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Stability of ertapenem in aqueous solutions

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Abstract

The kinetics of degradation of ertapenem was studied in aqueous solutions at 303, 313, 323 and 333 K and pH 0.42–12.5. Degradation was studied using two methods: HPLC (LiChrospher RP-18 column, 5 μm, 250 mm × 4 mm; mobile phase: methanol–phosphate buffer 25 mmol l^{-1}, pH 6.5 (15:85, v/v); flow rate—1.2 ml/min; detection UV—298 nm) and UV (294 nm). Specific acid–base catalysis involves: (a) hydrolysis of ertapenem, catalysed by hydrogen ions; (b) hydrolysis of ertapenem dianions catalysed by hydroxide ions; (c) spontaneous hydrolysis of zwitter ions and dianions of ertapenem under the influence of water. The thermodynamic parameters of these reactions—energy, enthalpy and entropy of activation were calculated. It was observed that buffer catalysis occurred in acetate, phosphate and borate buffers.

Keywords: Ertapenem; Stability in aqueous solutions; pH-rate profile; Catalytic rate constants; Thermodynamic parameters

1. Introduction

Ertapenem (Fig. 1) is a new antibiotic with a very broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria [1–6].

Ertapenem, like other carbapenems such as meropenem and imipenem [6], is an antibiotic for parenteral application. The hydrolysis of the β-lactam ring occurs in dilute aqueous solutions of ertapenem (<1 mg ml^{-1}). When the concentration of ertapenem is high (≥100 mg ml^{-1}) dimerization products (*I–V*) are formed [7]. During the manufacture and purification of ertapenem, a methanolysis product, an oxazinone derivative and an acetic acid adduct were also observed [8]. To separate ertapenem and its degradation products the gradient HPLC method was used [7,9].

The stability of ertapenem in solution of sodium chloride, sodium lactate, sodium bicarbonate, mannitol, dextrose and Ringer's solution, at 25 and 4 °C, were also studied [10].

The aim of this study was to analyse general and specific acid–base hydrolysis of ertapenem at pH 0.42–12.5, at 303, 313, 323 and 333 K. To determine the reaction rate, the isocratic HPLC and spectrophotometric (UV) methods were used [11].

2. Experimental

2.1. Materials and reagents

Ertapenem for injection—INVANZ (Merck & Co. Inc., Whitehouse Station, NJ, USA) is a sterile, synthetic, white to off-white hygroscopic, weakly crystalline powder. Each vial contains 1.046 g of ertapenem sodium (equivalent to 1 g of ertapenem) and inactive ingredients: 175 mg of sodium bicarbonate and sodium hydroxide to adjust pH to 7.5. Diprophylline (conforming to FP VI Poland) was used as an internal standard (IS). All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical or high-performance liquid chromatographic grade.

2.2. Methods

2.2.1. HPLC analysis

Chromatographic separation and quantitative analysis were performed by using a high-performance liquid chromatograph equipped with an LC-6A pump (Shimadzu), a UV–vis (SPD-6AV) detector (Shimadzu) and a Rheodyne with a 50 μl loop. An LiChrospher RP-18 analytical column, 5 μm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) was used as the stationary phase. The mobile phase consisted of 15 volumes of methanol and 85 volumes of phosphate buffer 25 mmol l^{-1} (pH

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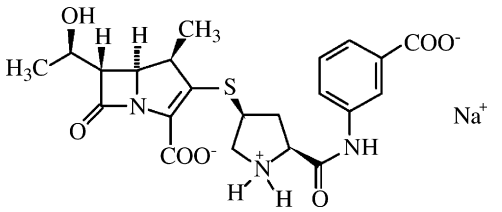

Fig. 1. Ertapenem = [4*R*-[3(3*S**,5*S**),4α,5β,6β(*R**)]]-3-[[5-[[(3-carboxyphenyl)amino]-carbonyl]-3-pyrrolidinyl]thio]-6-(1–hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt.

6.5). The flow rate was 1.2 ml min^{-1} and UV detection was performed at 298 nm.

2.2.2. UV analysis

A UV–vis Lambda 20 spectrophotometer (Perkin-Elmer) was used (λ = 294 nm, 1.0 cm quartz cells). UV WinLab software was used for absorbance measurements.

The methods applied were validated and their suitability for kinetics studies had been demonstrated in the previous study [11].

2.3. Kinetics procedures

The degradation of ertapenem in aqueous solutions was studied at 303, 313, 323 and 333 K in hydrochloric acid (pH 0.42–2.04), phosphate buffer (pH 1.90–2.98 and 5.96–7.21), acetate buffer (pH 3.61–5.19), borate buffer (pH 7.60–10.05), carbonate buffer (pH 10.55) and in sodium hydroxide (pH 11.98–12.70) at 303, 298, 293 K. The pH values of the reaction solutions and those of the buffer standards used to calibrate the pH-meter were measured at reaction temperatures. The pH values of the reaction solutions in HCl and NaOH were calculated from the equations $pH = -\log f_{HCl}$ [HCl] or $pH = pK_w + \log f_{NaOH}$ [NaOH]. The activity coefficients f_{HCl} and f_{NaOH} were obtained from the literature or calculated by interpolation of literature data [12]. 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}).

Degradation was initiated by dissolving an accurately weighed 5.0 mg (HPLC) and 10.0 mg (UV) of INVANZ for injection in 25 ml (HPLC) or 50 ml (UV) of reaction solution equilibrated to required temperatures in stoppered flasks. At selected times, samples of reaction solutions (1.0 ml, HPLC and 2.0 ml, UV) were collected and instantly cooled with a mixture of ice and water and neutralized with1.0 ml (HPLC) or 2.0 ml (UV) of HCl or NaOH solutions of suitable concentrations and assayed. In the HPLC method, to each sample 2.0 ml of the internal solution (diprophylline 0.4 mg ml^{-1}) were added.

3. Results and discussion

3.1. Observed rate constants

At a pH range from 0.42 to 7.62 the observed rate constants (Fig. 2) were determined by HPLC method. The observed first-order rate constants (k_{obs}) for the degradation of ertapenem were

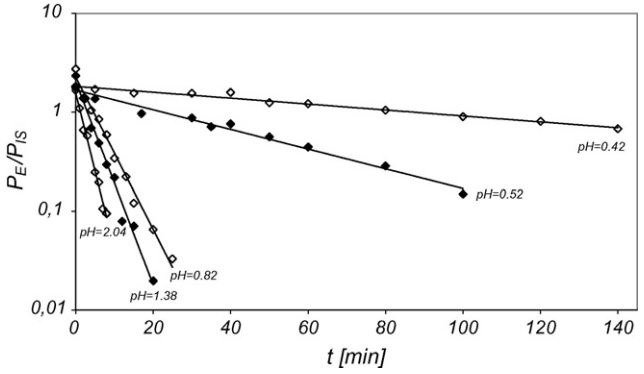

Fig. 2. Semilogarithmic plots $P_E/P_{IS} = f(t)$ for the degradation of ertapenem in HCl (pH 0.42–2.04), $\mu = 0.5$ mol l^{-1} at 303 K.

calculated from the following equation:

$$\ln\left(\frac{P_E}{P_{IS}}\right)_t = \ln\left(\frac{P_E}{P_{IS}}\right)_0 - k_{obs}t \quad (1)$$

P_E and P_{IS} are the areas of the peaks of ertapenem and the internal standard, at time $t = 0$ and t, respectively.

At a pH range from 7.63 to 12.70 the first-order rate constants of the degradation of ertapenem were determined from measurements of absorbance at 294 nm using the subtraction technique (Fig. 3):

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - k_{obs}t \quad (2)$$

A_0, A_t and A_∞ are the absorbance at $t = 0$, t and ∞, respectively.

3.2. Buffer catalysis

At constant pH, ionic strength ($\mu = 0.5$ mol l^{-1}) and temperature, in the presence of excess buffer, the rate constant k_{obs}, for the degradation of ertapenem increased as the total concentrations of acetate, phosphate or borate buffers increased (Fig. 4a and b).

The observed first-order rate constants k_{obs} under the conditions of general acid–base catalysis were calculated from the following equation:

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

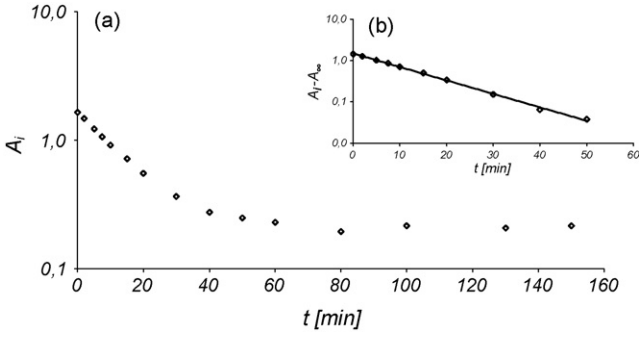

Fig. 3. Semilogarithmic plots $\ln A_i = f(t)$ (a) and $\ln(A_i - A_\infty) = f(t)$ (b) characterizing the degradation of ertapenem in borate buffer at 303 K.

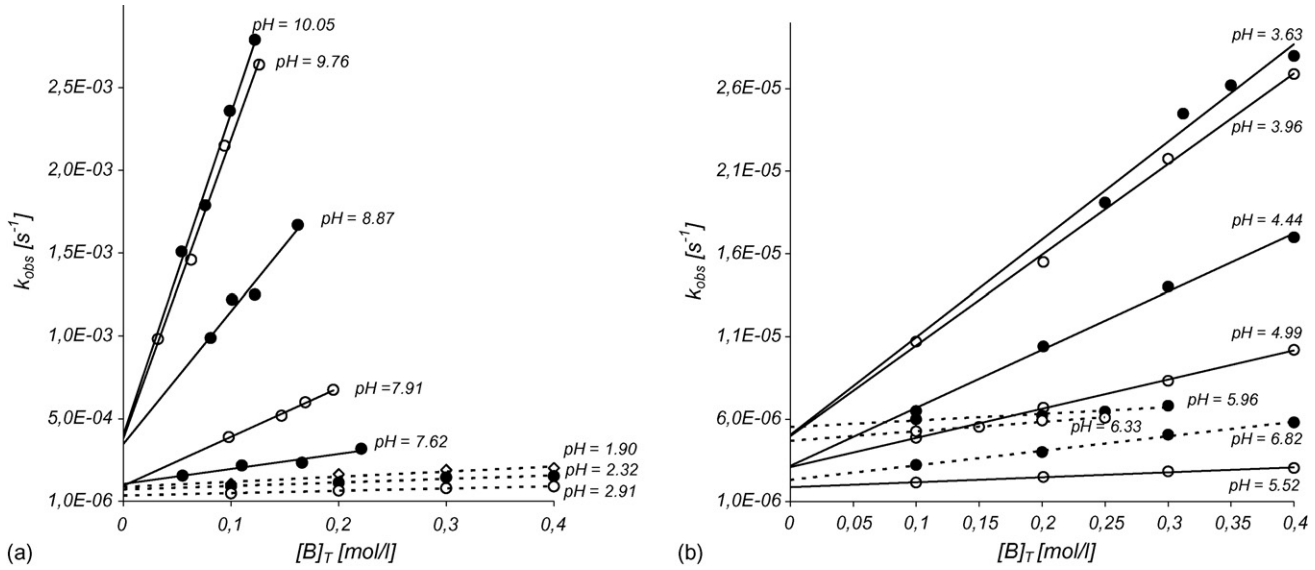

Fig. 4. Plots $k_{obs} = f([B]_T)$ for the degradation of ertapenem in: (a) phosphate (pH 1.90–2.91; - - -) and borate (pH 7.62–10.05; —) buffers at 303 K and (b) acetate (pH 3.63–5.52; —) and phosphate (pH 5.96–6.82; - - -) buffers at 303 K.

$[B]_T$ is the total buffer concentration, k_{pH} the rate constant at zero buffer concentration and k_B represents the catalytic effect of buffer.

The plots $k_{obs} = f([B]_T)$ obtained for the acetate, phosphate and borate buffers were linear and their slopes equaled k_B. The values of k_{obs} for $[B]_T = 0$ equaled k_{pH}.

No significant buffer catalysis was observed in the carbonate buffer. In the reaction solutions in HCl and NaOH and carbonate buffer the values of $k_{obs} = k_{pH}$.

3.3. pH-rate profiles

The rate constants k_{pH} determined in hydrochloric acid, sodium hydroxides and carbonate buffer solutions and calculated from Eq. (3) in the case of general acid–base catalysis were used to calculate the relationship $\log k_{pH} = f(pH)$ (Fig. 5).

The semilogarithmic relationship k–pH indicates that in water solutions at pH 0.42–12.5 the following reactions are possible:

- degradation of ertapenem catalysed by hydrogen ions:

$$E + H^+ \rightarrow \text{products},\ k_{H^+}$$

- spontaneous hydrolysis of zwitter ions E^{+-} and dianions E^{2-} of ertapenem under the influence of the water:

$$E^{+-} \xrightarrow{H_2O} \text{products},\ k_{H_2O}$$

$$E^{2-} \xrightarrow{H_2O} \text{products},\ k'_{H_2O}$$

- degradation of dianions of ertapenem catalysed by hydroxyl ions:

$$E^{2-} + OH^- \rightarrow \text{products},\ k_{OH^-}$$

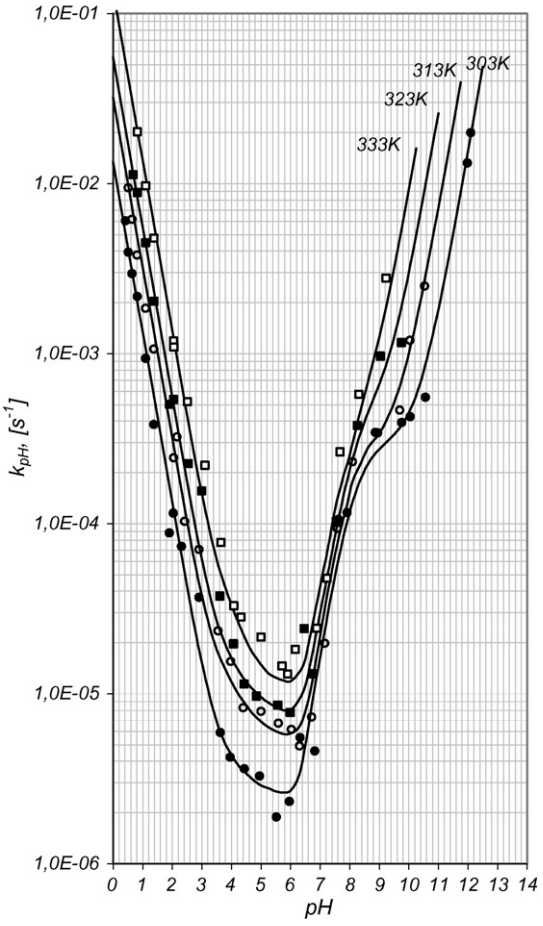

Fig. 5. pH-rate profiles for the degradation of ertapenem at 303 K (●), 313 K (◯), 323 K (■) and 333 K (□). The points are determined experimentally. The lines were calculated from the Eq. (4).

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

Catalytic rate constants	Temperature (K)	$k \pm \Delta k$ (s^{-1})	Statistical evaluation $k_i = f(1/T)$	Thermodynamic parameters
k_{H^+} ($mol^{-1}l\,s^{-1}$)	303	$(1.34 \pm 0.51) \times 10^{-2}$	$r = -0.9936$	$E_a = 64.83 \pm 22.36$ kJ mol^{-1}
	313	$(3.21 \pm 0.63) \times 10^{-2}$	$a = -7797 \pm 2690$	$\Delta H^{\neq} = 62.35 \pm 24.84$ kJ mol^{-1}
	323	$(5.54 \pm 0.70) \times 10^{-2}$	$b = 21.4 \pm 8.5$	$\Delta S^{\neq} = -69.91 \pm 174.49$ J K^{-1} mol^{-1}
	333	$(14.7 \pm 2.2) \times 10^{-2}$		
k_{H_2O} (s^{-1})	303	$(0.328 \pm 0.137) \times 10^{-5}$	$r = -0.9616$	$E_a = 50.18 \pm 43.55$ kJ mol^{-1}
	313	$(1.00 \pm 0.21) \times 10^{-5}$	$a = -6035 \pm 5237$	$\Delta H^{\neq} = 47.70 \pm 46.03$ kJ mol^{-1}
	323	$(1.16 \pm 0.73) \times 10^{-5}$	$b = 7.45 \pm 16.50$	$\Delta S^{\neq} = -182.98 \pm 107.73$ J K^{-1}mol^{-1}
	333	$(2.27 \pm 1.56) \times 10^{-5}$		
k'_{H_2O} (s^{-1})	303	$(3.03 \pm 0.13) \times 10^{-4}$	$r = -0.9510$	$E_a = 19.80 \pm 19.57$ kJ mol^{-1}
	313	$(3.23 \pm 4.37) \times 10^{-4}$	$a = -2381 \pm 2354$	$\Delta H^{\neq} = 17.32 \pm 22.05$ kJ mol^{-1}
	323	$(5.12 \pm 1.25) \times 10^{-4}$	$b = -0.30 \pm 7.42$	$\Delta S^{\neq} = -247.41 \pm 183.22$ J K^{-1}mol^{-1}
	333	$(5.71 \pm 4.35) \times 10^{-4}$		
k_{OH^-} ($mol^{-1}l\,s^{-1}$)	293	0.474 ± 0.156	$r = -0.9961$	$E_a = 58.13 \pm 7.14$ kJ mol^{-1}
	298	0.920 ± 0.824	$a = -6990 \pm 859$	$\Delta H^{\neq} = 55.65 \pm 9.62$ kJ mol^{-1}
	303	1.04 ± 0.50	$b = 23.20 \pm 2.78$	$\Delta S^{\neq} = -52.02 \pm 221.80$ J K^{-1}mol^{-1}
	313	2.38[a]		
	323	4.66[a]		
	333	9.18[a]		

$\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated for 298 K.
[a] The value extrapolated.

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

$$k_{pH} = k_{H^+}a_{H^+} + k_{H_2O}f_{E^{+-}} + k'_{H_2O}f_{E^{--}} + k_{OH^-}a_{OH^-}f_{E^{--}} \quad (4)$$

a_{H^+} and a_{OH^-} are the hydrogen and hydroxide ion activity and $f_{E^{+-}}$ and $f_{E^{--}}$ are the fractions of zwitter ions and dianions of ertapenem. The values $f_{E^{+-}}$ and $f_{E^{--}}$ were calculated after taking into account the values of pK_a of ertapenem that were 2.9, 6.0 and 8.2.

The catalytic rate constants k_{H^+} (Fig. 6, Table 1) at 303, 313, 323 and 333 K were calculated from the equation:

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

using the values of k_{pH} at pH 0.52–2.04.

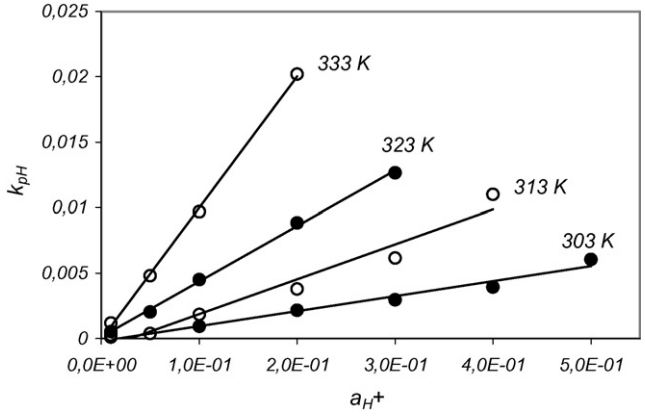

Fig. 6. Plots $k_{pH} = f(a_{H^+})$ for the degradation of ertapenem in HCl at 303, 313, 323, 333 K.

The catalytic rate constants k_{OH^-} (Table 1) at 303, 298 and 290 K were calculated from the equation:

$$k_{pH} = k_{OH^-}a_{OH^-} \quad (6)$$

using the values of k_{pH} above pH 11.98. At this pH range the value of $f_{E^{--}} \rightarrow 1$. The catalytic rate constants k_{OH^-} at 313, 323 and 333 K were obtained from the Arrhenius equation.

The plots $k_{pH} = f(a_{H^+})$ and $k_{pH} = f(a_{OH^-})$ were linear, with the positive slope that equaled k_{H^+} or k_{OH^-}, respectively.

The values of the partial reaction rates k_{H_2O} and k'_{H_2O} (Table 1), which describe spontaneous hydrolysis of ertapenem under the influence of water, were calculated from the equation:

$$k'_{pH} = k_{H_2O}f_{E^{+-}} + k'_{H_2O}f_{E^{--}} = k_{pH} - (k_{H^+}a_{H^+} + k_{OH^-}a_{OH^-}f_{E^{--}}) \quad (7)$$

The catalytic rate constants k_{H_2O} and k'_{H_2O} (Table 1) were calculated as the mean values of k'_{pH} at the relevant pH range (k_{H_2O} in the pH range 4.44–5.52 at 303 K, 5.00–6.03 at 313 K, 4.43–5.90 at 323 K, 3.65–5.90 at 333 K, where $f_{E^{--}} \rightarrow 0$, and k'_{H_2O} in the pH range 6.82–8.82 at 303 K, 7.58–9.69 at 313 K, 7.54–8.18 at 323 K and 7.38–5.79 at 333 K, where $f_{E^{+-}} \rightarrow 0$, respectively).

The correct choice of Eq. (4) was verified by comparing the calculated theoretical profile of $\log k = f(\text{pH})$ and the experimental results (Fig. 5).

3.4. Temperature dependence

Based on the Arrhenius relationship $\ln k = \ln A - E_a/RT$, linear plots of $\ln k$ versus $1/T$ were used to calculate the energy of activation (E_a) and pre-exponential coefficient (A) for the partial reactions (Table 1). The lowest energy of activation was observed

in the reaction $E^{2-} \xrightarrow{H_2O}$ products. The energy of activation of the other reactions did not demonstrate such significant differences and was between 50.18 and 64.83 kJ mol^{-1}. The entropy of activation of all reactions was negative, which may suggest the bimolecular character of reactions.

4. Summary

The specific acid–base catalysis involves: (a) hydrolysis of ertapenem, catalysed by hydrogen ions; (b) hydrolysis of ertapenem dianions, catalysed by hydroxide ions; (c) spontaneous hydrolysis of zwitter ions and dianions of ertapenem under the influence of the water. Ertapenem has the greatest stability at pH 5–6. The components of a bicarbonate buffer used in the injection of INVANZ do not demonstrate a catalytic effect, while the components of phosphate, acetate and borate buffers have such an effect.

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