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THERMAL AND SPECTROSCOPIC ANALYSIS OF FLORFENICOL IRRADIATED IN THE SOLID-STATE

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The study has been undertaken to check the effect of ionising radiation on the physical and chemical properties of florfenicol, an antibiotic of a wide range of antibacterial activity. The solid-state samples were subjected to an electron beam generated by accelerator corresponding to the doses of 25, 100 and 400 kGy, and the effect of the exposure was analysed by the methods not requiring changes in the state (with no preliminary treatment), such as SEM, DSC, FTIR, XRD, EPR and HPLC.

Florfenicol irradiated with a dose of 25 kGy has not changed the form or colour, however, a small increase in intensity of some absorption bands in the FTIR spectrum and of some peaks in the XRD pattern, a decrease in the melting point by 0.6°C, the appearance of free radicals and a loss in the FF content within the error of the method (0.91%) have been observed. After irradiation with greater doses (100 and 400 kGy) the changes have intensified, yellow discolouration appeared and the loss of FF content has increased to 6.39%. As follows from the results, the compound studied in solid-state undergoes radiolysis after e-beam irradiation in the doses ≥25 kGy, but lower doses (15–20 kGy) can be applied for its decontamination or sterilization with no adverse effect on its physico-chemical properties.

Keywords: *DSC, e-beam radiation sterilization, free radicals, FTIR, HPLC, radiochemical stability, SEM, XRD*

Introduction

Florfenicol (FF) is a new analogue of thiamphenicol and chloramphenicol used in the form of powders and injections. The structural difference between FF and thiamphenicol is the replacement of the hydroxyl group at C_1 atom in the 1,3-propanediol chain by the fluorine atom. This replacement has resulted in endowing FF with a much greater antibacterial activity than that of its analogues and with an additional effectiveness in treatment of inflammations caused by pathogens resistant to chloramphenicol, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salomonella typhimurium* or *Proteus vulgaris* [1, 2].

To be used in injections FF must satisfy the requirements of microbiological purity applied to parental drugs. The European Pharmacopoeia Ph. Eur. 5.0 [3] recommends a few methods of drug sterilization, among them the radiation sterilization. In this method is based on the bactericidal properties of ionising radiation. The radiation produces lethal changes in micro-organisms but on the other hand, it can induce damaging changes in the medical therapeutic substance by generation of free radicals, damage to the crystalline lattice or appearance of radiolysis products [4–6].

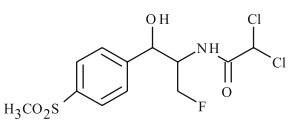
The aim of this study was to check the possibility of applying radiation sterilization to FF, so to establish the radiochemical stability of FF. The methods used to evaluate the effect of radiation were: DSC, EPR, XRD, FTIR and SEM and HPLC for determination of the content of the compound after irradiation.

Experimental

Materials

Characterization of the investigated FF, in the form of white, loose powder, obtained from Sigma-Aldrich (serial number T0216) is presented in Table 1.

Table 1 Characterisation of florfenicol (FF)

Chemical name	N-[1-(Fluoromethyl)-2-hydroxy-2-(4-(methylsulfonyl)phenyl)-ethyl]-2,2-dichloroacetamide
Chemical structure	
Molecular formula	$C_{12}H_{14}Cl_2FNO_4S$
Molar mass/g mol^{-1}	358.21
Melting point/°C	152–154

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Methods

Exposure to irradiation

Approximately 0.1 g of FF was placed in a colourless glass vial of 5 mL in 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 had absorbed a dose of 25, 100 and 400 kGy.

Organoleptic analysis

Before and after irradiation the compound was subjected to organoleptic analysis comparing its colour against a white background, and observations of its 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 made using a SEM 515 (Philips) electron microscope with 14 mm working distance and 3–10 kV accelerating voltage.

Fourier transforms infrared spectroscopy (FTIR)

FTIR study was performed for non-irradiated and irradiated samples weighed in 1 mg portions of the FF and for 300 mg of potassium bromide dried in 600°C for 4 h 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} against the reference sample.

X-ray diffractometry (XRD)

The X-ray diffraction patterns were obtained in the 2θ=5–50° range for powdered samples, using the CuK_α radiation and HZG-3 powder diffractometer, controlled by IBM PC unit.

Differential scanning calorimetry (DSC)

The measurements were performed using a DSC Haas XP-10 instrument. The samples of about 3–5 mg were sealed in aluminium cells with pierced lids. The measurement was 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, 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 the accuracy of 0.001 mT. Microwave frequency was measured to the accuracy of 0.001 GHz. The spectra were double integrated over the magnetic field range 334–354 mT, which gives a figure proportional to the number of radicals in the sample.

High performance liquid chromatography (HPLC)

The HPLC system consisted of a Waters Model 616 solvent pump system equipped with a Photodiode Array UV-Vis Waters 996 detector set at 225 nm (corresponding to λ_{max} of FF). Chromatographic separation was performed with a Waters Symmetry C18 reverse phase column (3.9 mm·250 mm, 2.5 μm particle size). Two different eluents were employed: phosphate buffer-acetonitrile 90:10 *v*/*v* (phase A), and acetonitrile (phase B). Gradient elution with phase A and phase B was made at a flow rate of 1 mL min^{-1}. The following gradient program was used: 100% phase A–0% phase B for 11 min, changing to 80% phase A–20% phase B over 10 min. The separation was conducted at room temperature. The precision of the HPLC method was characterised by relative standard deviation of 2.00%. The quantification limit was 0.61 mg L^{-1} and the limit of detection was 0.22 mg L^{-1} [7].

Results and discussion

The antibiotic studied (FF) in the solid-state was irradiated with a beam of electrons of 9.96 MeV in the doses of 25, 100 and 400 kGy. According to the European Norm EN 552 [8], sterilization of medical therapeutic substances is performed with the dose of 25 kGy, the 4 and 16 times greater doses were applied to help detecting even the smallest changes related to the irradiation.

As follows from the organoleptic analysis, FF irradiated with the dose of 25 kGy remained white powder, while after irradiation with 100 kGy the colour of the powder changed into pale yellow which deepened into yellow after irradiation with 400 kGy. Such parameters as the form, smell, solubility and clarity of methanol solutions were found to remain unchanged after irradiation with all doses studied. No significant changes were also detected in the FTIR

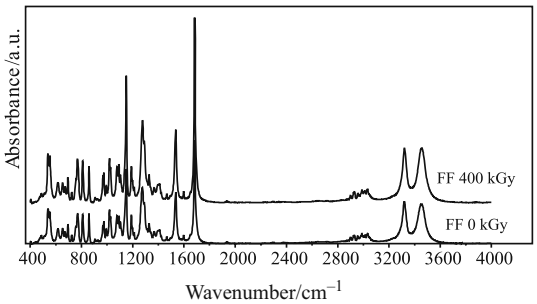

Fig. 1 FTIR spectra of florfenicol (FF) before and after irradiation with the dose 400 kGy

spectrum recorded after the irradiation with 400 kGy relative to that of the non-irradiated sample, only the intensities of some peaks were slightly changed, Fig. 1. The greatest change in intensity was observed for the peak at 1685 cm^{-1}, suggesting either a formation of an intermediate product or a radiolysis product with an additional C=O type chromophore (oxidation of the secondary alcohol group at C_1 carbon of the side chain) or the location of the free radical charge causing an increase in absorbancy at this wavelength.

The discolouration of the irradiated FF can appear as a result of formation of a colourful product of radiolysis, damage to the crystal lattice or generation of colourful free radicals. To verify the first hypothesis the DSC study was made as it is known that the presence of contaminants in the form of the products of decomposition (including those of radiolysis) [9–18] leads to a decrease in the melting point of a compound studied. The DSC curves obtained are presented in Fig. 2. For the non-irradiated compound the DSC curve showed two endothermic peaks at 151.6 and 155.9°C, corresponding to the melting point, and two broad exothermic peaks at about 250°C, corresponding to the thermal decomposition of FF. The obtained values of T_{onset}, T_{peak} and ΔH of the phase transition are given in Table 2. With increasing dose of irradiation the melting point decreased. The changes were linear (Fig. 3) and described by high correlation coefficients: for T_{onset} as a function of the dose and T_{peak} as a function of the dose the coefficients were r=0.9946 and 0.9996, while for ΔH as a function of the dose r=0.9684. Changes in the exothermic peaks were also noted; as a result of irradiation with 100 and 400 kGy, the first peak at about 242°C became sharper and more intense, while the peak at 250°C got lowered. With increasing dose of irradiation the two exothermic peaks appeared at lowered temperatures. The DSC results indicate that the ionising radiation causes decomposition of the compound studied and the radiolysis product (or products) are responsible for the changes in DSC curves and can be responsible for the drug discolouration.

Another probable reason for discolouration can be damaged to the crystal lattice. This possibility was

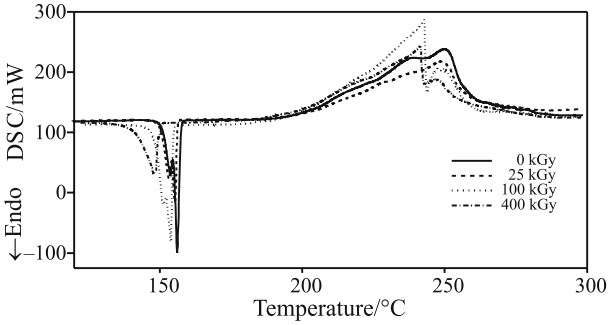

Fig. 2 DSC curves of florfenicol (FF) before and after irradiation

Table 2 Melting points and values melting enthalpy of florfenicol (FF) determined by DSC before and after irradiation with the doses 25, 100 and 400 kGy

Dose/kGy	0	25	100	400
T_{onset}/°C	150.7	149.4	146.9	139.9
T_{peak}/°C	155.9	155.3	153.7	148.0
ΔH/J g^{-1}	232.7	225.8	210.8	188.3

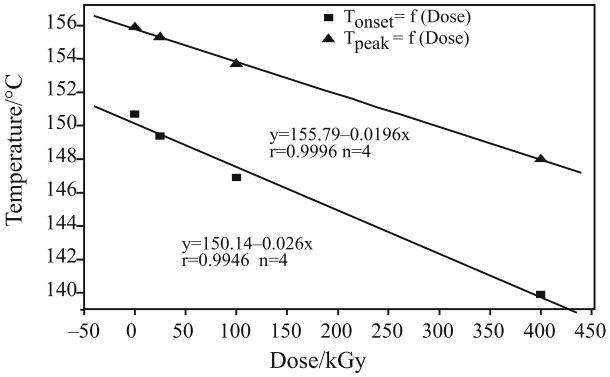

Fig. 3 Melting point of florfenicol (FF) *vs.* the dose of irradiation

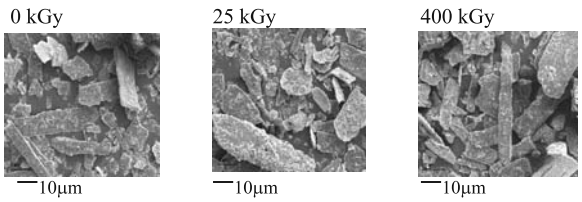

Fig. 4 SEM microphotographs of florfenicol (FF) before and after irradiation

tested on the basis of the SEM photographs taken before and after irradiation (Fig. 4, Table 3). The differences in the morphology and appearance of the powder grains were insignificant and the absolute differences in refinement were of the order of 0.5–5.0%. The relative differences indicated a decrease in the contribution of the smallest size particles (0–150 μm) at the expense of increasing contribution of the largest particles (350–500 μm). This phenomenon can be re-

Table 3 Particle size distribution (%) of florfenicol (FF) determined from SEM micrographs

Particle size/μm	Content/%		Differences/%	
	0 kGy	400 kGy	Absolute*	Relative**
0–49	21.7	22.2	0.5	2.3
50–99	26.3	21.2	5.1	19.3
100–149	21.3	24.1	2.8	13.1
150–199	12.3	16.3	4.0	32.5
200–249	7.7	7.0	0.7	9.1
250–299	4.7	4.2	0.5	10.6
300–349	2.5	3.1	0.6	24.0
350–399	1.4	0.8	0.6	42.8
400–449	0.8	0.4	0.4	50.0
450–500	1.3	0.5	0.8	61.5

*Absolute differences/%=content$_{0\ kGy}$–content$_{400\ kGy}$
**Relative differences/%=absolute differences·100%/content$_{0\ kGy}$

lated to aggregation of particles as a result of the free radicals appearance.

The EPR spectrum (Fig. 5) of irradiated FF revealed the hyperfine structure, whereas in the non-irradiated samples no free radicals were detected. The signal recorded after irradiation with 25 kGy was intense and the number of unpaired electron spins per a gram of the substance studied was $1.83 \cdot 10^{15}$. The colour of the sample did not change, which may indicate that the radicals formed are colourless and cannot cause discolouration of the compound studied.

The effect of irradiation on the lattice was also checked by X-ray diffraction study (XRD). The powder XRD patterns of the non-irradiated FF and the drug irradiated with the doses of 25, 100 and 400 kGy were compared. Small differences were noted in the 2θ range 5–50° (Fig. 6). For the drug irradiated with 25 kGy, the XRD pattern showed a slight increase in intensity of a few peaks and disappearance of peak of low intensity at about 20°, along with some changes

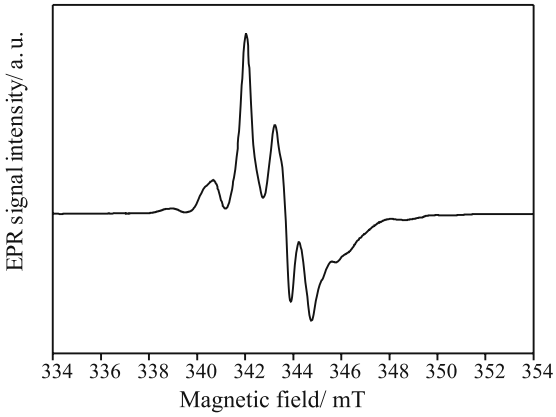

Fig. 5 EPR spectra of florfenicol (FF) immediately after irradiation with the dose of 25 kGy

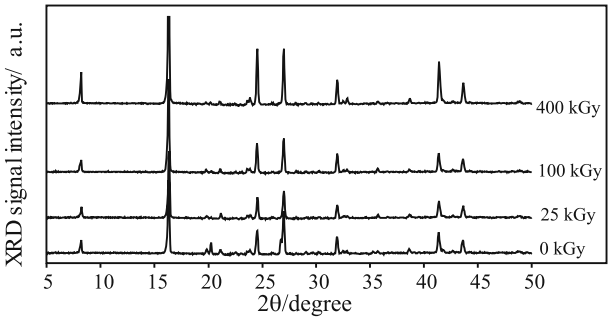

Fig. 6 XRD spectra of florfenicol (FF) before and after irradiation

in the character at about 27°. After irradiation with the dose of 100 kGy, a significant increase in the intensity of the peak at ~16° was observed, while after the irradiation with the dose of 400 kGy this increase was almost twofold. The changes in the crystal refinement observed in SEM images and in the course of the XRD patterns are significant enough to conclude that under the effect of ionising radiation, in particular in the doses >25 kGy, the crystal lattice of FF changes.

In view of the above results, the discolouration of FF caused by irradiation with doses >25 kGy in

Table 4 Characterization of irradiated florfenicol (FF) and its analogues [7, 9, 10]

Compound	Dose/ kGy	Colour	T_{peak}–$T_{peak\ 0\ kGy}$/ °C	EPR/ spin g^{-1}	Amount changes by HPLC/%
Florfenicol	25	white	–0.6	$1.83 \cdot 10^{15}$	–0.91
	100	pale-yellow	–2.2	nd	–1.98
	400	yellow	–7.9	nd	–6.39
Thiamphenicol	25	white	–0.5	$5.80 \cdot 10^{15}$	–0.81
	100	pale-salmon	–1.7	nd	–2.03
	400	salmon	–4.0	nd	–10.45
Chloramphenicol	25	white	–0.2	$1.03 \cdot 10^{9}$	–0.85
	100	yellow	–2.0	$6.73 \cdot 10^{9}$	–3.50
	400	yellow	–6.5	$8.77 \cdot 10^{9}$	–11.60

solid-state can be a result of free radicals captured in crystal lattice defects [19, 20] or a colour product of radiolysis.

In order to check if the changes observed may also lead to the loss of FF content, the HPLC analysis was made. In the non-irradiated drug the content of FF was 99.00%, after irradiation with 25 kGy the loss of FF content was 0.91%, accompanied by the appearance of three new peaks, after irradiation with 400 kGy observed nine peaks of radiolysis products and the initial content of FF was reduced by 6.39% (Table 4). As follows from the above results, the discolouration of the irradiated FF is caused by a product of radiolysis, which will be confirmed by the HPLC-MS study [7]. However, already at this stage of the analysis we can conclude that the radiochemical stability of FF is greater than those of the earlier studied chloramphenicol and thiamphenicol [9, 10].

It is therefore reasonable to conclude that irradiation with a dose of 25 kGy, which is a standard dose used for sterilization, or with lower doses 15–20 kGy, can be used for sterilization of FF, with no risk that the content of FF will decrease by a value greater than the error of the method of its determination, of course on condition that the radiolysis products do not prove to be toxic, which will be checked in further study.

Conclusions

The most effective methods in investigation of FF radiolysis, providing quantitative results, proved to be DSC, HPLC and EPR, whereas the qualitative data on the process can be also obtained by FTIR, SEM and XRD.

The irradiation of FF with ionising radiation in the doses >25 kGy results in discolouration, changes in the FTIR spectra and XRD patterns, the appearance of free radicals and loss of FF content. After irradiation with the dose of 25 kGy, the above described changes are very small so it can be expected that it will be possible to use the doses <25 kGy for decontamination and sterilisation of FF without changes in its physical and chemical properties.

A comparison of the above discussed results with those obtained for the radiochemical stability of chloramphenicol and thiamphenicol [9, 10] shows that the radiochemical stability of FF is greater than that of its above-mentioned analogues.

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