

Kinetics of cefamandole nafate degradation in solid phase

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Abstract

The influence of temperature and relative humidity (RH) on the stability of cefamandole (CM) nafate sodium in the solid phase was investigated. Changes in the concentration of cefamandole nafate sodium were recorded using HPLC with UV detection. The method was validated for the following parameters: selectivity, linearity, precision, limit of detection and sensitivity. It showed good linearity ($r=0.9996$) in the range 0.4×10^{-4} – 5.6×10^{-4} g ml⁻¹ using a LiChrospher RP-18 column and as mobile phase acetonitrile–triethylamine (10% v/v, adjusted to pH 2.5 with phosphoric acid (84%) and diluted with water) (35:65). The degradation of CM occurring at 0% RH of the ambient air and at air humidity RH > 50% is a first-order reaction relative to substrate concentration. The first-order rate constants (k) were determined for CM degradation in dry air at 373, 383, 388 and 393 K, at air humidity RH = 76.4% at 323, 333, 343 and 353 K, and at 353 K at air humidity RH > 50%. The kinetic and thermodynamic parameters of the decomposition were calculated.

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1. Introduction

Cefamandole (CM) belongs to the second generation of cephalosporines. It is characterised by a considerable resistance against the action of plasmid-mediated β -lactamases, among these also against staphylococcus penicillinase. CM is used either in the form of a sodium salt or in the form of the biologically inactive CM nafate sodium salt, the latter being readily hydrolysed in the body to free CM [1].

Previous studies of the stability of CM dealt with the determination of CM in parenteral solutions stored in polyvinyl chloride infusion bags [2], the stability of CM in the amorphous state, in the crystalline state, and in aqueous solutions as a function of water content at 25DG C and 40DG C [3]. The stability of CM injection in various parenteral solutions and with secondary additives [4], and the chemical stabilities of CM and metronidazole when mixed together in aqueous solutions for intravenous injection [5] were also investigated.

The aim of the study was to evaluate the stability of CM in the solid phase and to estimate the effect of temperature and relative humidity (RH) on the degradation of CM.

2. Experimental

2.1. Chemicals and reagents

Sodium salt of CM nafate, the lyophilised substance for injection, made by Polfa, Tarchomin, Poland. Other chemical substances and reagents were products of the Sigma Chemical Co.

2.2. Chromatographic conditions

The method used in our experiments is a modification of the procedure for CM nafate detection given in the Ph.Eur 4.

The chromatographic separation and quantitative determination were performed on a high-performance liquid chromatograph equipped with a pump model L-6000, and an LC-2UV detector was used for detection.

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A Merck analytical column (LiChrospher RP-18; 5 μ m particle size, 250 \times 4 mm) was used as the stationary phase. The injector was the Rheodyne 7120 with a noose of 20 μ l. The mobile phase consisted of acetonitrile–triethylamine (10% v/v, adjusted to pH 2.5 with phosphoric acid (84%) and diluted with water) (35:65). The flow rate was 0.6 ml min⁻¹. UV detection was carried out at 254 nm.

The internal standard was a solution of salicylic acid in methanol at a concentration of 0.8 mg ml⁻¹.

2.3. Calibration curve

The calibration curve for CM was taken in a mixture of acetonitrile and water (1:1 v/v), for solutions of concentrations varying from 0.4×10^{-4} to 5.6×10^{-4} g ml⁻¹. The internal standard was a solution of salicylic acid in methanol at a concentration of 0.8 mg ml⁻¹. To 1.0 ml of the so obtained solution of CM 1.0 ml of a solution of internal standard was added and the so obtained solution was analysed by means of HPLC. Twenty microliter of the samples to be analysed were fed to the HPLC-column and the emerging signals recorded and analysed:

- the CM—emerged with a retention time of ca. 6.9 min;
- the internal standard—emerged with a retention time of ca. 5.5 min.

The chromatograms were interpreted using the following equation: $h_i/h_s = f(c)$; where h_i is the value of the CM signal and h_s represents the value of the internal standard signal.

2.4. Conditions of the kinetic studies

For the experimental, 10 mg samples of CM were weighed into 5 ml open vials. To assess the stability of CM in dry air, the vials containing the studied substances were immersed in a sand bath that was placed in a heat chamber adjusted to temperatures of 373, 383, 388 and 393 K. Samples destined for the study of the impact of humidity were placed into exicators in heat chambers set to the desired temperatures: 323, 333, 343 and 353 K. The exicators contained saturated solutions of inorganic salts, which safeguarded the desired RH of the ambient air sodium bromide (RH \sim 50.9%), potassium iodide (RH \sim 60.5%), sodium nitrate (RH \sim 66.5%) and sodium chloride (RH \sim 76.4%) [6]. Each series comprised 12–21 samples. After definite time intervals, determined by the rate of degradation, the respective vials were taken out of the chamber, cooled to room temperature and the contents dissolved in a mixture of acetonitrile and water (1:1 v/v). The so obtained solution was quantitatively transferred into

measuring flasks and refilled to a total volume of 25.0 ml with the above mentioned solvent. To 1.0 ml of the so obtained solution of CM 1.0 ml of internal standard was added. These samples were analysed according to the procedure described for calibration curves (Section 2.3).

3. Results and discussion

Concentration changes of CM under the experimental conditions were assessed using HPLC. The method applied was validated by the following parameters: selectivity, precision, linearity, detection limit and sensitivity. For the validation test, the following substances were used:

- a comparative CM sample;
- a sample of CM heated to 393 K in dry air;
- a sample of CM heated to 333 K in a humid atmosphere of 76.4% RH.

3.1. Selectivity

The method is selective for CM, its products of decomposition and the internal standard, as shown by the chromatograms (Fig. 1). In the chromatograms taken over a period of 0–10 min, the following peaks

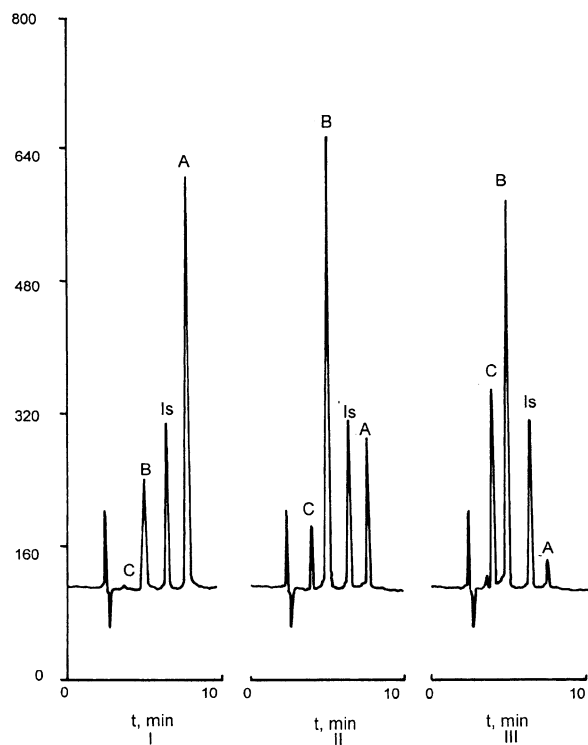


Fig. 1. HPLC chromatograms of the sodium salt of CM nafate (I) and of a mixture of a solid phase preparation of CM and of its degradation products following incubation at 333 K in a humid atmosphere (RH = 76.4%) for: 90 min (II) and 330 min (III). A—sodium salt of CM nafate, Is, internal standard, B and C—degradation products.

emerged: peak A corresponding to the investigated substance, with a retention time of ca. 6.9 min, peak I_s corresponding to the internal standard, with a retention time of ca. 5.5 min and peaks B and C corresponding to degradation products of CM, with a retention time of ca. 4.9 and 3.9 min, respectively (Fig. 1).

3.2. Linearity

In the conditions studied, a linear dependence was obtained between the height of the peaks and CM concentration over a range of 0.4×10^{-4} – 5.6×10^{-4} g ml $^{-1}$ ($y = (8075.4 \pm 134.9)x$, $r = 0.9996$, $n = 13$).

3.3. Precision

Method precision was obtained on the different days by making injections of eight standard preparations of CM (0.4 mg ml^{-1}). The retention times and the ratios of the CM peak size to that of the internal standard in the injections are: $x = 6.85 \pm 0.09$, %RSD 0.65 and $x = 3.22 \pm 0.20$, %RSD 1.51, respectively.

3.4. Detectability limit and sensitivity of the method

The concentration of CM at which the height of the peak of the analysed substance is ten-times higher than the noise level was taken as the limit of detectability. In our experimental conditions this parameter was 0.4×10^{-4} g ml $^{-1}$.

The slope of the curve plotted from the relationship $y = ax$ (see Section 3.2) indicates a significant sensitivity of the applied method.

During the experimental reaction products were formed exhibiting a yellow, light brown and dark brown coloration; all products of degradation were soluble in a mixture of acetonitrile and water (1:1 v/v). When kept in either hot dry or humid atmospheres, the compound underwent an appreciable degradation as demonstrated by the decline of the CM peak, starting from $h_i \rightarrow 0$ (peak height) over a period ranging from $t_0 \rightarrow t_\infty$. At the same time, the formation of product B was observed. The value of h_i/h_s of the product rises, to achieve the value of $(h_i/h_s)_\infty$. Next, degradation product B and the resultant formation of product C were observed. The concentration of product C rises to reach the asymptotic value of $c_\infty = (h_i/h_s)_\infty$.

The degradation of CM in the solid phase occurs in compliance with the model of the first-order rate reaction with regard to substance concentration and results from the consecutive reaction: $A \rightarrow B \rightarrow C$. The velocity constants of the first-order rate reaction of CM degradation relative to substrate concentration were calculated from the following equation:

$$\ln H_t = \ln H_0 - k_{\text{obs}} \cdot t \quad (1)$$

where: $H = h_i/h_s$, k_{obs} , the observed first-order rate reaction velocity relative to substrate concentration, t , time.

In accordance with equation 1, the semilogarithmic plots $\ln H_t = f(t)$ are straight lines (Figs. 2–4) and their slopes are a measure of the reaction velocity. For the interpretation of the straight curves $y = ax + b$ the following statistical parameters of the respective equations were computed by means of the minimal square method: $a \pm \Delta a$; $b \pm \Delta b$, standard errors S_a , S_b , S_y and the coefficient of linear correlation r . The values of $\pm \Delta a$ and $\pm \Delta b$ were computed for $f = n - 2$, with $\alpha = 0.05$.

The so determined velocity constants of the degradation of CM served for calculating the thermodynamic parameters of the reaction. The relationship $\ln k_i = f(1/T)$ complies with the Arrhenius equation $\ln k_i = \ln A - a/T$ (Table 1).

In hot dry air, $\ln k_i = \ln(1.47 \pm 8.96) - (-6648 \pm 5073)/298$, while under conditions of an increased RH (RH $\sim 76.4\%$) the respective $\ln k_i = \ln(13.61 \pm 1.94) - (-7509 \pm 987)/298$.

The effect of humidity on the stability of CM is described by the following equation:

$$\ln k_i = a(\text{RH}\%) + b \quad (2)$$

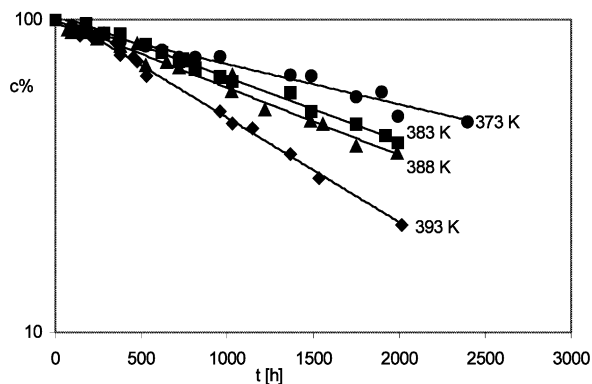


Fig. 2. Semilogarithmic plots $H_i = f(t)$ for the decomposition reaction of CM in the solid phase in dry air.

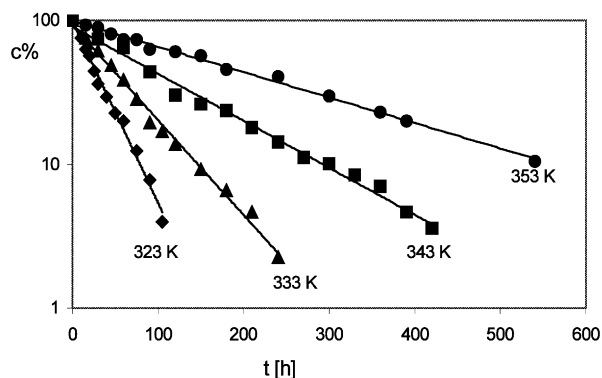


Fig. 3. Semilogarithmic plots $H_i = f(t)$ for the decomposition reaction of CM in the solid phase at RH = 76.4%.

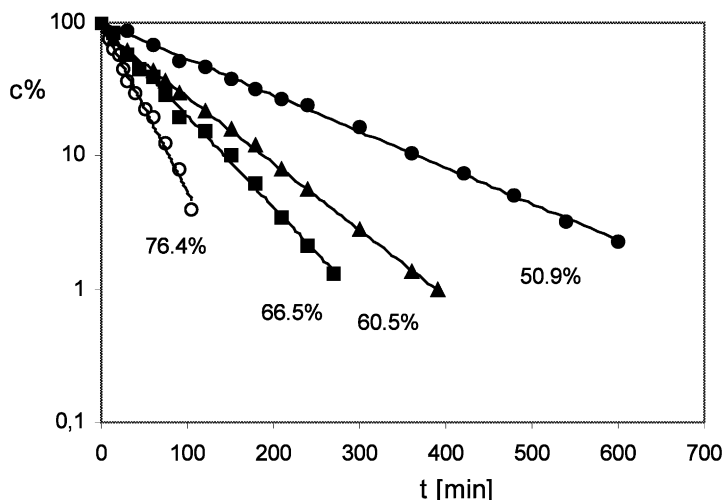


Fig. 4. Semilogarithmic plots $H_i = f(t)$ for the decomposition reaction of CM in the solid phase at various humidities at 353 K.

Table 1

Thermodynamic parameters of the reaction of CM decay in solid phase occurring in an anhydrous, dry atmosphere at various temperatures and at a constant RH (RH = 76.4%)

T (K)	$(k \pm \Delta k) \times 10^6$ (s^{-1})	n	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
RH = 0%				
373	0.0812 ± 0.0065	18	$S_a = 1179$	$E_a = 55 \pm 42$ kJ mol $^{-1}$
383	0.1297 ± 0.0042	21	$b = 1.5 \pm 13.0$	$\Delta H^{\ddagger b} = 53 \pm 45$ kJ mol $^{-1}$
388	0.1380 ± 0.0076	21	$S_b = 3.1$	$\Delta S^{\ddagger b} = -233 \pm 135$ J K $^{-1}$ mol $^{-1}$
393	0.2159 ± 0.0075	21	$r = -0.9699$	
298 ^a	8.98×10^{-10}		$S_y = 0.12$	
RH = 76.4%				
323	67.78 ± 2.19	15	$S_a = 229$	$E_a = 62 \pm 8$ kJ mol $^{-1}$
333	125.43 ± 5.02	16	$b = 13.6 \pm 2.9$	$\Delta H^{\ddagger b} = 60 \pm 11$ kJ mol $^{-1}$
343	252.85 ± 12.22	13	$S_b = 0.7$	$\Delta S^{\ddagger b} = -131 \pm 220$ J K $^{-1}$ mol $^{-1}$
353	482.80 ± 25.64	12	$r = -0.9991$	
298 ^a	9.28×10^{-6}		$S_y = 0.05$	

n , Number of experiments; $\Delta k = S_a t_{\text{rel}}$; E_a , activation energy; ΔH^{\ddagger} , enthalpy; ΔS^{\ddagger} , entropy; $E_a = -aR$ (J mol $^{-1}$); $\Delta H^{\ddagger} = E_a - RT$ (J mol $^{-1}$); $\Delta S^{\ddagger} = R(\ln A - \ln(k_B T)/h)$ (J K $^{-1}$ mol $^{-1}$), where: k_B stands for the Boltzmann constant (1.3807×10^{-23} J K $^{-1}$); h , Planck's constant (6.6256×10^{-24} J s); R , universal gas constant (8.314 J K $^{-1}$ mol $^{-1}$); T , temperature in K ($t + 273$ K); a , vectorial coefficient of the Arrhenius relationship and A , stands for the frequency coefficient.

^a The reaction velocity constant calculated from the Arrhenius equation: $\ln k_i = \ln A - (E_a/R) \times 1/T$.

^b Calculated for 298 K.

The semilogarithmic plot of $\ln k_i = f(\text{RH}\%)$ is a straight line (Fig. 5), and the slope of this curve characterises the effect of humidity on the stability of CM at 353 K, while the value of $b = k$ represents the stability of CM at 353 K and at RH = 0%.

The value of $k_0 = 5.01 \times 10^{-6}$ s $^{-1}$, calculated from equation 2 differs from $k'_0 = 2.90 \times 10^{-8}$ s $^{-1}$ for the temperature 353 K under dry air conditions, calculated from the Arrhenius equation. Such results indicate differences in the mechanism of degradation of CM occurring in the presence or absence of ambient humidity.

The relationships presented above show that CM is more stable in a dry atmosphere, and humidity constitutes the factor that determines the velocity of the degradative reaction.

4. Conclusions

Various derivatives of $\Delta 2$ -cefem: cefepime dichloride monohydrate [7], ceftazidime disodium salt [8], cefotax-

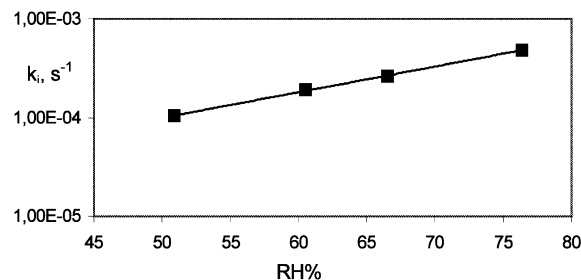


Fig. 5. A plot of the dependence $\ln k_i = f(\text{RH}\%)$ characterising the reaction of CM degradation at 353 K.

ime sodium salt [9], ceftazidime pentahydrate [10], ceftriaxone sodium salt [11], cefuroxime sodium salt [12], depending on their chemical structure and experimental conditions decompose according to the kinetics of either a first-order reaction, a reversible first-order reaction or to an autocatalytic first-order reaction with respect to substrate concentration.

The search for respective relationships between the chemical structure of Δ^2 -cefem derivatives and thermodynamic parameters is the subject of our future studies.

The kinetic mechanism of CM degradation is independent of storage conditions. Degradation of CM 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 concentration. The reaction of CM degradation in the solid state bears the characteristics of the consecutive reaction $A \rightarrow B \rightarrow C$. The degradation of CM is favoured in an increased RH of the ambient air.

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