THERMAL STUDY OF FOUR IRRADIATED IMIDAZOLINE DERIVATIVES IN SOLID STATE

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Four imidazoline derivatives: antazoline (AN), naphazoline (NN), tymazoline (TM), xylometazoline (XM), in the form of hydrochlorides in solid phase have been subjected to high energy e-beam irradiation from an accelerator (~10 MeV) at a dose varied from 25 to 200 kGy. The effects of the irradiation have been assessed by DSC, X-ray diffraction, FTIR, EPR and TLC.

The standard sterilisation dose of 25 kGy has been found to produce changes in the properties of one derivative (XM), two other ones (AN and TM) have been found sensitive to doses >100 kGy, whereas NN has been resistant to irradiation in the whole range studied (25–200 kGy). EPR results indicated that the changes taking place in the therapeutic substances studied are related to radical formation. The irradiation induced changes in colour, a decrease or increase in the melting point, changes in the XRD pattern, small changes in the shape of FTIR peaks and the presence of radiolysis products. The XM compounds cannot be sterilised by irradiation because of the radiation induced changes in its physico-chemical properties.

Keywords: *antazoline, DSC, EPR, FTIR, naphazoline, TLC, tymazoline, XRD, xylometazoline*

Introduction

Differential scanning calorimetry (DSC) has been recently often used in analysis of medicaments [1–19]. For many years it has been an important tool in the study of drug polymorphism [2–6]. This area of study is very important not only from the technological but also from the pharmacological point of view as different polymorphous forms can have different physical properties such as density, solubility, melting point and hence may be characterised by different bioavailability [7]. Many authors recommend this method for investigation of phase equilibria in multicomponent systems and for detection of inconsistencies between two biologically active substances [8] or between the therapeutic substance and emollients used in drug formulation technology [9–12]. The DSC method has been also appreciated by the authors working on evaluation of stability of therapeutic substances [13–19]. It has been successfully applied in estimation of the thermal [13], photochemical [14] and radiochemical stability of drugs [15–21]. Determination of the radiochemical stability of therapeutic substances (and emollients) has become of increasing importance in view of growing application of radia-

tion sterilisation, an effective method of sterilisation and decontamination of drugs [22]. Particularly important is determination of the radiochemical stability of therapeutic substances being components of eye drops and ophthalmic ointments [21], injections and infusion fluids and drugs applied to open injuries and burns as they must be sterile [22, 23]. The four selected derivatives of imidazoline show antihistamine activity (AN, TM) or α-adrenergic activity (NN, XM). They are components of popular eye drops, nose drops, gels and aerosols, or injections (AN). The aim of the study was assessment of these compounds resistance to e-beam irradiation used for radiation sterilisation.

Experimental

Materials

The material studied were four commercially available imidazoline derivatives in substantia, satisfying the pharmacopoeia [24, 25] demands.

Methods

Exposure to irradiation

Approximately 0.5 g of substance was placed in colourless glass jar of 5 mL volume 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 dose of 25, 50, 100 and 200 kGy [24–26].

Organoleptic analysis

Before and after irradiation the compound was subjected to organoleptic analysis comparing its colour (against a white background), form, odour, solubility and clarity of solution to those of the non-irradiated sample [24, 25].

Scanning electron microscopy (SEM)

SEM analysis was performed on a SEM 515 (Philips) electron microscope with 14 mm working distance and 3–10 kV accelerating voltage.

X-ray diffraction (XRD)

X-ray analysis was performed on a Philips powder diffractometer model PW 1070 controlled by an IBM PC unit, using CuK$_\alpha$ radiation (35 kV, 20 mA) and a nickel filter. The patterns were recorded for $5°<2\theta<50°$ for the gaps of $1°/1°$ at a counter step $0.02°$ s^{-1}. All XRD measurements were carried in an air atmosphere.

Infrared spectroscopy (FTIR)

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.

Electron paramagnetic resonance (EPR)

The EPR experiments were carried out for non-irradiated and irradiated samples, in standard EPR quartz sample tubes from Wilmad. The measurements were performed with a Bruker EPR EMX-10 spectrometer working at 9.4 GHz (X-band) at room temperature (293 K). The sensitivity of the spectrometer is $1 \cdot 10^{10}$ spins per gram. Induction of the magnetic field was measured to an accuracy of 0.001 mT. Microwave frequency was measured to an accuracy of 0.001 GHz. The spectrometer operating conditions during the experiment were: modulation amplitude of 0.1 mT, microwave power of 2 mW and sweep width of 20 mT.

Thin layer chromatography (TLC)

Plates of the size 5.0×20.0 cm, covered with silica gel Kisegel 60 F$_{254}$ were used. The mobile phases were used:

A. benzene – acetone – 25% ammonia (3:25:3);
B. 2-propanol – toluene – 25% ammonia (3:2:1);
C. 1,4-dioxan – xylene – toluene – 2-propanol – 25% ammonia (1:2:1:4:2). The traces were set with a quartz lamp working at λ=254 nm.

Differential scanning calorimetry (DSC)

The measurements were performed using a Netzsch model 204 Phoenix instrument. The samples of about 3–5 mg were sealed in aluminium cells with pierced lids. The measurement were performed in helium atmosphere in temperatures from 20 to 300°C at a scanning rate of 5°C min^{-1}. The results were processed using TA (Netzsch) program. For the determination of the enthalpy values of the representative phase transitions, linear or tangent-sigmoidal baselines were used.

Results and discussion

Four selected derivatives of imidazoline in the form of solid-state hydrochlorides have been subjected to e-beam irradiation in the doses of 25, 50, 100 and 200 kGy.

The first noted effect of irradiation was a change in colour of three of them: XM, AN and TM. XM changes its colour from white to pale yellow already upon irradiation with a dose of 25 kGy, and the intensity of the yellow colour increases with increasing dose of irradiation. Upon irradiation with doses up to 100 kGy AN and TM did not change colour and remained white, after irradiation with 100 kGy AN became pale pink and TM became pale yellow. No changes in colour were observed for the fourth derivative that is NN, even upon irradiation with a dose of 200 kGy.

No changes attributable to irradiation were observed in the phase, smell, solubility and clarity of water solutions of all four irradiated compounds.

As evidenced by the SEM photographs (Fig. 1), the compounds studied in solid phase occur in crystallites of different shape and size. NN and AN occur in the form of the best developed and largest crystals, XM and TM appear as poorer developed and more refined crystals. A comparison of the SEM photos taken of the initial samples and those
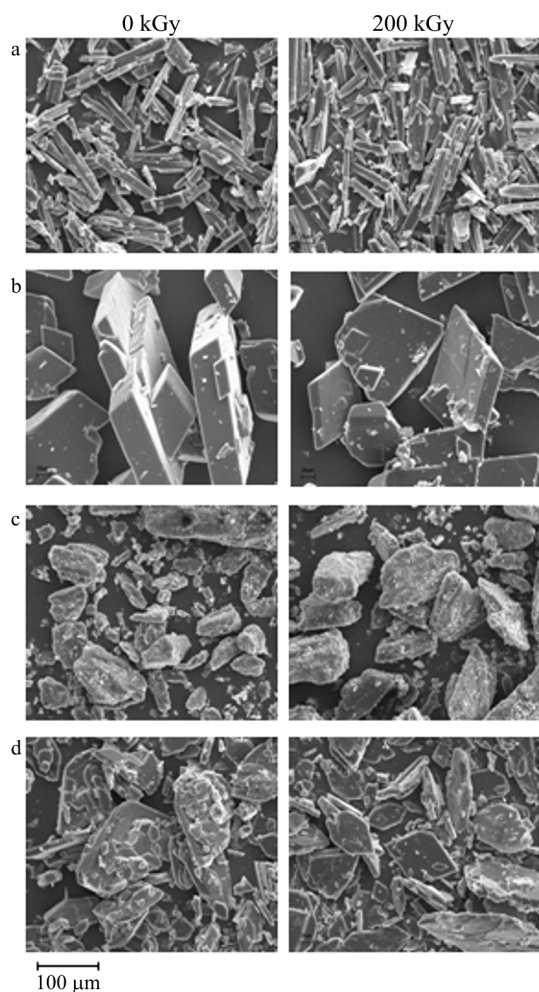
0 kGy   200 kGy

Fig. 1 SEM photographs of the compounds examined before and after irradiation (200 kGy): a – antazoline, b – naphazoline, c – tymazoline, d – xylometazoline

irradiated with a dose of 200 kGy, revealed no changes induced by irradiation.

To check the effect of irradiation on the crystal structure of the derivatives studied, they were subjected to XRD analysis. The XRD patterns were recorded for the initial substances and for the same substances exposed to irradiation with a dose of 200 kGy (Fig. 2). The general character of the patterns remains unchanged. Small changes in the intensity of certain peaks could be observed for AN and XM, while slightly more profound changes for TM and NN. The XRD pattern of irradiated NN also revealed a change in the shape of one peak (Fig. 2).

As the changes appear only as a decrease or increase in intensity of single peaks it is reasonable to conclude that ionising radiation has only small effect on the crystalline structure of the compounds studied.

At the next step of the study, the compounds before and after irradiation with a dose of 100 kGy were subjected to FTIR measurements. The character
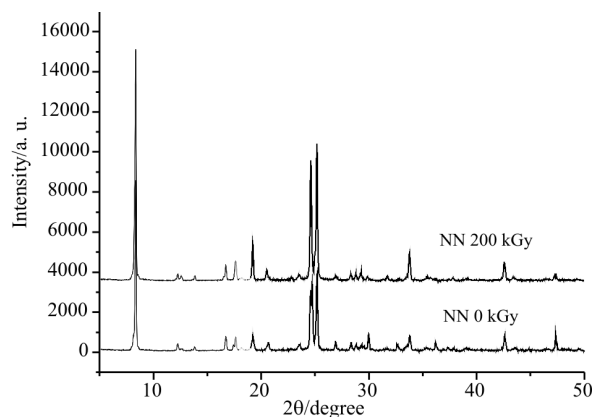

Fig. 2 X-ray powder diffraction patterns of naphazoline before and after irradiation

of the FTIR spectra of the compounds studied, recorded before and after irradiation, in the range 4000 to 400 cm^{-1}, was unchanged, that is no significant changes manifested as shifts, changes in the shape or intensity of peaks were observed. Small changes were observed in the spectrum of naphazoline in the range 2800–3000 cm^{-1} and in that of TM in the same range and additionally in the range 400–1000 cm^{-1}. They could be a result of changes in the position of the methylene bridge and thus in the position of the imidazoline ring with respect to the plane of the other rings (2800–3000 cm^{-1}) and in the mutual position of the benzene ring substituents (1000–500 cm^{-1}) in TM.

In order to explain the character of changes taking place in the process of radiodegradation, the substances studied were subjected to electron paramagnetic resonance (EPR) measurements. The EPR spectra showed no signals from the non-irradiated crystalline imidazoline derivatives. Figure 3 shows exemplary EPR spectra of AN, NN, TM and XM taken immediately after their irradiation with a dose of 25 kGy. The EPR spectra were measured immediately after irradiation and subsequently several times over a period of six months. Changes over time were observed in the line intensities and shapes. The stability of the radicals in the irradiated imizadoline derivatives samples was also studied. The signal intensity decay data obtained for a sample irradiated at the dose 25 kGy were used to get the decay characteristics of the radicals. In AN, NN, TM and XM upon absorption of 25 kGy, the amounts of $7.23 \cdot 10^{14}$, $1.43 \cdot 10^{14}$, $2.00 \cdot 10^{14}$ and $61.81 \cdot 10^{14}$ spins per gram were generated, respectively, as measured directly after irradiation.

To find out whether the presence of free radicals could lead to subsequent reactions and appearance of radiolysis products the thin layer chromatography (TLC) was applied. Three products of radiolysis were

detected in irradiated XM (R_f=0.14, R_f=0.57, R_f=0.90, phase A) and one product in irradiated AN (R_f=0.92, phase A). In irradiated NN and TM no radiolysis products were detected even after their irradiation with a dose of 200 kGy.

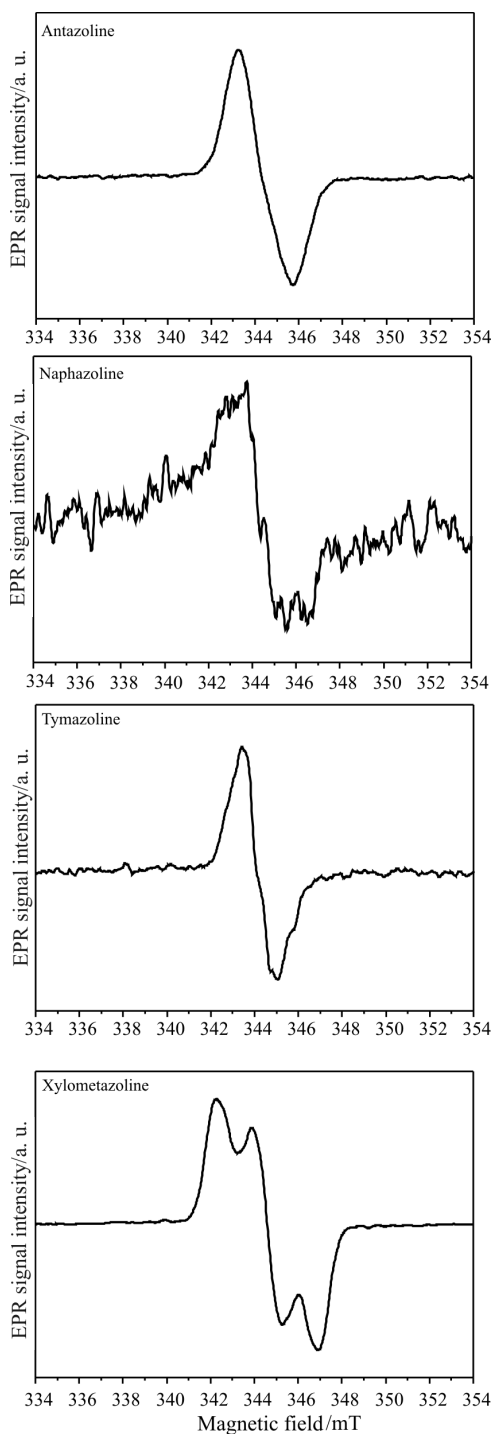The results obtained for AN and XM are consistent with those of organoleptic analysis, assuming that the changes in colour can be related to the appearance of the radiolysis products. However, no such correlation was found for TM, which becomes yellow as a result of irradiation but no products of its radiolysis were detected by the TLC method.

At this stage of our investigation the compounds studied were subjected to differential scanning calorimetry. This very sensitive method was applied to confirm no radiolysis of TM, if yes the DSC spectrum of TM taken before and after irradiation should be the same and the melting point of this compound should be unchanged. The DSC spectra of TM were recorded before irradiation and after irradiation with each of the doses considered. The spectra taken after irradiation were, however, found to show a distinctly notable decrease in the melting point proportional to the dose applied: from 0.1°C for the dose of 25 kGy to the value of 3.1°C for the dose of 200 kGy (Fig. 4, Table 1). This result proves that TM undergoes radiolysis and the radiolysis products are responsible for the decrease in its melting point, but probably the TLC method is not sensitive enough to detect their presence. The DSC spectrum of irradiated NN shows insignificant changes in the melting point with respect to that in the spectrum of non-irradiated compound, which confirm that this substance is resistant to ionising irradiation and does not undergo radiolysis as evidenced by the TLC results. The DSC spectrum of XM shows an increase in its melting point, while that of AN a decrease in its melting point. A considerable increase in the melting point of irradiated XM is a phenomenon we have observed for the first time in our investigation of radiolysis of therapeutic substances [16–21]. It is supposed that this increase is related to a considerable radiolysis of this compound (three radiolysis products detected by TLC) and a high concentration of the radicals formed (the highest from among the compounds studied in this work). For antazoline an additional effect was observed.
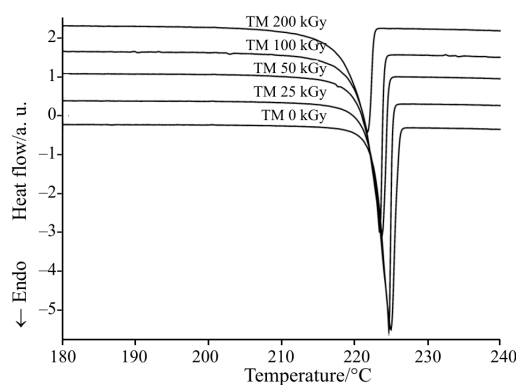

Fig. 3 EPR spectra of antazoline, naphazoline, tymazoline and xylometazoline after irradiation (25 kGy)

Fig. 4 The DSC curves obtained for tymazoline before and after irradiation

Table 1 Melting enthalpies and characteristic temperatures from DSC data

Compound	T_{peak}/°C				
	0 kGy	25 kGy	50 kGy	100 kGy	200 kGy
AN	247.3; 248.1; 249.1	247.1; 248.0	247.1	247.1	–
NN	259.7	259.3	259.4	259.9	–
TM	224.9	224.8	223.8	223.5	221.8
XM	314.1	318.7	318.1	323.3	317.5
	T_{onset}/°C				
AN	245.4	245.2	245.1	244.8	–
NN	257.5	257.5	257.4	257.9	–
TM	222.6	221.9	220.6	220.6	218.2
XN	nd	nd	–	nd	nd
	ΔH/J g^{-1}				
AN	−179.5	−174.2	−170.1	−161.8	–
NN	−165.7	−166.3	−159.1	−158.9	–
TM	−144.9	−145.3	−136.4	−140.2	−122.5
XM	nd	nd	–	nd	nd

nd – no determined

The DSC curve of the initial sample shows three maxima (T_{peak}=247.3; 248.1; 249.1°C). For the sample irradiated with a dose of 25 kGy the DSC curve had only two maxima (at 247.1 and 248.0°C), and the DSC curves of the samples irradiated with higher doses had only one maximum at about 247.1°C.

For the samples studied we also determined changes in the enthalpy of melting accompanying the irradiation. Similarly as in our earlier studies, with increasing irradiation dose the enthalpy of melting decreased. The most profound change was observed for TM for which the difference between the non-irradiated sample and the sample irradiated with a dose of 100 kGy reached 15.5%. For XM the determination of the change in the enthalpy of melting was impossible because the melting occurred together with decomposition.

Conclusions

According to the results collected in Table 2, XM is the least resistant (the most sensitive) to ionising radiation, while NN is the most resistant (the least sensitive) to it. Taking into account the amount of free radicals and the changes observed for the compounds studied by the other methods it can be reasonably supposed that these changes are related to the concentration of the free radicals, which is the highest in XM and the lowest in NN. Assuming that the concentration of free radicals can be a criterion of radiochemical stability, the compounds studied can be ordered as follows according to increasing resistance to ionising irradiation: XM<AN<TM<NN.

In conclusion, it is highly probable that the radiochemical stability of the imidazoline derivatives studied is determined mainly by their chemical

Table 2 Compilation of the results obtained after irradiation the imidazoline derivatives

Compound	Analytical results						
	SEM	IR	TLC	Organoleptic analysis	DSC	XRD	EPR
XM	–	–	+ 3 radiolysis products	white → yellow	↑ T_{peak} +	+	+
AN	–	–	+ 1 radiolysis products	white → pink	↓ T_{peak} +	+	+
TM	–	– +	–	white → yellow	↓ T_{peak} +	+	+
NN	–	– +	–	–	–	+	+

'–' no changes, '+' changes, '– +' small changes

structure, crystallinity and the concentration of free radicals formed upon irradiation.

NN characterised by the simplest structure, forming large crystals and producing the lowest concentration of free radicals on irradiation is the most radiochemically stable, while XM of the most spatially developed structure, forming fine crystallites and producing the highest concentration of free radicals is the lest radiochemically stable.

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