simplest equation that best calculated the observed k_{SUM} values was empirically selected by testing pH-rate equations that describe profiles with inflections in the acidic region (Connors et al., 1985; Carstensen, 1995). For monocarboxylic acids, these profiles are generally described by Eq. (2) or a kinetic equivalent where $k_{(pH)}$ is k_{SUM} in the absence of buffer catalysis.

$$k_{(pH)} = k_{H1}(a_{H+})f_1 + k_{H2}(a_{H+})f_2 + k_{H_2O} + k_{OH}(a_{OH-}) \quad (2)$$

In Eq. (2), k_{H1} and k_{H2} are catalytic rate constants (M^{-1}/min) for hydrogen ion activity (a_{H+}), k_{OH} is the catalytic rate constant (M^{-1}/min) for hydroxyl ion activity ($a_{OH-} = K_W/[a_{H+}]$) and k_{H_2O} is the first-order rate constant (min^{-1}) for spontaneous hydrolysis. The values for K_W as a function of temperature were obtained from Harned and Owen (1958). The fraction of substrate as the carboxylic acid (f_1) and the carboxylate (f_2) were calculated from Eqs. (3) and (4) where the apparent dissociation constant, K_a, was an adjustable parameter together with the rate constants in Eq. (2).

$$f_1 = \frac{a_{H+}}{(a_{H+} + K_a)} \quad (3)$$

$$f_2 = \frac{K_a}{(a_{H+} + K_a)} \quad (4)$$

Eqs. (2)–(4) achieved their best fit with pK_a = 3.53. The experimental $k_{(pH)}$ values were calculated with $\leq 10\%$ error for 70% of the values and with errors of 15–23% for the remaining values.

Eq. (5) was successful when two pK_a values were required to describe the pH-rate profiles for ceftazidime hydrolysis (Zhou and Notari, 1995).

$$k_{(pH)} = k_{H1}(a_{H+})f_1 + k_{H2}(a_{H+})f_2 + k_{H3}(a_{H+})f_3 + k_{H_2O} + k_{OH}(a_{OH-}) \quad (5)$$

In Eq. (5), k_{H1}, k_{H2} and k_{H3} are catalytic rate constants for hydrogen ion activity. Temocillin has two carboxylic acid groups (Scheme 1). Potentiometric titration of the temocillin sample indicated pK_a values of 2.14 and 3.53 when the data were analyzed by the method of Niebergall et al. (1973). The fractions of temocillin as the dicar-

boxylic acid (f_1), the monocarboxylate (f_2) and the dicarboxylate (f_3) were calculated from Eqs. (6)–(8) with the dissociation constants (K_{a1} and K_{a2}) determined from the pK_a values.

$$f_1 = \frac{a_{H+}^2}{(a_{H+}^2 + a_{H+}K_{a1} + K_{a1}K_{a2})} \quad (6)$$

$$f_2 = \frac{(a_{H+}K_{a1})}{(a_{H+}^2 + a_{H+}K_{a1} + K_{a1}K_{a2})} \quad (7)$$

$$f_3 = \frac{(K_{a1}K_{a2})}{(a_{H+}^2 + a_{H+}K_{a1} + K_{a1}K_{a2})} \quad (8)$$

Eqs. (5)–(8) predicted 92% of the $k_{(pH)}$ values with $\leq 10\%$ error and the remaining values with errors of 15–23%. Therefore, Eqs. (5)–(8) were selected for $k_{(pH)}$ predictions.

Buffer catalysis was not detected in the neutral or acidic pH region. Hydrolysis rates were accelerated by the basic component of the borate buffer ($B_4O_7^{2-}$). The temocillin hydrolysis rate constant in borate buffer was defined as:

$$k_{SUM} = k_{(pH)} + k_{B_4O_7}[B_4O_7^{2-}] \quad (9)$$

The values for the general-base catalytic constant ($10^2\ k_{B_4O_7}$ in M^{-1}/min) were 0.613 at 40°C, 1.77 at 50°C and 5.99 at 60°C.

As anticipated from the work of Kirsch and Notari (1984), catalysis was not observed for the carbonate buffer. The unit slope of the pH-rate profiles indicated that specific hydroxyl ion catalysis was the dominant kinetic term in the pH region 9–11. Kirsch and Notari (1984) have shown that general-base catalysis by carbonate buffer cannot effectively compete with specific hydroxyl ion catalysis.

3.3. Rate constants as a function of pH and temperature

The Arrhenius equation was used to define k_{H1}, k_{H2}, k_{H3}, k_{H_2O}, k_{OH} and $k_{B_4O_7}$ as a function of temperature:

$$k = Ae^{-E/RT} \quad (10)$$

In Eq. (10), A is the pre-exponential term, E is the energy of activation, R is 1.987 cal/mol-deg and T is absolute temperature (Connors, 1990). The A and E values for $k_{B_4O_7}$ were determined by linear regression based on the logarithmic form of Eq. (10). The A and E values for k_{H1}, k_{H2}, k_{H3}, k_{H_2O}, and k_{OH} (Table 3) were determined as follows. Each rate constant was expressed as $Ae^{-E/RT}$ and each function was substituted in Eq. (5) to give:

$$\begin{aligned} k_{(pH)} = {} & (A_{H1}e^{-EH1/RT})(a_{H+})(f_1) \\ & + (A_{H2}e^{-EH2/RT})(a_{H+})(f_2) \\ & + (A_{H3}e^{-EH3/RT})(a_{H+})(f_3) \\ & + (A_{H_2O}e^{-EH_2O/RT}) \\ & + (A_{OH}e^{-EOH/RT})(a_{OH-}) \end{aligned} \quad (11)$$

The absolute temperatures °K corresponding to 25, 30, 40, 50 and 60°C were substituted for T. The resulting five equations were solved by simultaneous nonlinear regression with the A and E values as adjustable parameters. This procedure was applied to the k_{SUM} values obtained from the four borate intercepts and the remaining 58 experimentally observed k_{SUM} values in Tables 1 and 2.

The A and E values from Table 3 were used in Eq. (11) to calculate k_{SUM} values for all conditions in Tables 1 and 2 with the exception of those in borate buffer. In the presence of borate buffers, the A and E values for $B_4O_7^{2-}$ (Table 3) were substituted in Eq. (10) to calculate $k_{B_4O_7}$ and this value was used in Eq. (9) with the $k_{(pH)}$ value calculated from Eq. (11). An excellent linear correlation (slope = 1.01 and $r^2 = 0.99$) was found between the 72 experimentally determined k_{SUM} values (including the two values in Section 4) and the calculated values (Fig. 4).

Table 3
Pre-exponential factors, A (M^{-1}/min except for k_{H_2O} in min^{-1}) and activation energies, E (cal/mol)[a]

k_I	A	E
k_{H1}	1.706×10^{12}	18 023
k_{H2}	2.625×10^{15}	21 343
k_{H3}	5.109×10^{13}	17 327
k_{H_2O}	1.296×10^{11}	21 561
k_{OH}	1.209×10^{10}	13 352
$k_{B_4O_7}$	2.733×10^{15}	25 393

[a] Reported digits exceed experimental precision to minimize rounding errors in predictions (Connors, 1990).

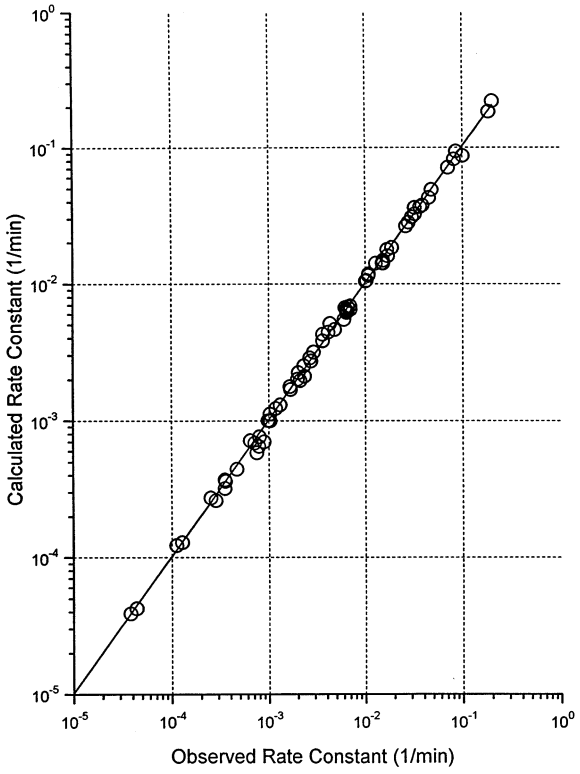


Fig. 4. Linear correlation (slope = 1.01, $r^2 = 0.99$) between temocillin hydrolysis first-order rate constants (k_{SUM}) calculated with Eqs. (9) and (11) and the 72 experimental values determined at 25–60°C in formate, phosphate, borate and carbonate buffers and in HCl. The log–log scale was used solely to facilitate visualization.

4. Discussion

4.1. Competing rates of degradation and epimerization

The concentration of temocillin remaining in solution as a function of time is [TEM] = [R] + [S]. Scheme 2 illustrates three cases for the hydrolysis kinetics of a mixture of R and S isomers where k_{rs} and k_{sr} are the first-order rate constants for interconversion of the two isomers and k_{dr} and k_{ds} are the degradation rate constants for the R and S isomer, respectively. The first-order rate constant for epimerization of R to S or S to R is $k_{epi} = k_{rs} + k_{sr}$ (Bird et al., 1984). If $k_{dr} \approx k_{ds}$, a first-order plot for [TEM], [R] or [S] will be linear with a negative slope $\approx k_{dr} \approx k_{ds}$. The following analysis examines the consequences when $k_{dr} \neq k_{ds}$.

Case 1 occurs when k_{epi} is insignificant relative to k_{dr} and k_{ds}. The R and S isomers degrade independently and epimerization plays no role. If k_{dr} and k_{ds} are sufficiently different, temocillin loss is described by the biexponential equation:

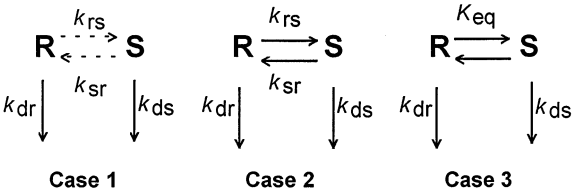
$$[TEM] = [R_0]e^{-(k_{dr})t} + [S_0]e^{-(k_{ds})t} \quad (12)$$

where $[R_0]$ and $[S_0]$ are the initial concentrations. A semilog plot of [TEM] versus time is linear during the terminal phase because the exponential with the larger rate constant becomes insignificant. Such data can be quantitatively analyzed to obtain values for $[R_0]$, $[S_0]$, k_{dr} and k_{ds} as illustrated for the hydrolysis of a diethyl-*t*-butylcarbinyl chloride isomer mixture (Moore and Pearson, 1981). When k_{dr} and k_{ds} are not sufficiently different, a semilog plot of [TEM] versus time is linear as observed for cefuroxime axetil isomers (Nguyen, 1991).

Case 3 is the opposite extreme where k_{epi} is sufficiently large, relative to k_{dr} and k_{ds}, to maintain a constant [R]/[S] ratio while the [R] and [S] values decrease with time. The [R]/[S] ratio will be the same as the equilibrium ratio, $K_{eq} = k_{sr}/k_{rs} \approx$ [R]/[S]. The rate of loss of temocillin is defined by:

$$-\frac{d[TEM]}{dt} = k_{SUM}[TEM] = (f_R k_{dr} + f_S k_{ds})[TEM] \quad (13)$$

where f_R = [R]/[TEM] and f_S = [S]/[TEM]. A first-order plot for [TEM], [R] or [S] will be linear with



Scheme 2. Three cases illustrating first-order hydrolysis of R and S isomers in a mixture. The rate constant k_{rs} represents conversion of R to S and k_{sr} represents S to R. The rate constant for degradation of the R isomer is k_{dr} and of the S isomer is k_{ds}. The epimerization rate constant is $k_{epi} = k_{rs} + k_{sr}$.

a negative slope defined as:

$$-\text{slope} = f_R k_{dr} + f_S k_{ds} \quad (14)$$

In case 2, all rate constants (k_{rs}, k_{sr}, k_{dr}, and k_{ds}) represent competing processes. If k_{dr} and k_{ds} are sufficiently different from each other, and f_R or f_S does not approach unity, the loss of temocillin is described by a biexponential equation,

$$[TEM] = [C_1]e^{-\alpha t} + [C2]e^{-\beta t} \quad (15)$$

where C_1 and C_2 are the initial values associated with each exponential and the apparent rate constants, α and β, are complex functions that cannot be solved for k_{rs}, k_{sr}, k_{dr}, or k_{ds} (Gibaldi and Perrier, 1982).

4.2. Shelf-life definitions

The shelf-life for temocillin in aqueous solution was defined as the time during which its concentration equaled or exceeded 90% of its initial concentration [TEM_0]. When hydrolysis is first order, shelf-life is commonly calculated from $T_{90} = -\ln 0.90/k$ (Connors et al., 1985; Connors, 1990; Carstensen, 1995). Fig. 1 illustrates the misinterpretation of shallow biexponential data as first order. The true time (T_{true}) at which [TEM] becomes 90% of [TEM_0] cannot be explicitly solved for biexponential loss (Eq. (12) or Eq. (15)). However, T_{true} can be observed from the temocillin time course simulated with Eqs. (16)–(18).

$$\frac{d[R]}{dt} = k_{sr}[S] - k_{rs}[R] - k_{dr}[R] \quad (16)$$

$$\frac{d[S]}{dt} = k_{rs}[R] - k_{sr}[S] - k_{ds}[S] \quad (17)$$

$$[TEM] = [R] + [S] \quad (18)$$

The T_{true} and T_{90} values may or may not agree depending on the relative values for k_{rs}, k_{sr}, k_{dr}, and k_{ds}.

4.3. Shelf-life calculations based on temocillin first-order loss (k_{SUM}) and the time-course for the sum of the isomers

Eqs. (16)–(18) were used to simulate typical sampling protocols for [R], [S] and [TEM] as a function of time. Each simulation provided ten assays spaced to provide changes of ~0.1[C_0] per sampling interval. The values for k_{rs}, k_{sr}, k_{dr}, and k_{ds} were systematically varied to examine their influence on the T_{true} and T_{90} values. The experimentally observed k_S values were usually similar to or somewhat larger than k_R. This observation was simulated in Scheme 2 by assigning values for k_{ds} that were $\geq k_{dr}$. The relative values of k_{rs} and k_{sr} for a mixture of 68% R isomer and 32% S isomer were defined by $k_{sr} = (K_{eq})(k_{rs}) = (2.125)(k_{rs})$ (Bird et al., 1984).

The k_{epi}/k_{dr} ratios were varied to represent all potential kinetic situations from case 1 to 3 by gradually increasing the ratio from 0 (case 1) to 3000 (case 3). The k_{ds}/k_{dr} ratio was varied from 1 to 4 for each k_{epi}/k_{dr} ratio. All simulations with these selected k_{rs}, k_{sr}, k_{dr}, and k_{ds} values provided [TEM] data that were adequately described by a first-order plot with residual errors $< 8\%$. These conditions produced first-order plots with less curvature than the example shown in Fig. 1.

The calculated T_{90} values were equal to or greater than the T_{true} values depending on the relative values for k_{rs}, k_{sr}, k_{dr}, and k_{ds}. The degree ($\Delta\%$) to which T_{90} overestimated T_{true} was calculated from:

$$\Delta\% = \frac{100\% \times (T_{90} - T_{true})}{T_{true}} \quad (19)$$

Fig. 5 illustrates several trends for the $\Delta\%$ values. Case 3 provided linear first-order plots with no residual errors and slopes defined by Eq. (14). Therefore, $\Delta\%$ was zero ($T_{90} = T_{true}$) for case 3 independent of the k_{ds}/k_{dr}. When k_{dr} was equal to k_{ds} (i.e. the k_{ds}/k_{dr} ratio = 1), $\Delta\%$ was zero ($T_{90} = T_{true}$) independent of the k_{epi}/k_{dr} values.

For the remaining simulations, $\Delta\%$ was greatest when temocillin data had the most biexponential character. For each k_{epi}/k_{dr} ratio, biexponential character increased as the k_{ds}/k_{dr} ratio increased from 1 to 4 as shown in Fig. 5. For any fixed k_{ds}/k_{dr} ratio, the biexponential character increased as k_{epi}/k_{dr} decreased and the kinetics approached that of case 1. Consequently, as illustrated in Fig. 5 when $k_{ds}/k_{dr} = 4$, $\Delta\%$ increased from 0% for $k_{epi}/k_{dr} = 3000$ (case 3) to 30% for $k_{epi}/k_{dr} = 0$ (case 1).

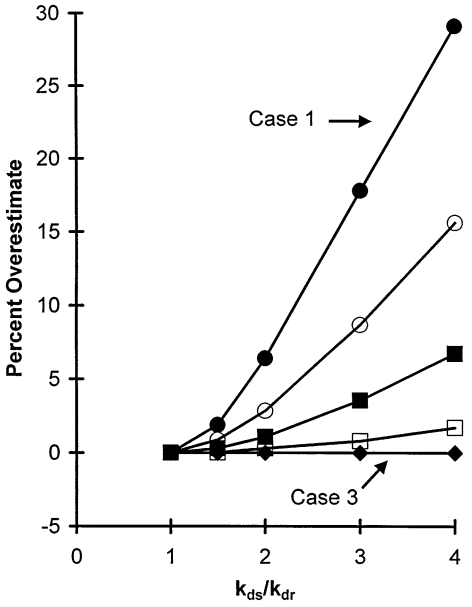


Fig. 5. The percent overestimate (Δ% in Eq. (19)) that results from using $T_{90} = -\ln 0.90/k_{SUM}$ instead of the actual time (T_{true}) for 10% loss of total drug in a mixture of R and S isomers. The Δ% values are shown as a function of degradation rate constant ratios for the S and R isomers (k_{ds}/k_{dr}). The epimerization constant was altered to produce five different k_{epi}/k_{dr} ratios equal to 0 (●), 3 (○), 6 (■), 30 (□), and 3000 (◆). The [R], [S], and [TEM] time-courses for this analysis were generated with Eqs. (16)–(18) to simulate the cases in Scheme 2.

4.4. Temocillin shelf-life predictions

There were no differences between the experimental temocillin T_{90} and T_{true} values. Therefore, temocillin shelf-life was predicted with $T_{90} = -\ln 0.9/k_{SUM}$. This can be rationalized based on Fig. 5. The largest experimentally observed k_S/k_R ratio was 1.4 (Table 2, 0.09 M phosphate, pH 5.4 at 60°C). At this k_{dr}/k_{ds} ratio, the total range for Δ% in Fig. 5 is 0–2% from case 3 to case 1. This difference is too small to be experimentally observable.

Simulated data for [R], [S], or [TEM] were described by case 3 when $k_{epi} \geq (30) \times (k_{dr})$ (Fig. 5). Bird et al. (1984) studied temocillin epimerization at pH 5 and 7, by initiating reactions with each isomer at 25 and 37°C. The values for k_{epi} were the same with either starting material because $k_{epi} = k_{rs} + k_{sr}$. These k_{epi} values were 30–400 times greater than the k_{SUM} values calculated under these conditions with Eq. (11). This rules out cases 1 and 2 because k_{SUM} cannot be less than the smaller of k_{dr} and k_{ds}. Therefore, k_{epi} must be at least 30–400 times greater than the smaller of k_{dr} and k_{ds}. This ratio is sufficiently large to cause the hydrolysis kinetics for the temocillin isomer mixture to behave like case 3. This case results in similar slopes for first-order plots of total temocillin or either isomer. Most of the observed k_R and k_S values were similar to one another. Furthermore, the relative R and S values were constant and time-independent at each temperature from 25°C (68% R, 32% S) to 60°C (60% R, 40% S).

The predicted rate constants at 30°C in 0.2 M phosphate buffer (pH 6.5) and 0.1 M phosphate buffer (pH 7.5) were 4.20×10^{-5} and 3.85×10^{-5} min^{-1}, respectively. These rate constants differ by $< 8\%$ because they are in the pH-independent region. The experimentally determined k_{SUM} values at 30°C (4.21×10^{-5} min^{-1} at pH 6.5 and 3.79×10^{-5} min^{-1} at pH 7.5) differed from predicted values by 0.2 and 1.6%, respectively.

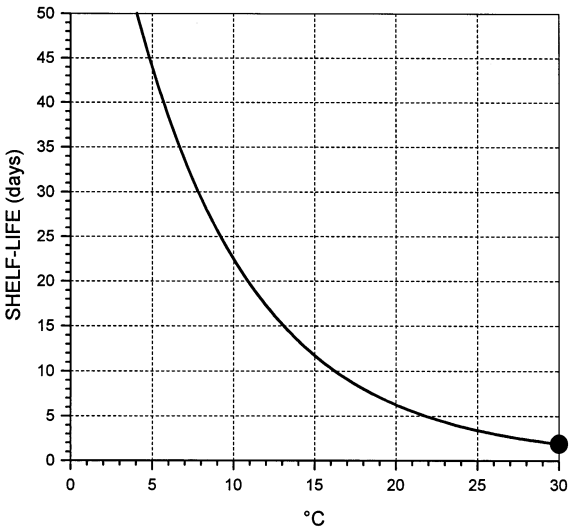


Fig. 6. The predicted shelf-life ($T_{90} = -\ln 0.90/k_{SUM}$) as a function of temperature for an aqueous temocillin solution in the pH-independent range, 6.5–7.5, in the presence or absence of phosphate buffer. The T_{90} (1.8 ± 0.1 days) at 30°C (●) was experimentally verified at pH 6.5 and 7.5.

The maximum stability for an aqueous temocillin solution occurred in the pH range, 6.5–7.5 (Fig. 3). Phosphate buffer did not accelerate hydrolysis. Therefore, the shelf-life was predicted from $T_{90} = -\ln 0.90/k_{SUM}$ by using Eq. (11) to calculate k_{SUM}. Fig. 6 shows the predicted shelf-life as a function of temperature in the pH range 6.5–7.5 where typical values were 1.8 days (30°C), 3.5 days (25°C), 12 days (15°C), and 44 days (5°C).

Acknowledgements

We wish to thank S. Trowbridge, Compound Bank Supervisor, P.G. Treagust, Manager of Reference Materials Group and SmithKline Beecham Pharmaceuticals, UK, for supplying temocillin. S. Craig Dyar gratefully acknowledges the support of an American Foundation for Pharmaceutical Education Predoctoral Fellowship.

References

Ariens, E.J., 1983. Stereochemistry and Biological Activity of Drugs. Blackwell, Oxford, pp. 1–204.

Ariens, E.J., Wuis, E.W., Veringa, E.J., 1988. Stereoselectivity of bioactive xenobiotics. A pre-Pasteur attitude in medicinal chemistry, pharmacokinetics and clinical pharmacology. Biochem. Pharmacol. 37, 9–18.

Bird, A.E., Charsley, C., Jennings, K.R., Marshall, A.C., 1984. High-performance liquid chromatographic assay of temocillin and epimerisation of its diastereoisomers. Analyst 109, 1209–1212.

Burton, G., Dobson, C.R., Everett, J.R., 1986. The degradation of temocillin, a 6α-methoxypenicillin, and identification of the major degradation products. J. Pharm. Pharmacol. 38, 758–761.

Carstensen, J.T., 1995. Drug Stability, 2nd ed. Marcel Dekker, New York, pp. 17–121.

Connors, K.A., 1990. Chemical Kinetics. VCH, New York, pp. 17–58, 245–291.

Connors, K.A., Amidon, G.L., Stella, V.L., 1985. Chemical Stability of Pharmaceuticals, 2nd ed. Wiley, New York, pp. 8–31, 43–62, 302–321.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, New York, pp. 89–92.

Guest, E.A., Horton, R., Mellows, G., Slocombe, B., Swaisland, A.J., Tasker, T.C.G., 1985. Human pharmacokinetics of temocillin (BRL 17421) side chain epimers. J. Antimicrob. Chemother. 15, 327–336.

Harned, H.S., Owen, B.B., 1958. The Physical Chemistry of Electrolytic Solutions, 3rd ed. Reinhold, New York, pp. 638, 748.

Hutt, A.J., O'Grady, J., 1996. Drug chirality: a consideration of the significance of the stereochemistry of antimicrobial agents. J. Antimicrob. Chemother. 37, 7–32.

Kirsch, L.E., Notari, R.E., 1984. Theoretical basis for the detection of general-base catalysis in the presence of predominating hydroxide catalysis. J. Pharm. Sci. 73, 724–727.

Moore, J.W., Pearson, R.G., 1981. Kinetics and Mechanism. Wiley, New York, pp. 286–288.

Nguyen, N.T., 1991. The relationship of diastereomer hydrolysis kinetics to shelf-life predictions for cefuroxime axetil. Pharm. Res. 8, 893–898.

Niebergall, P.J., Schnaare, R.L., Sugita, E.T., 1973. Potentiometric determination of overlapping dissociation constants. J. Pharm. Sci. 62, 656–659.

Shand, D.G., Rango, R.E., 1972. The disposition of propranolol. I. Elimination during oral absorption in man. Pharmacology 7, 159–168.

Simonyi, M., 1984. On chiral drug action. Med. Res. Rev. 4, 359–413.

Walker, G., 1994. ABPI Data Sheet Compendium 1993–1994. Datapharm, London.

Zhou, M., Notari, R.E., 1995. Influence of pH, temperature, and buffers on the kinetics of ceftazidime degradation in aqueous solutions. J. Pharm. Sci. 84, 534–538.