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THERMAL ANALYSIS IN EVALUATION OF THE RADIOCHEMICAL STABILITY OF SOME FUNGICIDAL DRUGS

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Abstract

Four azole derivatives showing antimycotic activity (Miconazole, Ketoconazole, Clotrimazole, Fluconazole) in solid phase were exposed to beta irradiation at the dose of 20–200 kGy and then alterations in the physicochemical properties of the above derivatives were studied using the methods: scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analysis.

It was found that the compounds irradiated with sterilising doses (20–50 kGy) showed no significant alterations in their physicochemical properties, while application of doses >50 kGy resulted in small changes in the X-ray diffraction patterns and in the course of DSC curves, including a decrease in the melting points and enthalpy of the process.

For Miconazole and Fluconazole, a linear and relatively strong correlation was found (from r =0.9782 to r =0.9003) between the size of the dose of irradiation and the decrease in the melting point and enthalpy value.

Keywords: clotrimazole, DSC, fluconazole, ketoconazole, miconazole, SEM, X-ray powder diffractometry

Introduction

In order to assure the required microbiological purity of drugs they are subjected to various methods of sterilisation. The Polish Pharmacopoeia VI and European Pharmacopoeia recommend drugs sterilisation by thermal techniques (saturated water vapour and dry hot air), chemical techniques (ethylene oxide), by filtration or ionising radiation [1, 2].

Sterilisation by irradiation has several advantages (high efficiency of the process, potential for sterilisation of thermolabile and packed materials), but on the other hand it can be destructive to some chemical compounds.

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In order to find out whether a given therapeutic substance can be subjected to sterilisation by irradiation, it should be proved that the irradiation does not induce changes either in its contents or in its physicochemical properties and, thus, in its pharmacological activity [3, 4].

Frequently, in order to detect all changes that might appear on irradiation, the radiation doses considerably exceeding the sterilising dose (25 kGy) are applied. This procedure should allow detection of the radiolysis products produced in minimum quantities.

This report presents results of analytical tests, including those of DSC, performed to establish the effect of irradiation on four azole derivatives known for their fungicidal activity (currently in use in therapy), by the sterilising (20–50 kGy) or higher (100–200 kGy) doses.

The experiments were aimed at answering the question whether the examined compounds can be subjected to sterilisation by irradiation as well as at determining their radiochemical stability.

Experimental

Materials

Four azole derivatives of antifungial activity: Miconazole (1-(2,4-dichloro -[(2,4-dichlorobenzyl)oxy]phenethyl)imidazole), nitrate salt, Ketoconazole (*cis*-1-acetyl -4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl)-methoxy] phenyl] piperazine), Clotrimazole (1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole), Fluconazole (2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazolyl)-2-propanol) in solid phase were examined. The compounds are characterised by the data given in Table 1.

Methods

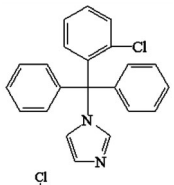
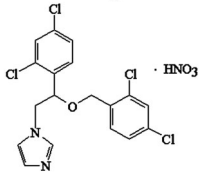
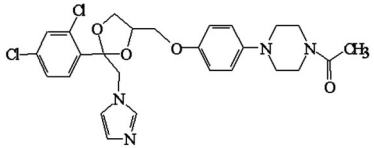
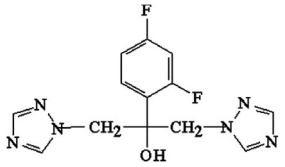
Exposure to beta radiation

Approximately 0.1 g of each substance was placed in colourless glass vials of 3 mL capacity and closed with plastic stoppers. The samples in the vials were exposed to beta irradiation in a linear electron accelerator LAE 13/9 (electron beam 9.96 MeV and current intensity 6.2 μA) till they absorbed a dose of 20, 50, 100 and 200 kGy.

Scanning electron microscopy (SEM)

Scanning electron microscopic (SEM) investigation of the initial and irradiated substances (dose of 200 kGy) was conducted on a Philips SEM 515 microscope. To be able to do this the samples were sputtered with gold in an ionising sputtering chamber. Observations were made with working distance 14 mm and at the exciting voltage of 3–10 kV.

Table 1. Chemical characteristics of the compounds

Compound	Symbol	Molecular mass	Melting temperature/°C	Formula	Manufacturer
Clotrimazole	CK	344.8	141–145		Jelfa SA Jelenia Góra LOT: 807002
Miconazole	MK	479.1	178–184		Institue of Pharmaceutical Sciences Warsaw LOT: 971204
Ketoconazole	KK	531.4	148–152		Polfa SA Warszawa LOT: A-05-9627
Fluconazole	FK	306.2	-		Pliva SA Cracow LOT: FL02004

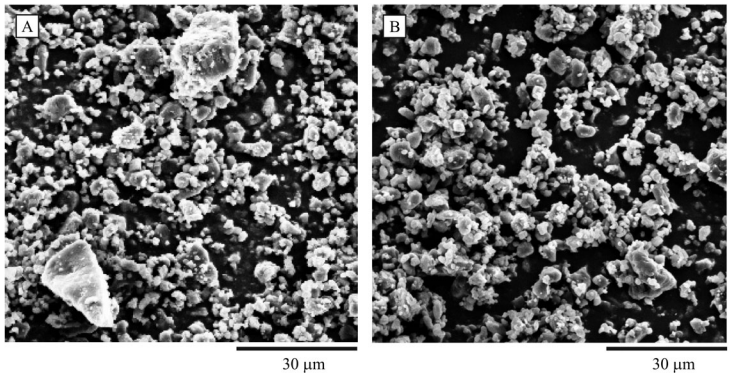


Fig. 1 SEM micrographs of A – Clotrimazole before and B – after irradiation (200 kGy)

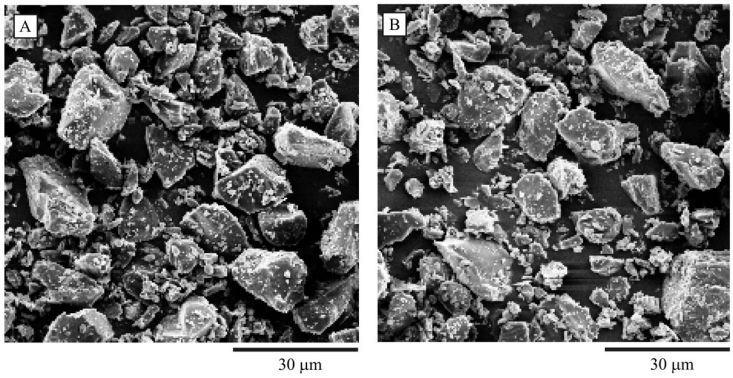


Fig. 2 SEM micrographs of A – Ketoconazole before and B – after irradiation (200 kGy)

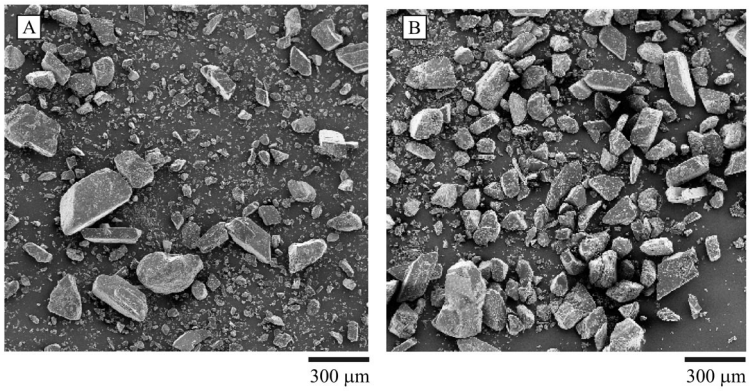


Fig. 3 SEM micrographs of A – Fluconazole before and B – after irradiation (200 kGy)

X-ray diffractometry

The samples were subjected to grinding in an agate mortar and delicately compacted in a sample holder. The measurements were conducted using a modernized HZG-3 system. X-ray tube with a copper anode (λ=1.54178 Å), fed by a TUR M-62 generator, was the source of radiation. The measurement was performed within the 2θ angle range of 4° to 60°, at 0.05° min^{-1} scanning rate.

Differential scanning calorimetry (DSC)

Measurements were performed on an apparatus DSC-204 made by Netzsch. Samples of 3 mg ±5% were closed in aluminium crucibles with pierced lid. Prior to measurements, the samples were thermally equilibrated at 20°C for 5 min, and the measurements were performed at the heating rate of 5°C min^{-1} in the helium. For each sample three independent measurements were performed and the results were averaged. The data were analysed by using a computer program TA (Netzsch). For the determination of the enthalpy values characterising phase transitions, the base line was estimated by the linear or tangent-sigmoidal method.

Results and discussion

All the compounds examined were fine crystalline powders and did not change their morphology after irradiation, although two of them, namely KK and FK changed their colour. KK became creamy in colour following the dose of 50 kGy, which turned salmon-coloured when exposed to the dose of 200 kGy. In the case of FK, the creamy colour appeared already after the dose of 20 kGy. It became salmon-coloured on exposure to the dose of 50–100 kGy and after being irradiated with the dose of 200 kGy, its colour changed to orange (Table 2).

Table 2 Changes in the colours of the compounds after irradiation

Dose/kGy	Compound			
	Clotrimazole	Miconazole	Ketoconazole	Fluconazole
0	white	white	white	white
20	white	white	white	cream-coloured
50	white	white	cream-coloured	cream-coloured
100	white	white	cream-coloured	salmon-coloured
200	white	white	salmon-coloured	orange

However, the drastic changes in colour were not accompanied by changes in SEM patterns (Figs 1–4), which were recorded both for the compounds that changed their colour and for those with no change in their original colour. For all four compounds studied the SEM image obtained was almost identical before and after irradiation.

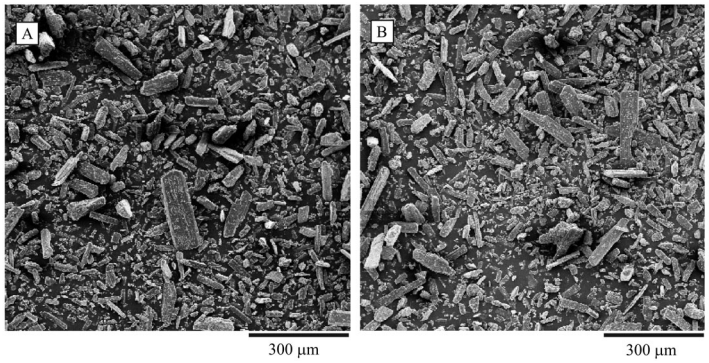


Fig. 4 SEM micrographs of A – Miconazole before and B – after irradiation (200 kGy)

In such a situation, we have decided to compare the X-ray diffraction patterns obtained for all compounds studied. Following irradiation, only small changes in the diffraction patterns were observed. In the case of compounds that changed their colour, the alterations were more evident than in those that maintained their original colour. The alterations could be noted most clearly in the X-ray diffraction pattern of FK, in which

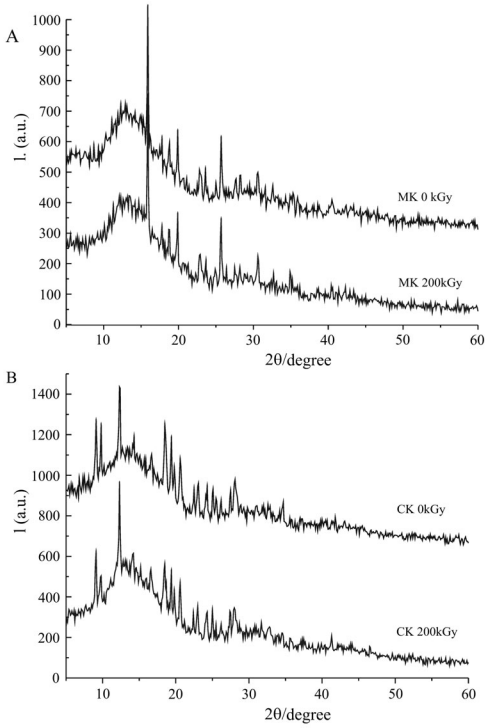


Fig. 5 X-ray powder diffraction patterns of A – Miconazole and B – Clotrimazole before and after irradiation

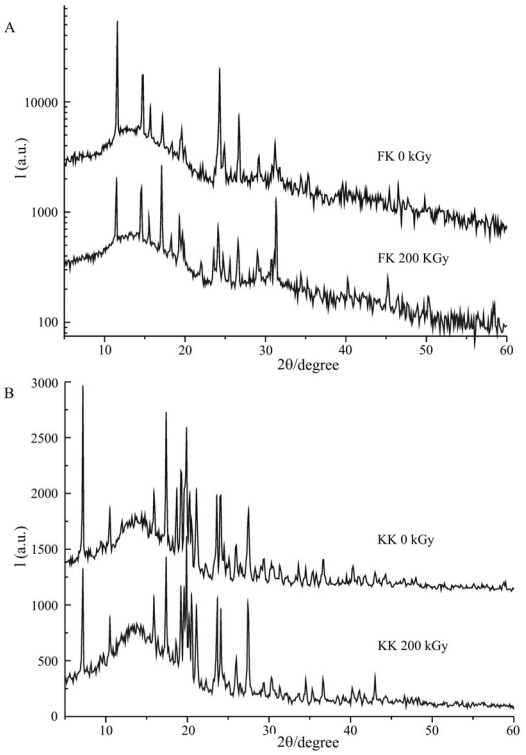


Fig. 6 X-ray powder diffraction patterns of A – Fluconazole and B – Ketoconazole before and after irradiation

most peaks changed their intensities and, sporadically, also their shape, in contrast to the X-ray diffraction patterns of MK in which, apart from an increase in the intensity of the main peak, other changes were observed only in the range from 15 to 30° (Figs 5, 6).

An increase in the intensity of the amorphous phase appearing in the diffraction pattern of the samples after the irradiation was also observed. However, no changes suggesting a polymorphous change as a result of the irradiation were detected. Taking into regard the sensitivity of the diffraction method used allowing identification of new phases only from a level of a few percent – the results are rather of qualitative importance.

The compounds examined were subjected to DSC measurements and the DSC curves for all initial compounds and those exposed to a high dose (100–200 kGy) of beta radiation were recorded. The DSC curves for all the substances subjected to beta irradiation are shown in Figs 7 and 8. The thermal parameters characterising phase transitions such as T_{onset} and T_{peak} are presented in Table 3 and the enthalpy values are given in Table 4.

Comparison of the courses of the DSC curves for irradiated and non-irradiated compounds, in each case points to a shift in peaks corresponding to melting toward lower temperatures. The extent of the shift amounted, on the average, to 2°C; its

Table 3 Characteristic temperatures evaluated from DSC data. The values in parentheses are standard deviations

Comp.	0 kGy			200 kGy β			ΔT_{peak}[1]/°C	ΔT_{onset}[2]/°C
	T_{peak}/°C	T_{onset}/°C	$T_{peak}-T_{onset}$/°C	T_{peak}/°C	T_{onset}/°C	$T_{peak}-T_{onset}$/°C		
KK	150.0(2)	148.3(1)	1.7	149.3(1)	147.1(2)	2.2	0.7	1.2
CK	144.8(2)	142.9(1)	1.9	142.7(1)	140.7(1)	2	2.1	2.2
FK	140.5(1)	139.2(0)	1.3	138.4(2)	136.9(1)	1.5	2.1	2.3
MK	184.4(1)	183.3(1)	1.1	181.5(0)	179.5(0)	2	2.9	3.8

[1] $\Delta T_{peak}=T_{peak}^{0kGy}-T_{peak}^{200kGy}$
[2] $\Delta T_{onset}=T_{onset}^{0kGy}-T_{onset}^{200kGy}$

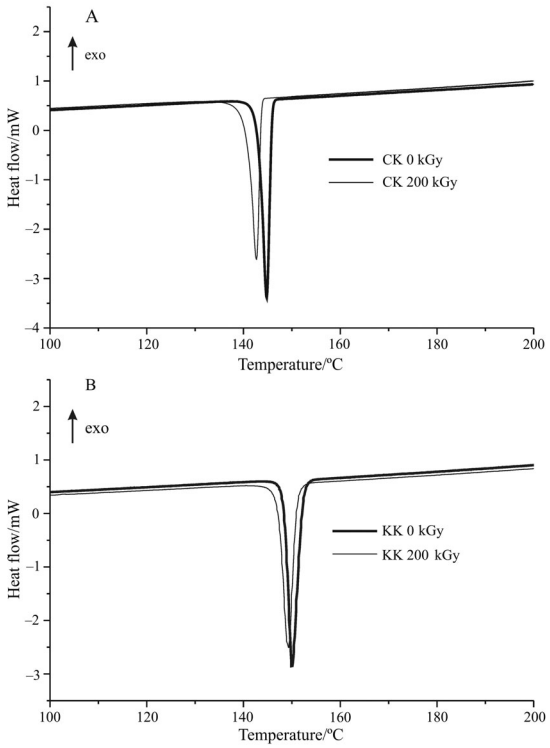


Fig. 7 The DSC curves obtained for A – Clotrimazole and B – Ketoconazole before and after irradiation

lowest value was recorded for KK (0.7°C) and the highest one for MK (2.9°C). A similar effect was observed in relation to temperatures characterising the onset of the phase transition (T_{onset}). On the other hand, the temperature ranges in which the melting process took place did not expand. The significant shift in the temperature of the transition onset (T_{onset}) and the increase in ($T_{peak} - T_{onset}$) difference have been noted earlier in our studies of radiostability of selected steroids [5] and have been reported by other investigators as well [6, 7]. The most pronounced difference

Table 4 Melting enthalpies of reference (ΔH_0) and irradiated (ΔH_{200}) samples evalueted from DSC data. The values in parentheses are standard deviation

Compound	ΔH_0/J g^{-1}	ΔH_{200}/J g^{-1}	$[\Delta H_0 - \Delta H_{200}]/\Delta H_0$/%
KK	–103.6(4)	–96.1(9)	7.21
CK	–94.3(5)	–87.4(7)	7.37
FK	–122.8(9)	–106.0(1)	13.68
MK	–129.0(3)	–101.0(4)	21.77

Table 5 The DSC data for Fluconazole and Miconazole determined for different doses of beta radiation. The values in parentheses are standard deviation

Sample	ΔH/J g^{-1}	T_{peak}/°C	T_{onset}/°C
FK 0 kGy	–122.8(9)	140.5(1)	139.2(1)
FK 20 kGy	–117.9(3)	139.9(1)	138.4(1)
FK 50 kGy	–112.0(1)	139.5(2)	138.0(1)
FK 100 kGy	–109.1(4)	139.3(1)	137.5(2)
FK 200 kGy	–106.0(1)	138.4(2)	136.9(1)
MK 0 kGy	–129.0(3)	184.4(1)	183.3(1)
MK 20 kGy	–127.0(2)	183.7(1)	181.5(1)
MK 50 kGy	–119.0(2)	183.0(1)	181.0(1)
MK 100 kGy	–111.0(3)	182.7(1)	180.7(1)
MK 200 kGy	–101.0(4)	181.5(0)	179.5(0)

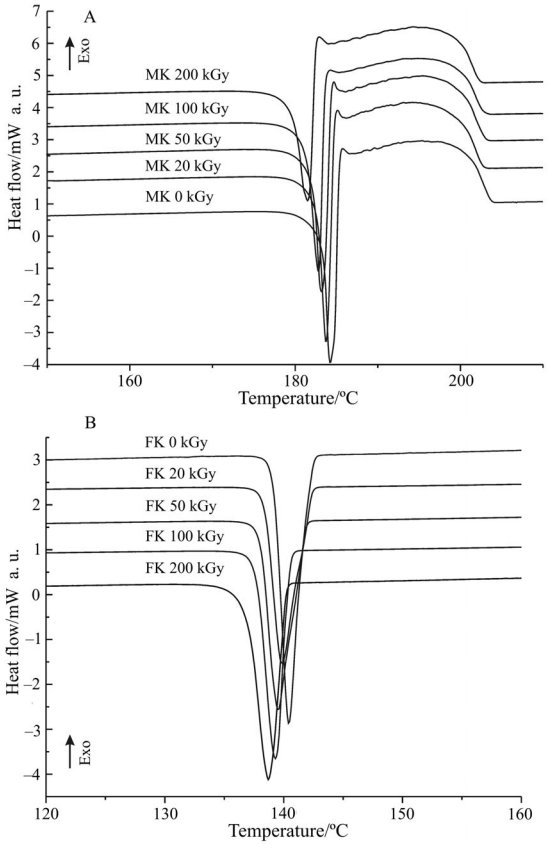


Fig. 8 DSC curves obtained for A – Miconazole and B – Fluconazole before and after irradiation

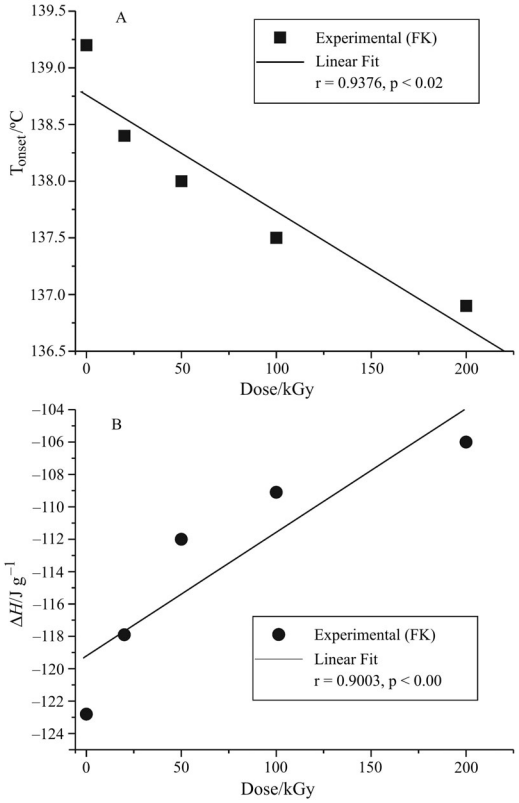


Fig. 9 T_{onset} *vs.* the dose of A – irradiation and enthalpy of melting *vs.* the dose of B – irradiation for Fluconazole

between the phase transition onset temperatures before and after irradiation (ΔT_{onset}) was recorded for MK (ΔT_{onset}=3.8°C) while the smallest one for KK (ΔT_{onset}=1.2°C).

Another change accompanying the exposure to beta radiation, apart from the shift in the phase transition temperature, was a decrease in the melting enthalpy [5, 8].

In the substances studied, the change was from 7.21% (KK) to 21.77% (MK) as related to the values of the initial substance melting enthalpy. The detailed values are listed in Table 4. As can be concluded from the results of X-ray diffraction analysis (Figs 5, 6), the changes are most probably a result of distortions to the crystal lattice caused by the irradiation. Although no significant changes were observed in the diffraction patterns in the peaks positions, some of the peaks became broader and changed their intensities, when compared to those for the non-irradiated samples. This would indicate a disturbed crystal lattice in the samples tested after the irradiation.

The next stage of the studies consisted in monitoring tendencies in changes of thermal parameters of the substances exposed to various doses of beta radiation. The study was performed for MK, which revealed the strongest quantitative changes in DSC and for FK showing the most pronounced changes in the colour and XRD spec-

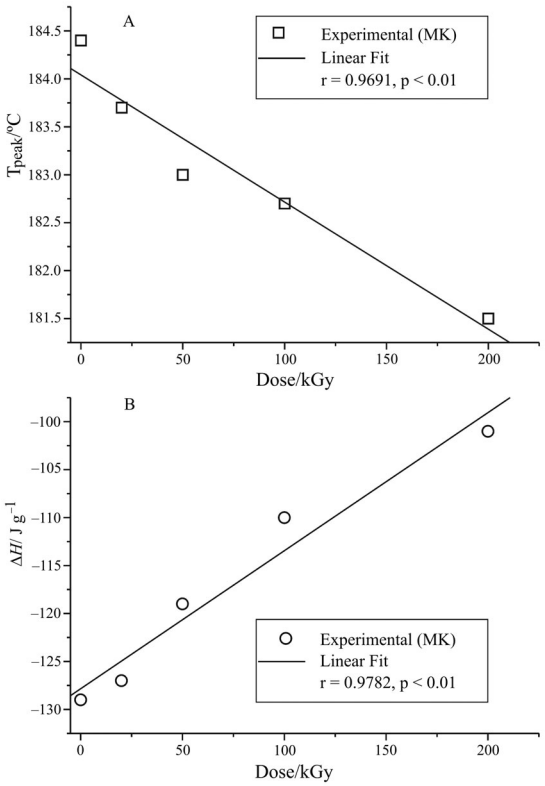


Fig. 10 T_{onset} *vs.* the dose of A – irradiation and enthalpy of melting *vs*. the dose of B – irradiation for Miconazole

trum. MK and FK were exposed to the doses of 20, 50, 100 and 200 kGy. The results of DSC studies are presented in Table 5 and in Fig. 8. Taking advantage of the obtained parameters (T_{onset} and enthalpy), their relation to the applied dose of beta radiation was examined. The estimated correlation coefficients for the linear relationship between T_{onset} and the enthalpy of the melting process are 0.9376 and 0.9003 for FK and 0.9782 and 0.9691 for MK (Figs 9, 10).

Conclusions

Summing up, we wish to emphasise that sterilisation of azole derivatives showing anti-fungal activity using beta radiation affects their physicochemical properties, as proved by results of organoleptic and thermal analysis and X-ray diffraction patterns. First of all, the compounds studied manifest variable sensitivity to the applied radiation dose, evidenced by changes in their physical and chemical parameters as a result of irradiation. The greatest changes in the colour and XRD pattern are observed for FK, but the greatest changes in the DSC, melting point and melting enthalpy are

noted for MK whose colour does not change even after the irradiation with the dose of 200 kGy. Therefore, in order to establish which compound is the most and which the least sensitive to ionising radiation, further, quantitative studies are needed by other analytical methods such as chromatographic ones (HPLC, GC) or spectrophotometric ones (UV, IR). Such studies are underway and they are expected to bring a final answer to the question whether the above compounds or some of them could be safely subjected to sterilisation by irradiation.

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References

1 Pharmacopoea Polonica, Editio VI, (2002).
2 European Pharmacopeia 3rd Edition (1997), Supplement (2000).
3 W. Bőgl, Radiat. Phys. Chem., 25 (1985) 425.
4 N. G. S. Gopal, K. M. Patel, G. Sharma, H. L. Bhalla, P. A. Wills and N. Hilmy, Radiat. Phys. Chem., 32 (1998) 619.
5 B. Marciniec, M. Kozak, L. Wachowski and M. Ogrodowczyk, J. Therm. Anal. Cal., 73 (2003) 473.
6 F. Zeegers, A. S. Crucq, M. Gibella and B. Tilquin, J. Chim. Phys. PCB, 90 (1993) 1029.
7 F. Zeegers and B. Tilquin, J. Chim. Phys. PCB, 88 (1991) 1137.
8 B. Marciniec, Z. Płotkowiak, L. Wachowski, M. Kozak and M. Popielarz-Brzezińska, J. Therm. Anal. Cal., 68 (2002) 423.