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The influence of radiation sterilisation on some β-blockers in the solid state

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ABSTRACT

Six derivatives of aryloxyalkylaminopropanol of known β-adrenolytic activity (acebutolol, alprenolol, atenolol, metoprolol, pindolol, propranolol) in solid phase were exposed to the ionising radiation generated by e-beam of high-energy electrons from an accelerator (~10 MeV) in doses from 25 to 400 kGy. To establish the effects of irradiation on their physico-chemical properties, the compounds were then analysed by differential scanning calorimetry (DSC), scanning electron microscope (SEM), X-ray diffraction (XRD) and FT-IR spectrometry. The standard sterilisation dose (25 kGy) was found to cause no changes in only one derivative – acebutolol, whereas in the other derivatives the irradiation caused colour changes, differences in X-ray diffraction patterns and in the character of DSC curves, including a decrease in the melting point. For each derivative one clear peak corresponding to the process of melting was observed and its position shifted towards lower temperatures with increasing dose of irradiation. For all compounds studied the value of the shift was between 0.1 and 1.0 °C. For alprenolol, propranolol and metoprolol linear relations were found between the irradiation dose and the decrease in the melting point, described by the correlation coefficient (between 0.9446 and 0.9864). No changes were observed in the FT-IR spectra and in the SEM images of the compounds studied.

1. Introduction

β-Blockers, which act by blocking β receptors of the sympathetic nervous system, comprise a large number of pharmaceutical preparations used for the treatment of many conditions of the circulatory system, such as in coronary heart disease, arrythmia and hypertension. These medications are often administered in the form of intravenous infusions or injections and hence they should be completely sterile, i.e. should contain no microorganisms or their spores. The accepted content of microorganisms in therapeutic drugs is regulated by pharmacopoeia [1–2]. The methods of sterilisation recommended by FP VIII (Pharmacopeia Polonica VIII) and European Pharmacopoeia include those based on the use of hot saturated steam, dry air, ethylene oxide, and ionising radiation. The latter has many advantages (can be performed in any temperature, is highly efficient and can be used for terminal sterilisation) but also the drawback of possible damages induced by this radiation (decrease in content of the active substance, decrease in the activity, the appearance of products of radiolysis and changes in the physico-chemical properties).

As the therapeutic compounds in solid phase are more resistant to ionising radiation than their solutions [3–5], the majority of the compounds are subjected to radiation sterilisation *in substantia*, and then injections are prepared in sterile conditions (production lines), under the sterile air blow.

The effect of radiation sterilisation on drugs is assessed by fast and accurate analytical methods, including thermal methods. One of such methods is differential scanning calorimetry (DSC), which provides information on the stability of chemical compounds, including radiostability [6–10]. This is because drugs of low radiochemical stability show a decrease in their melting point due to the fact that products of radiolysis act as impurities.

For drugs of high radiochemical stability the DSC results show only a small decrease in the melting point between 0.2 and 0.4 °C. In this paper the DSC method was applied to evaluate the radiochemical stability of selected β-blockers in solid phase. The low stability of practolol and propranolol was observed as early as 1970s. The gamma irradiation of both compounds to doses 5–50 kGy resulted in a slight discolouration and for practolol specifically two products of radiolytic decomposition were detected by the TLC method. One of the products was identified as N,N-bis-(3-p-acetaminephenoxy-2-hydroxy-propylo)-izopropyloamine, which is an impurity formed in the process of production and the others occurred in trace amounts [11]. Only recently the effect of irradiation on β-blockers was studied by thermal (DSC, DTA, TG) and X-ray diffraction methods [12,13]. Gamma irradiation of atenolol in the dose of 20 kGy resulted in changes in atenolol structure and a decrease in the melting point by 1.1 °C. Tymolol irradiated with

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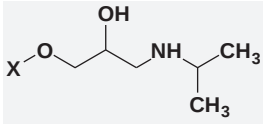
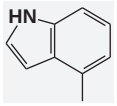
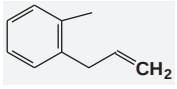
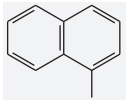
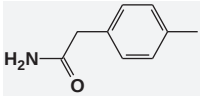
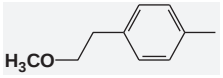
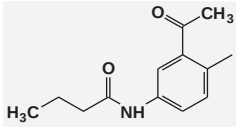
Table 1
Characterisation of examined pharmaceuticals.



Compound	Molecular weight [g/mol]	Symbol	Melting point [°C]	Substituent X=
Pindolol	248.32	PD	171–173	
Alprenolol hydrochloride	249.34	AL	108–111	
Propranolol hydrochloride	259.34	PR	163–166	
Atenolol	266.34	AT	146–148	
Metoprolol tartrate	267.36	MT	120–123	
Acebutolol hydrochloride	336.42	AC	141–143	

a dose of 50 kGy was found to be stable and suitable for radiation sterilisation [13].

In the current work we have investigated whether other sources of ionising radiation such as e-beam would have the same effect on the selected β-blockers. Six selected derivatives of aryloxyalkylaminopropanol were subjected to irradiation by a beam of high-energy electrons (9.96 MeV) with the doses varied from 25 to 400 kGy, and the effects of the irradiation were detected by DSC, FT-IR, X-ray and SEM methods.

2. Experimental

2.1. Materials

Six commonly used in medical therapy β-blockers were studied. Two of them were used in the form of bases (atenolol – AT and pindolol – PD), three in the form of hydrochlorides (acebutolol – AC, alprenolol – AL and propranolol – PR) and one in the form of tartrate (metoprolol – MT). The substances are characterised in Table 1.

2.2. Methods

2.2.1. Exposure to irradiation

Approximately 0.1 g of substance was placed in a colourless 5 mL glass jar and closed with a plastic stopper. The vials were then exposed to radiation from a linear electron accelerator LAE 13/9 (electron beam 9.96 MeV and current intensity 6.2 μA) till they absorbed doses of 25, 50, 100, 200 and 400 kGy.

2.2.2. Organoleptic analysis

Before and after irradiation the compounds were subjected to organoleptic analysis comparing their colour (against a white background), form, odour, solubility and clarity of solution to those of the non-irradiated sample.

2.2.3. Scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM 515, Philips) was used to study irradiated and non-irradiated compounds. Samples were placed on specimen stubs and fixed with carbon tabs; they were then sputter coated with gold in a sputter coater type SCD 050 Balzers. The stubs were next placed in SEM 515 (Philips) working at 15 kV and magnifications 50×, 250×, 500× and 1000×. Chosen pictures were processed by DISS (Digital Image Scanning System). Particle size was measured manually. About 1000 particles were sampled.

2.2.4. X-ray diffractometry

The samples were subjected to grinding in an agate mortar and delicately compacted in a sample holder. X-ray diffraction studies were performed on a Philips PW1050 instrument equipped with a Cu Kα source (λ = 1.54178 Å) and Ni filter in the range of 4–60° 2Θ at 0.05/2 s scanning rate.

2.2.5. Differential scanning calorimetry (DSC)

Measurements were performed on an apparatus DSC-204 made by Netzsch. Samples of 3 mg ± 5% were closed in hermetic aluminium capsules. Prior to measurements the samples were thermally incubated at 20 °C for 5 min, and the measurements were performed at the heating rate of 5 °C/min in the helium atmosphere. For each sample three independent measurements were performed

Table 2
Changes in colour as a result of irradiation of β-blockers.

Compound	0 kGy	25 kGy	50 kGy	100 kGy	200 kGy	400 kGy
AT	White	White	White	White	Almost white	Light cream
MT	White	White	White	White	Almost white	Light cream
AC	White	White	White	Almost white	Light cream	Cream
PR	White	White	White	Light cream	Cream	Dark cream
AL	White	Cream	Light yellow	Yellow	Yellow	Dark yellow
PD	White	Light cream	Light cream	Cream	Cream	Beige

and the results were averaged. The data were analysed by a computer program TA (Netzsch). For determination of the enthalpy values characterising phase transitions, the base line was estimated by the linear or tangent-sigmoidal method.

2.2.6. Infrared spectroscopy (FT-IR)

A KBr disc was prepared by mixing 1.00 mg of a substance with 300 mg of KBr and compressing it with Pye Unicam minipress. The spectra were recorded using a Bruker FT-IR spectrometer in the range of 400–4000 cm^{-1} with KBr as a blank.

3. Results and discussion

All compounds were white fine grain crystallite powders prior to irradiation. After irradiation no changes were observed in their formulation, smell, clarity, solubility in water and in methanol. However, there were some changes in the colour. For two derivatives: AL and PD a change from white to creamy colour was observed for the lowest dose of 25 kGy. For AC and PR changes in colour were observed after exposure to a dose of 100 kGy, while for AT and MT after a dose of 200 kGy. The discolouration was more intense with increasing the dose of irradiation (Table 2), although water and methanol solutions of all irradiated substances remained clear and colourless. There were no accompanying changes in the SEM image. According to the SEM photos taken prior to irradiation, all the derivatives studied were crystalline compounds. AC, AL and AT showed the largest and well-developed crystals (50–300 μm), while MT, PD and PR were in the form of fine-grain crystallites (0–175 μm) (Table 3). For all 6 derivatives studied, the SEM images were almost unchanged after the irradiation with no differences in the shape and size of crystallites (Fig. 1).

Table 3
Particle size distribution determined from SEM micrographs.

Particle size [μm]	AC	AL	AT	MT	PD	PR
0–25	–	–	–	98.2	93.9	21.5
25–50	–	–	–	1.8	6.1	21.5
50–75	5.0	17.5	–	–	–	11.5
75–100	10.3	21.3	–	–	–	16.6
100–125	16.7	18.6	33.1	–	–	12.6
125–150	20.1	18.9	33.9	–	–	14.8
150–175	14.9	10.0	26.0	–	–	1.5
175–200	15.1	8.6	4.9	–	–	–
200–225	8.0	2.7	2.1	–	–	–
225–250	5.1	1.7	–	–	–	–
250–275	3.0	0.7	–	–	–	–
275–300	1.8	–	–	–	–	–

At the next stage of the study we compared the XRD patterns recorded for substances in the powder form before and after the irradiation to doses of 25 and 400 kGy. For all the compounds studied there were some small changes in the XRD patterns, i.e. an increase in the main peak intensity or changes in the ratios of intensities of some bands observed as a result of irradiation. The most profound changes were noted for AL and PR, and for them additional XRD powder patterns were

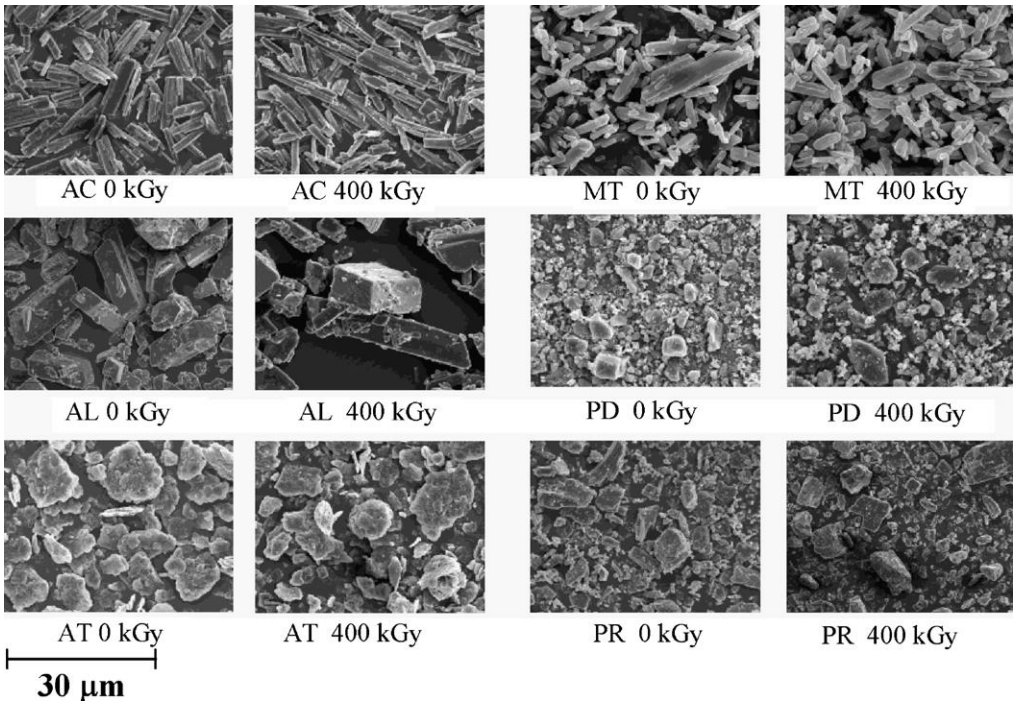


Fig. 1. SEM microphotographs of investigated β-blockers before and after irradiation.

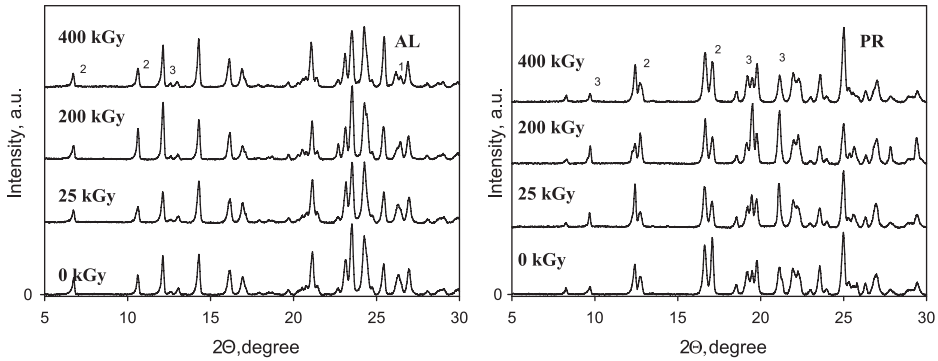


Fig. 2. XRD spectra of AL and PR before and after irradiation. 1, new pattern; 2, increase in strain; 3, decrease in strain.

recorded after irradiation to 200 kGy. As shown in Fig. 2, for AL the greatest changes occur in the range >25°. The reflexes in this range changed their shapes and sometimes even their positions. For PR such changes were observed in the whole range of the XRD pattern from 10° to 30°. The differences could suggest some damage to the crystal lattice, which probably is the reason for the colour changes, which has also been reported in [14,15].

The differences noted in the X-ray diffraction patterns of AL and PR samples (see Fig. 2) are mainly in the intensity of the signals and their FWHM (width of the peak at half height). In the sample labelled as AL a new pattern was observed at values of 26.8 2Θ for samples irradiated to 200 and 400 kGy. In the same sample reflexes occurring at angles 6.7°, 10.6° and 12.6° were accompanied by changes in the FWHM of peaks. For the first two reflexes, an increase in the FWHM from 0.073 and 0.075 (for sample 0 kGy) to 0.151 and 0.165 (for sample 400 kGy) was observed. The changes are related to the II category strains. On the other hand, in the third reflex there was a decrease in FWHM from 0.180 to 0.116. Similar changes were observed for sample designated as PR. Comparing the observed FWHM reflections for this sample, it was concluded that these changes are related to inner stress of the second order generated in the process of sample irradiation. Observed changes are not related to the change in the size of crystallites, forming a mosaic structure. If that had been the case then a reduction in the crystallite size along one axis and an increase along the other axis would have been observed. A similar pattern of changes was observed for a sample labelled PR. When half-widths of peaks were compared, there was an increase in the FWHM for peaks characterised by angles 12.75, 17.07 2Θ and a decrease for peaks at 9.68, 19,178 and 21.13 2Θ. High values of FWHM of the diffraction lines for this sample can be due to the inner stress of second order generated in the process of the radiation of tested samples.

To detect possible differences in the structure of the compounds studied before and after irradiation, the FT-IR spectra were recorded. In the range from 4000 to 400 cm^{-1} the course of the FT-IR spectra did not reveal any significant changes in the shape, intensity or positions of the absorption bands. No bands were also observed to disappear or appear, Fig. 3.

At the next stage of the study, the compounds studied were examined using thermal analysis and their DSC curves were recorded before and after irradiation. After irradiation with the standard dose of 25 kGy all samples with the exception of AC showed a shift in melting points towards lower temperatures. Fig. 4 presents the DSC curves for AC and MT before and after irradiation with the doses of 25, 100 and 400 kGy. There were no changes observed in DSC course after irradiation for the AC sample. The sharp signal in the same temperature range was observed after each dose of irradiation. As it can be seen on the basis of data collected in Table 3, the above sample had the lowest ΔT values, compared with other investigated samples. In case of MT some changes in DSC curves can be noticed. After irradiation to 400 kGy the melting point value was radically shifted towards lower temperatures and, moreover, the intensity of the above signal was significantly lower as compared with the others presented for MT. MT samples (Table 3) were found to be characterised by the higher ΔT values than all the samples investigated in this study.

It is worth to notice that in case of some samples the DSC curves showed more than one single peak (Fig. 5). The peak observed for AL at doses 0, 25 and 100 kGy, as a single sharp peak, became broad and double after irradiation to 400 kGy and, additionally, shifted towards lower temperature. However, for MT a 25 kGy dose was sufficient to observe two sharp peaks. After a 400 kGy dose it became broad and double as well as shifted towards lower temper-

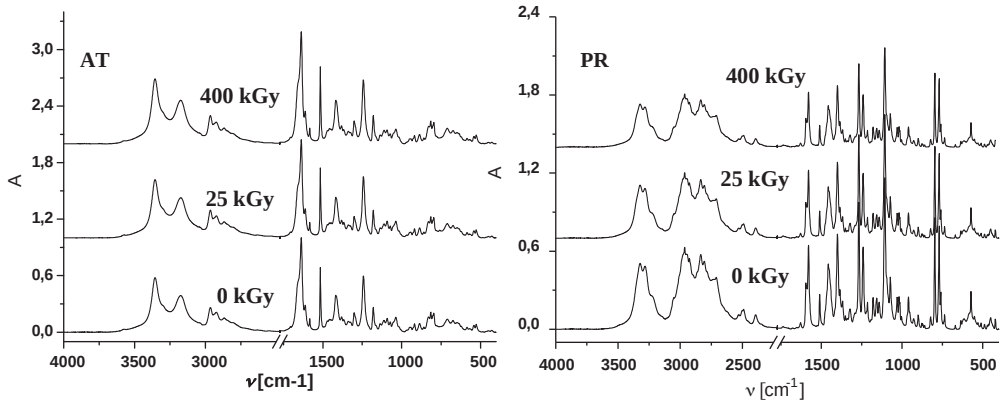


Fig. 3. FT-IR spectra of β-blockers before and after irradiation.

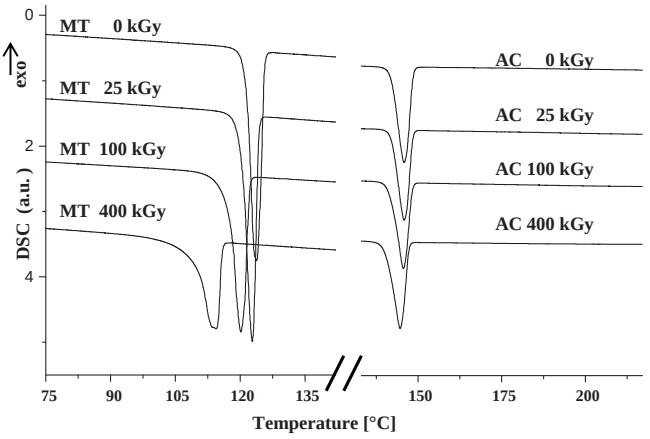


Fig. 4. DSC curves of AC and MT before and after irradiation.

atures, in a manner similar to AL samples. The above could be the result of radiolysis processes taking place during irradiation of the sample. Presence of products generated as the result of this process can be confirmed by changes observed in DSC curves obtained for the samples after irradiation. The changes in melting point values mentioned above result from radiolysis of molecules. The radiolysis leads to formation of products which act as impurities, causing the melting point values variability. This is an interesting observation because of a fact that no significant changes were observed in SEM images and FT-IR spectra as well as in XRD patterns carried out for these samples. Changes indicated in XRD patterns are not related to the ones observed in DSC curves obtained for the same samples.

With increasing dose of irradiation a slight shift towards lower temperature values in melting points could be noticed. The courses of the DSC curves and changes in the melting point of all β-blockers are shown in Fig. 4 and Table 4. The melting point determined by the DSC method and the dose were found to be linearly related for AL, PR and MT, for which the correlation coefficients were 0.9446, 0.9810 and 0.9864 (Fig. 6). Similar relations have been noted for other groups of therapeutic drugs [16–18].

Table 4
DSC results for irradiated β-blockers.

Compound	Dose/kGy	DSC		
		T_{max} (°C)	ΔT_{peak} (°C)	T_{onset} (°C)
AC	0	145.2 ± 0.2		140.2 ± 0.2
	25	145.2 ± 0.2	–	140.5 ± 0.2
	100	144.9 ± 0.2	−0.3	140.1 ± 0.2
	400	144.0 ± 0.2	−1.2	140.1 ± 0.2
PR	0	164.2 ± 0.3		163.2 ± 0.3
	25	163.8 ± 0.3	−0.4	162.4 ± 0.3
	100	163.6 ± 0.3	−0.6	162.9 ± 0.3
	400	162.4 ± 0.3	−1.6	161.3 ± 0.3
PD	0	170.6 ± 0.3		169.1 ± 0.3
	25	170.3 ± 0.3	−0.3	168.2 ± 0.3
	100	169.6 ± 0.3	−1.0	168.4 ± 0.3
	400	168.8 ± 0.3	−1.8	166.9 ± 0.3
AT	0	154.1 ± 0.2		152.6 ± 0.2
	25	152.8 ± 0.2	−1.3	151.4 ± 0.2
	100	153.1 ± 0.2	−1.0	151.2 ± 0.2
	400	151.5 ± 0.2	−2.6	150.1 ± 0.2
AL	0	110.4 ± 0.2		108.4 ± 0.2
	25	110.3 ± 0.2	−0.1	107.5 ± 0.2
	100	108.4 ± 0.2	−2.0	101.8 ± 0.2
	400	106.3 ± 0.2	−4.1	103.2 ± 0.2
MT	0	123.0 ± 0.5		121.4 ± 0.5
	25	122.0 ± 0.5	−1.0	119.1 ± 0.5
	100	119.6 ± 0.5	−3.4	117.6 ± 0.5
	400	114.1 ± 0.5	−8.9	108.7 ± 0.5

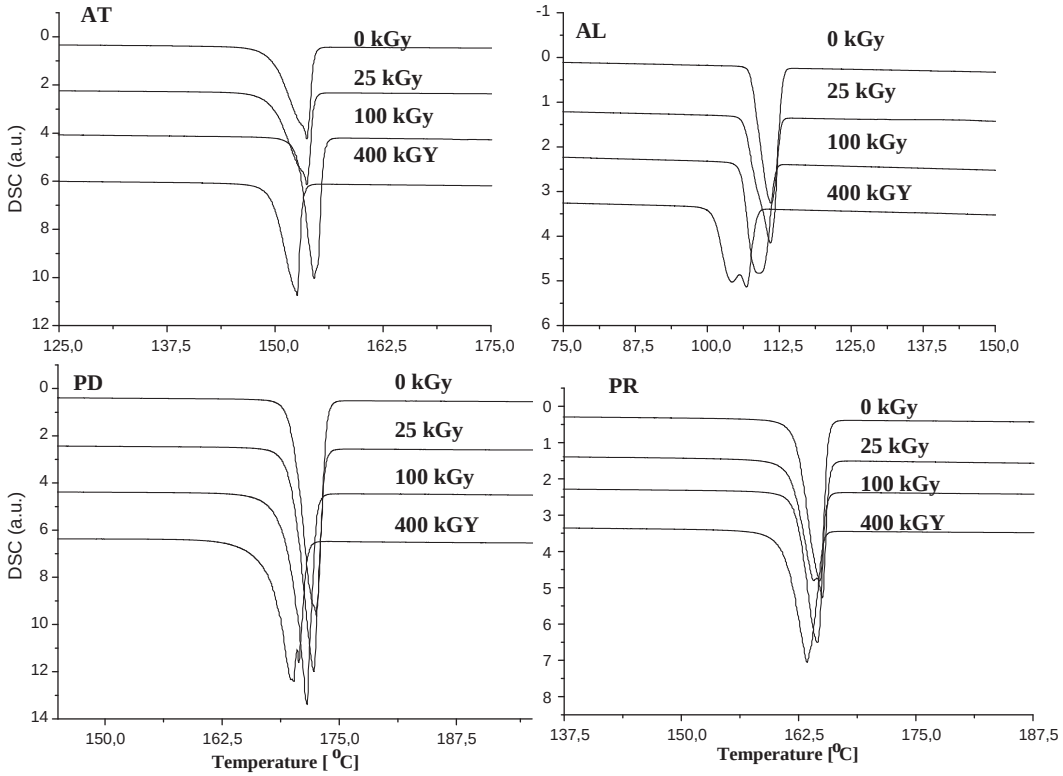


Fig. 5. DSC curves of investigated β-blockers before and after irradiation.

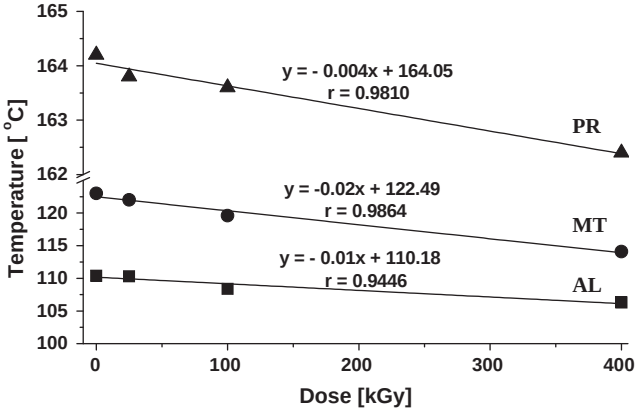


Fig. 6. The T_{peak} versus the dose of irradiation for PR, MT and AL.

4. Conclusions

The results presented above imply that the radiation sterilisation (by a beam of electrons) of six β-blockers affects their physico-chemical properties. The changes were observed in the solid state using organoleptic methods and direct methods such as thermal and XRD analysis. Different drugs showed different sensitivity to irradiation. The greatest changes in the colour and in the XRD pattern were noted for AL, whereas the greatest changes in the DSC course and melting point were detected for MT whose colour did not change until the dose of 200 kGy was reached. It can be concluded therefore that the compounds MT and AL are most sensitive to irradiation, while AT and AC are found to be most resistant to it. To draw reliable conclusions on their sensitivity to irradiation, the derivatives of interest should be studied by quantitative methods such as chromatography (HPLC) or UV spectrophotometry and only then it would be possible to indicate which of them could be sterilised by ionising irradiation.

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