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Final product analysis in the e-beam and gamma radiolysis of aqueous solutions of metoprolol tartrate

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Abstract

The radiostability of metoprolol tartrate aqueous solutions and the influence of the absorbed dose (0–50 kGy), dose rate (e-beam (EB) vs. gamma (γ)) and radioprotectors (pharmaceutical excipients) are investigated by HPLC-UV analyses and through computer simulations. The use of radioprotecting excipients is more promising than an increase in the dose rate to lower the degradation of metoprolol tartrate aqueous solutions for applications such as radiosterilization. The decontamination of metoprolol tartrate from waste waters by EB processing appears highly feasible.

Keywords: Radiosterilization; Radioprotection; Drug aqueous solution; Drug degradation; Metoprolol tartrate

1. Introduction

The radiolysis of drugs in aqueous solution, with the use of high doses of irradiation, has not extensively been studied. Different parameters influence the degradation of drugs in aqueous solution, these include the conditions of irradiation: such as the choice of the ionizing radiation, the absorbed dose and the dose rate; as well as those of the drug formulation such as: the drug solute concentration and the presence of pharmaceutical excipients (Allen, 1961; Spinks and Woods, 1990). More research is needed on the radiolysis of drugs in aqueous solution because these parameters could be optimized for radioprotection or radiodestruction, depending on the application.

Two potential applications of the radiolysis of drugs in aqueous solution, at high absorbed doses, are the radiosterilization of drugs and the decontamination of nonbiodegradable drug molecules from waste waters. There is a consensus that radiosterilization is not applicable to drugs in aqueous media because of the greater degradation compared to the solid state (Boess and Bögl, 1996; Jacobs, 1995; Gopal et al., 1988). The European Agency for the Evaluation of Medicinal Products (EMEA, 2000) decision trees for the choices of sterilization methods do not even propose radiosterilization for aqueous products, whereas in the solid state it is the first choice for thermosensitive drugs. Several studies have proposed that e-beam (EB) processing could be a good method to remove nonbiodegradable pharmaceutical products from waste waters, as these are not efficiently removed by conventional waste water plant treatments (Getoff, 1996; Kimura et al., 2004; Kurucz et al., 1995).

The drug solute chosen for this study is metoprolol tartrate, whose chemical structure is in Fig. 1. Meto-

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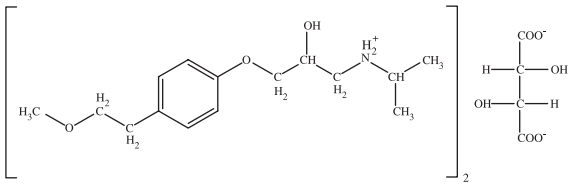


Fig. 1. Chemical structure of metoprolol tartrate.

prolol tartrate is a β-blocker that is highly soluble in water and stable in aqueous solutions. β-blockers are currently sterilized by aseptic filtration because of their heat sensitivity and therefore, an alternative sterilization method could be the exposure to ionizing radiation. β-blockers are also present in μM concentrations in waste water effluents because they are not efficiently removed by waste water plants (Huggett et al., 2003). Therefore, the degradation of β-blockers in aqueous solution by radiation processing would be worthwhile to investigate.

This work is complementary to the first study on the radical mechanisms in the radiosterilization of metoprolol tartrate aqueous solutions, which studied the reactions of the radicals from the water radiolysis with metoprolol tartrate by pulse radiolysis (Slegers et al., 2003). The hydroxyl radical was found to be the main reactive species responsible for the degradation of the metoprolol molecule. Computer simulation with a simplified radiolysis model suggested that the dose rate was the most important parameter influencing the degradation of drugs in aqueous solution (Slegers et al., 2003; Slegers and Tilquin, 2005).

In this study, the loss in metoprolol tartrate as a function of absorbed dose is quantified by HPLC-UV with a calibration curve and is compared to the results of the computer simulation program. Two different ionizing radiation sources, those of high-energy electrons and γ rays, are used in order to study the effect of the dose rate. The influence of pharmaceutical excipients on the radiolysis of metoprolol tartrate aqueous solutions is also studied. Sodium chloride is chosen because it is in the commercialized injectable drug; the other two excipients, 1,2-propanediol and mannitol, are potential radioprotectors since they are $^{\bullet}$OH scavengers. The use of radioprotectors has no effect on the destruction of microorganisms for applications such as radiosterilization, since their inactivation is essentially by the direct effect of the ionizing radiation on their genome (Tubiana et al., 1990; Nordhauser and Olson, 1998). The EB and γ radiolytic products are also quantified by HPLC-UV and their UV–VIS spectra are recorded with an HPLC-diode array detector.

2. Materials and methods

2.1. Samples for irradiation

Metoprolol tartrate aqueous solutions of $1\,mg\,ml^{-1}$ are prepared. Metoprolol tartrate with minimum 99% purity is purchased from Sigma. Pharmaceutical excipients are added in isotonic concentrations: sodium chloride 0.9% (m/v), mannitol 5% (m/v) and 1,2-propanediol 2% (v/v). Sodium chloride (European Pharmacopoeia specifications) is purchased from UCB. D-mannitol (US Pharmacopeia specifications) and 1,2-propanediol (American Chemical Society specifications) are purchased from Sigma. High-purity nitrogen, supplied by Air-Liquide®, is used to saturate the solutions prior to irradiation. Tri-distilled water is used to prepare all the solutions (Allen, 1961).

2.2. E-beam irradiations

A double-beam linear electron accelerator is used for the EB irradiations (Mölnlycke Beta Plant, Waremme, Belgium). The samples are irradiated at room temperature with the following doses: 0, 10, 15, 25 and 50 kGy. The absorbed doses are measured with ceric sulfate dosimeters (Spinks and Woods, 1990). The average dose rate is calculated as $8.9\times10^{3}\,Gy\,s^{-1}$ for the single EB and $1.8\times10^{4}\,Gy\,s^{-1}$ for the double EB. The irradiations are performed in triplicates.

2.3. Gamma irradiations

A panoramic chamber with a cobalt-60 source is used for the γ irradiations (UCL, Louvain-La-Neuve, Belgium). The samples are irradiated at room temperature with the following doses: 0, 0.5, 2.5, 5, 7.5, 10, 15, 25 and 50 kGy. The absorbed doses are measured with ceric sulfate dosimeters (Spinks and Woods, 1990). The average dose rate is $8.9\times10^{-2}\,Gy\,s^{-1}$. The irradiations are performed in triplicates.

2.4. pH measurements

The pH measurements are performed with a Hanna Instruments HI 9025 microcomputer pH-meter at 25 °C. The pH measurements are repeated twice on new samples.

2.5. Samples for the calibration curve

A stock solution of $2400\,\mu g\,ml^{-1}$ metoprolol tartrate is prepared and dilutions are performed to obtain different concentrations ranging from 4.80 to $1200\,\mu g\,ml^{-1}$. Each dilution is injected three times and the calibration curve is repeated three separate times. Pre-validation and validation studies are performed

using e-noval® and new-daily® softwares from Arlenda (Hubert et al., 2003; http://www.arlenda.be).

2.6. Samples for analysis

The samples from three separate irradiations are each injected three times. The non-irradiated samples, of known metoprolol tartrate concentration, serve as quality control standards to validate the quantification results using the new-daily® software from Arlenda (Hubert et al., 2003; http://www.arlenda.be).

2.7. HPLC-UV

The system is composed of the following Kontron Instruments: a 422 pump, a 560 autosampler with a 20 μL sample loop and a 433 Capillary UV detector fixed at 223 nm. Borwin® software version 1.21 is used to control the autosampler and for the data acquisition.

2.8. HPLC-diode-array

The system is composed of a Merck-Hitachi L-6200 Intelligent Pump, a Rheodyne® manual injector with a 20 μL sample loop and a Merck-Hitachi L-4500 UV–Visible Diode Array Detector with scans of 200–700 nm. Hitachi D-7000 Manager (HSM) software version 4.0 is used to control the HPLC components and for the data acquisition.

2.9. Chromatographic separation

A Macherey-Nagel 250 × 4 mm Nucleodur® C18 endcapped column of 5 μm particle size with an 8 × 4 mm pre-column is used. The mobile phase consists of 20% HPLC gradient grade acetonitrile and 80% aqueous solution composed of 0.025 M acetate and 0.025 M phosphate buffer adjusted to pH 2.5 with NaOH 2 M, 40 mM pentane-sulfonate ion-pairing reagent and 20 mM triethylamine. The flow-rate is 1 ml min^{-1}.

2.10. Computer simulation

Chemsimul® is used to simulate the radiolysis of metoprolol tartrate solutions. Chemsimul® solves the non-linear differential equations of the reaction rates of all the species in the irradiated solution. The simulation program is adapted to the radiolysis of aqueous solutions as it allows the input of the radiation chemical yields, the absorbed dose and the dose rate of the ionizing radiation (Kirkegaard and Bjergbakke, 1999; http://www.risoe.dk/ita/chemsimul). The 30 or more reactions of the water radiolysis used in the computer simulation program are well documented in other publications (Bjergbakke et al., 1984). The reaction rates of metoprolol tartrate with the products of the water radiolysis are published in a previous work (Slegers et al., 2003).

2.11. Statistical analysis

A statistical analysis on the loss of metoprolol tartrate as a function of the absorbed dose in the absence and presence of pharmaceutical excipients is performed using a three-way partially nested analysis of covariance model. Natural logarithmic transformations of the metoprolol tartrate concentration are used in the models. JMP version 3.1.4 is used to perform the statistical analyses.

3. Results and discussion

3.1. pH measurements

The pH of the non-irradiated metoprolol tartrate 1 mg ml^{-1} solutions varies around 7.5 because no buffer is present. The pH of the samples is lowered by a maximum of 3 units (from pH 7.5 to 4.5) for both EB and γ irradiations in the absence and presence of pharmaceutical excipients. The water radiolysis mechanism is independent of the pH (from pH 3 to 11) and therefore no influence of the pH is expected (Spinks and Woods, 1990).

3.2. Chromatograms at 223 nm

The chromatograms, at 223 nm, of non-irradiated samples are superimposed to those of samples with 12.8 kGy EB and 10.0 kGy γ irradiations, as may be seen in Fig. 2. No impurities/degradation products are detected in the non-irradiated solutions of metoprolol tartrate.

The overlay of all the chromatograms (not illustrated in the figure) shows that the loss of metoprolol, with retention time 20 min, increases with increasing absorbed doses.

A zoom on the radiolytic products, shown in Fig. 2, reveals three main products with retention times 13.1, 13.7 and 17.3 min for EB irradiations and one main product with retention time 17.3 min for γ irradiations. Many other degradation peaks are detected for both EB and γ irradiations of metoprolol tartrate solutions, as illustrated by Tables 1 and 2, respectively. This is unexpected for several reasons. First of all, past studies on drugs irradiated in aqueous solution with doses of 500 Gy, showed only one radiolytic product, anti-cefotaxime for cefotaxime (Crucq and Tilquin, 1996a), and the dehydrodimer for chloramphenicol (Zeegers and Tilquin, 1991). For the γ irradiation of metoprolol tartrate with 500 Gy, 12 radiolytic products are already

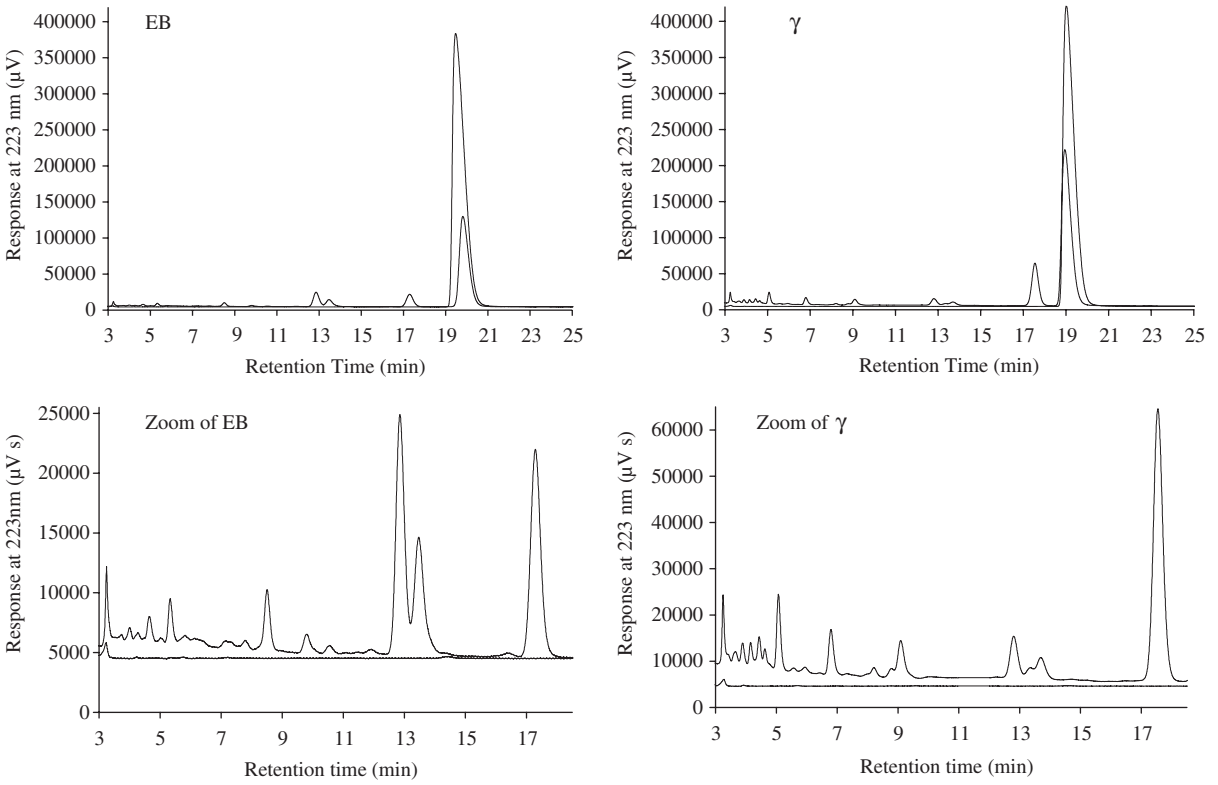


Fig. 2. Overlay of non-irradiated and irradiated chromatograms, at 223 nm, of metoprolol tartrate (1 mg ml^{-1}) solutions, saturated in nitrogen. Zoom on radiolytic products prior to metoprolol peak. EB: e-beam irradiation of 12.8 kGy. γ: gamma irradiation of 10 kGy.

detected as may be seen in Table 2. Second, only the hydroxyl radical is highly reactive towards metoprolol, with a reaction rate constant of $5.2 \times 10^9\,M^{-1}\,s^{-1}$ (Slegers et al., 2003). Metoprolol reacts slowly with the hydrated electron with a reaction rate constant of $6.8 \times 10^7\,M^{-1}\,s^{-1}$ (Slegers et al., 2003). Therefore, for the same concentration in metoprolol and not taking into account the competing reactions for hydroxyl radicals and hydrated electrons from the water radiolysis, the proportion of hydroxyl radicals to hydrated electrons reacting with metoprolol is 76–1. The hydroxyl radical is responsible for the formation of the majority of radiolytic products detected and many sites of $^{\bullet}$H-abstraction and $^{\bullet}$OH-addition are possible on the metoprolol molecule, whose chemical structure is in Fig. 1.

3.3. UV–VIS spectra

The absorption spectra, ranging from 200–700 nm, of metoprolol and the main radiolytic products with retention times 13.1, 13.7 and 17.3 min are shown in Fig. 3. The main EB and γ radiolytic products all have spectra similar to metoprolol with maxima around 223 and 275 nm. The similarities in the UV–VIS spectra and the retention times of the products eluting at 13.1 and 17.3 min suggest they are the same for γ and EB irradiations, whereas the EB radiolytic product at 13.7 min seems unique.

The spectra of all the other radiolytic products detected (not shown in the figure) are also highly similar to metoprolol's spectrum. Radiolytic products have been found to contain the same chromophore as the parent compound and to have similar structures (Barbarin and Tilquin, 2001; Crucq and Tilquin, 1996b; Barbarin et al., 2001).

The diode-array detector showed a peak purity of 1.000 for all retention times in the chromatogram for both EB and γ irradiations, even though through the method development we know some minor peaks co-elute. The UV detection does not seem the best choice to detect radiolytic products as even diode-array scans fail to discriminate between them. An on-line HPLC system with a mass detector would provide mass and spectral information, as well as differentiate between co-eluting radiolytic products (Barbarin et al., 2001; Görög et al., 1997). Therefore, an LC-MS-MS method has been developed to analyze and fragment the radiolytic products in order to elucidate their structure. These results will be presented in a separate article.

Table 1
Quantification of metoprolol tartrate and radiolytic products by the calibration curve

Retention time (min)	Absorbed dose (kGy)				
	0	13.0	15.9	27.8	56.9
3.6	.	+	+	.	.
3.8	.	+	+	.	.
4.0	.	++	+	+	+
4.3	.	+	+	+	.
4.7	.	+++	++	++	+
5.1	.	+	+	.	.
5.4	.	++++	+++	++	.
6.0	.	+	+	+	.
6.3	.	+	.	.	.
6.9	.	+	.	.	.
7.2	.	++	++	++	.
7.7	.	+	+	+	.
8.5	.	+++	+++	+++	.
9.9	.	+++	+++	++	+
10.0	.	++	.	+	.
12.2	.	+	+	+	.
13.1	.	**21.02** (±2.93)	**19.40** (±3.29)	**10.45** (±1.66)	+
13.7	.	**7.22** (±3.12)	**7.40** (±2.87)	+++++	.
17.3	.	**32.42** (±2.62)	**28.37** (±0.65)	**10.96** (±0.22)	.
Metoprolol	**1037.87** (±19.51)	**274.36** (±4.52)	**185.26** (±13.32)	**30.43** (±0.66)	.
With NaCl	**1033.78** (±15.66)	**306.63** (±39.10)	**196.93** (±8.31)	**39.84** (±8.43)	.
With 1,2-propanediol	**1055.20** (±40.91)	**951.67** (±31.77)	**909.39** (±30.96)	**838.84** (±29.12)	**715.44** (±52.18)
With mannitol	**1034.34** (±36.50)	**883.77** (±13.87)	**868.70** (±31.47)	**745.15** (±31.77)	**572.15** (±7.62)

E-beam irradiations of metoprolol tartrate ($1\,mg\,ml^{-1}$) solutions, saturated in nitrogen.
+ $0.14\,\mu g\,ml^{-1} = LOD \leqslant [product] < 1\,\mu g\,ml^{-1}$.
++ $1\,\mu g\,ml^{-1} \leqslant [product] < 2\,\mu g\,ml^{-1}$.
+++ $2\,\mu g\,ml^{-1} \leqslant [product] < 3\,\mu g\,ml^{-1}$.
++++ $3\,\mu g\,ml^{-1} \leqslant [product] < 4\,\mu g\,ml^{-1}$.
+++++ $4\,\mu g\,ml^{-1} \leqslant [product] < LOQ = 4.91\,\mu g\,ml^{-1}$.

3.4. Calibration curve

The EB and γ radiolytic products have similar structures and chromophores as metoprolol and therefore, they may be quantified by the response of metoprolol at 223 nm. The European Pharmacopoeia (Ph. Euro., 2005) recommends this method for related substances and tolerates 0.8–1.2 difference in the response factor. The International Conference on Harmonization guidelines also recommends this method for new impurities in new drug substances (ICH, 1995) and products (ICH, 1996).

A calibration curve is chosen to quantify the loss in metoprolol tartrate and the radiolytic products because of the vast range of concentrations that need to be covered, thus avoiding tedious dilutions of non-irradiated samples to obtain the same magnitude in area under the curve (AUC) as irradiated samples (The European Pharmacopoeia, 2005). A square-root linear regression, with intercept −26.57 and slope 121.5, represented the best fit for the chosen acceptation limits of 10% and the maximum risk of 5%. The limit of detection (LOD) is $0.14\,\mu g\,ml^{-1}$, the lower limit of quantification (LLOQ) is $4.91\,\mu g\,ml^{-1}$ and the upper limit of quantification (ULOQ) is $1227\,\mu g\,ml^{-1}$ of metoprolol tartrate; these are determined by the e-noval® software from Arlenda (Hubert et al., 2003; http://www.arlenda.be).

Table 2
Quantification of metoprolol tartrate and radiolytic products by the calibration curve

Retention time (min)	Absorbed dose (kGy)								
	0	0.5	2.5	5	7.5	10	15	25	50
3.6	.	+	+	+	+	+	++	+++	+++
3.8	.	.	.	.	+	+	+	+	.
4.0	.	+	++++	+++++	++	++	++	++	+
4.3	.	+	.	.	++	+	+++	**6.05** (±1.68)	**6.38** (±2.72)
4.5	.	++	+	+	+	++	.	+	.
4.7	.	++	+++	+++++	**5.75** (±1.68)	**5.58** (±0.45)	**8.62** (±1.80)	**9.11** (±0.89)	**6.05** (±2.47)
5.1	.	.	.	+	+	+	+	+	+
5.4	.	++	+++++	**8.41** (±1.64)	**8.63** (±1.55)	**10.83** (±0.46)	**10.38** (±1.52)	**9.11** (±2.86)	+++
6.0	.	.	.	+	+	+	+	+	+
6.3	.	+	+	++	++	++	++	+	.
6.9	.	.	.	.	.	.	+	+	.
7.2		++++	+++++	**10.95** (±5.40)	**8.83** (±4.11)	**10.68** (±3.38)	**11.99** (±6.50)	**10.33** (±6.45)	+++++
8.5	.	.	+	+++	+++	++++	**5.24** (±4.93)	**8.81** (±5.32)	**11.17** (±4.73)
9.5	.	++	+++	++++	+++	++	++	+	+
9.9		++	+++++	**5.06** (±1.77)	**7.11** (±2.19)	**8.91** (±0.75)	**6.30** (±2.11)	+++++	++
11.0	.	.	.	.	.	.	.	+	+
12.2	.	.	.	.	+	++	++	+++	+++
13.1		++	++++	**5.05** (±0.84)	**5.81** (±0.81)	**6.77** (±2.73)	**7.80** (±0.29)	**7.75** (±2.67)	+++
14.0			+++	++++	**6.99** (±1.86)	**7.53** (±1.07)	**7.00** (±1.70)	**4.98** (±0.97)	++
17.3		**6.31** (±0.46)	**32.14** (±2.83)	**51.22** (±15.62)	**72.49** (±18.19)	**76.67** (±11.12)	**93.26** (±28.18)	**92.26** (±24.95)	**35.05** (±12.59)
Metoprolol	**1054.41** (±48.71)	**1032.07** (±37.45)	**898.38** (±67.78)	**712.25** (±28.04)	**599.45** (±26.85)	**498.27** (±32.75)	**317.56** (±6.82)	**134.33** (±26.40)	**16.96** (±8.50)
With NaCl	**1076.07** (±30.65)	**1041.02** (±37.48)	**928.72** (±22.33)	**777.14** (±59.60)	**696.79** (±28.50)	**574.92** (±37.80)	**388.35** (±67.44)	**230.40** (±29.33)	**38.93** (±13.68)
With 1,2-Propanediol	**1050.02** (±3.70)	**1046.54** (±28.28)	**1025.86** (±13.64)	**1012.49** (±40.60)	**983.06** (±36.62)	**962.53** (±29.71)	**935.52** (±48.84)	**807.65** (±34.43)	**631.44** (±29.98)
With Mannitol	**1057.34** (±2.05)	**1036.52** (±16.54)	**1018.78** (±10.85)	**1014.03** (±21.80)	**979.12** (±8.15)	**977.54** (±13.66)	**936.03** (±7.22)	**863.70** (±11.80)	**746.98** (±18.63)

Gamma irradiations of metoprolol tartrate (1 mg ml^{-1}) solutions, saturated in nitrogen.
+ 0.14 μg ml^{-1} = LOD ≤ [product] < μg ml^{-1}.
++ 1 μg ml^{-1} ≤ [product] < 2 μg ml^{-1}.
+++ 2 μg ml^{-1} ≤ [product] < 3 μg ml^{-1}.
++++ 3 μg ml^{-1} ≤ [product] < 4 μg ml^{-1}.
+++++ 4 μg ml^{-1} ≤ [product] < LOQ = 4.91 μg ml^{-1}.

3.5. Area under the curve

Fig. 4 shows the total % area under the curve (AUC) of chromatograms obtained at 223 nm, as a function of the absorbed dose for EB and γ irradiations of metoprolol tartrate solutions. The % AUC is relative to the total AUC of the non-irradiated solutions and is further divided into that of metoprolol and the sum of the radiolytic products. No impurities/degradation products are found in the non-irradiated samples and therefore metoprolol represents 100% of the total AUC of the chromatogram.

The total % AUC decreases as a function of absorbed dose for both EB and γ irradiations. The total UV–VIS absorbance, without any chromatographic separation, also decreases with increasing absorbed doses for both

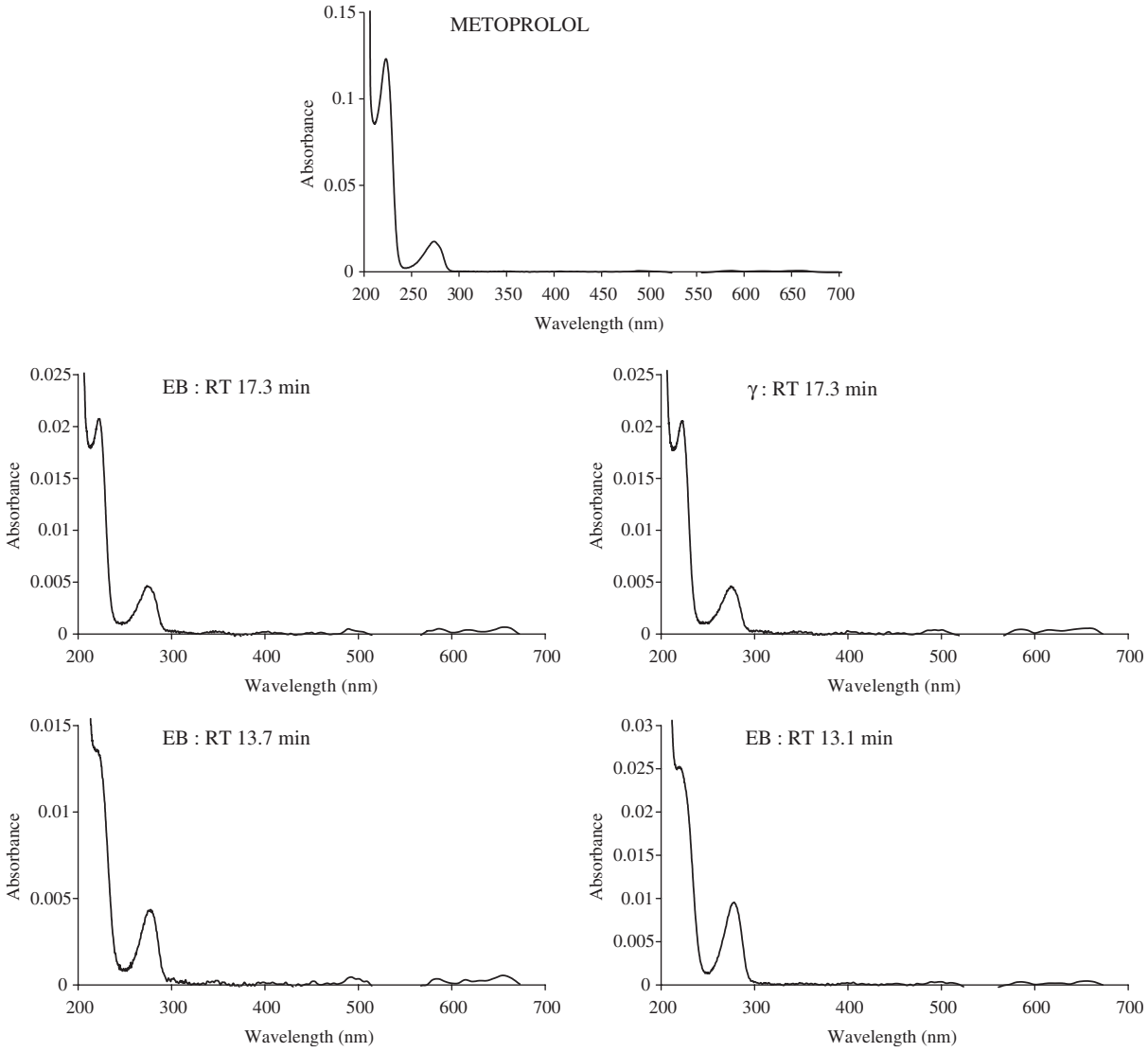
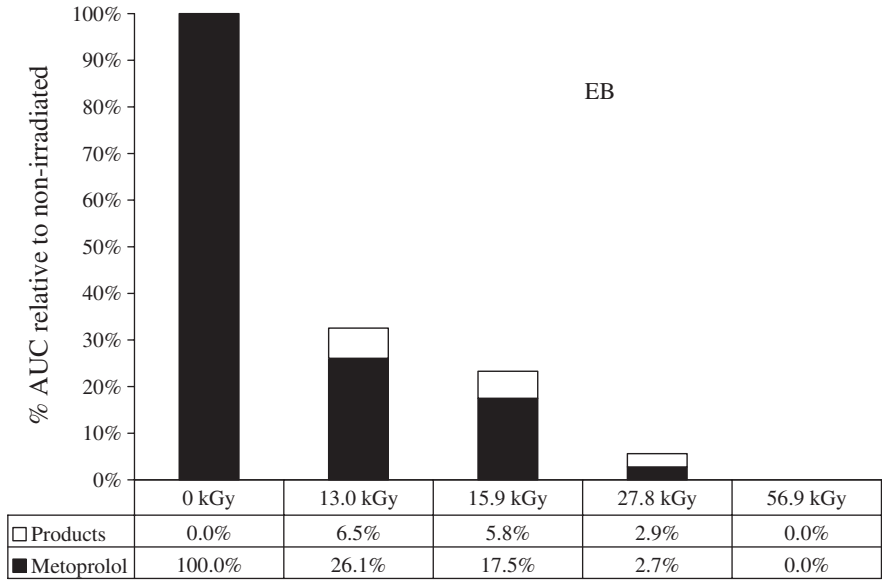


Fig. 3. DAD spectra of metoprolol with retention time 20 min and main radiolytic products with retention times 17.3, 13.7 and 13.1 min. EB: electron-beam irradiation of 12.8 kGy. γ: gamma irradiation of 10 kGy. Metoprolol tartrate (1 mg ml^{-1}) solutions, saturated in nitrogen.

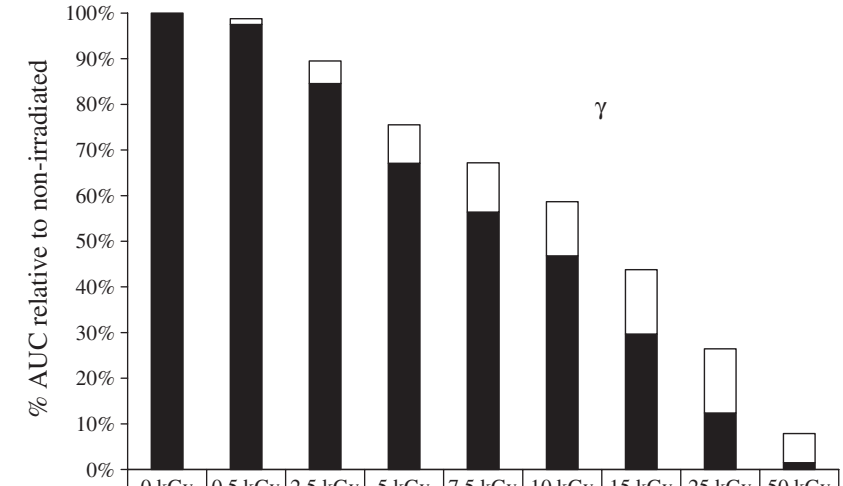
EB and γ irradiations. This suggests a loss in the chromophore by the breakage of metoprolol into smaller molecules that do not absorb at 223 nm.

The % AUC of metoprolol decreases as a function of the absorbed dose for both EB and γ irradiations. The degradation of metoprolol is faster for EB compared to γ irradiations as may be seen by comparing the % AUC of metoprolol at similar absorbed doses. At 15–25 kGy, doses needed to achieve sterility, the degradation of metoprolol is in the range of 70–80% AUC, which is too high for applications such as radiosterilization but adequate to destroy the molecule in waste waters.

The EB radiolytic products detected are degraded, since the sum of their % AUC decreases with an increase in absorbed dose, as may be seen in Fig. 4. The absorbed dose corresponding to the maximum % AUC of EB radiolytic products cannot be extrapolated since the minimum absorbed dose is 13 kGy. The minimum absorbed dose is limited by the maximum speed of the conveyor belt of the EB facility. The γ radiolytic products increase in % AUC and reach a plateau around 15–25 kGy, before decreasing with an increase in absorbed dose, they are also degraded. The degradation of the radiolytic products means they also react with the



	0 kGy	13.0 kGy	15.9 kGy	27.8 kGy	56.9 kGy
□ Products	0.0%	6.5%	5.8%	2.9%	0.0%
■ Metoprolol	100.0%	26.1%	17.5%	2.7%	0.0%



	0 kGy	0.5 kGy	2.5 kGy	5 kGy	7.5 kGy	10 kGy	15 kGy	25 kGy	50 kGy
□ Products	0.0%	1.2%	5.0%	8.4%	10.8%	11.9%	14.1%	14.0%	6.4%
■ Metoprolol	100.0%	97.5%	84.6%	67.1%	56.4%	46.8%	29.7%	12.4%	1.5%

Fig. 4. Area under the curve (AUC) in % relative to non-irradiated, at 223 nm, as a function of absorbed dose in kGy for metoprolol tartrate (1 mg ml^{-1}) solutions, saturated in nitrogen. The AUC is divided into that of metoprolol (■) and the sum of radiolytic products (□). EB: electron-beam irradiations. γ: gamma irradiations.

water radiolysis radicals, thus competing with metoprolol tartrate. The reactions of radiolytic products with the water radiolysis reactive species are favored as the concentration of metoprolol tartrate decreases with an increase in absorbed dose.

For the highest EB irradiation, the % AUC of metoprolol and its degradation products reaches 0%, whilst for the highest γ irradiation, there is still a 1.5% AUC in metoprolol and 6.4% AUC in degradation products. Since radiation-induced degradation products have similar structures, they may have similar pharmacological properties as the parent compound, as in the case of Estradiols (Kimura et al., 2004). Therefore, for the ionizing radiation treatment of non-biodegradable pharmaceutical products in waste waters, the radiolytic products should also be degraded. In these regards, EB irradiations are more suited than γ irradiations for the removal of metoprolol tartrate from waste waters.

3.6. Radiolysis of metoprolol tartrate

The loss in metoprolol tartrate is quantified in $\mu g\,ml^{-1}$ and expressed as a function of the absorbed dose in kGy, for EB and γ irradiations of $1\,mg\,ml^{-1}$ solutions, saturated in nitrogen, as may be seen in Fig. 5. The quantification results for metoprolol tartrate are in Tables 1 and 2 for EB and γ irradiations, respectively.

The loss in metoprolol tartrate follows an exponential decay for both EB and γ irradiations. The loss in metoprolol tartrate is significantly faster ($p<0.0001$) for EB compared to γ irradiations. This seems to be in contradiction to the protective effect expected by an increase in the dose rate by a factor of 10^5, when going from γ rays ($\sim 10^{-1}\,Gy\,s^{-1}$) to high-energy electrons ($\sim 10^{4}\,Gy\,s^{-1}$), favoring radical–radical reactions and lowering radical–drug solute reactions (Allen, 1961; Spinks and Woods, 1990; Slegers et al., 2003; Slegers and Tilquin, 2005). In order to determine the extent of the dose rate effect, a computer simulation under the experimental irradiation conditions is performed using Chemsimul®.

The computer simulation results on the EB and γ radiolysis of metoprolol tartrate solutions are added to the experimental curves, as is shown in Fig. 5. The computer simulation shows no significant difference ($p>0.85$) in the loss of metoprolol tartrate between the EB and γ irradiations. An increase in the dose rate by 10^5 is not enough to lower the degradation of the drug through radical recombination because metoprolol tartrate completely scavenges hydroxyl radicals at a concentration of $3\times10^{-3}\,M$ ($1\,mg\,ml^{-1}$).

Theoretical drug simulations have demonstrated that the dose rate effect protects through radical recombination for intensities of $10^{12}\,Gy\,s^{-1}$ for similar solute concentrations and reactivity with the products of the water radiolysis as metoprolol tartrate. The dose rate effect is more pronounced for lower drug solute concentrations (Slegers et al., 2003; Slegers and Tilquin, 2005).

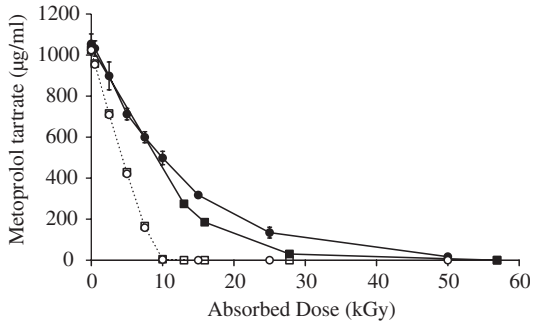


Fig. 5. Metoprolol tartrate concentration in $\mu g\,ml^{-1}$ as a function of absorbed dose in kGy, for irradiations of metoprolol tartrate ($1\,mg\,ml^{-1}$) solutions, saturated in nitrogen. E-beam irradiation (–■–), gamma irradiation (–●–), e-beam simulation (--□--) and gamma simulation (--○--).

The difference in the loss of metoprolol tartrate between EB and γ irradiations may not be explained by the dose rate. Therefore, the difference is attributed to the radiolysis of the radiolytic products. Since the radiolytic products are present in greater quantities and numbers for γ compared to EB irradiations, they scavenge more reactive species from the water radiolysis and radioprotect the drug solute. This hypothesis is supported by the fact that both γ and EB irradiations show the radiolysis of degradation products, as may be seen in Tables 1 and 2.

The radiolysis of the degradation products is not included in the reactions of the computer simulation and this explains why the computer simulation predicts a significantly greater degradation of metoprolol tartrate as a function of the absorbed dose than the experimental curves for both EB ($p<0.0002$) and γ ($p<0.0001$) irradiations. The simplified model in the simulation program already contains a reaction of hydroxyl radicals with the hydroxyl–metoprolol transients in order to reproduce the pulse radiolysis results (Slegers et al., 2003). However this is not enough to simulate the radiolysis of metoprolol tartrate at high-absorbed doses and longer irradiation times. Moreover, there are so many radiolytic products formed that it would be an enormous task to identify and synthesize or isolate them, in sufficient quantity and purity, to measure their reaction rate constants with the water radiolysis intermediates by pulse radiolysis. It is not possible to include all the reactions of the radiolytic products in the simulation program because the radiolysis mechanism is too complex.

3.7. Radiolysis of degradation products

The profile of the EB radiolytic products as a function of the absorbed dose is that of a formation and then a degradation as can be seen in Table 1. At the lowest EB absorbed dose of 13.0 kGy, 19 peaks are detected in total. The number of peaks detected progressively falls to 16, 14 and 4 present in traces, with absorbed doses of 15.9, 27.8 and 56.9 kGy, respectively.

The profile of the γ radiolytic products as a function of the absorbed dose is complex, as can be seen in Table 2. The number peaks detected increases from 12, at 0.5 kGy, to a maximum of 20 at 25 kGy. The peaks detected show different tendencies as a function of absorbed dose, some show a rise and fall with their maxima at different absorbed doses, while others continually increase up to 50 kGy.

The radiolysis of the degradation products shows many differences between EB and γ irradiations. First of all, the EB peaks detected fall after 13 kGy, whilst the γ peaks detected increase to a maximum at 25 kGy before decreasing, and this in accordance with their % AUC as a function of absorbed dose. Secondly, the EB radiolytic

products follow a rise and fall, whereas γ radiolytic products appear and disappear at different absorbed doses; the profile is more complex for γ compared to EB irradiations and this suggests differences in the reactivity of the radiolytic products with the water radiolysis radicals. Lastly, 16 radiolytic products are common to both EB and γ irradiations, however their distribution and quantities are very different as may be seen in Tables 1 and 2. The peaks detected with retention times 7.7, 10.5 and 13.7 min seem unique to EB irradiations and those with retention times 4.5, 9.5, 11.0 and 14.0 min to γ irradiations.

The radiolytic products are not present in sufficient quantity to radioprotect metoprolol tartrate for applications such as radiosterilization. An effective radioprotector must be present at a significantly greater concentration than the drug solute so that its radical scavenging strength (k[S] in s^{-1}) is greater by 100 times fold. Moreover, a radioprotector should react with the water radiolysis radicals to form transients and/or products that do not react with the drug solute. Therefore, a few pharmaceutical excipients that are potential radioprotectors are investigated to lower the degradation of metoprolol tartrate irradiated in aqueous solution for applications such as radiosterilization.

3.8. Influence of pharmaceutical excipients

The influence of the following pharmaceutical excipients: sodium chloride, 1,2-propanediol and mannitol, on the loss of metoprolol tartrate in $\mu g\,ml^{-1}$ as a function of the absorbed dose in kGy is represented in Fig. 6. The quantification results are in Tables 1 and 2 for EB and γ irradiations, respectively.

Metoprolol tartrate is commercialized as an injectable drug with 0.9% sodium chloride (0.1540 M) as an isotonicity agent and that is why this excipient is studied. In the presence of sodium chloride, the loss in metoprolol tartrate is not significantly different as that with no excipients for EB irradiations ($p > 0.37$), and is very significantly different for γ irradiations ($p < 0.0001$). However, sodium chloride does not protect metoprolol tartrate from degradation for both EB and γ irradiations. Since sodium chloride does not react efficiently with hydroxyl radicals at neutral pH and reacts slowly with the hydrated electron and the hydrogen atom, with reaction rate constants of 7×10^6 and $1 \times 10^5\,M^{-1}\,s^{-1}$, respectively (Buxton et al., 1988), no radioprotection was expected.

1,2-propanediol is a pharmaceutical excipient that is commonly used as a co-solvent and preservative in various parenteral formulations and can be added in 10–60% concentration (Rowe et al., 2003). Moreover, it reacts rapidly with hydroxyl radicals with a reaction rate constant of $1.7 \times 10^9\,M^{-1}\,s^{-1}$ (Buxton et al., 1988) and that is why this excipient is studied. The concentration isotonic to serum is 2% (0.2718 M) and at such a concentration the fraction of hydroxyl radicals reacting with 1,2-propanediol to metoprolol is 29 to 1. As may be seen in Fig. 6, 1,2-propanediol significantly reduces ($p < 0.0001$) the loss in metoprolol tartrate for both EB and γ irradiations.

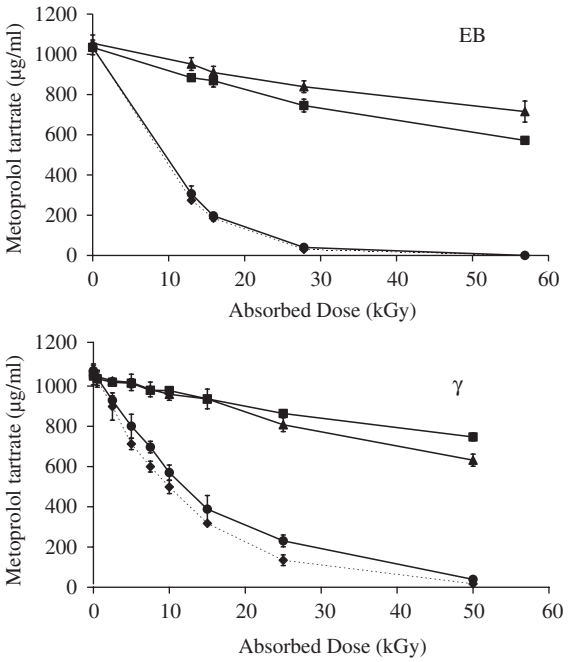


Fig. 6. Metoprolol tartrate concentration in $\mu g\,ml^{-1}$ as a function of absorbed dose in kGy for e-beam (EB) and gamma (γ) irradiations of metoprolol tartrate ($1\,mg\,ml^{-1}$) solutions, saturated in nitrogen and containing the following excipients: 0.9% NaCl (–●–), 2% 1,2-propanediol (–▲–), 5% mannitol (–■–), no excipient (–♦–).

Mannitol also significantly reduces ($p < 0.0001$) the loss in metoprolol for both EB and γ irradiations. Mannitol is a pharmaceutical excipient that is commonly used in tablets, lyophilized drugs, as a thickening agent, as a carrier in dry powder inhalers and can be administered parenterally (Rowe et al., 2003). Mannitol is chosen because it reacts quite well with hydroxyl radicals with a reaction rate constant of $1.7 \times 10^9\,M^{-1}\,s^{-1}$; it reacts slowly with hydrogen atoms, with a reaction rate constant of $7 \times 10^6\,M^{-1}\,s^{-1}$ (Buxton et al., 1988). A 5% solution (0.2745 M) is isotonic to serum and at such a concentration, the fraction of hydroxyl radicals reacting with mannitol compared to metoprolol is 29 to 1.

Comparisons between the EB and γ irradiations of the metoprolol tartrate solutions with pharmaceutical excipients also show very significant differences ($p < 0.001$), as was the case without excipients. For example 1,2-propanediol is the best radioprotector for EB irradiations, whilst mannitol for γ irradiations.

4. Conclusion

The differences in the loss of metoprolol tartrate in the absence and in the presence of sodium chloride, 1,2-propanediol and mannitol, as well as the differences in the number, formation, degradation, distribution and quantities of radiolytic products detected, suggest that the EB and γ radiolysis mechanisms are different. In the radiolysis of drugs in the solid-state, differences are also observed between EB and γ irradiations (Crucq et al., 2005).

A dose rate of $10^4\,Gy\,s^{-1}$, from an EB industrial irradiator, is not high enough to lower the degradation of metoprolol tartrate at the reference sterilization dose of 25 kGy. The lesser degradation of metoprolol tartrate as a function of absorbed dose for γ compared to EB irradiations is attributed to the protection by the radiolytic products which are present in greater quantities and numbers.

Radiosterilization of metoprolol tartrate aqueous solutions, with a validated sterilization absorbed dose lower than 25 kGy (The United States Pharmacopeia, 2000; ISO 11137, 1995) and the appropriate choice of radioprotecting pharmaceutical excipients, appears to be feasible. Since metoprolol tartrate is degraded at lower doses and less radiolytic products are detected, for EB compared to γ irradiation, EB irradiators would be more suited for the removal of metoprolol tartrate from waste waters. Moreover, EB irradiators may treat large quantities of waste water effluents in a few seconds.

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