

THERMAL STABILITY OF 1,4-DIHYDROPYRIDINE DERIVATIVES IN SOLID STATE

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Abstract: The effect of temperature and air humidity on the stability of 7 derivatives of 1,4-dihydropyridine (nifedipine, nisoldipine, nitrendipine, nimodipine, nicardipine, felodipine and amlodipine) in solid state has been studied by accelerated testing. Quantitative analysis of the compounds studied was made by UV spectrophotometry, identification of the thermodegradation products and reference to the standard were made by thin layer chromatography (TLC), UV spectra and the reaction with KMnO_4 . Thermodegradation of the derivatives studied was found not to occur in dry air, whereas at air humidity it occurred according to the first order reaction at a similar rate for all derivatives. The main product of thermodegradation of the derivatives with the nitro substituent was a nitrozoderivative formed as a result of dihydropyridine ring aromatisation accompanied by water molecule elimination.

Keywords: thermostability, nifedipine, nitrendipine, nisoldipine, nimodipine, nicardipine, felodipine, amlodipine, UV, TLC

The derivatives of 1,4 dihydropyridine (1,4-DHP) introduced in the 1970s have been used for the treatment of hypertension (1-3), coronary disease (4-5) and supraventricular rhythm disorder (5). The chemical structure of the derivatives based on the dihydropyridine unit with a nitro or chlorophenyl substituent at position 4 determines their high sensitivity to light and UV irradiation (6-10). The problems related to this high photosensitivity have been the subject of interest of many authors, but not much attention has been devoted to their stability on exposition to elevated temperatures and relative humidity. The aim of this study was to establish the effect of these two factors. From a large group of 1,4-DHP derivatives the study was performed for the 7 most often used ones. Five of them contained nitrophenyl substituent: nifedipine (NF), nisoldipine (NS), nitrendipine (NT), nimodipine (NM) and nicardipine (NC), while the other two contained a chlorophenyl substituent: amlodipine (AL) with one chlorine atom and felodipine (FD) with two (Table 1).

The measurements were performed by the test of accelerated ageing at temperatures within the range 70–90°C, in the dry atmosphere and in the atmospheres of elevated relative humidity of 50.9 – 90.9%. The degree of degradation of the compounds studied was measured by the UV spectrophotometry, the detection and identification of the thermodegradation products was made by thin layer chromatography (TLC).

The results were expected to show whether these highly photosensitive derivatives would be resistant to elevated temperature and humidity like perazine derivatives (11) or more sensitive to these factors.

EXPERIMENTAL

Materials and methods

Nifedipine – 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester; SIGMA series no.: LOT 57H1139; active substance content: 99.42%.

Nitrendipine – 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid ethyl methyl ester Pharmaceutical Institute in Warsaw (Poland), series no. 060796; active substance content: 99.60%.

Nisoldipine – 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid butyl methyl ester Pharmaceutical Institute in Warsaw (Poland), series no.: 230191; active substance content: 98.70%.

Nimodipine – 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid isopropyl-2-methoxyethyl ester; Pharmaceutical Institute in Warsaw (Poland), series no.: 0112/95; active substance content: 98.99%.

Nicardipine – 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl-2-[methyl(phenylmethyl)amino] ester; SIGMA,

series no.: LOT 28H0977; active substance content: 98.88%.

Felodipine – 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylic acid ethyl methyl ester; Pharmaceutical Institute in Warsaw (Poland), series no.: 960430; active substance content: 98.99%.

Amlodipine – 1,4-dihydro-6-dimethyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3,5-pyridinedicarboxylic acid 3-ethyl-5-methyl ester; Pharmaceutical Institute in Warsaw (Poland), series no.: AB-8050019; active substance content: 98.95%.

Reference Substances:

Nitro derivative of nifedipine, The United States Pharmacopeia Reference Substance, 2,6-

dimethyl-4(2'-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester.

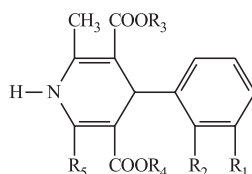
Nitroso derivative of nifedipine, The United States Pharmacopeia Reference Substance, 2,6-dimethyl-4(2'-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester.

Kinetic study at elevated humidity

Portions of 0.01 g of each compound studied, carefully weighted on an analytical balance to an accuracy of about 0.0001 g, were placed in glass vials of 4 cm³ in capacity. The vials were put into glass desiccators with air of different relative humidity. The desiccators were placed in a universal thermostat (KBC 65 W i KBC 25 G) at different temperatures.

● At 80°C three different relative humidity values obtained by conducting the reaction in the

Table 1. Compilation of the examined 1,4-DHP derivatives.



Compound	Symbol	R ₁	R ₂	R ₃	R ₄	R ₅
Nifedipine	NF	-H	-NO ₂	-CH ₃	-CH ₃	-CH ₃
Nitrendipine	NT	-NO ₂	-H	-CH ₃	-CH ₂ -CH ₃	-CH ₃
Nisoldipine	NS	-H	-NO ₂	-CH ₃	-CH ₂ -CH-(CH ₃) ₂	-CH ₃
Nimodipine	NM	-NO ₂	-H	-CH(CH ₃) ₂	-(CH ₂) ₂ -O-CH ₃	-CH ₃
Nicardipine	NC	-NO ₂	-H	-CH ₃	-(CH ₂) ₂ -N(CH ₃)-CH ₂ -C ₆ H ₅	-CH ₃
Felodipine	FD	-Cl	-Cl	-CH ₃	-CH ₂ -CH ₃	-CH ₃
Amlodipine	AL	-H	-Cl	-CH ₃	-CH ₂ -CH ₃	CH ₂ -O-(CH ₂) ₂ NH ₂

Table 2. Analytical parameters for UV method.

Analytical parameters	Compound						
	NF	NT	NS	NM	NC	FD	AL
Linearity range [mol/L] × 10 ⁵	0.41 – 1.39	0.34 – 1.10	0.35 – 1.19	0.34 – 0.97	0.29 – 0.79	0.39 – 0.99	0.34 – 1.07
Correlation coefficient r	0.9996	0.9995	0.9999	0.9915	0.9955	0.9978	0.9994
Equation of calibration curve							
Slope a	137.3751	148.8838	126.8948	124.5511	128.6571	179.0491	115.0809
Intercept b	1.31 × 10 ⁻²	0.35 × 10 ⁻²	0.59 × 10 ⁻²	3.33 × 10 ⁻²	3.34 × 10 ⁻²	0.86 × 10 ⁻²	0.98 × 10 ⁻²
LOD (mol/L) × 10 ⁻⁴	1.85	2.49	1.21	14.00	8.18	4.46	5.07
LOQ (mol/L) × 10 ⁻⁴	5.61	7.56	3.67	42.47	25.38	13.5	15.3
RDS	0.36	1.48	0.90	0.07	1.32	1.23	1.10

LOD = 3.3 × (SD/slope)

LOQ = 10 × (SD/slope)

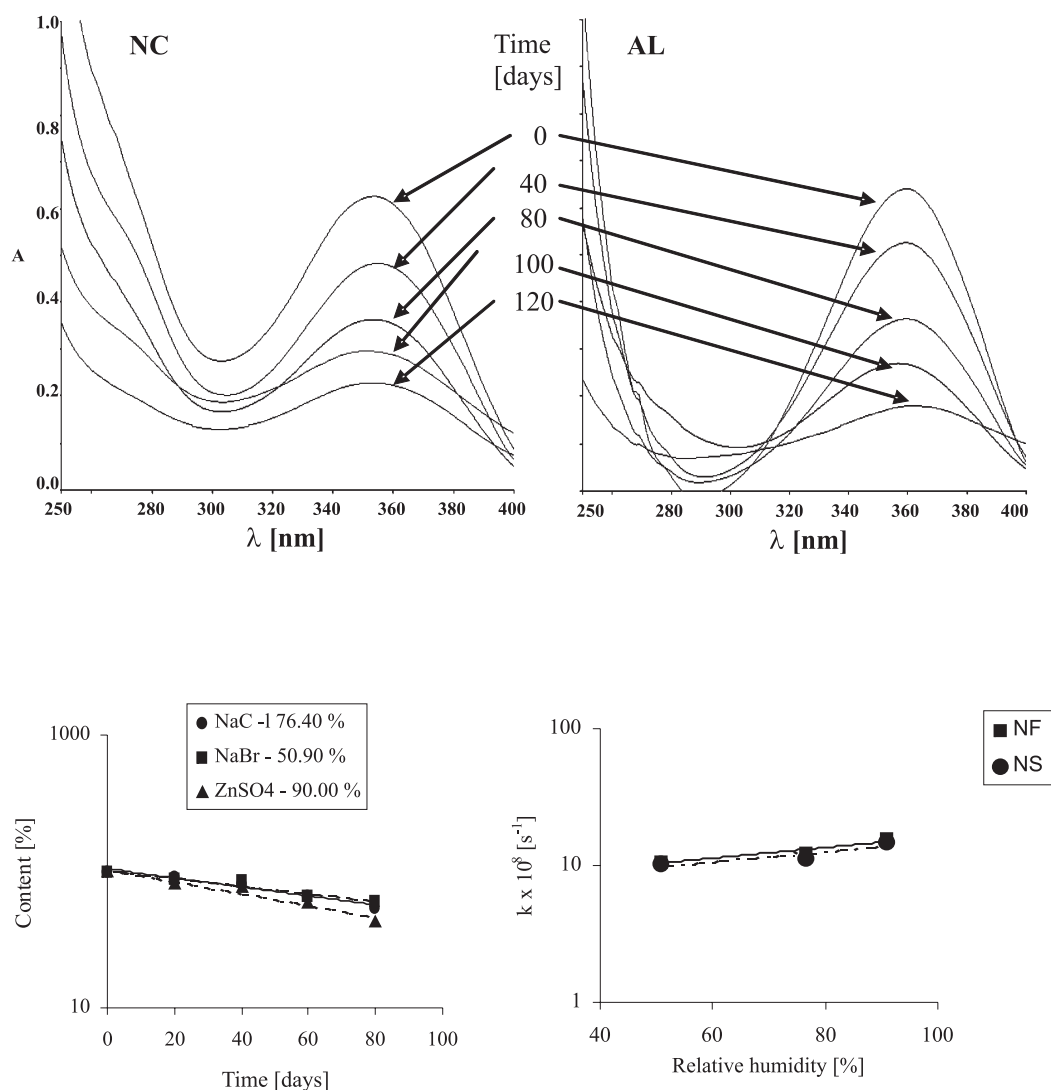


Figure 1. Changes in the UV spectra on thermal decomposition of the 1,4-DHP studied at 80°C and at different relative humidities and the semi-logarithmic dependence of $t_{0.1}$ time on the relative humidity, for NS and NF.

atmosphere of saturated solutions of appropriate salts: NaBr (relative humidity 50.90%); NaCl (76.40%) and ZnSO₄ (90.90%).

● At 70, 80 and 90°C at the relative humidity of 76.40%.

At certain intervals the samples were checked for the presence of the initial compound by UV spectrophotometry.

UV spectrophotometry

The samples thermostated for a certain period of time at a given temperature and containing initially 0.01 g of the compound studied were quantita-

tively moved to measuring vessels of 5.0 cm³ in capacity, dissolved in methanol and the content of the substance was determined by the earlier validated method (Table 2) (12). From the initial solutions portions of 0.3 – 1.5 cm³ were taken out and transferred to measuring flasks of 10 – 50 cm³ in capacity and the flasks were filled with methanol. The absorbance of the samples was measured on a UV/VIS spectrometer PERKIN ELMER Lambda 20 at the analytical wavelengths characteristic for a given derivative and the reference sample was the reagent used for sample preparation.

Thin layer chromatography (TLC)

Chromatographic analysis of the compounds studied was performed on plates covered with silica gel Kieselgel 60F₂₅₄ (Merck) in a layer of 0.25 mm in thickness and the size of 4 × 15 cm. The methanol solutions of the 1,4-DHP derivatives studied in the amount of 50 and 250 mL which corresponded to 0.05×10^{-3} and 0.25×10^{-3} g of the substance analyzed, were placed on the plates using Hamilton syringe. The plates were developed over 3.0 cm in 0.5 to 3 h with a mixture of benzene – methanol at the ratio 6:1 (v/v) (7). After drying at room temperature the spots were observed in the light of an analytical quartz lamp of $\lambda = 254$ nm.

RESULTS AND DISCUSSION

Kinetic study of thermodegradation of the selected 1,4-DHP derivatives in solid state were performed using the direct UV spectrophotometry for determination of the content of non decomposed initial substance. All the derivatives studied were found not to decompose in dry air atmosphere (the content of the therapeutic substance did not change for about 120 days at 90°C) but they underwent thermodegradation at elevated temperature in the humid atmosphere. The thermodegradation occurred

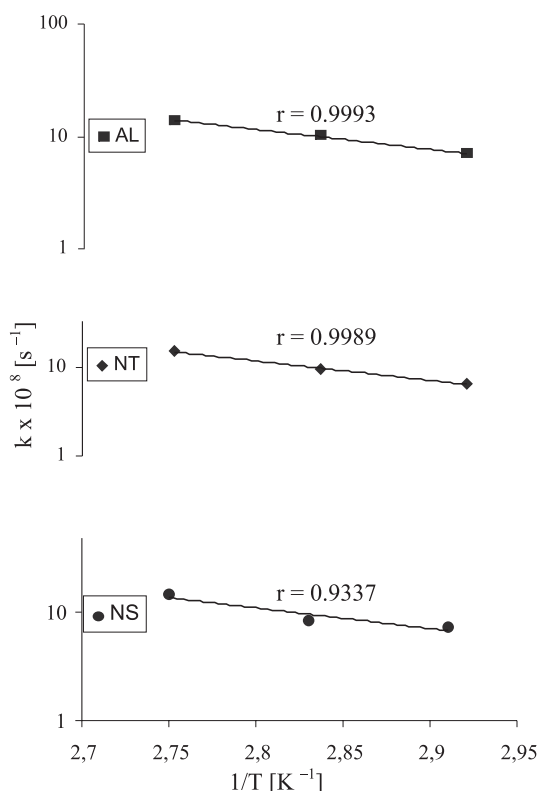


Figure 2. The Arrhenius dependence of the thermal decomposition of NS, NT and AL at relative humidity of 76%.

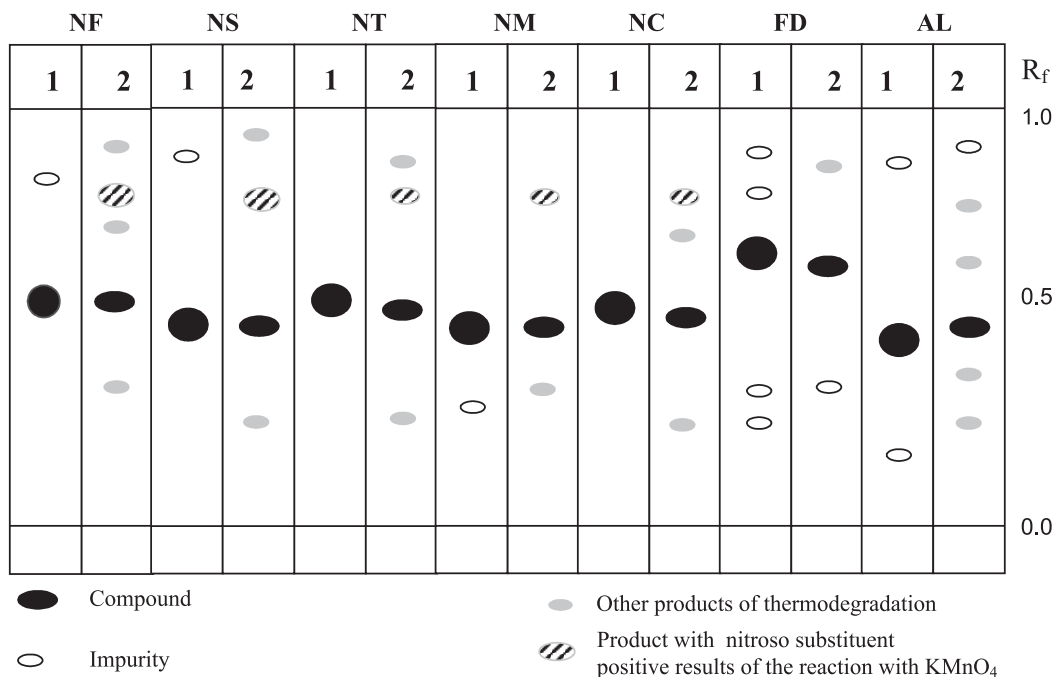
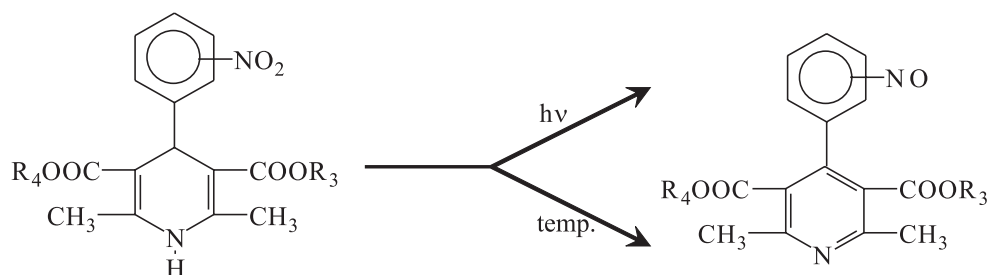


Figure 3. Chromatographic patterns of 1,4-DHP derivatives prior to storage (1) and after 120 days of storage at 80°C and relative humidity of 90.0% (2); stationary phase: Kieselgel 60 F₂₅₄ mobile phase: benzene-methanol (6:1, v/v) detector: UV lamp $\lambda = 254$ nm.



Scheme 1. A scheme of formation of the nitroso-derivative in the processes of photo and thermodegradation in the solid state of 1,4-DHP derivatives with nitrophenyl substituent.

Table 3. Kinetic parameters of thermal decomposition of 1,4-DHP derivatives on storage at 80°C at different relative humidity values (H_R), $n = 5$.

Compound	Kinetic parameters	Temperature 80°C		
		H_R 50.9%	H_R 76.4%	H_R 90.9%
NS	$k \times 10^{-8}$ [s ⁻¹]	10.71	12.29	15.58
	$t_{0.1}$ [days]	11.39	9.92	7.83
	$t_{0.5}$ [days]	74.83	65.74	51.48
	r	0.9797	0.9953	0.9584
NF	$k \times 10^{-8}$ [s ⁻¹]	10.24	11.34	14.66
	$t_{0.1}$ [days]	11.90	10.75	8.43
	$t_{0.5}$ [days]	78.33	70.98	54.71
	r	0.9668	0.9846	0.9884
AL	$k \times 10^{-8}$ [s ⁻¹]	9.63	10.50	12.01
	$t_{0.1}$ [days]	12.67	11.62	10.15
	$t_{0.5}$ [days]	83.29	76.39	66.78
	r	0.9769	0.9966	0.9868
NC	$k \times 10^{-8}$ [s ⁻¹]	9.55	10.21	11.07
	$t_{0.1}$ [days]	12.77	11.94	11.01
	$t_{0.5}$ [days]	83.99	78.63	72.45
	r	0.9742	0.9901	0.9732
NT	$k \times 10^{-8}$ [s ⁻¹]	9.16	9.39	9.82
	$t_{0.1}$ [days]	13.25	12.98	12.42
	$t_{0.5}$ [days]	87.56	85.35	81.67
	r	0.9780	0.9943	0.9892
NM	$k \times 10^{-8}$ [s ⁻¹]	9.20	9.29	9.49
	$t_{0.1}$ [days]	13.32	13.12	12.85
	$t_{0.5}$ [days]	86.99	86.29	84.52
	r	0.9795	0.9906	0.9778
FD	$k \times 10^{-8}$ [s ⁻¹]	8.47	8.26	8.87
	$t_{0.1}$ [days]	14.40	14.76	13.75
	$t_{0.5}$ [days]	94.69	94.62	90.43
	r	0.9962	0.9989	0.9899

according to the first order reaction at the reaction rates similar for all compounds, while the reaction rate constants vary from $8.26 \times 10^{-8} \text{ s}^{-1}$ for FD (temp. 80°C, H_R 76.4%) to $15.58 \times 10^{-8} \text{ s}^{-1}$ for NS (temp.

80°C, H_R 90.9%) (Table 3, Figure 1). The measurements performed at different humidities at a constant temperature proved that the reaction rate constant was only slightly dependent on the relative

Table 4. Kinetic parameters of thermal decomposition of 1,4-DHP derivatives on storage at different temperatures at the relative humidity of 76%, n = 5.

Compound	Kinetic parameters	Relative humidity 76.4%			
		Temp. 70°C	Temp. 80°C	Temp. 90°C	Temp. 20°C
NS	$k \times 10^{-8}$ [s ⁻¹]	7.41	12.22	14.82	8.9×10^{-9}
	$t_{0.1}$ [days]	16.48	9.99	8.24	137.07
	$t_{0.5}$ [days]	108.39	65.74	54.19	901.22
	r	0.9908	0.9797	0.9751	0.9688
NF	$k \times 10^{-8}$ [s ⁻¹]	7.21	11.32	14.41	8.5×10^{-9}
	$t_{0.1}$ [days]	16.89	10.79	8.47	143.52
	$t_{0.5}$ [days]	102.72	70.98	55.70	943.63
	r	0.9675	0.9668	0.9832	0.9852
AL	$k \times 10^{-8}$ [s ⁻¹]	6.92	10.53	13.87	7.9×10^{-9}
	$t_{0.1}$ [days]	17.48	11.62	8.79	154.42
	$t_{0.5}$ [days]	114.91	76.39	57.79	1015.29
	r	0.9911	0.9769	0.9778	0.9934
NC	$k \times 10^{-8}$ [s ⁻¹]	6.91	10.23	13.26	7.8×10^{-9}
	$t_{0.1}$ [days]	17.65	11.94	9.33	156.39
	$t_{0.5}$ [days]	116.05	78.63	60.45	1028.31
	r	0.9942	0.9901	0.9434	0.9978
NT	$k \times 10^{-8}$ [s ⁻¹]	6.39	9.39	13.11	6.7×10^{-9}
	$t_{0.1}$ [days]	19.09	12.98	9.32	182.07
	$t_{0.5}$ [days]	125.50	85.35	61.23	1197.14
	r	0.9933	0.9966	0.9765	0.9993
NM	$k \times 10^{-8}$ [s ⁻¹]	6.27	9.29	12.90	6.4×10^{-9}
	$t_{0.1}$ [days]	19.44	13.12	9.46	190.61
	$t_{0.5}$ [days]	127.81	86.29	62.18	1253.25
	r	0.9926	0.9795	0.9775	0.9985
FD	$k \times 10^{-8}$ [s ⁻¹]	5.61	8.47	12.46	4.6×10^{-9}
	$t_{0.1}$ [days]	21.72	14.39	9.79	256.19
	$t_{0.5}$ [days]	142.81	94.62	64.37	1743.66
	r	0.9949	0.9962	0.9816	0.9999

humidity value. For instance, for the compounds NT, NM and FD, whose decompositions were the slowest, the reaction rate constants increased only by 5-7% with increasing humidity. Only for NS and NF the rate constant values changed significantly with increasing relative humidity and these changes could be described by a linear relation: $\lg t_{0.1} = f(H_R)$, characterized by the correlation coefficients from 0.9185 for NF to 0.9478 for NS (Figure 1).

As follows from Figure 1 and Table 3, the increase in the reaction rate constant with increasing humidity was close to 40% for both compounds for the relative humidity increasing from 50 to 90%. These results indicate that NS and NF, so the compounds whose thermodegradation is the fastest are also the most sensitive to humidity.

At the next stage, the measurements were performed at a constant relative humidity of ~76% at three different temperatures of 70, 80 and 90°C. The rate constants determined are presented in Table 4.

The values imply an exceptionally small temperature coefficient of the reaction on the average not exceeding 50%, i.e. $Q \leq 1.5$, which confirms and explains the high resistance of the compounds studied to elevated temperature. The same conclusion follows from the thermodynamic parameters of the process obtained from the Arrhenius equation and the rate constants at room temperature (Table 5, Figure 2).

The activation energy of the thermodegradation of the derivatives studied is on the average ~35 kJ/mol, while the enthalpy (ΔH) ~29.58 kJ/mol, and entropy (ΔS) ~255.35 J/K \times mol.

The rate constants determined for room temperature and relative humidity of 76.4% have values in the range $4.5-8.9 \times 10^{-9}$ [s⁻¹] and confirm that even at high humidity the selected 1,4-DHP derivatives in the solid state are highly stable on storage in the dark. A comparative analysis of the results suggests that their thermal stability depends on the chemical structure and in particular on the position of the nitro

Table 5. Coefficients of the Arrhenius equation $\ln k = a \times 1/T + b$ describing thermal decomposition of 1,4-DHP derivatives at relative humidity 76% in the liquid phase.

Kinetic parameters	Compound						
	NF	NS	NC	AL	NM	FD	NT
n	3	3	3	3	3	3	3
r	0.9889	0.9337	0.9998	0.9993	0.9999	0.9996	0.9989
a	-3521.098	-3679.7063	-4063.9878	-4290.0962	-4591.3266	-4795.6304	-5244.6489
*Sa	259.6649	1411.2203	70.8415	154.4596	167.2289	132.1927	245.7112
b	-6.3596	-5.7751	-4.6624	-3.9869	-3.2155	-2.2111	-1.3155
*Sb	1.4994	3.9948	0.2005	0.4372	0.4734	0.3742	0.6955
**Sy	0.06	0.16	0.06	0.02	0.02	0.01	0.03
Ea (kJ/mol)	29.27	30.59	33.79	35.67	38.17	39.87	43.61
ΔH (kJ/mol)	26.83	28.15	31.35	33.23	35.73	37.43	41.17
ΔS (J/K \times mol)	250.55	297.55	249.44	248.76	247.99	246.99	246.09

* Sa, Sb – the standard deviation of intercept a and slop b, respectively;

** Sy – the standard deviation of y-residuals

group. The derivatives with the NO₂ group at the *meta* position (NC, NT, NM) are much more resistant to the effect of temperature than those with NO₂ group at the *ortho* position (NS, NF). The compounds devoid of this group: FD and AL, are more thermally stable than NS and NF, but their stabilities differ from those of the other derivatives. FD containing two chlorine atoms is the most stable from all the derivatives studied, whereas AL containing one chlorine atom is more stable than NS and NF (derivatives with NO₂ at the *ortho* position), but it is less stable than NC, NT and NM (derivatives with NO₂ at the *meta* position). These data suggest that the replacement of the NO₂ group in the 1,4-DHP derivatives by one chlorine atom increases their thermal stability relative to that of the compounds with NO₂ at the *ortho* position, but the replacement of NO₂ by a chlorine atom and additional introduction of another chlorine atom makes it even more stable, relative to the derivatives with NO₂ both at the *ortho* and *meta* positions.

Of course this is just a supposition as it has been drawn on the basis of only 2 chloro-derivatives and should be confirmed on a larger body of data. According to increasing thermal stability the 1,4-DHP derivatives studied can be arranged in the following sequence:



This sequence holds for all temperatures and humidities studied. The least thermally stable are the nitrophenyl derivatives with the NO₂ group at the *ortho* position (NF and NS), which decompose by about 30% faster than those with NO₂ at the *meta*

position, by about 10% faster than the derivative with one chlorine atom (AL) and by 50% faster than that with two chlorine atoms (FD).

The detection and identification of the products formed on thermal decomposition of the derivatives studied was performed by a comparison with the standards of the photolysis products (nitrozo- and nitro-derivative) for NF, and for NS and the other compounds by thin layer chromatography, UV spectrometry and reaction with KMnO₄. Only two of the derivatives studied were found chromatographically pure (NT and NC), while the others contained from 1 to 4 initial impurities (Figure 3). The chromatograms of the compounds subjected to accelerating ageing procedure at 80°C and at relative humidity of 90% for 120 days revealed additional spots testifying the formation of the decomposition products. In the chromatograms of five derivatives with the nitrophenyl group: NF, NS, NT, NC and NM, a common product of decomposition characterized by R_f of about 0.77, was identified. Similar character of the UV spectra of the thermodegradation product of R_f ~0.77 (obtained after elution of the spot) appearing on decomposition of all derivatives to the spectrum of the nitrozo-derivatives form NF, positive results of the reaction with KMnO₄ (pink color) and with Ehrlich reagent (yellow hue), indicate that this product is a nitrozo-derivative. It is a compound forming by the redox reaction leading to aromatization of the 1,4-DHP ring accompanied with elimination of the water molecule (Scheme 1). Color reactions with these reagents permit distinction of the nitrozo-derivative from the nitro-derivative as for

the latter the color reactions are negative. The product is the same as that identified in the earlier study of photodegradation of these derivatives (6,7,9,12). In view of the above it is reasonable to suppose that the thermal decomposition occurs according to the same mechanism as the photolytic decomposition, but the former is much slower. The results obtained also suggest that the highly photosensitive compounds are exceptionally resistant to the effect of elevated temperature, which has been also indicated in our earlier study of photolysis and thermodegradation of perazine (11). The photosensitivity and thermal resistance of some chemical structures e.g. NO₂ or phenothiazine ring, is a direct consequence of the fact that such a structure can be much easier disturbed by a quantum of light providing corpuscular photon able to eject an electron than by thermal energy provided in the form of molecular vibrations.

CONCLUSIONS

1. 1,4-DHP derivatives in solid state studied by the test of accelerated ageing do not undergo thermodegradation in dry air atmosphere, at extremely low humidity.

2. Thermal decomposition of all 1,4-DHP derivatives studied in the range 70–90°C in humid air atmosphere takes place according to the first order reaction.

3. All the derivatives studied respond to elevated temperature very slowly and at similar rates, which means that their thermal stability is high and similar.

4. The main product of thermal decomposition of 1,4-DHP derivatives with nitrophenyl substituent

is the nitroso-derivative formed as a result of the DHP ring aromatization.

5. The main product of thermodegradation of the 1,4-DHP derivatives with the nitrophenyl substituent is the same as the main product of photodegradation of these derivatives as a result of UV irradiation.

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