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ANALYSIS

THE STABILITY OF *N*-[2-(4-*o*-FLUOROPHENYLPIPERAZIN-1-YL)ETHYL]-2,5-DIMETHYL-1-PHENYLPYRROLE-3,4-DICARBOXIMIDE IN AQUEOUS-ORGANIC SOLUTIONS

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Abstract: The first-order reaction of solvolysis of *N*-[2-(4-*o*-fluorophenylpiperazin-1-yl)ethyl]-2,5-dimethyl-1-phenylpyrrole-3,4-dicarboximide (PDI) was investigated as a function of pH at 333, 328, 323, 318 and 308 K in the pH range 1.11 - 12.78. The decomposition of PDI was followed by the HPLC method (Nucleosil 10-C8 column (250 × 4 mm I.D., dp = 10 µm), mobile phase: 0.018 mol/L ammonia acetate - acetonitrile (40 : 60 v/v), UV detector: 240 nm, flow rate: 1 mL/min. Specific acid-base catalysis involves solvolysis of the undissociated molecules of PDI catalyzed by hydroxide ions and spontaneous solvolysis of the undissociated and monoprotonated forms of PDI under the influence of solvents. The thermodynamic parameters of the reactions - activation energy (E_a), enthalpy ($DH^{\#}$), entropy ($DS^{\#}$) - were calculated.

Keywords: 3,4-pyrroledicarboximides, HPLC, stability

Some derivatives of *N*-[4-heteroaryl(aryl)piperazin-1-ylalkyl]imides represent a group of compounds which are analogues of buspirone (anxiolytic agent), tiospirone (antipsychotic agent) or antagonist of postsynaptic receptors 5-HT_{1A} NAN-190. In the literature the preparation and pharmacological properties of series of pyrrole-3,4-dicarboximide (Fig. 1) are described. Most of them show moderate acute toxicity (LD_{50}), suppress spontaneous and amphetamine-induced locomotor activity in mice (central nervous system depressive action) and additionally the analgesic activity (1, 2).

Based on structure-activity relationships (SAR) studies, it was estimated that one of the more promising compounds is *N*-[2-(4-*o*-fluorophenylpiperazin-1-yl)ethyl]-2,5-dimethyl-1-phenylpyrrole-3,4-dicarboximide (PDI) (Fig. 2). This compound has analgesic activity (ED_{50} = 9.35 mg/kg) approx. 4 times greater than that of acetylsalicylic acid (ED_{50} = 39.15 mg/kg) in the "writhing test" and low toxicity (LD_{50} > 2000 mg/kg) (2, 3).

The authors of the synthesis observed the methanolysis during the purification of PDI. It indicates that PDI is unstable under these conditions (3). The results of these investigations encouraged us to test the stability of PDI. The knowledge of the stability of compounds in the wide pH range is essential because it allows establishing their dosage forms or storage conditions and developing or modifying their chemical structure. Most of compounds are fairly stable in the neutral pH value found in the intestine but can be unstable at the pH value found in the stomach.

Our previous tests showed that the decomposition of this compound in the pH range 11.63 – 12.78, at 293, 298, 303 and 308 K, is a pseudo-first-order reaction catalyzed by hydroxide ions and described by the equation (4):

$$k_{pH} = k_{OH^-} \cdot a_{OH^-} \quad (1)$$

Because PDI is insoluble in water, all studies were conducted in the mixture of acetone-ethanol-water (4 : 46 : 50, v/v/v). At the beginning, it was established that the concentration of the solvents (acetone in the range of 4 – 30% and ethanol 36 – 61%) did not influence the constant rates of PDI degradation (4).

Apart from these observations, the literature does not refer to research into the kinetics of PDI.

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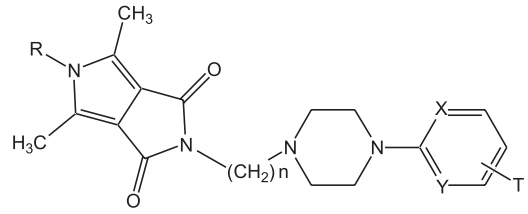

n = 1-4

IA R = Ar, n-C_4H_9, X, Y = CH or N, T = H

IB R = Ar, n-C_4H_9, X, Y = CH, T = o-Cl, o-CH_3O, m-Cl, m-CF_3

IC R = Hetar., X = N, Y = N or CH, T = H

Figure 1. Structure of derivatives of pyrrole-3,4-dicarboximide (2)

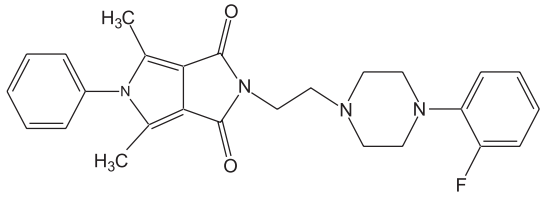

Figure 2. Structure of *N*-[2-(4-o-fluorophenylpiperazin-1-yl)ethyl]-2,5-dimethyl-1-phenylpyrrole-3,4-dicarboximide (PDI)

The aim of these studies was to evaluate the stability of PDI in aqueous-organic solutions, depending on pH of solutions and temperature.

To separate PDI and its degradation products our previously designed HPLC method was used (4).

EXPERIMENTAL

Materials

The compound, *N*-[2-(4-*o*-fluorophenylpiperazin-1-yl)ethyl]-2,5-dimethyl-1-phenyl-pyrrole-3,4-dicarboximide (PDI), was synthesized in the Department of Chemistry of Drugs, Wrocław Medical University, Poland. All other chemicals and solvents were of analytical or high-performance liquid chromatographic grade.

Analytical method

The reversed phase high performance liquid chromatography was applied for the determination of PDI, its degradation products and the internal standard. The analytical system consisted of an LC-6A pump (Shimadzu), UV-VIS detector SPD-6AV (Shimadzu) and a Rheodyne 7120 (50 µL fixed-loop) injector. In the HPLC method the following parameters were applied: column Nucleosil 10-C8 (250 × 4 mm I.D., dp = 10 µm), mobile phase: 0.018 mol/L ammonia acetate - acetonitrile (40 : 60, v/v), flow rate: 1 mL/min, UV detector: 240 nm and the internal standard – papaverine hydrochloride (0.07 mg/mL).

Validation of the method

The selectivity of the HPLC method was examined for non-degraded and degraded samples of PDI. The degraded samples of compound PDI were incubated in aqueous-organic solutions of sodium hydroxide (0.03 mol/L) at 298 K. The samples were collected at specified time intervals. It was found that the HPLC method is selective for the active compound in the presence of its degradation products and IS (Fig. 3) because on the chromatograms the following separate peaks were observed:

– PDI, with the retention time ca. 7.57 min,

– IS with the retention time ca. 6.03 min,

– P_I and P_{II}, corresponding to the degradation products I and II of compound PDI, with the retention times ca. 3.80 and 10.61 min, respectively.

The linearity between P/P_{IS} (P and P_{IS} – areas of PDI and IS) and concentrations of PDI ranging

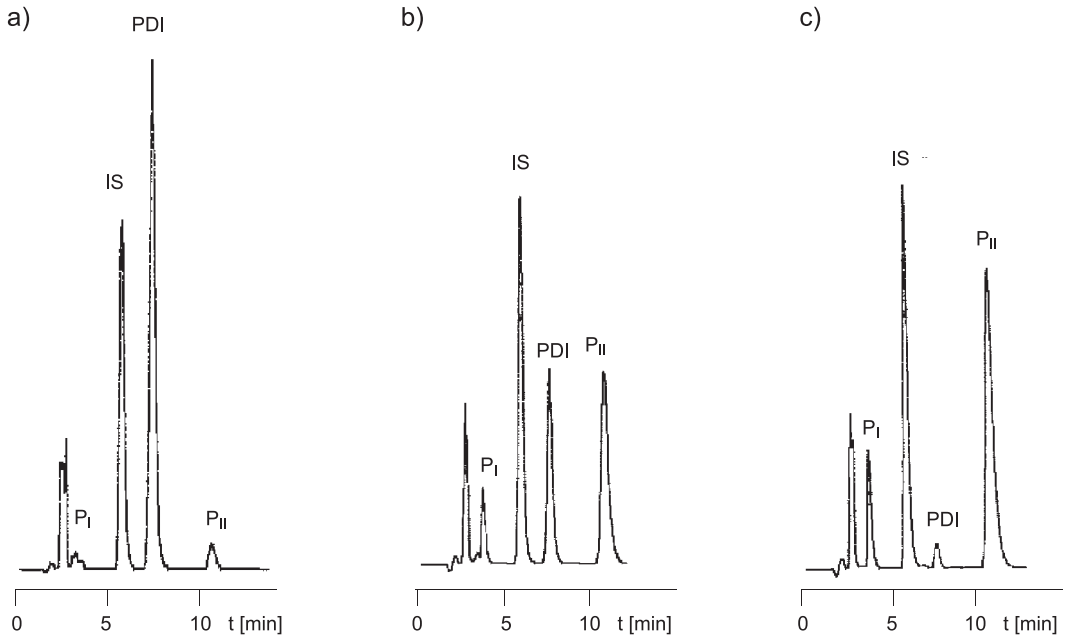

Figure 3. Chromatograms of PDI, products of its degradation (P_I , P_{II}) and internal standard (IS) after solvolysis in aqueous-organic solutions of sodium hydroxide (0.03 mol/L) at 298 K after: a) t = 1 min; b) t = 12.5 min; c) t = 38 min

from 20 mg/mL to 120 mg/mL was evaluated. The parameters of regression were as follows: $y = (23.33 \pm 0.61)x$; $r = 0.9994$; n = 10 (the value b calculated from the equation $y = ax + b$ ($b = -0.00881$) was statistically insignificant).

The precision of the method was determined by the analysis of 6 replicate injections of standard solution containing 20 mg/mL, 40 mg/mL, 80 mg/mL, 104 mg/mL of PDI. The precision of the measurements was adequate because the relative standard deviation (RSD) was – for repeatability 0.56% for 20 mg/mL; 1.11% for 40 mg/mL; 2.10% for 80 mg/mL; 0.68% for 104 mg/mL (n = 6) and for intermediate precision RSD = 1.91% (80 mg/mL, n = 12).

The limits of detection (LOD) and quantitation (LOQ) were calculated from the formulas LOD = $3.3S_y/a$ and LOQ = $10S_y/a$, where S_y is the standard deviation of the blank signal and a is the slope of the corresponding calibration curve. Under the conditions of this study the limit of detection was 3.61 µg/mL of PDI and the limit of quantitation was 10.93 µg/mL.

The influence of the method parameters changes (robustness) on the chromatographic separation of the tested compound was investigated. The following parameters were taken into account:

Table 1. Apparent first-order rate constants k_{obs} for the degradation of compound PDI in aqueous-organic Britton-Robinson's buffer at 333 K

pH	Britton-Robinson's buffer	
	B_T [mol/L]	$10^5\ k_{obs}$ [s^{-1}]
5.31	0.0622	(1.38 ± 0.06)
	0.0533	(1.49 ± 0.04)
	0.0444	(1.41 ± 0.08)
	0.0356	(1.42 ± 0.05)
7.82	0.0525	(2.24 ± 0.07)
	0.0450	(2.13 ± 0.13)
	0.0375	(2.29 ± 0.08)
	0.0300	(2.34 ± 0.06)

- the quantitative composition of the mobile phase
- concentration of ammonia acetate from 0.016 mol/L to 0.020 mol/L,
- concentration of acetonitrile 55 – 65%,

the flow rate 0.8 mL/min; 1.0 mL/min; 1.2 mL/min,

- the kind of the stationary phase (Hypersil RP-18, 250 × 4 mm; Lichrosorb RP-18, 250 × 4 mm; Nucleosil 10–C8, 250 × 4 mm).

The composition of the mobile phase and the flow rate influenced the retention time, whereas the kind of the stationary phase influenced the shape

Table 2. Catalytic rate constants and thermodynamic parameters for the degradation of compound PDI in aqueous-organic solutions

Catalytic rate constans k_i	Temperature [K]	$k_i \pm \Delta k$ [s^{-1}]	Statistical evaluation $k_i = f(1/T)$	Thermodynamic parameters
$k_{R\text{-}OH}$ [s^{-1}]	298	2.33×10^{-9} [a]	r = -0,9720 a = -11717 ± 8616 b = 19.44 ± 26.48	E_a = 97.42 ± 71.64 kJ/mol $\Delta H^{\#}$ = 94.94 ± 71.64 kJ/mol* $\Delta S^{\#}$ = -83.29 ± 220.16 J/K mol*
	318	2.98×10^{-8}		
	323	4.92×10^{-8}		
	328	6.68×10^{-8}		
	333	1.71×10^{-7}		
$k'_{R\text{-}OH}$ [s^{-1}]	298	1.44×10^{-6} [a]	r = -0.9933 a = -7641 ± 1638 b = 12.19 ± 5.09	E_a = 63.53 ± 13.62 kJ/mol $\Delta H^{\#}$ = 61.05 ± 13.62 kJ/mol* $\Delta S^{\#}$ = -143.56 ± 42.32 J/K mol^{-1}*
	308	$(3.06 \pm 0.51) \times 10^{-6}$		
	318	$(7.87 \pm 0.93) \times 10^{-6}$		
	323	$(1.14 \pm 0.07) \times 10^{-5}$		
	328	$(1.51 \pm 0.04) \times 10^{-5}$		
	333	$(1.96 \pm 0.11) \times 10^{-5}$		
k_{OH^-} [L/mol s] (4)	293	$(4.12 \pm 0.54) \times 10^{-2}$	r = -0.9955 a = -4166 ± 1208 b = 11.03 ± 4.02	E_a = 34.63 ± 10.04 kJ/mol $\Delta H^{\#}$ = 32.15 ± 10.04 kJ/mol* $\Delta S^{\#}$ = -153.21 ± 33.42 J/ K mol*
	298	$(5.31 \pm 1.47) \times 10^{-2}$		
	303	$(6.32 \pm 0.82) \times 10^{-2}$		
	308	$(8.40 \pm 2.93) \times 10^{-2}$		
	318	12.56×10^{-2} [a]		
	323	15.41×10^{-2} [a]		
	328	18.74×10^{-2} [a]		
	333	22.70×10^{-2} [a]		

$\Delta H^{\#} = E_a - RT$ [J/mol]; $\Delta S^{\#} = R$ [lnA – ln (k × T/h)] [J/K mol]; k – Boltzmann's constant ($1{,}3805 \times 10^{-23}$) [J/K], h –Planck constant ($6{,}6256 \times 10^{-34}$) [J s]; *Calculated values for 298 K; [a] Extrapolated values

and symmetry of the peaks. On the basis of the results of this study the best conditions were chosen.

Kinetic procedures

All studies were carried out in aqueous-organic solutions (acetone - ethanol - water; 4 : 46 : 50, v/v/v) of hydrochloric acid (pH 1.11 – 1.39) at 333 K, Britton-Robinson's buffer (mixture: acetic acid, boric acid, *o*-phosphoric acid and sodium hydroxide; pH 2.27 – 10.0) at 333, 328, 323, 318 and 308 K and sodium hydroxide solutions in the pH range 11.63 - 12.78 at 293, 298, 303, 308 K. The results of studies in sodium hydroxide solutions and the influence of acetone and ethanol on the stability of compound PDI were presented in the previous paper (4). The apparent pH values for hydrochloric acid and sodium hydroxide solutions were calculated from the equations, respectively:

$$pH = -\log f_{HCl}\,[HCl] \text{ and } pH = pK_w + \log f_{NaOH}\,[NaOH] \qquad (2, 3)$$

The activity coefficients f_{NaOH} or f_{HCl} were taken from the literature or obtained by the extrapolation of literature data (5). Other apparent pH values were measured with a potentiometric pH-meter (CD-401, Elmetron, Zabrze, Poland) at the reaction temperature.

The constant ionic strength (μ) 0.5 mol/L was adjusted for each solution by adding a calculated amount of sodium chloride solution (4.0 mol/L).

The degradation was initiated by adding a dissolved sample of PDI (2.5 mg in 0.5 mL of acetone) to an aqueous-organic solution of specific pH, equilibrated to the required temperature in a stoppered flask.

The initial concentration of PDI in the samples to be examined was ~0.2 mg/mL. At specified time intervals 0.5 mL of the reaction solution was collected and neutralized, if necessary, and instantly cooled with a mixture of water and ice. 0.25 mL of the IS solution was added to each sample and analyzed.

RESULTS AND DISCUSSION

First-order rate constants

The degradation of PDI, as a result of solvolysis, is a pseudo-first-order reaction described by the following equation:

$$\ln (P_t/P_{IS}) = \ln (P_0/P_{IS}) - k_{obs} \cdot t \qquad (4)$$

where: P_0, P_t - peak areas of PDI at time zero and time t, respectively; P_{IS} - peak area of IS; k_{obs} - the observed pseudo-first-order reaction rate constants of degradation of PDI.

General acid-base catalysis

The catalytic effect was determined by measuring the rate of degradation of PDI at a constant pH (7.82 or 5.31), ionic strength (μ = 0.5 mol/L) and

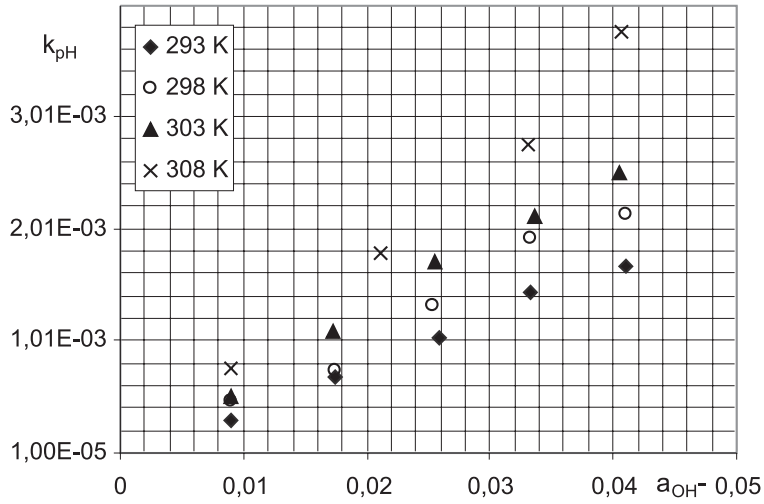

Figure 4. Plots $k_{pH} = f(a_{OH^-})$ for the degradation of compound PDI at the temperature 293 – 308 K

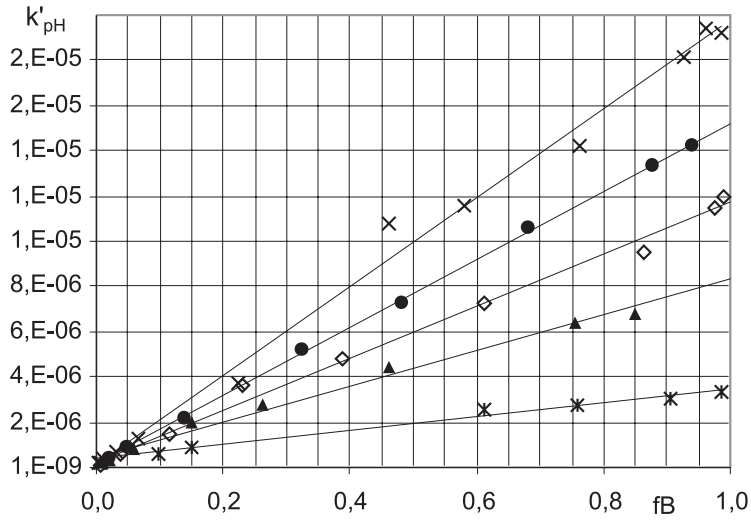

Figure 5. Plots $k'_{pH} = f(f_B)$ for the degradation of compound PDI at the temperature 308 – 333 K

temperature (333 K). Only the buffer concentration at a specific pH was different. The results obtained did not show any significant statistical differences (Table 1). Thus, the components of the Britton-Robinson's buffer at concentrations applied did not demonstrate any catalytic effect. Therefore, the rate constant (k_{obs}) for the degradation of PDI is independent from the concentrations of the Britton-Robinson's buffer and is equal to k_{pH}.

Therefore, it was established that under the conditions of the study only specific acid-base catalysis occurred.

Specific acid-base catalysis

The semilogarithmic plots of $k_{pH} = f(pH)$ indicate that specific acid-base catalysis of PDI involves the following reactions:

- solvolysis of the undissociated molecules of PDI catalyzed by hydroxyl ions,
- spontaneous solvolysis of the undissociated molecules and monoprotonated forms of PDI under the influence of solvents.

The total rate of solvolysis of PDI is equal to the sum of microscopic reaction rates:

$$k_{pH} = k_{R\text{-}OH}\, f_{BH^+} + k'_{R\text{-}OH}\, f_B + k_{OH^-}\, a_{OH^-}\, f_B \qquad (5)$$

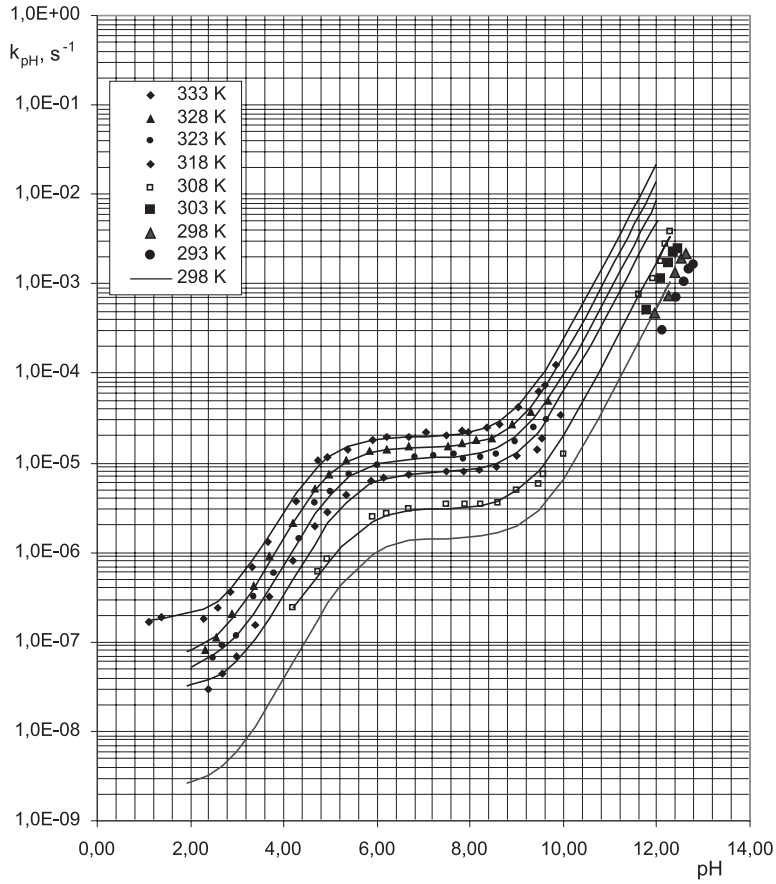

Figure 6. Rate-pH profiles for the degradation of compound PDI at the temperature 308 – 333 K. The points are experimental data and the lines are calculated from equation 5

where: R = H or C_2H_5-; a_{OH^-} – hydroxide ion activity; f_{BH^+}, f_B – fractions of monoprotonated or undissociated forms of PDI, respectively.

The pKa values were appointed from the semilogarithmic plots of $k_{pH} = f(pH)$ (pKa = 5.50 at 308 K, 5.40 at 318 K, 5.20 at 323 K, 5.00 at 328 K, 4.80 at 333 K). The pKa value at 298 K was calculated by extrapolation of the above data and it amounts to 5.87.

The catalytic rate constants k_{OH^-} (Table 2) at 293, 298, 303 and 308 K were calculated from the equation $k_{pH} = k_{OH^-}\ a_{OH^-}$ using the values of k_{pH} above pH 11.5. In this pH range the value of $f_B \rightarrow 1$. The plots $k_{pH} = f(a_{OH^-})$ were linear and their slopes equal to k_{OH^-} (Fig. 4). The catalytic rate constants k_{OH^-} at 318, 323, 328 and 333 K were obtained from the Arrhenius equation, by extrapolation.

The values $k_{R\text{-}OH}$ and $k'_{R\text{-}OH}$ were calculated from the values k'_{pH} in the pH ranges: 2.27 – 6.69 at 333 K; 3.35 – 6.21 at 328 K; 2.98 – 7.22 at 323 K; 3.40 – 6.15 at 318 K; 4.74 – 7.50 at 308 K, where:

$$k'_{pH} = k_{pH} - (k_{OH^-}\ a_{OH^-}\ f_B) = k_{R\text{-}OH} f_{BH^+} + k'_{R\text{-}OH} f_B \quad (6)$$

The plots $k'_{pH} = f(f_B)$ are linear and $k'_{R\text{-}OH}$ are equal to the values k'_{pH} for $f_B = 1$ (Fig. 5).

The values $k_{R\text{-}OH}$ were calculated from the values k''_{pH} in the pH ranges: 2.39 – 3.00 at 318 K; 2.50 – 2.67 at 323 K; 2.33 – 2.88 at 328 K; 1.11 – 1.39 at 333 K, where:

$$k''_{pH} = k'_{pH} - k'_{R\text{-}OH} f_B = k_{R\text{-}OH} f_{BH^+} \quad (7)$$

Therefore:

$$k_{R\text{-}OH} = k''_{pH} / f_{BH^+}. \quad (8)$$

The correctness of equation 5 was verified by the correspondence between the calculated theoretical profile of log $k_{pH} = f(pH)$ and the experimental results (Fig. 6).

Thermodynamic parameters

The values of catalytic rate constants k_{OH^-}, $k_{R\text{-}OH}$ and $k'_{R\text{-}OH}$ were used to calculate the slopes (a) of the plots $\ln k_i = f(1/T)$ and the values of lnA (A –

coefficient of frequency). From these values the thermodynamic parameters were calculated: activation energy (E_a), enthalpy (DH^a) and entropy (DS^a) (Table 2).

CONCLUSION

The knowledge of the chemical and physical stability of pharmacologically active compounds is essential in the development and evaluation of pharmaceuticals. On the basis of the research the following conclusions can be drawn:

During the degradation of *N*-[2-(4-*o*-fluorophenylpiperazin-1-yl)ethyl]-2,5-dimethyl-1-phenylpyrrole-3,4-dicarboximide (PDI) in aqueous-organic solutions in the pH range 1.11 – 12.78 at 293 – 333 K only specific acid-base catalysis is observed. It involves solvolysis of undissociated molecules catalyzed by hydroxyl ions and spontaneous solvolysis of undissociated and monoprotonated forms under the influence of solvents.

The lowest activation energy concerns solvolysis of undissociated PDI molecules catalyzed by hydroxide ions.

The negative value of entropy for all reactions investigated may suggest the bimolecular character of reactions.

The highest stability of PDI was observed in the pH range of 1.11 – 2.50.

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