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Short communication

The influence of pH, temperature and buffers on the degradation kinetics of cefetamet pivoxil hydrochloride in aqueous solutions

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Abstract

The first-order hydrolysis kinetics of cefetamet pivoxil (CP) were investigated as a function of pH, temperature and buffers. The degradation was followed by HPLC. Buffer catalysis was observed in acetate and phosphate buffers. The pH–rate profiles for hydrolysis of cefetamet pivoxil were obtained at 333, 343, 353 and 363 K. The pH–rate expression was $k_{pH} = k_{H^+}a_{H^+} + k_{H_2O}k_{OH^-}a_{OH^-}$, where k_{H^+} and k_{OH^-} are the second-order rate constants ($mol^{-1}\,l\,s^{-1}$) for hydrogen ion activity and for hydroxyl ion activity respectively, and k_{H_2O} is the pseudo-first-order rate constant (s^{-1}) for spontaneous reaction under the influence of water. The pH–rate profile was characteristically U-shaped. Maximum stability was observed in the pH region from 3 to 5.

Keywords: Cefetamet pivoxil hydrochloride; Stability in aqueous solutions; pH–rate profile; Catalytic rate constants; Thermodynamic parameters

1. Introduction

Cefetamet pivoxil hydrochloride (Scheme 1) is an oral prodrug of cefetamet that after oral administration is rapidly hydrolysed by nonspecific mucosal esterases to its free acid in blood and in body tissues with high yield [1–5]. Similarly to third generation cephalosporins, cefetamet demonstrates significant activity against Gram-negative organisms such as *Klebsiella* spp., *Proteus mirabilis* and *P. vulgaris*, *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Haemophilus influenzae*, *Moraxella catarralis*, *Neisseria* spp., *Vibrio* spp. and Gram-positive organism such as *Streptococcus* spp.

The stability of cefetamet pivoxil hydrochloride (CP) in solid state and the levels of degradation of cefetamet pivoxil in 0.6 mol l^{-1} phosphate buffer and human intestinal juice at 37 °C over 24 h have been reported in the literature [6,7]. The degradation of CP occurring at air humidity RH >50% is an autocatalytic first-order reaction with respect to substrate concentration, while at 0% relative humidity of the ambient air it

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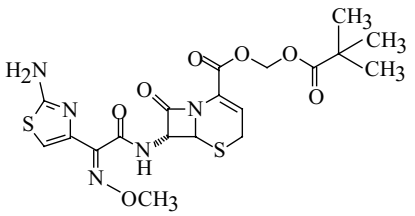

Scheme 1.

is a first-order reaction relative to substrate concentration [6]. Degradation of cefetamet pivoxil progressed much faster in the intestinal juice than in the phosphate buffer. In the phosphate buffer, the major degradation product was the Δ3-cephalosporin (61%), while in the intestinal juice it was the Δ2-cephalosporin (86%) [7].

The literature does not refer to research into the kinetics of cefetamet pivoxil hydrochloride. Therefore, the aim of this study was to investigate the kinetics of CP degradation in aqueous solution as a function of pH, temperature and buffer concentration and to develop equations that predict cefetamet pivoxil stability at any pH and temperature, in the presence or absence of buffer.

2. Experimental

2.1. Chemicals and reagents

Cefetamet pivoxil hydrochloride was obtained from the Institute of Biotechnology and Antibiotics in Warsaw, Poland. Acetanilide (serving as the internal standard (IS)) was a product of Sigma. All other chemicals were analytical or HPLC grade.

2.2. Chromatographic conditions

The compound studied was determined by means of HPLC. The analytical system consisted of a Rheodyne 7120 20 μl fixed-loop injector, an LC-2UV detector (Shimadzu), an L-6000 pump (Hitachi). An LiChrosorb 100 RP-18 column (250 mm × 4 mm i.d., dp = 7 μm (Merck)) was used. The flow rate was 1.0 ml min^{-1}. UV detection was carried out at 265 nm. The mobile phase consisted of 0.1 mol l^{-1} phosphate buffer (pH 6.5)–acetonitryle (6:4). The internal standard was a solution of acetanilide in a mixture of acetonitryle and water (1:1) at a concentration of 0.1 mg ml^{-1}.

2.3. Linearity

Linearity between P/P_{IS} (P and P_{IS}—areas of CP and IS) and concentrations of CP in water ranging from 0.025 to 0.300 mg ml^{-1} were evaluated. The internal standard was added to the solutions under investigation at a constant concentration of 0.1 mg ml^{-1}. Linearity was also examined for three consecutive days in solutions of the same concentration prepared from the stock solution.

2.4. Precision and accuracy

The precision of the method was determined through the analysis of eight injections of the standard solution containing 0.05, 0.1 and 0.2 mg ml^{-1} of the substance dissolved in water.

2.5. Kinetic measurements

The degradation of CP as a result of hydrolysis was studied at 333, 343, 353 and 363 K in hydrochloric acid (pH 0.44–1.40), phosphate buffers (pH 2.07–3.47 and 5.86–7.32), acetate buffer (pH 3.92–5.62) and borate buffer (pH 7.46–8.00). The compositions of the buffer solutions were calculated from the Henderson–Hasselbalch equation. The pH values of the buffer reactions were measured before and after each reaction at the experimental temperatures. The pH values for the reactions in HCl were calculated from the equation:

$$\mathrm{pH} = -\log f_{\mathrm{HCl}}[\mathrm{HCl}] \quad (1)$$

The coefficient of activity f_{HCl} was taken from literature [8]. The ionic strength (μ) of all the solutions was adjusted to 0.5 mol l^{-1} with a solution of sodium chloride (4 mol l^{-1}). The solutions of appropriate pH and ionic strength of 0.5 mol l^{-1} were heated to the desired temperatures, and then a sample of CP was added. The initial concentration of CP was 0.2 mg ml^{-1}. Samples of reaction mixtures (1 ml) were collected at time intervals depending on the respective reaction rates for a given pH. They were instantly cooled with mixture of ice and water and neutralized if necessary. To each

such sample 1.0 ml of the internal standard solution was added and subjected to analysis.

3. Results and discussion

Changes of in CP concentration under experimental conditions were measured using the HPLC method previously described by Wyss and Bucheli [9] and modified for the purpose of this study. The method applied was validated with respect to selectivity, linearity, precision and accuracy. Peak A corresponding to the investigated substance, with a retention time of ca. 9.93 min and peak IS corresponding to the internal standard, with a retention time of ca. 3.16. emerged in chromatograms taken over a period of 0–15 min. The degradation products appeared before or at the beginning of the solvent front. Under the conditions of the study, a linear dependence was obtained between the heights of the peaks and concentration. The equation for the calibration curve is: $y = (5.04 \pm 0.15)x$; (for the equation $y = ax + b$, the value b is insignificant). The calculated correlation coefficient was >0.999, thus indicating high linearity. Accuracy and precision of the measurement were also satisfying, because the R.S.D. < 2.5% (0.26–2.26%). Therefore, the proposed procedure allows to satisfactory determinations for the kinetic studies.

3.1. Determination of first-order rate constants

The degradation of cefetamet pivoxil as a result of hydrolysis is a pseudo-first-order reaction described by the following equation:

$$\ln c = \ln c_0 - k_{obs}t \quad (2)$$

where c and c_0 are the time-dependent concentration and the initial concentration of cefetamet pivoxil, at the times: $t > 0$ and $t = 0$ respectively, k_{obs} is the observed rate constant of the pseudo-first-order reaction of CP degradation. The semilogarythmic plots $c\% = f(t)$ obtained according to the above equation are linear (Fig. 1) and their slope is equal to the rate constant of the reaction with the negative sign ($-k_{obs}$). The number of measurements of c_t taken over the time span from $t_0 \rightarrow t_i$ ranged from 8 to 12.

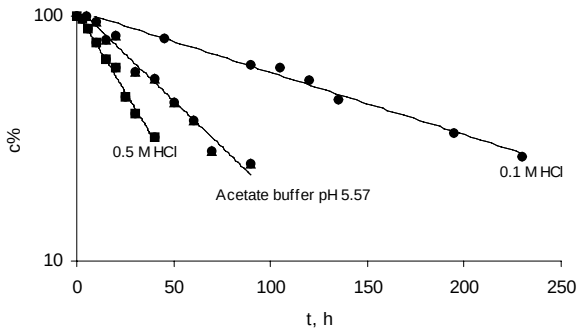

Fig. 1. Semilogarithmic plots of the percentage of not degraded CP at 363 K in 0.5 mol l^{-1} HCl, 0.1 mol l^{-1} HCl and 0.4 mol l^{-1} acetate buffer, pH 5.57.

3.2. Buffer catalysis

An increase of the rate constant for cefetamet pivoxil degradation was observed while when buffer concentration increased at constant pH and temperature. For each buffer solution determination was performed at three to four different pH values. The catalytic effect was observed for the components of acetate (pH 3.92–5.62) and phosphate (pH 2.07–3.47 and 5.86–7.32) buffers, so

$$k_{obs} = k_{pH} + k_B[B]_T \quad (3)$$

where k_{pH} is the rate constant at zero buffer concentration, $[B]_T = [HA] + [A^-]$ represents total buffer concentration.The plots $k_{obs} = f([B]_T)$ obtained for the acetate and phosphate buffers are linear and the slope is equal to k_B. The ordinate at $[B]_T = 0$ is equal to k_{pH}. $k_B = k_{HA}f_{HA} + k_{A^-}f_{A^-}$ represents the catalytic effect of acetate buffer, f_{HA} and f_{A^-} refer to the molar fractions of the acetate buffer species; $f_{A^-} = [A^-]/[B]_T = K_a/(K_a + a_{H^+})$. Since $f_{HA} + f_{A^-} = 1$, plots of k_B against f_{HA} should be linear with intercept k_{A^-} when f_{HA} is zero, and k_{HA} when f_{HA} is unity.

The catalytic rate constants for the phosphate buffers were determined in a manner analogous to that used in the interpretation of acetate buffer catalysis. For the acetate buffer, the acetate was catalytic but no significant catalysis by the acetic acid component was found. For the phosphate buffer in the pH region 2.07–3.47, both the phosphoric acid and the $H_2PO_4^-$ alike were catalytic. In the pH region 5–7.32, only the HPO_4^{2-} was catalytic.

3.3. Rate constants as a function of pH and temperature

The observed rate constants at each pH were corrected for buffer catalysis using linear regression of k versus total buffer concentration to determine the intercepts at each constant ratio. The first-order rate constants determined as a function of pH in HCl and borate buffer and from buffer intercepts at 333, 343, 353 and 363 K were used to obtain the pH–rate profile (Fig. 2) for cefetamet pivoxil degradation. The pH–rate profile showed a characteristic U-shape. As follows from the semilogarythmic dependence k–pH the possible reactions in the water solution in the pH range from 0.44 to 8.0 are:

- hydrolysis of cefetamet pivoxil catalysed by hydrogen ions

  $\mathrm{CP} + \mathrm{H^+} \xrightarrow{\mathrm{H_2O}} \text{products},\ k_{\mathrm{H^+}}$

- spontaneous hydrolysis of cefetamet pivoxil under the influence of water

  $\mathrm{CP} \xrightarrow{\mathrm{H_2O}} \text{products},\ k_{\mathrm{H_2O}}$

- hydrolysis of cefetamet pivoxil catalysed by hydroxyl ions

  $\mathrm{CP} + \mathrm{OH^-} \xrightarrow{\mathrm{H_2O}} \text{products},\ k_{\mathrm{OH^-}}$

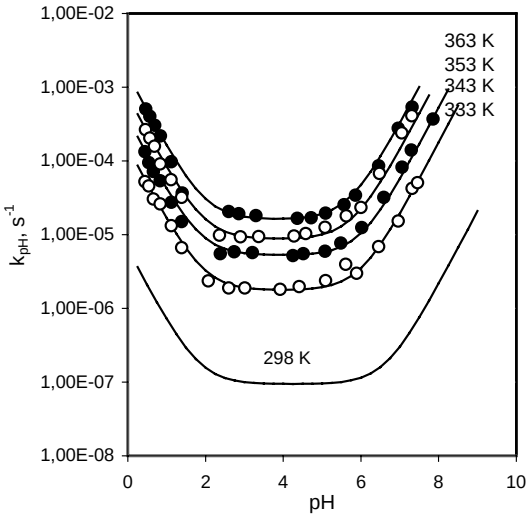

Fig. 2. pH–rate profiles for degradation of CP at 333, 343, 353 and 363 K. The symbols represent experimental points. The profile at 298 K was calculated from the values in Table 1.

The total rate of the reaction is equal to the sum of partial reaction rates:

$$k_{\mathrm{pH}} = k_{\mathrm{H^+}} a_{\mathrm{H^+}} + k_{\mathrm{H_2O}} + k_{\mathrm{OH^-}} a_{\mathrm{OH^-}} \quad (4)$$

The catalytic rate constants $k_{\mathrm{H^+}}$ and $k_{\mathrm{OH^-}}$ were calculated using k_{pH} value from the appropriate pH range. The plots $k_{\mathrm{pH}} = f(a_{\mathrm{H^+}})$ and $k_{\mathrm{pH}} = f(a_{\mathrm{OH^-}})$ are linear, of a positive slope equal to $k_{\mathrm{H^+}}$ or $k_{\mathrm{OH^-}}$, respectively. The catalytic rate constants $k_{\mathrm{H_2O}}$ was calculated as the

Table 1
Catalytic rate constants and thermodynamic parameters for degradation of CP in aqueous solutions

Catalytic rate constant	Temperature (K)	$(k \pm \Delta k)$ $\ln k_i = f(1/T)$	Statistical evaluation	Thermodynamic parameters
$k_{\mathrm{H^+}}$ ($\mathrm{mol^{-1}\,l\,s^{-1}}$)	333	$(1.51 \pm 0.22) \times 10^{-4}$	$r = -0.9990$	$E_a = 75.9 \pm 10.4\,\mathrm{kJ\,mol^{-1}}$
	343	$(3.70 \pm 0.38) \times 10^{-4}$	$a = -9124 \pm 1251$	$\Delta H^{\neq} = 73.41 \pm 12.9\,\mathrm{kJ\,mol^{-1}}$
	353	$(7.50 \pm 0.80) \times 10^{-4}$	$b = 18.6 \pm 3.6$	$\Delta S^{\neq} = -89.9 \pm 215.0\,\mathrm{J\,K^{-1}\,mol^{-1}}$
	363	$(14.7 \pm 0.90) \times 10^{-4}$		
$k_{\mathrm{H_2O}}$ ($\mathrm{s^{-1}}$)	333	$(1.75 \pm 0.15) \times 10^{-6}$	$r = -0.9853$	$E_a = 73.0 \pm 38.5\,\mathrm{kJ\,mol^{-1}}$
	343	$(5.34 \pm 0.22) \times 10^{-6}$	$a = -8777 \pm 4631$	$\Delta H^{\neq} = 70.5 \pm 41.0\,\mathrm{kJ\,mol^{-1}}$
	353	$(9.66 \pm 1.18) \times 10^{-6}$	$b = 13.3 \pm 13.3$	$\Delta S^{\neq} = -134.8 \pm 134.1\,\mathrm{J\,K^{-1}\,mol^{-1}}$
	363	$(16.0 \pm 1.2) \times 10^{-6}$		
$k_{\mathrm{OH^-}}$ ($\mathrm{mol^{-1}\,l\,s^{-1}}$)	333	18.5 ± 0.9	$r = -0.9979$	$E_a = 52.5 \pm 10.4\,\mathrm{kJ\,mol^{-1}}$
	343	34.6 ± 4.7	$a = -6311 \pm 1245$	$\Delta H^{\neq} = 50.0 \pm 12.8\,\mathrm{kJ\,mol^{-1}}$
	353	58.3 ± 5.5	$b = 21.9 \pm 3.6$	$\Delta S^{\neq} = -62.8 \pm 215.2\,\mathrm{J\,K^{-1}\,mol^{-1}}$
	363	88.4 ± 7.8		

$\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated for 298 K.

mean value from the equation:

$$k_{pH} = k_{pH} - (k_{H^+}a_{H^+} + k_{OH^-}a_{OH^-}) = k_{H_2O} \quad (5)$$

The correct choice of Eq. (4) was verified by the correspondence between the calculated theoretical profile of log $k = f(\text{pH})$ and the experimental results. High linear correlation ($r = 0.997$) was found between the 71 experimentally determined k values and k-values calculated with Eq. (4).

Using the catalytic rate constants derived from the Arrhenius equation ($\ln k_i = \ln A - a1/\text{T}$), the slope ($a$) of the plots $\ln k_i = f(1/\text{T})$ and the values of ln A (A—coefficient of frequency) for particular reactions were calculated. These values were used to determine of the activation energy, enthalpy and entropy (Table 1).

In the acidic solution, the degradation of cefetamet pivoxil is the effect of ester bond hydrolysis with a yield of 100%. The degradation product, cefetamet, degraded further with the rate constant approximately five-fold lower than the rate constant of ester bond hydrolysis. In neutral solution, either hydrolysis of ester bond of the cefetamet pivoxil and further isomerization of acid have been observed.

4. Conclusions

Within the limits of this study, the following conclusions were drawn:

1. The degradation of cefetamet pivoxil in aqueous solutions occurs as a result of three simultaneous processes. The first one is a reaction catalysed by hydrogen ions, the second is a spontaneous reaction catalysed by water molecules, and the third is a reaction catalysed by hydroxide ions.
2. The hydrolysis of cefetamet pivoxil involves general catalysis and specific acid–base catalysis.
3. The catalytic effect was observed for the components of acetate and phosphate buffers.
4. Cefetamet pivoxil exhibits the greatest stability in the pH range from 3 to 5.

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