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THE EFFECT OF IONIZING RADIATION ON CHLORAMPHENICOL

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The effect of ionizing radiation on the physicochemical properties of chloramphenicol in solid state has been studied. The compound was e-beam irradiated with doses from the range 25–400 kGy and the possible changes were detected in the organoleptic methods (colour, form, odour, solubility and clarity), by SEM observations, X-ray, chromatography (TLC), spectrophotometry (UV, IR, EPR) and thermal (DSC) methods.

No significant changes relative to the unirradiated sample were observed as a result of irradiation with the dose of 25 kGy – a standard dose for radiation sterilization, besides free radicals generation. Higher doses were found to produce a change in colour, increase in absorbance (UV), changes in the XRD spectra and appearance of products of radiolysis. The presence of the radiolysis products was confirmed by the TLC method, indirectly by DSC showing a decrease in the melting point from 0.2 to 4.5°C and enthalpy from 3.8 to 23.3 J g^{-1}, respectively. A linear relationship was obtained between the irradiation dose (25–400 kGy) and the melting point of chloramphenicol, characterised by the correlation coefficient r=0.9968.

The EPR signal intensity increased with increasing dose of irradiation and the lifetime of the free radicals was longer than 6 months. No changes were detected in SEM and IR spectra.

As follows from our results, the DSC method is most suitable for a fast monitoring of the drugs subjected to sterilization by irradiation as it permits detection of changes occurring even on irradiation with low doses and their quantitative description.

Keywords: *drug analysis, DSC, EPR, IR, radiation sterilization, radiolysis in the solid state, SEM, TLC, UV, X-ray*

Introduction

Chloramphenicol (CHF) is an antibiotic with a wide spectrum of antibacterial activity. It was isolated for the first time from *Streptomyces venezuele* medium by Burkholder *et al.*, in 1947 [1]. Now it is chemically synthesised. Because of its high toxicity, its application in general inflammations is limited, but it is often administered for topical use (ointments, eye drops) and hence it must meet specific Pharmacopoeia demands, e.g. must be sterile.

One of the possible methods of sterilization is the ionizing irradiation based on the use of β, γ and X-ray radiation or electron beam. The method is fast and effective, however, the exposure to ionizing radiation can lead to changes in the physicochemical properties of a given therapeutic substance of ready pharmaceutical formulation and to the appearance of the products of radiolysis, which is equivalent to the loss in the active substance and can induce changes or decrease its pharmacological activity. Therefore, prior to the application of this sterilization method, each substance must be subjected to analytical tests checking if it can be sterilized in this way. The appearance of possible changes occurring as a result of irradiation is studied by the organoleptic, chemical, instrumental and biological methods. It is also important to compare the possible radiolysis products with those appearing as a result of decomposition under the activity of other factors [2].

The effect of ionizing radiation on the physicochemical properties of chloramphenicol was already studied [3–10]. The results proved that exposure to ionizing irradiation causes the appearance of radiolysis products, however, none of the authors of the hitherto published works identified all the radiolysis products and determined the safe dose of irradiation.

In the study reported the effects of irradiation were tested by the classical methods (organoleptic analysis), spectrophotometric methods (UV, IR, EPR), microscope observations (SEM), X-ray method (XRD), chromatographic (TLC) and thermal (DSC) methods. The analytical methods were chosen to minimise the need of preliminary treatment of samples studied and to perform majority of the tests in solid state, without the necessity of transforming it into liquid phase. This approach reduces the time of analyses, facilitates detection of changes in solid state and increases the precision and accuracy of results.

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Experimental

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 gamma 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, 100 or 400 kGy.

Organoleptic analysis

Before and after irradiation the compound was subjected to organoleptic analysis [11], comparing its colour (against a white background), form, odour, solubility and clarity of solution (0.005 g of the substance was dissolved in 5 mL of a properly chosen solvent) to those of the non-irradiated sample.

Scanning electron microscopy (SEM)

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

Infrared spectrophotometry (IR)

IR study was performed for non-irradiated and irradiated samples weighted in 1 mg portions of the chloramphenicol and for 300 mg of potassium bromide dried in 600°C for four hours and compressing it with Pye Unicam minipress. The spectra were recorded using a Bruker IR spectrometer in the range of 400–4000 cm^{-1} *vs.* the reference sample.

Ultraviolet spectrophotometry (UV)

Carefully weighted portions of 0.015 of chloramphenicol before and after irradiation were dissolved in 25 mL of methanol and diluted with water in portion 0.5:10 (v:v). The absorption spectra were taken for 1 cm layers and recorded using a PerkinElmer Lambda 20 in the range of 400–200 nm using water as reference.

X-ray diffractometry

The X-ray diffraction patterns were obtained in the 2θ=4–60° range for powdered samples, using the CuK_α radiation and HZG-3 powder diffractometer, controlled by IBM PC unit.

Thin layer chromatography (TLC)

Plates of the size 5.0·20.0 cm, covered with silica gel Kieselgel 60 F_{254} were used. The mobile phase was ethyl acetate and ethyl acetate – methanol (99:1, 98:2 and 95:5). The traces were set with a quartz lamp working at λ=254 nm [12].

Differential scanning calorimetry (DSC)

The measurements were performed using a DSC – 204 Netzsch 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 baseline was used.

Electron paramagnetic resonance (EPR) spectroscopy

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 spectra were doubly integrated over the magnetic field range 334–354 mT which gives a figure proportional to the number of radicals in the sample.

Results and discussion

Chloramphenicol (CHF) irradiated in solid state by 25 kGy dose remained a white fine-crystalline odourless powder, but under irradiation with higher doses its colour changed to bright yellow (100 kGy) and to deeper yellow tone on irradiation with 400 kGy. No changes in the smell, solubility or clarity of the solutions of the irradiated compound in methanol were observed. No changes were detected in the SEM pictures and IR spectra between the irradiated and non-irradiated samples (Figs 1 and 2). The compound remained crystalline with no changes in the size and shape of the crystals. Similarly, in the IR spectra no line shifts, changes in intensity or shape of the lines were noted. As to the UV spectrum, in the range of 200–400 nm (Fig. 3) no changes were observed, but at λ_{max}=266 nm an increase in absorbance was noted with increasing irradiation dose (3.3% on irradiation

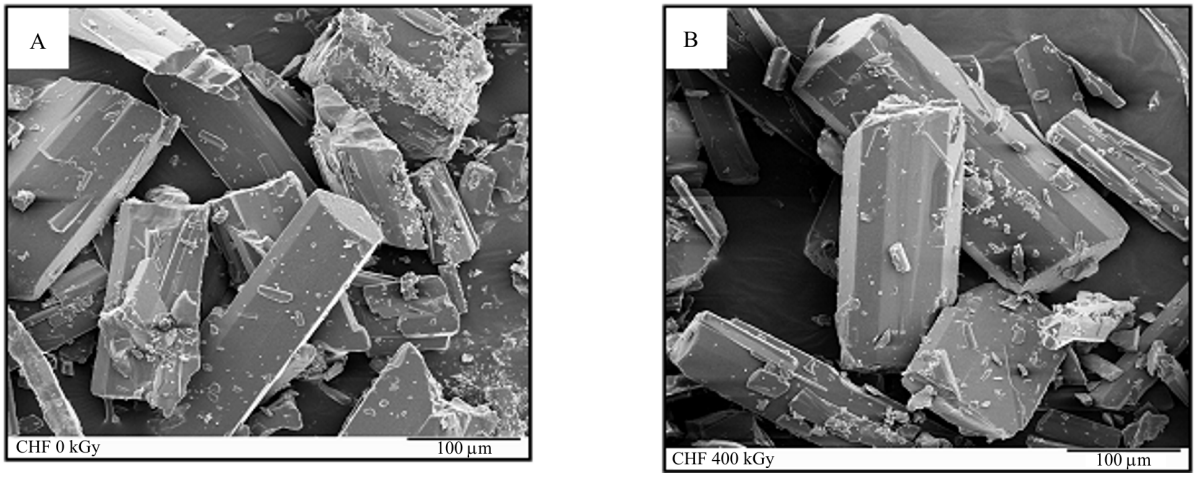

Fig. 1 SEM photographs of CHF A – before and B – after irradiation with the dose 400 kGy

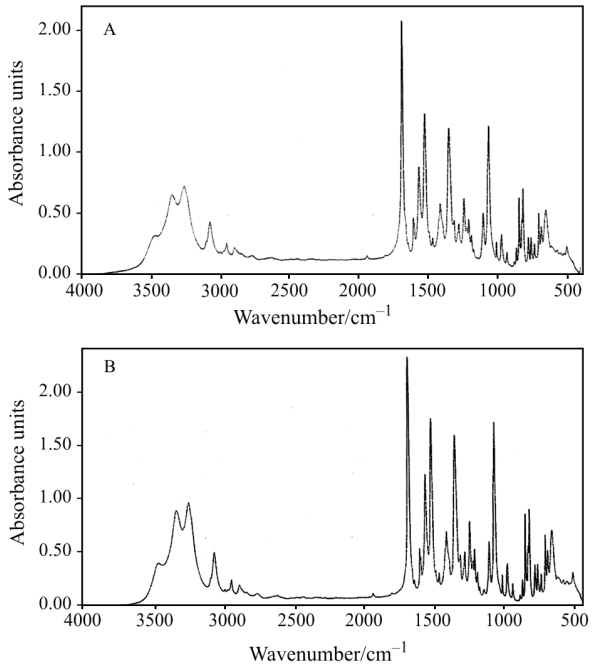

Fig. 2 IR spectra of CHF A – before and B – after irradiation with the dose 400 kGy

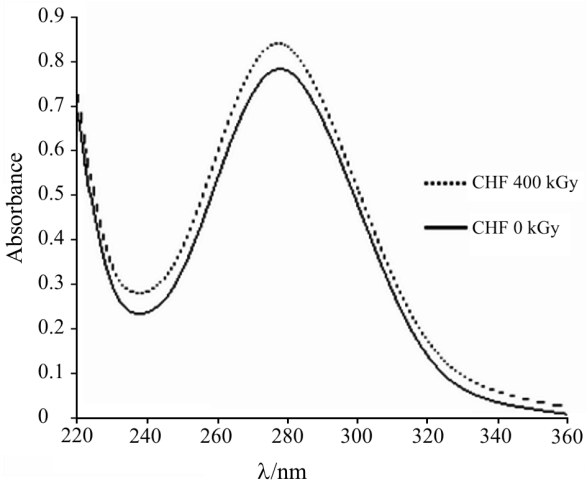

Fig. 3 UV spectrum of CHF before and after irradiation

with 400 kGy). This effect was probably caused by a combined determination of CHF and the radiolysis products formed, which means that the UV method is poorly specific and cannot be used for determination of the content but only to confirm the formation of radiolysis products.

To find the reason for the colour changes in the CHF samples irradiated with 100 and 400 kGy, the XRD method was applied. No changes in the XRD pattern were noted between the non-irradiated sample and the one irradiated with 25 kGy. After irradiation with 100 and 400 kGy, significant changes appeared in the peak intensities. These changes can indicate alterations in the crystal lattice of irradiated CHF as a probable reason for the colour changes.

Another reason for the changes in the colour of the compound irradiated with 100 and 400 kGy can be the appearance of radiolysis products or free radicals. The presence of radiolysis products was confirmed by TLC method in the compound irradiated by 100 kGy. The mobile phase used (ethyl acetate and methanol at 99:1) permitted detection of two new spots characterised by R_f 0.82 and 0.36 on the chromatogram of CHF irradiated with 100 kGy and three new spots (R_f 0.82, 0.36 and 0.47) on that of CHF irradiated with 400 kGy (Fig. 4).

At the next stage the effect of irradiation was studied by DSC method for the samples irradiated with 25, 100 and 400 kGy. The DSC curves obtained for CHF (Fig. 5) were characterised by a single sharp endothermic peak, shifting to lower temperatures with increasing dose of irradiation. The observed decrease in the melting point was accompanied by a decrease in the peak height. The temperature range of the melting process was not changed. The shift of the DSC peaks for irradiated CHF and in particular the

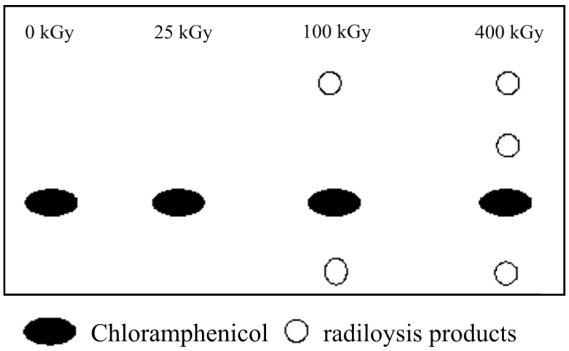

Fig. 4 TLC chromatogram before and after irradiation

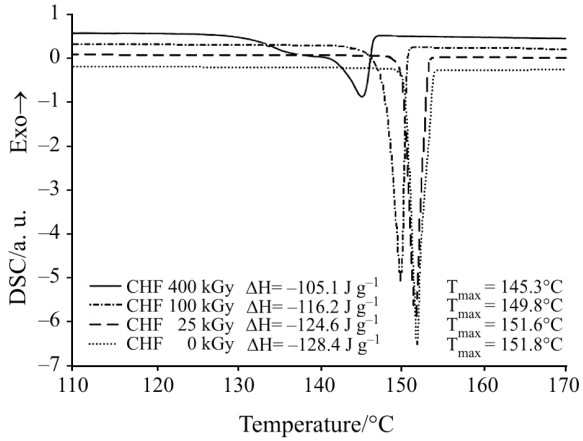

Fig. 5 DSC curves of CHF before and after irradiation

second peak appearing after irradiation with the dose 400 kGy, indicates a decrease in the purity of the compound, probably as a result of the appearance of the radiolysis products and indirectly confirms the TLC results.

For the compound irradiated with 25 kGy the difference in the maximum temperatures (T_{peak}) before and after irradiation was only 0.2°C, but it increased to 2.0 and 4.5°C for the compound irradiated with 100 and 400 kGy, respectively. Moreover, the value of melting enthalpy decreased with the dose of the compound irradiation. The greatest change >20 J g^{-1} was noted for the dose of 400 kGy. The values of the melting point and enthalpy of melting are given in Table 1. Statistical analysis of the results revealed a good correlation between the dose of irradiation and the DSC parameters: T_{peak} and the enthalpy of melting, characterised by the correlation coefficients of r=0.9968 and r=0.9959, respectively (Fig. 6). The correlations between ΔT_{peak} and the dose as well as ΔH–ΔH_0 and the dose were characterised by similarly high correlation coefficients: r=0.9988 and 0.9734, respectively. Linear relations between the DSC parameters and the dose of irradiation were already reported in [13–15] for therapeutic substances from other pharmacological and chemical groups. The decrease in the melting point and in the enthalpy of melting revealed by DSC and testifying to diminished purity of the sample, can be related to the change in the sample colour, the appearance of the radiolysis products (TLC), increased absorbance (UV) and changed in the XRD pattern. As the TLC spots corresponding to the products of radiolysis were not coloured, the change in colour could be related to the crystal lattice changes, suggested by XRD, or the appearance of free radicals. To verify the latter, the irradiated compound was subjected to EPR study. The EPR spectra recorded a few hours after irradiation of CHF with 25 kGy and 121 days later are shown in Figs 7 and 8. The signal suggests the formation of a nitric radical [10] with the unpaired electron on the nitrogen atom. 121 days after the irradiation the relative intensity of the signal decreased from $1.03 \cdot 10^9$ to $1.52 \cdot 10^8$ spin/g. On the other hand, with increasing dose of irradiation the relative intensity of the signal increased to $6.73 \cdot 10^9$ and $8.77 \cdot 10^9$ spin/g for the doses of 100 and 400 kGy, respectively. As the radicals do not change the colour of the CHF sample irradiated with 25 kGy, it is probable that the radicals are colourless. This assumption implies that the changes in colour of the irradiated CHF should be assigned to

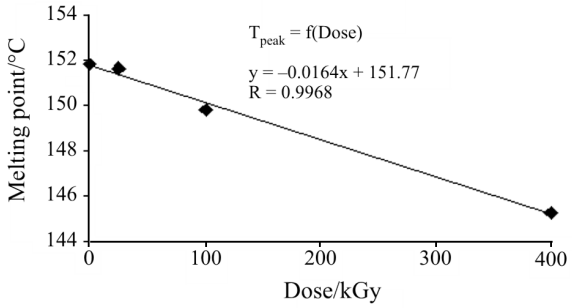

Fig. 6 Melting point of CHF *vs.* the dose of irradiation

Table 1 Melting points and enthalpy of melting of CHF determined by DSC

Dose/kGy	Melting point/°C			Enthalpy/J g^{-1}	
	Reference	T_{peak}	$T_{peak} - T_{0kGy}$	ΔH	$\Delta H - \Delta H_{0kGy}$
0	149–153	151.8	–	–128.4	–
25	–	151.6	–0.2	–124.6	3.8
100	–	149.8	–2.0	–116.2	12.2
400	–	145.3	–6.5	–105.1	23.3

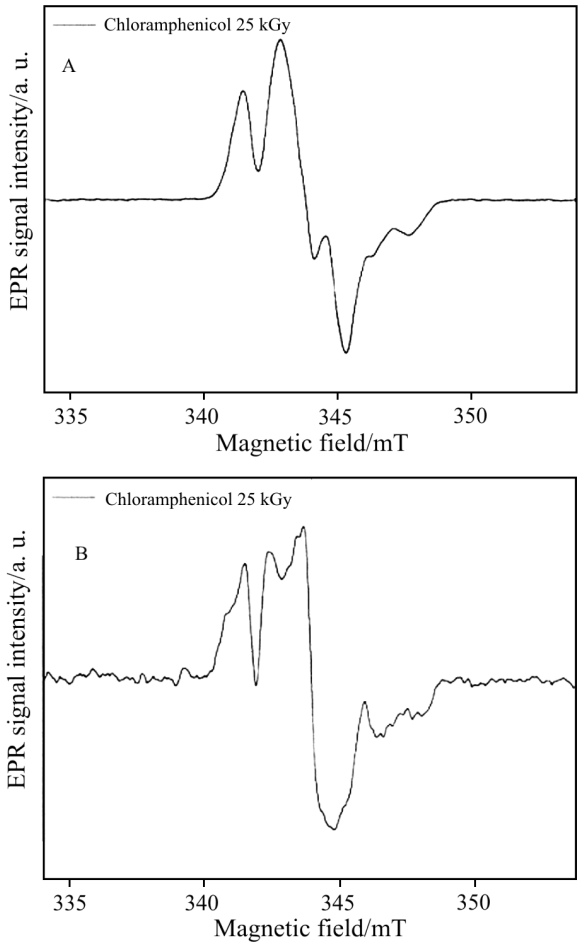

Fig. 7 EPR spectra of CHF immediately after irradiation with the dose of A – 25 kGy and B – 121 days after the irradiation

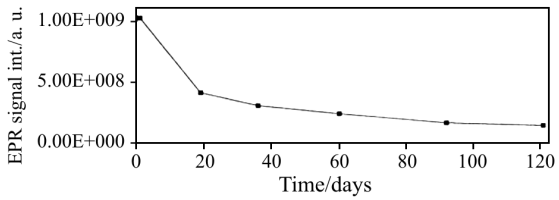

Fig. 8 Disappearance of free radicals of CHF irradiated with the dose 25 kGy

changes in the crystal lattice as suggested by the XRD data. However, the assumption of the colourless radicals should be verified. The colourless spots on the TLC chromatogram can originate from the yellow compounds as in this technique highly diluted solutions are used. Therefore, the explanation of the colour change will be a subject of further study. As to the free radicals, although their presence in therapeutic substances is not health threatening and no pharmacopoeia imposes the need for studying the coexisting reactive oxygen species in pharmaceutical products, the presence of free radicals can mask results of other measurements [16].

Analysis of the effectiveness of the instrumental methods used has shown by using IR and SEM methods it was impossible to detect any changes in the irradiated CHF samples in solid state. TLC and XRD methods permitted the detection of changes in the samples irradiated with higher doses, while UV, EPR and DSC methods were proven the most effective in detection the changes taking place in the compound studied on its irradiation with the dose of 25 kGy (Table 2). From the latter three methods DSC seems the most convenient perm,itting the monitoring of the changes in three parameters: the shape of DSC curve, melting point and melting enthalpy.

Table 2 Characterisation of different analytical methods used for investigation of irradiated CHF in solid state

Method		Dose/kGy		
		25	100	400
SEM		–	–	–
IR		–	–	–
TLC		–	2 spots	3 spots
XRD		–	+	+
UV		2.4%	2.7%	3.3%*
EPR		$1.03 \cdot 10^9$	$6.73 \cdot 10^9$	$8.77 \cdot 10^{9**}$
DSC	ΔT	0.2	2.0	6.5
	ΔH	3.8	12.2	23.3

– no changes observed, + changes observed, * increase of absorbance at λ_{max}, ** spin/g, ΔT – differences of melting point (°C), ΔH – differences of enthalpy (J g^{-1})

Conclusions

The above-discussed results indicate that the ionizing radiation is not neutral for the physicochemical properties of chloramphenicol. However, on irradiation with the dose of 25 kGy, usually applied in sterilization procedure, no significant changes in the compound properties have been noted, except the appearance of free radicals (EPR) the changes detected by the methods DSC and UV have been on the border of the error of the methods. Irradiation with the doses of 100 and 400 kGy resulted in changes in the compound colour (from white through bright yellow to deep yellow), decrease in the melting point (DSC), an increase in the absorbance (UV), the appearance of additional spots on the TLC chromatograms and changes in the XRD pattern. These changes have not been detected neither by IR spectroscopy nor SEM.

The most suitable methods for monitoring of these changes are EPR and DSC. However, by the EPR method it is not always possible to detect the presence of free radicals [15] (although the compound is known to undergo decomposition), the DSC method permits a detection and quantitative analysis

of the changes occurring upon irradiation with even low doses, i.e. it provides the values of the melting point and the enthalpy of melting.

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