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Radiostability of pharmaceuticals under different irradiation conditions ☆

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Abstract

In this paper, the products studied are cefazolin, glucagon and dobutamine HCl. The radioresistance of pharmaceuticals may depend on the conditions of irradiation. The best is to irradiate the drugs in solid state and the chemical transformations can be reduced also by lowering the temperature of the liquid. In solid state, the dose rate has no influence on the decomposition for the selected molecules and it should be noted that drug excipients selected for bioavailability reasons are not always radioprotectors. These results are important from a technical point of view in pharmaceutical industry.

Keywords: Gamma irradiation; Electron-beam irradiation; Radiosensitivity; Cefazolin; Glucagon; Dobutamine HCl; Pharmaceuticals; Radiosterilization

1. Introduction

Gamma or electron (*e*)-beam radiation processing is an attractive, alternative method for sterilization. It will play an increasingly important role in the terminal sterilization of pharmaceutical products. The advantages of sterilization by ionizing radiation include high penetrating power for X- and γ-rays and high intensity for electron beams, low measurable residues, small temperature rise and fewer variables to control. Thus, sterilization can be carried out on the final packaged product and is applicable to heat-sensitive drugs (Reid, 1995).

Several studies on the effects of ionizing radiation in pharmaceutical systems have been performed (Schüttler and Bögl, 1993, 1994; Barbarin et al., 2001; Horsch et al., 2001; Kempner, 2001). There is relatively little information available concerning the difference in the radiolysis of drugs between irradiation by γ-rays compared to electrons, as well as the influence of the conditions of irradiation (Horsch et al., 2001).

The present investigation is aimed at studying the influence on the degradation of the drug of different parameters, such as radiation quality or dose rate (γ-rays, *e*-beam), temperature, physical state, and drug excipients. The radioinduced degradation of the drug was investigated by HPLC for purity determination and by visible spectroscopy for the color change. The purpose is to contribute to the description of the effect of the irradiation conditions.

☆This paper was written in memoriam to Christiane Ferradini.

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2. Materials and methods

Cefazolin, glucagon, and dobutamine HCl and their correspondent drug products Kefzol®, Glucagon® for injection and Dobutrex® solution were manufactured, sealed and supplied by Eli Lilly. Their formula are presented in Fig. 1. The standard compounds were mostly solid amorphous. The commercial drugs were lyophilized except for Dobutrex® solution that was an aqueous solution.

2.1. Irradiation

Triplicate samples in sealed glass vials were irradiated in independent experiments. Different γ-rays or *e*-beam facilities were used for irradiation. The γ-rays facility used was a ^{60}Co Gammacell 220 (Griffith-Mediris, Fleurus, Belgium). The dose rate was 1 kGy/h. The e-beam facility used was a double beam linear electron accelerator (LINAC) (Mölnlycke, Waremme, Belgium). The beam power of each electron generator is about 20 kW. The accelerated electrons were delivered in pulses of 474 and 478 Hz, respectively. The dose of 25 kGy was given in a few seconds; the dose rate of the double LINAC is in an order of magnitude of 10^8 Gy min^{-1}. An internal standard, a polymethylmethacrylate (PMMA) film, was used to control the delivered dose by being irradiated with the sample. Its absorbency is measured afterwards. The dosimetry was determined by the certified irradiation centers.

2.2. High-performance liquid chromatography (HPLC)

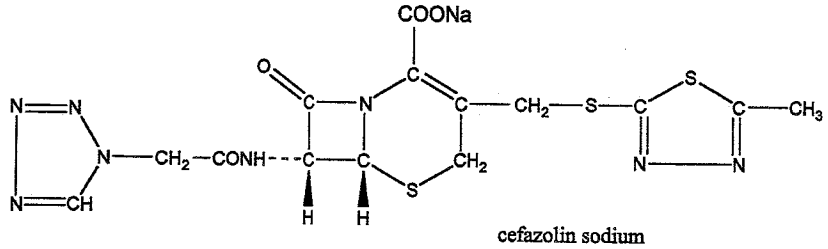
For purity determination, the following HPLC system was used: Pump Spectra Physics SP 8800; Autosampler SP 8880 with injection loop of 50 µl for glucagon and 20 µl for other molecules; Variable wavelength detector Spectra 100. Column heater: 30 °C. The analytical column, the mobile phase, the flow rate, the wavelength of the UV detection and the injected standard compounds are detailed in Table 1 for each molecule. As the concentrations of radiolysis products are very low (<ppm) (Barbarin et al., 2001), measurements of the loss of pharmaceuticals are more accurate than biological determination of the potency.

2.3. Visible spectra

The visible spectra were recorded with a UVIKON 933 spectrophotometer; water from Millipore Q was used as a reference. Samples were prepared as follows: 50 mg/ml for cefazolin sodium, 12.5 mg/ml for dobutamine HCl and 1 mg/ml for glucagon. Absorbance measurements are used to determine the discoloration and the loss of pharmaceuticals in the presence of excipients.

3. Results

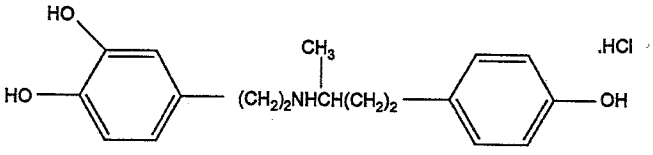
The related compounds (impurities) were not studied in this paper, only the purity of the initial compound



cefazolin sodium

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lyr-Tyr-Leu-Asp-Ser
Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr

glucagon



dobutamine HCl

Fig. 1. Formula of cefazolin sodium, glucagon and dobutamine HCl.

Table 1
Conditions for HPLC analysis

Injected standard samples	Analytical column	Mobile phase	Flow rate (ml/min)	Wavelength (nm)
Cefazolin sodium 0.5 mg/ml of mobile phase	Ultrasphere ODS 5 µm, 4.6 mm × 15 cm	Acetonitrile-triethylamine Solution (10 ml/L) adjusted at pH 2.5 with phosphoric acid (9-91)	2	220
Dobutamine HCl 0.7 mg/ml of mobile phase	Supercosil LC-18 5 µm, 4.6 mm × 25 cm	15.6 M 1-octanesulfonic acid pH 2.5-acetonitrile-methanol (60-24-16)	1	280
Glucagon 1 mg/ml of water	Zorbax 300 SB-C8 5 µm, 4.6 mm × 25 cm	Acetonitrile-water-0.2 M $NaH_2PO_4 \cdot H_2O$, 0.04 M cysteine (pH 2.6)(1-3-4)	1	214

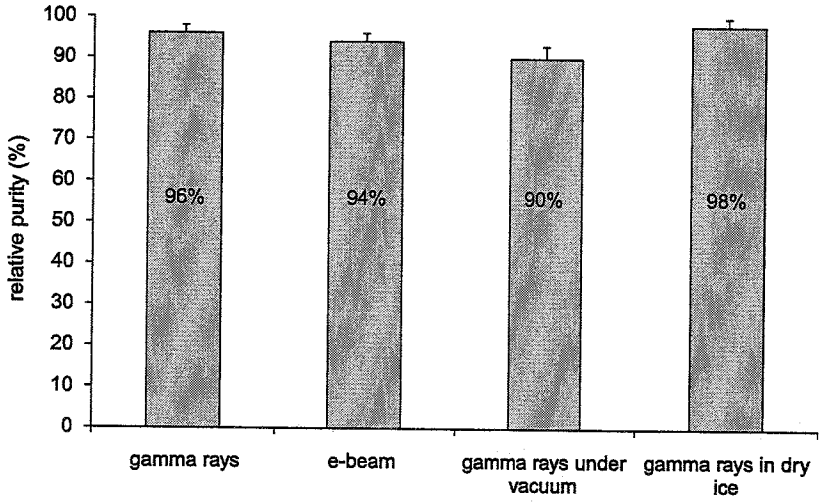


Fig. 2. Modification of the purity for Cefazolin sodium irradiated with a dose of 25 kGy.

was studied by UV spectrophotometry and by liquid chromatography.

3.1. *Influence of the irradiation conditions*

Cefazolin sodium samples were deoxygenated with a vacuum line or quenched in dry ice and were irradiated either by γ-rays or by *e*-beam. Purity was measured by liquid chromatography (Table 1) and discoloration was estimated by the absorbance at 450 nm, after an irradiation of 25 kGy. The results are shown in Figs. 2 and 3.

There was no significant difference observed when the irradiations were performed with γ-rays (dose rate about 3 $Gy\,s^{-1}$) and with *e*-beam (dose rate ca. 10 kGy/s). There was significant change when the drugs were irradiated under vacuum (free of oxygen).

The dose rate and oxygen atmosphere do not seem to play an important role in the radioinduced modifications of cefazolin. Only a lowering of the irradiation temperature reduces the yields of impurities and the yellow color change of the irradiated samples, which was still too great for medical use (European Pharmacopoeia, 1997).

3.2. *Influence of the physical state*

Dobutamine HCl was irradiated in the solid state and in an aqueous solution of 14 mg/ml. This concentration is the same as the reconstituted solution for medical use. The purity and the visible spectra were determined and the results are shown in Figs. 4 and 5.

3.3. *Influence of the drug excipients*

The radiostability of some drug compounds (glucagon and dobutamine HCl) were compared to the radiostability of their corresponding drug products (Gluca-

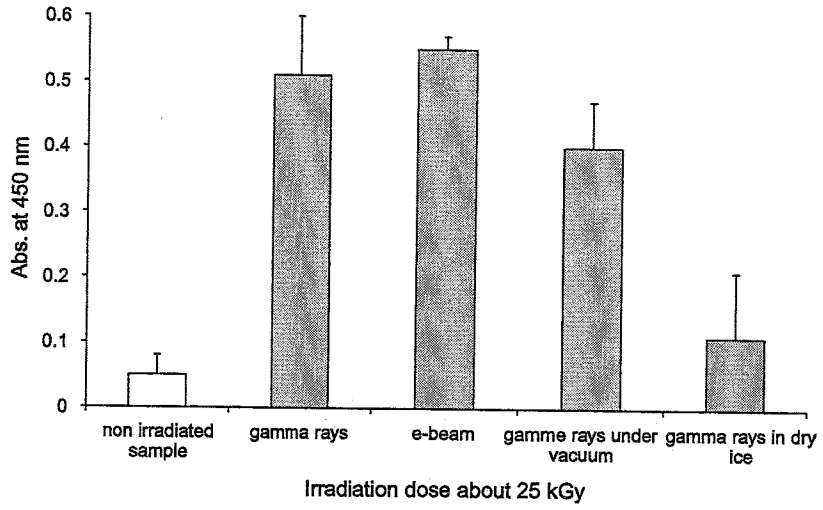


Fig. 3. Absorbance at 450 nm of Cefazolin sodium solutions non-irradiated and irradiated under different conditions.

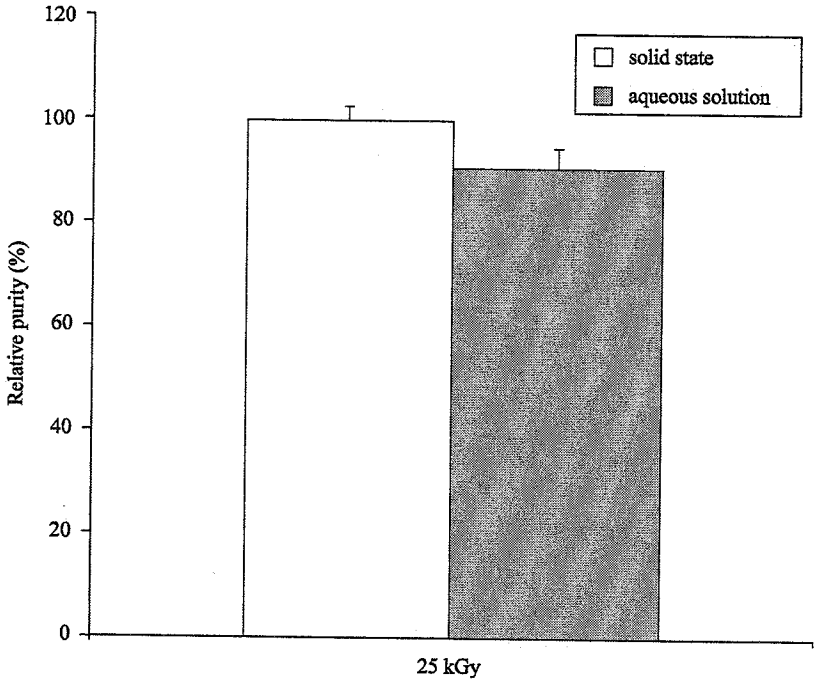


Fig. 4. Relative purity of Dobutamine HCl irradiated at 25 kGy.

gon® for injection and Dobutrex® solution). The stability was estimated by the purity and the visible spectrum after an irradiation of 25 kGy. The excipients present in the different drug products are respectively: o-lactose (98%) in Glucagon® for injection and sodium bisulfite (0.24 mg/ml) in Dobutrex® solution (dobutamine HCl 14 mg/ml). The purity results are expressed in Table 2 and the visible spectra are shown in Figs. 6 and 7.

4. Discussion

4.1. Influence of the irradiation conditions

The main difference between γ-rays and e-beam radiation is the dose rate. To obtain the same irradiation dose, gamma radiation needs several hours, whereas e-beam radiation requires only a few seconds. Therefore,

the number of tracks of ionizing particles will be different and overlapping of spurs is possible (Slegers and Tilquin, 2004). In the liquid state, the diffusion of the reactive species from the tracks may decrease with the dose rate as evidenced by the analysis of final products (Crucq et al., 2000). In the solid state, the "dose-rate" effect is low (Zeegers et al., 1997) or within the experimental error. For other drugs, in powder state, the degradation induced by e-beam irradiation was significantly smaller than by γ-irradiation (Horsch et al., 2001).

A vacuum line is used to remove air, especially oxygen, which generally amplifies the radical processes by forming peroxides. In the solid state, the radicals are trapped, and the diffusion of oxygen into the solid matrix is necessary to observe a chemical reaction. Diffusion of oxygen was observed in different vitro matrices (Tilquin, 1985, 1986), but not in amorphous or crystalline matrices. Nevertheless, the possibility of an "oxygen" amplification of the radiation chemistry is to be studied. In aqueous solution, O_2 molecules have time to migrate during gamma irradiation but not during electron irradiation.

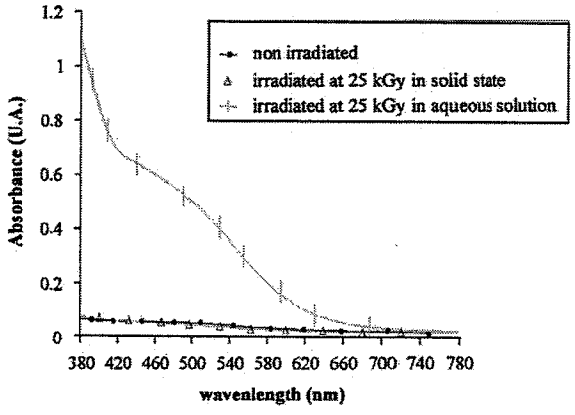


Fig. 5. UV spectra of Dobutamine HCl irradiated in solid state and in aqueous solutions.

By reducing the temperature (in dry ice), the rates of chemical reactions are reduced. At low temperatures, the radical–radical termination reactions with very low activation energy ($E_a \approx 0$) are favored instead of radical–molecule reactions ($E_a \geqslant 30$ kJ). Even the ratio of combination/dismutation of radical–radical reactions is temperature dependent (Tilquin, 1985).

4.2. Influence of the physical state

The aqueous solution under the effect of irradiation shows a significantly stronger decomposition than that of the drug in its solid form. The purity was lower after irradiation of the aqueous solution, whereas it was unaffected for the solid state. The visible spectra of the irradiated and non-irradiated solid drugs were similar, while absorbances were significantly higher when the drugs were irradiated in aqueous solutions. If, at the sterilization dose, vitamin B_{12} is destroyed in aqueous solution, the degree of decomposition was only 7% in the solid state (Juanchi et al., 2000).

The radiolysis of aqueous solutions produces reactive species (•OH, H•, e^-_{aq}, H_2O_2) that may react with the drug in the solution (Jay-Gerin and Ferradini, 2000; Ferradini and Jay-Gerin, 1999). As •OH is a strong oxidizing agent, oxidation of the drug solute generally occurs. The number of •OH radicals formed is a function of the absorbed dose; therefore, the irradiation dose determines the number of solute molecules that may be oxidized. Several simulation programs have been tested to predict the chemical change (Slegers and Tilquin, 2004).

Radiation sterilization of solid substances certainly poses fewer problems than that of aqueous solutions.

4.3. Influence of the drug excipients

The presence of excipients in Glucagon® for injection and in Dobutrex® solutions modifies the radioresistance. The lactose in glucagon samples (Glucagon® for injection) induces a significant coloration after irradiation and the presence of sodium bisulfite in dobutamine

Table 2
Influence of the excipients on the purity

Relative purity of the drug substance after an irradiation of 25 kGy (%)	Relative purity of the drug product after an irradiation of 25 kGy (%)
Glucagon	Glucagon® for injection Glucagon HCl 2%; lactose 98%
76±3	80±1
Dobutamine HCl in aqueous solution (14 mg/ml)	Dobutrex® solution Dobutamine HCl (14 mg/ml); sodium bisulfite (0.24 mg/ml)
91±4	95±2

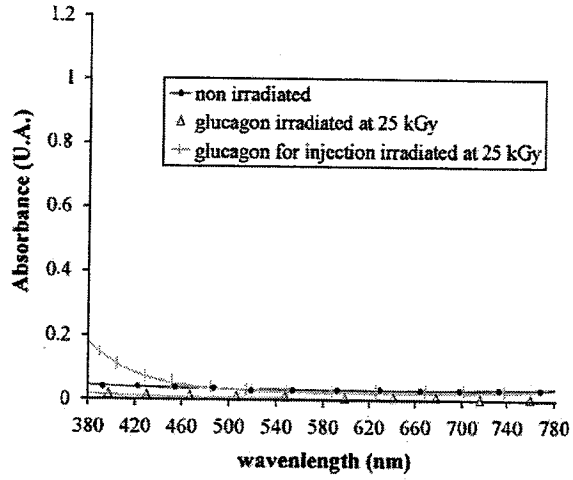


Fig. 6. UV spectra of Glucagon irradiated in solid state and in aqueous solutions.

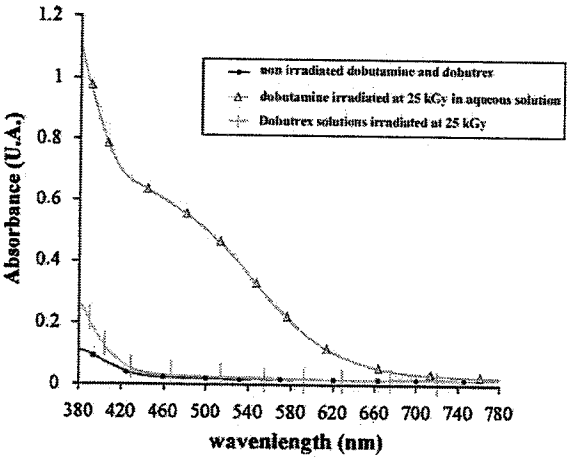


Fig. 7. UV spectra of Dobutamine HCl and Dobutrex® solutions.

samples (Dobutrex® solutions) protects the active compound.

The first question that arises concerns the quality of the mixture between the active solid drug compound and the excipients. When molecules of different compounds are mixed, an energy transfer is possible, in favor of those with low ionization potential. If the absorbed energy is divided as a function of the electron fraction between the compounds inside the microparticle, 98% lactose means that radiolysis of the lactose and not the glucagon will occur. For polymer microspheres, clonazepam had a radiostabilizing effect on the polymeric matrix. So, further work is needed (Montanari et al., 2001).

For aqueous solutions, the reactivity of the different solute molecules with the reactive species of the water radiolysis is predominant (Crucq et al., 2000), so an easily oxidizable solute may protect another solute molecule. Bisulfite is readily oxidized and can protect other solutes from the reactive species of the water radiolysis, however it protects also the microbes.

5. Conclusion

The dose rate and the oxygen atmosphere do not significantly modify the radioresistance of the studied solid drugs. The other tested parameters (irradiation temperature, physical state of the samples and the drug formulation) may play a key role in the radiosensitivity of the pharmaceuticals. These different parameters can be optimized in order to reduce degradation of the drug by ionizing radiation.

Acknowledgments

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