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DSC STUDY OF RADIOSTABILITY OF 1,4-DIHYDROPYRIDINE DERIVATIVES

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Abstract

The effect of sterilisation by irradiation has been studied for the seven most often used in medicine derivatives of 1,4-dihydropyridine (nifedipine, nisoldipine, nicardipine, nitrendipine, nimodipine, felodipine and amlodipine). The sterilisation was performed for the compounds in the solid phase with an electron beam of the energy 10 MeV, at room temperature, using the irradiation doses from 20 to 400 kGy.

The effects of the irradiation were studied by the methods SEM, DSC, XRD and TLC. The sterilisation with doses 20–100 kGy was found to cause no changes in the physico-chemical properties of the compounds, while the irradiation with higher doses (200–400 kGy) was found to induce changes in the colour, DSC spectrum and TLC picture. As follows from the TLC results, the main product of radiolysis of the compounds studied was a pyridine nitrozoderivative, which indicates the same mechanism of decomposition as in the process of photodegradation. The results prove that the 1,4-dihydropyridine derivatives being highly sensitive to visible and UV radiation are generally resistant to ionising radiation and thus can be subjected to sterilisation by irradiation.

Keywords: drug analysis, DSC, radiation sterilisation, SEM, XRD and TLC methods

Introduction

The heart and circulatory systems' diseases have become a twice more frequent cause of death than those related to neoplasmic changes [1]. This instance has stimulated intense search for new therapeutical drugs for treatment of the diseases. A good illustration of the successful search is a group of 1,4-dihydropyridine derivatives used in a number of new therapeutic drugs. The drugs usually used in the form of injections, must be sterilized. As they are resistant to elevated temperatures, they can be sterilized by the traditional thermal method [2, 3], but recently the thermal method has been replaced by a more effective sterilisation by irradiation. It should be emphasised that many known drugs cannot be sterilised by irradiation because of low radiochemical stability. Their irradiation leads to physical and chemical changes, decrease in the content of the therapeutic substance or complete decomposition [4–7]. Taking into regard the fact that 1,4-dihydropyridine derivatives were highly sensitive to the visible and ultraviolet radiation, it was necessary to check their behaviour under the sterilisation by irradiation [2, 8–10].

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The paper reports results of a study of the effect of ionising irradiation on the 1,4-dihydropyridine derivatives in solid-state performed by the analytical methods SEM, DSC, XRD, TLC. Some of the methods have been used for observation of the polymorphism [11, 12], solubility [13–16], release rate [17–18] and stability [19] of 1,4-dihydropyridine derivatives and in particular their complexes with cyclodextrines [11, 19, 20].

This study was performed on these of the 1,4-dihydropyridine derivatives that are most often used in medicine, differing in the substituent at the phenyl ring or the ester substituent.

The chemical formulae of the compounds studied are given in Table 1. The compounds in the solid phase were subjected to irradiation by doses used for sterilisation of medical substances 20–25 kGy, and much higher 100–400 kGy, to facilitate detection of possible changes in the physico-chemical properties of the substances studied caused by irradiation and to grasp possible relations between the size of the dose and intensity of the changes.

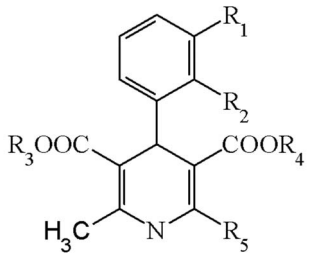
Experimental

Materials

Nifedipine (NF)	1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester; serial number: LOT 57H0977, active substance content: 98.78%
Nisoldipine (NS)	1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid butyl methyl ester; serial number 230191, active substance content: 98.93%
Nitrendipine (NT)	1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid ethyl methyl ester; serial number: 030496, active substance content: 98.83%
Nicardipine (NC)	1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methyl-2-[methyl(phenylmethyl)amino] ester; serial number: 28H0977, active substance content: 99.96%
Nimodipine (NM)	1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid isopropyl-2-methoxyethyl ester; serial number: 0112/95, active substance content: 99.36%
Felodipine (FD)	1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylic acid ethyl methyl ester; serial number: 9608, active substance content: 100.63%
Amlodipine (AL)	1,4-dihydro-6-dimethyl-2-[(2-amino-ethoxy)methyl]-4-(2-chlorophenyl)-3,5pyridine-dicarboxylic acid 3-ethyl 5-methyl ester; serial number:AB8056015 active substance content: 99.13%

Active substance contents for all compounds are converted and expressed per the mass of anhydrous substance.

Table 1 Structure of examined compounds



Compounds	Symbol	R_1	R_2	R_3	R_4	R_5
Nifedipine	NF	–H	$–NO_2$	$–CH_3$	$–CH_3$	$–CH_3$
Nisoldipine	NS	–H	$–NO_2$	$–CH_3$	$–CH_2–CH–(CH_3)_2$	$–CH_3$
Nitrendipine	NT	$–NO_2$	–H	$–CH_3$	$–CH_2–CH_3$	$–CH_3$
Nicardipine	NC	$–NO_2$	–H	$–CH_3$	$–(CH_2)_2–N(CH_3)–CH_2–C_6H_5$	$–CH_3$
Nimodipine	NM	$–NO_2$	–H	$–CH(–CH_3)_2$	$–(CH_2)_2–O–CH_3$	$–CH_3$
Felodipine	FD	–Cl	–Cl	$–CH_3$	$–CH_2–CH_3$	$–CH_3$
Amlodipine	AL	–H	–Cl	$–CH_3$	$–CH_2–CH_3$	$–O–(CH_2)_2NH_2$

Exposure to irradiation

Portions of approximately 0.1 g of each substance were placed in colourless glass jars of 3 mL capacity and closed with a plastic stopper. The samples in the vials were exposed to irradiation in a linear electron accelerator LAE 13/9 (electron beam 9.96 MeV and current intensity 6.2 μA) till they absorbed a total doses of 20, 100, 200 or 400 kGy.

Temperature measurement by the Boetius method

The melting point was measured by a heating table with a thermometer and optical microscope ensuring magnification from 60 to 100 times (the Boetius apparatus made by NAGEMA, Germany). The melting point can be determined to an accuracy of 0.5°C. The rate of sample heating was 5°C min^{-1}. The substance studied was placed between the microscope slides on the heating table at a spot allowing observations. The substance and the temperature indications were observed simultaneously.

Analysis by change in mass

The substances were weighted before and after irradiation on an analytical scale Mettler Toledo AG 204 (Switzerland) with an accuracy to four decimal digits. The loss or increase in mass was recorded if the difference was >±0.5 mg. The error in the mass determined was ±0.05%.

Water content determination using Karl Fischer reagent

Water content was determined in the substance studied before and after irradiation. The procedure was as follows: carefully weighted portions of 0.03 g of 1,4-dihydropiridines studied were dissolved in anhydrous methanol and water was titrated by using Karl Fischer reagent in a titrator Mettler Toledo DL 38 Karl Fischer titrator (Switzerland). The error in the water content determined by this method was ±0.01%.

Differential scanning calorimetry (DSC)

Samples of 4 mg ±10% were closed in aluminium crucibles with pierced lid. All DSC measurements were performed using DSC-204 (Netzsch). Prior to measurements the samples were isothermally incubated for 5 min at T=20°C. The measurements were performed in the temperature range 20–300°C at the heating rate of 5°C min^{-1} in the helium atmosphere. The data were analysed by a computer program TA (Netzsch). For determination of the enthalpy values characterising phase transitions, the linear or tangent-sigmoidal base line was used. For AL the processes of melting and decomposition occurred in the same temperature range and the method of peaks separation with the use of the Fraser–Suzuki profiles had to be applied [21].

X-ray diffraction measurements (XRD)

The crystalline phases were identified by the X-ray powder diffraction, with a modified powder diffractometer HZG-3 equipped with a sealed tube (CuK_α, λ=1.54178 Å) operating at 30 kV and 35 mA. Diffraction measurements were conducted within the 2θ angle from 5 to 50 degree, at the rate of 0.01° s^{-1}. All X-ray diffraction measurements were carried in air atmosphere.

Scanning electron microscopy (SEM)

Morphology of the samples studied was examined by using the SEM 515 (Philips) scanning electron microscope of a working distance 14 mm and accelerating voltage 3–10 kV. The powdered samples were deposited in the form of a thin layer onto the microscope tables (1 cm in diameter) and coated with gold in ionisation chamber SCD-050 (Balzers).

Thin layer chromatography (TLC)

Plates of the size 5.0×20.0 cm, covered with silica gel Kiesegel 60 F_{254} were used. The mobile phase was benzene-methanol (6:1). The traces were set with a quartz lamp working at λ = 254 nm [22].

Results and discussion

Organoleptic analysis of the compounds studied performed in 24 h after the irradiation with doses 20–100 kGy has shown no significant changes in their form or in their colour. Only the irradiation with higher doses (200–400 kGy) has resulted in an increased intensity of the yellow colour of all the compounds except AL, whose white colour (retained for lower doses) changed into cream (Table 2).

The compounds were weighted before irradiation and after the irradiation with the highest dose (400 kGy) immediately after the irradiation, and the content of water in them was determined by the Karl Fischer method [3].

The water content in all irradiated compounds studied was lower than that before the irradiation by from 0.02 to 0.07%, whereas the difference in their mass was ±0.01%.

Morphological analysis of the samples before and after the irradiation with the maximum dose performed by scanning electron microscopy (SEM) did not show significant morphological changes as a result of irradiation. The molecular size distribution in the 1,4-dihydropyridine derivatives studied obtained on the basis of the SEM photographs is described in Table 3 and selected photographs are shown in Fig. 1.

Despite insignificant differences in the mean size of the molecules, e.g. for NF and FD, microscopic observations did not show an increase in the amorphous phase content or a significant agglomeration of molecules on irradiation. Detail inspection of Fig. 1 reveals that after irradiation the molecules of NC (Fig. 1b) are slightly larger while those of FD slightly smaller (Fig. 1c).

Table 2 Changes in colour and in humidity as a result of irradiation of 1,4-dihydropiridine derivatives

Compound	Colour					Water content/%	
	0_{kGy}	20_{kGy}	100_{kGy}	200_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}
NS	yellow	no changes			deepening of colour	0.61	0.57
NC	yellow light	no changes			deepening of colour	0.64	0.60
FD	yellow light	no changes		deepening of colour		0.78	0.71
AL	white	no changes		cream		0.77	0.75
NM	yellow light	no changes		cream		0.56	0.53
NT	yellow light	no changes			deepening of colour	0.45	0.42
NF	yellow	deepening of colour				0.58	0.51

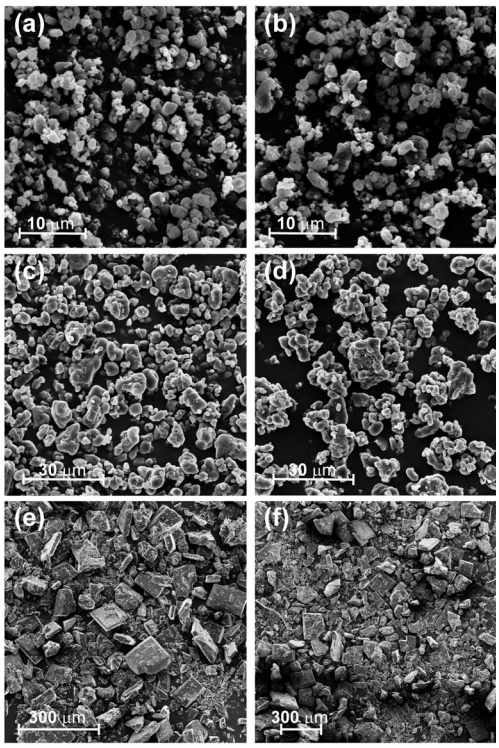


Fig. 1 SEM micrographs of a – nifedipine, c – nicardipine and e – felodipine before and b, d, f – after irradiation (400 kGy) respectively

Table 3 Particle size distribution determined from SEM micrographs

Particle size/μm	NF		NS		NT		NC		NM		FD		AL	
	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}	0_{kGy}	400_{kGy}
0–1	40	43												
1–2	34	29												
2–3	21	20	31	35			61	54						
3–4	4	7												
4–5	1	1			45	42								
5–7.5														
7.5–10			37	39			29	27	22	31	35	42	20	12
10–15			17	14			6	15						
15–20			10	8			4	3						
20–30			5	4			0	1						
30–50					21	30								
50–75	–	–			28	24								
75–100					5	3			34	23	37	43	25	31
100–150			–	–	1	1	–	–	19	21	12	9	32	35
150–200									10	18	10	5	15	13
200–300					–	–			12	5	6	1	6	8
>300									3	2	–	–	2	1

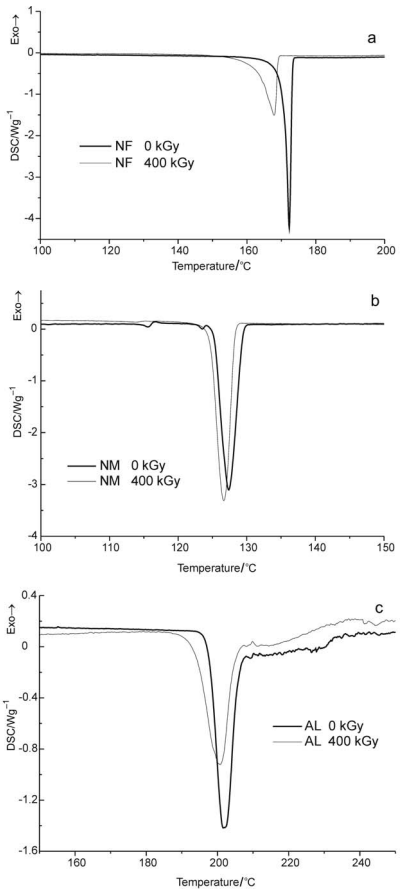


Fig. 2 The DSC curves obtained for a – nifedipine, b – nimodipine and c – amlodipine before and after irradiation (400 kGy)

The DSC results for the initial substances and those irradiated with the highest dose of 400 kGy are presented in Fig. 2. The DSC curves of the initial substances (before irradiation) most often revealed one significant phase transition corresponding to the melting process and sometimes the decomposition process in higher temperatures.

On the basis of the position of the initial point of transition (T_{onset}), the melting points of the non-irradiated substances were estimated and compared with literature values [3, 23, 24] (Table 4). Some differences were observed between the literature values of the melting points, those determined by the use of Boetius apparatus and those (T_{onset}) determined from the DSC curves. The differences are small and most probably related to the differences in the procedure of measurements such as: the rate of heating, the sample atmosphere and inaccuracies of the visual method of determination of the start of melting in the Boetius method.

Table 4 Comparison of melting points obtained by different methods

Compound	Melting temperature range / %		
	References [3, 23, 24]	Boetius apparatus	DSC method*
NM	125–129	125–127	124.7–129.4
FD	142–145	138–141	141.4–145.6
NS	148–149	142–146	148.1–151.7
NT	156–160	152–159	157.6–161.2
NF	171–175	170–174	170.6–173.3
NC	180–185	180–184	167.5–175.0
AL	197–200	211–213	197.7–206.1

* data of onset- and end-temperatures

The temperatures of the beginnings of the phase transitions (T_{onset}) and their maxima (T_{max}) for the initial samples and those irradiated with a maximum dose are given in Table 5. A comparison of the DSC curves of the irradiated and non-irradiated compounds has revealed a general tendency towards the shift of the peaks corresponding to melting point lower temperatures. The magnitude of the shift varied from about 4°C (NC 4.2°C, NF 4.5°C and NS 4.3°C) to 0.1°C (NT). For the NT derivative this difference being at the limit of the error of determination, indicates a high resistance of this compound even to a large dose of irradiation.

Another interesting effect was a broadening of the range of temperatures in which the melting process took place, evidenced as a significant shift of the temperature at which the transition starts (T_{onset}) and a significant increase in the difference $T_{max}-T_{onset}$. These values are presented in Table 5. The enthalpies of melting determined for the initial substances and those irradiated with the maximum dose of 400 kGy are given in Table 6. As follows from these data they are lower after the irradiation. The greatest changes in the enthalpy (above 20%) were noted for NS (28.1%) and NF (20.6%), while the smallest change of 0.6% were obtained for NM (3.8%) and NT (0.6%).

The four derivatives revealing the greatest changes in the thermal effects as a result of irradiation: FD, NC, NF and NS, were subjected to additional DSC analyses after irradiation with the intermediate doses of 20, 100 and 200 kGy. The values of the enthalpies, temperatures of the beginning of phase transition (T_{onset}) and the phase transition maxima (T_{max}) are given in Table 7. Statistical analysis revealed a significant linear correlation between the temperature of the beginning of the phase transition or the enthalpy of melting and the dose of irradiation, e.g. for FD the linear correlation obtained was characterised by the coefficient r=0.906, for NS the correlation between the enthalpy of melting and the dose of irradiation was characterised by the highest correlation coefficient of r=0.935 (Fig. 3).

The decrease in the melting point and the melting enthalpy observed for the substances studied on irradiation suggests that their sensitivity to ionising radiation is related

Table 5 Characteristic temperatures evaluated from DSC data

Compound	0_{kGy}			$400_{kGy}\beta$			ΔT_{max}[1]/°C	ΔT_{onset}[2]/°C
	T_{max}/°C	T_{onset}/°C	$T_{max}-T_{onset}$/°C	T_{max}/°C	T_{onset}/°C	$T_{max}-T_{onset}$/°C		
NF	172.3	170.6	1.7	167.8	163.1	4.7	4.5	7.5
NS	150.5	148.1	2.4	146.9	142.8	4.1	3.6	5.3
NC	172.4	167.5	4.9	168.2	163.0	5.2	4.2	4.5
AL	201.9	197.7	4.2	200.3	193.9	6.4	1.6	3.8
AL decomp.	214.5	194.4	20.1	212.8	189.1	23.7	1.7	5.3
FD	144.1	141.4	2.7	142.8	139.3	3.5	1.3	2.1
NT	160.0	157.6	2.4	159.9	157.3	2.6	0.1	0.3
NM	127.4	124.7	2.7	126.6	125.2	1.4	0.8	–0.5

[1] $\Delta T_{max}=T_{max}^{0}-T_{max}^{400}$
[2] $\Delta T_{onset}=T_{onset}^{0}-T_{onset}^{400}$

Table 6 Melting enthalpies of non-irradiated (ΔH_0) and irradiated (ΔH_{400}) samples evaluated from DSC data

Compound	ΔH_0/J g^{-1}	ΔH_{400}/J g^{-1}	$(\Delta H_0 - \Delta H_{400})/\Delta H_0$ / %
NS	–78.5	–56.5	28.1
NF	–105.9	–84.1	20.6
AL	–94.3/–65.5*	–84.1/–63.1*	10.8/3.6*
FD	–83.5	–75.0	10.2
NC	–95.9	–87.8	8.5
NM	–97.8	–94.0	3.8
NT	–110.0	–109.3	0.6

*data evaluated for decomposition process

Table 7 The DSC data for FD, NC, NF and NS obtained for different doses of irradiation

Sample	ΔH/J g^{-1}	T_{max}/°C	T_{onset}/°C
FD 0 kGy	–83.5	144.1	141.4
FD 20 kGy	–79.3	143.3	140.5
FD 100 kGy	–77.6	143.1	140.3
FD 200 kGy	–77.0	142.9	139.8
FD 400 kGy	–74.9	142.8	139.3
NC 0 kGy	–95.9	172.5	167.6
NC 20 kGy	–95.8	172.4	167.6
NC 100 kGy	–95.0	172.4	167.5
NC 200 kGy	–94.6	171.5	167.1
NC 400 kGy	–87.8	168.2	163.0
NF 0 kGy	–105.9	172.3	170.6
NF 20 kGy	–90.8	170.1	166.5
NF 100 kGy	–87.8	169.6	165.7
NF 200 kGy	–86.1	169.4	165.2
NF 400 kGy	–84.1	167.8	163.1
NS 0 kGy	–78.5	150.5	148.1
NS 20 kGy	–70.4	150.0	147.3
NS 100 kGy	–69.3	148.6	145.8
NS 200 kGy	–68.0	148.0	145.2
NS 400 kGy	–56.4	146.9	142.8

to the position of the NO_2 group in the phenyl substituent. The compounds with the NO_2 group in the meta position (NT, NM) are the most resistant, while those with the NO_2 group in the para position (NS, NF) are the least resistant to irradiation (Tables 5 and 6). The other compounds (NC, FD and AL) show intermediate resistance to irradiation, which can be related to the presence of a large volume ester substituents.

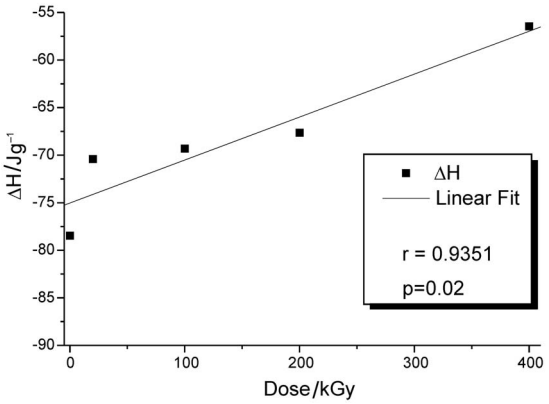


Fig. 3 The melting enthalpy of nisoldipine *vs.* the dose of irradiation

A similar decrease in the melting point and melting enthalpy caused by irradiation has been observed earlier for a series of antibiotics and steroids [10, 25]. Changes of similar character have been recorded by the DTA method for tolbutamide subjected to gamma irradiation [9]. Also studying the possibility of radiation sterilisation of the β-blocker of timolol with different doses of gamma irradiation (5–50 kGy) a decrease in the melting point with increasing irradiation dose has been reported [26].

Figure 4 presents the XRD study results for NS and NF, revealing most pronounced thermal changes, and for NT, characterised by the greatest resistance to irradiation. On the basis of the detailed analysis of the diffraction maxima before and after the irradiation, no evidence of significant polymorphous changes or decomposition of the compounds studied was found.

However, the compounds do undergo decomposition as a result of irradiation, which was proved by the TLC method study. The measurements performed for the initial compounds and after their irradiation with the highest dose of 400 kGy proved that the majority of compounds (NF, NS, NM, FD, AL) contained impurities before the irradiation. All the compounds after the irradiation with 400 kGy reveal the presence of one (FD, NC), two (AL) or three (NF, NS, NT) or even four (NM) products of decomposition (Fig. 5). Some of these products were identified as nitrozo-derivatives of pyridine, which would indicate that the radiolysis of the derivatives containing the NO_2 group gives the same products as their photolysis [2, 4, 6].

Interestingly, analysis of the results has shown that the effect of the ionising radiation applied as a beam of electrons is less harmful for the 1,4-dihydropyridine derivatives studied in the solid phase than the effect of the visible light or ultraviolet radiation of

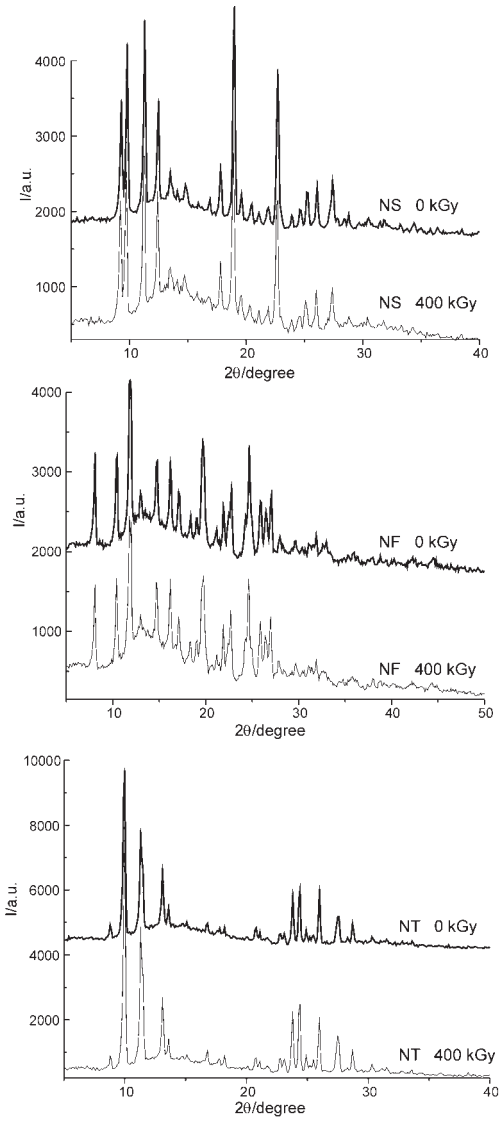


Fig. 4 X-ray powder diffraction patterns of a – nisoldipine, b – nifedipine and c – nitrendipine before and after irradiation (400 kGy)

much lower energy [2, 4, 6]. A similar observation of a greater effect of UV irradiation than gamma irradiation has been already made for proktolol and propranolol [7].

Most probably it is related to the mechanism of decomposition of these compounds involving oxidation of the pyridine ring (dehydrogenation at positions 1 and 4) with a simultaneous reduction of the nitric group $NO_2 \rightarrow NO$ and liberation of a water molecule [27]. It should be expected that the photon energy absorbed by the

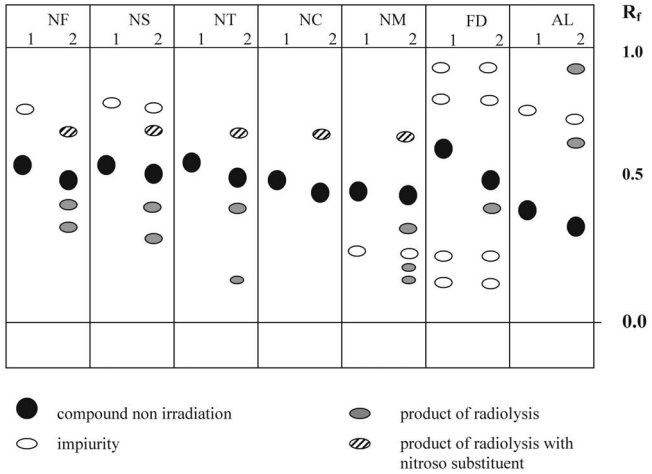


Fig. 5 Scheme of TLC chromatograms for 1,4-dihydropyridine before (1) and after (2) irradiation (400 kGy)

molecule matches the energy needed to initiate the first stage of oxidation ($NO_2 \rightarrow O = N–OH$) leading to a fast redox reaction and a decrease in the content of the substrate even to a greater degree than the decrease in the substrate caused by an attack of the beam of electrons. The results obtained in this paper suggest that FD is very sensitive to the effect of ionising radiation – similarly as NF, which is consistent with our earlier observations of its photosensitivity in the solid state [28, 29] but inconsistent with their resistivity to illumination with visible light in solutions [30].

Conclusions

The ionising radiation in the form of the beam of electrons, in the doses 20–100 kGy does not lead to notable changes in the colour, degree of refinement or morphology of the 1,4-dihydropyridine derivatives studied in the solid-state, and has no significant effect of their DSC and XRD spectra.

The ionising radiation in higher doses (200–400 kGy) leads to changes in the colour (more intense yellow or a change from white to cream), a decrease in the melting point (changes in the DSC curves) and the appearance of the radiolysis products indicated by the TLC results.

The process of radiolysis of 1,4-dihydropyridine derivatives can be observed by the DSC method as a decrease in the melting point (from 0.1 to 4.5°C) with increasing concentration of the products of radiolysis or increasing dose of irradiation.

For NS and NC a strong linear correlation ($r = 0.935$ and 0.942) has been found between the enthalpy of the melting process determined by the DSC method and the dose of ionising radiation. For FD and NF the correlation between the melting enthalpy and dose of ionising radiation was significantly lower ($r = 0.842$ and 0.707).

Although the differences in the chemical structures of the compounds studied are small, their resistance to the ionising radiation shows greater variation. The least sensitive to the ionising radiation are NT and NM having NO_2 groups in the phenyl substituent at the meta position, while the most sensitive are NF and NS, with the NO_2 group in the ortho position.

In the solid phase the 1,4-dihydropyridine derivatives studied are more resistant to irradiation with the electron beam of the energy of 10 MeV than to the UV irradiation and can be subjected to sterilisation by irradiation with no detriment of their physical and chemical properties.

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