PRACA ORYGINALNA

Stability of cefamandole nafate in aqueous solutions

Trwałość mrówczanu cefamandolu w roztworach wodnych

Beata Medenecka, Anna Jankowska

ABSTRACT

Cefamandole nafate is a semisynthetic second-generation cephalosporin antibiotic. Its antibacterial activity depends on presence of ß-lactamic bond, which is not stable on hydrolysis reactions. The products of these reactions may be caused different adverse effects.

The aim of research was to evaluate the stability of cefamandole nafate in TARCEFANDOL in aqueous solutions.

The degradation of cefamandole nafate was studied by using high-performance liquid chromatography with ultraviolet (UV) detection, as described in the monograph of cefamandole nafate in the European Pharmacopoeia. The method was modified and revalidated.

The stability of cefamandole nafate was investigated in aqueous solutions at 303, 313, 323, 333 K and pH 0.42–9.12. The degradation of cefamandole nafate under the conditions of general and specific acid-base catalysis was a first-order reaction depending on the substrate concentration. The catalytic effect was caused by the components of phosphate and acetate buffers. The values of k_{pH} and k_B, which describe the general acid-base catalysis (k_{pH}) and the catalytic effect of the components of the buffers (k_B), were obtained from the relationship $k_{obs} = f([B]_T)$. Whereas in the solutions of hydrochlochloric acid and borate buffer $k_{obs} = k_{pH}$. The semilogarythmic relationship $k_{pH} = f(pH)$ indicates that in water solutions at pH 0.42–9.12 the following reactions occur: the degradation of cefamandole nafate catalysed by hydrogen and hydroxide ions and spontaneous hydrolysis of cefamandole nafate under the influence of water. The catalytic rate constants of partial reactions and the thermodynamic parameters were calculated from suitable equations.

KEY WORDS

cefamandole nafate, HPLC, stability in aqueous solutions, pH-rate profile, kinetic and thermodynamic parameters

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STRESZCZENIE

Mrówczan cefamandolu należy do II generacji antybiotyków cefalosporynowych. Ich skuteczne działanie bakteriobójcze jest uwarunkowane obecnością wiązania ß-laktamowego, które jest podatne na czynniki hydrolityczne, a produkty reakcji hydrolizy mogą wywoływać działania niepożądane.

Celem badań była ocena trwałości mrówczanu cefamandolu, w preparacie TARCEFANDOL, w roztworach wodnych. W badaniach zastosowano metodę HPLC, polecaną przez Farmakopeę Europejską do oceny jakości mrówczanu cefamandolu. Metodę dostosowano do warunków badania trwałości oraz poddano rewalidacji. Badania trwałości mrówczanu cefamandolu w roztworach wodnych prowadzono w zakresie pH 0,42–9,12 w czterech temperaturach. Rozkład mrówczanu cefamandolu w warunkach ogólnej i właściwej katalizy kwasowo-zasadowej zachodził zgodnie z modelem reakcji pierwszego rzędu względem stężenia substratu. Efekt katalityczny wykazywały składniki buforu fosforanowego i octanowego. Z zależności $k_{obs} = f([B]_T)$ wyznaczono wartości k_{pH} i k_B, opisujące właściwą katalizę kwasowo-zasadową (k_{pH}) oraz efekt katalityczny składników buforu (k_B). W roztworach buforu boranowego i kwasu solnego występowała jedynie właściwa kataliza kwasowo zasadowa, czyli $k_{obs} = k_{pH}$. Na podstawie zależności $\log k_{pH} = f(pH)$ stwierdzono, że na rozkład mrówczanu cefamandolu w roztworach wodnych składają się reakcje: hydrolizy cząsteczek mrówczanu cefamandolu katalizowana jonami wodorowymi i wodorotlenowymi oraz hydrolizy spontanicznej pod wpływem wody. Z odpowiednich równań wyznaczono katalityczne stałe szybkości reakcji cząstkowych oraz parametry termodynamiczne reakcji.

SŁOWA KLUCZOWE

mrówczan cefamandolu, HPLC, trwałość w roztworach wodnych, parametry kinetyczne i termodynamiczne

INTRODUCTION

Cefamandole nafate (fig. 1) is a semisynthetic second-generation cephalosporin antibiotic. This antibiotic is active against a wide range of Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Streptococcus spp*., *Enterobacteriaceae spp*. and resistant to some β-lactamases produced by *Pseudomonas spp*. Cefamandole nafate is an antibiotic for parenteral administration in the treatment of urinary tract infections, skin and soft tissue infection and surgical prophylaxis. The side effects from cefamandole nafate are not common, but the chemical structure of this antibiotic, contains an N-methylthiotetrazole group, can cause hypoprothrombinemia and a reaction with etanol similar to that produced by disulfiram, due to inhibition of aldehyde dehydrogenase [1,2,3,4,5,6].

HPLC method and spectrophotometric methods (UV, IR, 1H-NMR and 13C-NMR) for the determination of cefamandole nafate have been reported in the literature [7,8]. The stability of cefamandole nafate in the solid state have been described. In studies of the solid state of cefamandole nafate the influence of temperature at 76.4% RH and 0% RH and relative air humidity on the stability of this substances have been established. The kinetic mechanism of cefamandole nafate degradation is independent of storage conditions. Degradation of cefamandole nafate occurs both when stored in a dry or humid atmosphere, and its course is that of the first-order rate reaction kinetics relative to substrate con-

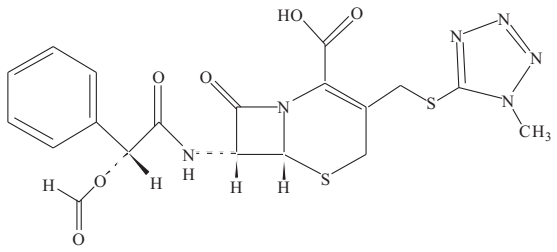

Fig. 1. Cefamandole nafate.

Ryc. 1. Mrówczan cefamandolu.

centration. The reaction of cefamandole nafate degradation in the solid state bears the characteristics of the consecutive reaction A→B→C. The degradation of cefamandole nafate is favoured in an increased relative humidity of the ambient air [9].
The aim of the present study was to analyse general and specific acid-base catalysis of cefamandole nafate at pH 0.43–9.12, at 303, 313, 323 and 333K. To determine the reaction rate HPLC method was used.

MATERIAL AND METHODS

REAGENTS

Cefamandole nafate for injection – TARCEFANDOL (TZF Polfa, Warszawa, Poland) is a sterile, synthetic, white and weakly crystalline powder. Salicylic acid (conforming to FP VIII) was used as an internal standard. All other chemical substances and solvents were the products of Sigma and Merck KgaA and were of analytical or high- performance liquid chromatographic grade.

EQUIPMENT

Chromatographic separation and quantitative analysis were performed by using a high-performance liquid chromatography equipped with an LC-6A pump, a Rheodyne 7120 injector with a 50 µl loop and a SPD-6AV UV-VIS detector set a 254 nm (all Shimadzu products). Separations was performed on a LiChrospher RP-18 column (250 -× 4 mm, 5 µm particle size; E. Merck).

CHROMATOGRAPHIC CONDITIONS

The method was a modification of the procedure in the European Pharmacopoeia VI for cefamandole nafate. In this study an internal standard (salicylic acid) was used to determine cefamandole nafate in TARCEFANDOL preparation.
The mobile phase consisted of 30 volumes of acetonitrile and 70 volumes of triethylamine 10% adjusted to pH 2.5 with phosphoric acid(V) (1.42 kg/l). The flow rate was 0.8 mL/min. The internal standard was a solution of salicylic acid (1.0 mg/ml) in the mobile phase. All chromatographic procedures were conducted at ambient temperature.

VALIDATION OF THE HPLC METHOD

The HPLC method was validated according to International Conference on Harmonisation Guidelines for Validation of Analytical Procedures [10].
The selectivity of the liquid chromatography method was examined for non-degraded and degraded samples. For the validation test the following substances were used: a comparative cefamandole nafate sample, a sample of cefamandole nafate incubated in phosphate buffer (pH 5.97) at 313K (fig. 2).

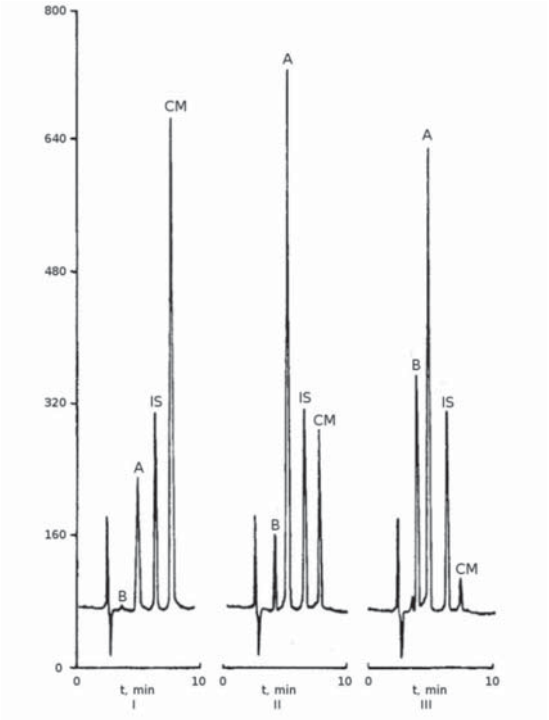

Fig. 2. HPLC chromatograms of cefamandole nafate (CM), its degradation products (A and B) and internal standard (IS) after incubation in phosphate buffer at 313 K: I at t = 0; II at t = 105 min; III at t = 120 min.

Ryc. 2. Chromatogramy HPLC mrówczanu cefamandolu (CM), produktów jego rozkładu i wzorca wewnętrznego (IS) w czasie t = 0 (I) oraz po 105 min (II) i 210 min (III) ogrzewania w temp. 313 K, w buforze fosforanowym o pH 5,97.

Calibration curves for HPLC analysis were determined by linear regression. The linearity between P/PIS (P and PIS – areas of cefamandole nafate and internal standard) and the concentrations of cefamandole nafate in a mixture of acetonitrile and water (3 : 7), ranging from 0.4 to 5.6 mg/ml, was evaluated. To 1.0 ml of cefamandole nafate solution 1.0 ml of internal standard solution was added and the so

obtained solutions were analyzed. 50 µl samples of these solutions were injected onto the column.
The precision of the method is expressed as the relative standard deviation (RSD) of replicate measurements. In order to evaluate the repeatability of the method (intra-day), eight samples of three different concentrations (low, c = 0.13 mg/ml; medium, c = 0.26 mg/ml; high, c = 0.48 mg/ml) were prepared and analyzed on the same day.
The LOD and LOQ parameters were determined from the regression equation, where: LOD = 3.3 Sy/a, LOQ = 10 Sy/a; Sy is a standard deviation and a is the slope of the calibration curve.

THE CONDITIONS OF KINETICS STUDIES

The degradation of cefamandole nafate in aqueous solutions was studied at 303, 313, 323 and 333K in hydrochloric acid (pH 0.43–2.04), phosphate buffer (pH 1.97–3.19 and 5.92–7.29), acetate buffer (pH 3.59–5.86) and borate buffer (pH 7.58–9.12). 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 hydrochloric acid were calculated from the equation: pH = $-\log f_{HCl}$ [HCl]. The activity coefficients f_{HCl} was obtained from the literature or calculated by interpolation of literature date [11]. The ionic strength μ of all the solutions was adjusted to 0.50 mol/l with a solution of sodium chloride (4 mol/l).
Degradation was initiated by dissolving an accurately weighed 10.0 mg of cefamandole nafate (TARCEFANDOL for injection) in 25 ml of reaction solution equilibrated to required temperatures in stoppered flasks. At selected times, samples (1.0 ml) of reaction solutions were collected and instantly cooled with a mixture of ice and water and neutralized with 1.0 ml of NaOH solutions of suitable concentrations and assayed. Next to each sample 1.0 ml of the internal standard solution (salicylic acid, 1.0 mg/ml) was added.

RESULTS AND DISCUSSION

Changes in the concentration of cefamandole nafate under the conditions of the study were evaluated using the HPLC method presented in the European Pharmacopoeia VI for cefamandole nafate and modified for this study. It was validated with respect to selectivity, precision and linearity. Detection and quantitation limits were also determined.
The HPLC method was found selective for the determination of cefamandole nafate in the presence of its degradation products and the internal standard, as shown in Figure 2. In the chromatograms taken over a period of 0 to 10 min, the following peaks emerged: peak CM, corresponding to cefamandole nafate, with retention time of approx. 8 min; peak IS, corresponding to the internal standard, with a retention time of approx. 6 min and peaks A and B, corresponding to the degradation products, with a retention time of approx. 3 and 4 min.
The linearity of the method was obtained between the areas of the peaks and the concentration of cefamandole nafate in the range of 0.4–5.6 mg/ml. The equation for the calibration curve is y = (0.325 ± 0.006) × x; r = 0.999; n = 13 (for the equation y = $ax + b$, the value b = 0.0003 is insignificant; $t_b = b/S_b$ = for f = n – 2). The precision of the method was adequate, because the RSD was less than 2.0%. Under the conditions of this study the detection limit was 0.46 mg/ml and the quantitation limit was suitable for kinetic studies.

OBSERVED RATE CONSTANTS

At a pH range from 0.43 to 9.12 the observed rate constants were determined by HPLC method. The observed first-order rate constants (k_{obs}) for the degradation of cefamandole nafate (fig. 3) were calculated from the following equation:

$$\ln (P/P_{IS})_t = \ln (P/P_{IS})_0 - k_{obs} \qquad (1)$$

P and P_{IS} are the areas of the peaks of cefamandole nafate and the internal standard, at time t = 0 and t, respectively.

BUFFER CATALYSIS

At constant pH, ionic strength (μ = 0.5 mol/l) and temperature, in the presence of excess buffer, the rate constant k_{obs}, for the degradation of cefamandole nafate increased as the total concentrations of acetate and phosphate buffers increased (fig. 4).

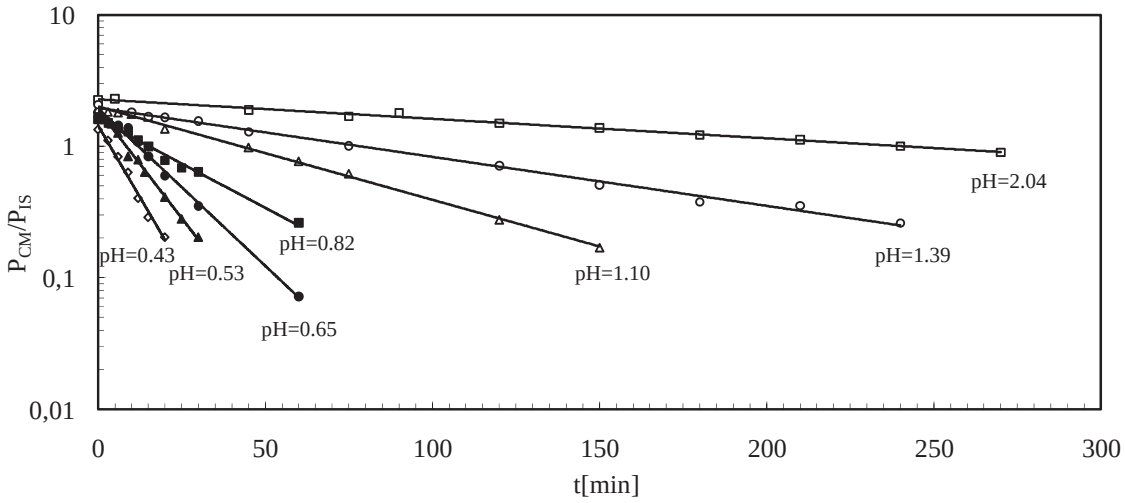

Fig. 3. Semilogarithmic plots P_{CM}/P_{IS} = f(t) for the degradation of cefamandole nafate in HCl (pH 0.43–2.04), μ = 0.5 mol/l at 313 K.
Ryc. 3. Półlogarytmiczny wykres zależności PCM/PIS = f(t) reakcji rozkładu mrówczanu cefamandolu w kwasie solnym (pH 0,43–2,04) w temp. 313 K.

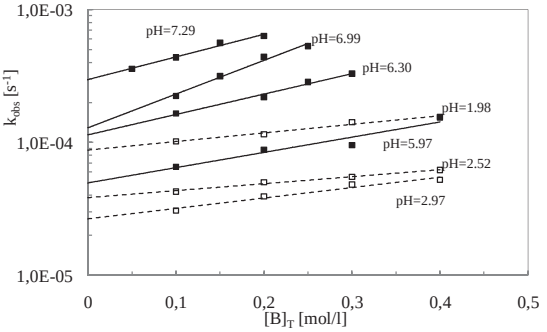

Fig. 4. Plots k_{obs} = f($[B]_T$) for the degradation of cefamandole nafate in: (a) phosphate (pH 1.98 – 2.97(□ and ...) and pH 5.97 – 7.29(■ and —) buffers and (b) acetate (pH 3.69 – 5.67) buffer at 313 K.

Ryc. 4. Katalityczny wpływ (a) buforu fosforanowego (pH 1,98––2,97(□ i ...) i pH 5,97 – 7,29 (■ and —) oraz (b) buforu octanowego (pH 3,69 – 5,67) na obserwowane stałe szybkości reakcji rozkładu mrówczanu cefamandolu w temp. 313 K.

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

$$k_{obs} = k_{pH} + k_B[B]_T \qquad (2)$$

$[B]_T$ is the total 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 and phosphate 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 borate buffers and in the reaction solutions in the borate buffer and in hydrochloric acid, the values $k_{obs} = k_{pH}$.

PH-RATE PROFILE

The rate constants k_{pH} determined in hydrochloric acid and borate buffer solutions and calculated in the case of general acid-base catalysis were used to calculate the relationship log k_{pH} = f(pH) (fig. 5).

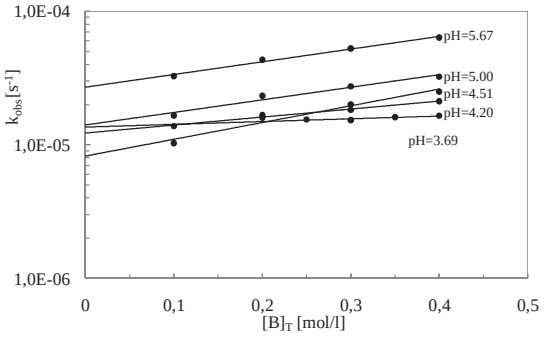

Fig. 5. pH-rate profiles for the degradation of cefamandole nafate at 303 (■), 313 (□), 323 (●), 333 K (○). The points are determined experimentally. The lines were calculated from the Eq. (3).

Ryc. 5. Półlogarytmiczna zależność kpH = f(pH) reakcji rozkładu mrówczanu cefamandolu w 303 (■), 313 (□), 323 (●), 333 K (○). Punkty przedstawiają wartości kpH wyznaczone doświadczalnie. Linia ciągła przedstawia profil obliczony na podst. równ. (3).

This semilogarithmic relationship indicates that in water solutions at pH 0.42–9.12 the following reactions are possible:

- degradation of cefamandole nafate catalysed by hydrogen ions:
  CME + H^+ → products, k_{H^+}

- spontaneous hydrolysis of cefamandole nafate under the influence of the water:
  CME → products, k_{H2O}
- degradation of cefamandole nafate catalysed by hydroxyl ions:
  CME + OH^- → products, k_{OH^-}

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

$$k_{pH} = k_{H^+} a_{H^+} + k_{H2O} + k_{OH^-} a_{OH^-} \qquad (3)$$

a_{H^+} and a_{OH^-} are hydrogen and hydroxide ion activity.

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

$$k_{pH} = k_{H^+} a_{H^+} \qquad (4)$$

using the values of k_{pH} below pH 2.05.

The catalytic rate constants k_{OH^-} (tab. II) at 303, 313, 323 and 333K were calculated from the equation:

$$k_{pH} = k_{OH^-} a_{OH^-} \qquad (5)$$

using the values of k_{pH} above pH 6.0.

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

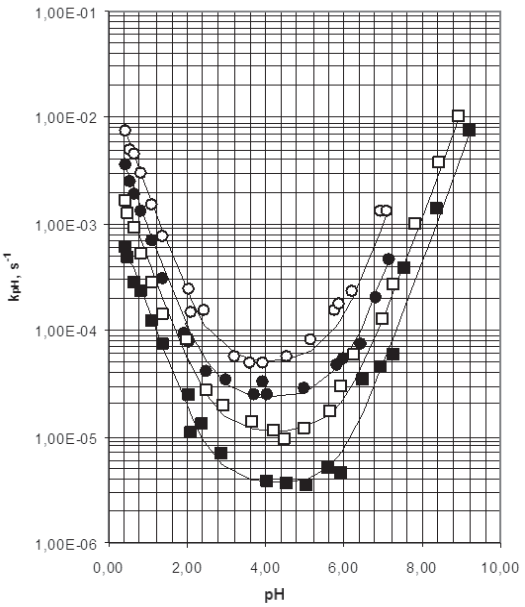

Fig. 6. Plots $k_{pH} = f(a_{H^+})$ for the degradation of cefamandolu nafate in HCl at 303, 313, 323 and 333 K.

Ryc. 6. Zależność kpH = f(aH+) reakcji rozkładu mrówczanu cefamandolu w roztworach wodnych, w temp. 303, 313, 323 i 333 K.

The catalytic rate constants k_{H2O} (Table 1) was calculated, as the mean value of k'_{pH} at the pH range 4.0–6.0, from the equation:

$$k'_{pH} = k_{pH} - (k_{H^+} a_{H^+} + k_{OH^-} a_{OH^-}) \qquad (6)$$

Table I. Catalytic rate constants and thermodynamic parameters for degradation of cefamandole nafate in aqueous solutions
Tabela I. Parametry kinetyczne i termodynamiczne reakcji rozkładu mrówczanu cefamandolu w roztworach wodnych

T, K	1/T	k_{H^+} [$mol^{-1} \cdot l \cdot s^{-1}$]	Statistical evaluation $k_{H^+} = f(1/T)$	Thermodynamic parameters
303	0,0033	$1,56 \cdot 10^{-3}$	$a \pm \Delta a =$	
313	0,0032	$4,34 \cdot 10^{-3}$	- 8379,9 ± 1490,1	E_a = 69,7 ± 12,4 [kJ · mol^{-1}]
323	0,0031	$9,28 \cdot 10^{-3}$	$b \pm \Delta b =$	$\Delta H^{\neq}$ = 67,2 ± 12,3 [kJ · mol^{-1}]^a
333	0,0030	$19,2 \cdot 10^{-3}$	21,3 ± 4,7	$\Delta S^{\neq}$ = - 68,1 ± 38,9 [J · K^{-1} · mol^{-1}]^a
T, K	1/T	k_{H_2O} [s^{-1}]	Statistical evaluation $k_{H_2O} = f(1/T)$	Thermodynamic parameters
303	0,0033	$0,25 \cdot 10^{-5}$	$a \pm \Delta a =$	
313	0,0032	$1,09 \cdot 10^{-5}$	- 9282,8 ± 6257,1	E_a = 77,2 ± 52,0 [kJ · mol^{-1}]
323	0,0031	$1,50 \cdot 10^{-5}$	$b \pm \Delta b =$	$\Delta H^{\neq}$ = 74,7± 52,0 [kJ · mol^{-1}]^a
333	0,0030	$4,80 \cdot 10^{-5}$	17,9 ± 19,7	$\Delta S^{\neq}$ = - 96,1 ± 163,9 [J · K^{-1} · mol^{-1}]^a
T, K	1/T	k_{OH^-} [$mol^{-1} \cdot l \cdot s^{-1}$]	Statistical evaluation $k_{OH^-} = f(1/T)$	Thermodynamic parameters
303	0,0033	297,0	$a \pm \Delta a =$	
313	0,0032	398,0	- 4072,7 ± 3021,8	E_a = 33,9 ± 25,1 [kJ · mol^{-1}]
323	0,0031	539,4	$b \pm \Delta b =$	$\Delta H^{\neq}$ = 31,4± 25,1 [kJ · mol^{-1}]^a
333	0,0030	1039,0	19,1 ± 9,5	$\Delta S^{\neq}$ = - 86,4 ± 79,2 [J · K^{-1} · mol^{-1}]^a

The correct choice of Eq. (3) was verified by comparing the calculated theoretical profile of log k = f(pH) and experimental results (fig. 6).

TEMPERATURE DEPENDENCE

The values of reaction rate constants k_{obs} were used to calculate the energy of activation (E_a) and pre-exponential coefficient (A) from the Arrhenius relationship $\ln k = \ln A - E_a/RT$ (tab. I). The lowest energy of activation was observed in the reaction of degradation of cefamandole nafate catalysed by hydroxyl ions. The energy of activation of the other reactions did not demonstrate such significant differences. The entropy of activation of all reactions was negative, which may suggest the bimolecular character of reactions.

CONCLUSIONS

The specific acid-base catalysis involves: the degradation of cefamandole nafate, catalysed by hydrogen and hydroxide ions and spontaneous hydrolysis of cefamandole nafate under the influence of water. Cefamandole nafate has the greatest stability at pH 3.5–5. The catalytic effect was caused by the components of phosphate and acetate buffers, while the components of borate buffer do not demonstrate such an effect.

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