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Radiosterilization of drugs in aqueous solutions may be achieved by the use of radioprotective excipients

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Abstract

The aim of this study was to assess the feasibility of radiosterilization of drugs aqueous solutions and to evaluate the effects of some additives, such as mannitol, nicotinamide and pyridoxine, which might protect the drug from degradation. Metoclopramide was selected as a model drug. The structures of the degradation products were determined to gain insight on the radiolysis mechanisms in aqueous solution in order to design strategies to lower the drug degradation.

Metoclopramide hydrochloride aqueous solutions with and without excipients were irradiated either with gamma rays or high-energy electrons. HPLC-DAD was used to measure the loss of chemical potency and to quantify the degradation products which were also characterized by LC–APCI–MS-MS. Metoclopramide recovery for gamma and electron beam-irradiated solutions containing either mannitol, pyridoxine or nicotinamide meets the pharmacopoeial specifications for metoclopramide content up to a 15 kGy irradiation so that metoclopramide solutions containing these excipients might be radiosterilized at 15 kGy either with gamma rays or high-energy electrons. Structures are proposed for the majority of radiolysis products. Similar radiolysis products were detected for gamma and electron beam irradiations but the chromatographic profiles were different (differences in the distribution of radiolysis products).

Keywords: Metoclopramide hydrochloride; Radiolysis; HPLC-DAD; LC–MS; Radioprotective excipients

1. Introduction

Radiosterilization is a safe method recommended for the sterilization of thermosensitive solid drugs (EMEA, 1999; European Pharmacopoeia, 2005; USP, 2005). However, it is not considered for the sterilization of drugs in aqueous solutions (EMEA, 1999) as, compared to the solid state, a higher degradation is reported after irradiation of drug solutions (Jacobs, 1985; Boess and Bögl, 1996; Angelini et al., 1998). In irradiated aqueous solutions, the reactive species generated by the radiolysis of water (mainly $^{\bullet}OH$, $^{\bullet}H$ and e_{aq}^{-} in deaerated water) react with the solute (indirect effect), giving rise to radiolytic products. Up to now, few studies have been carried out on the irradiation of aqueous solutions at radiosterilization doses (Slegers and Tilquin, 2006). Previous experiments have shown that the drug degradation could be reduced by the addition of appropriate excipients (Boess and Bögl, 1996; Moore et al., 1996; Slegers and Tilquin, 2006) that are able to scavenge some reactive species from irradiated water, protecting the drug from degradation. Optimal irradiation conditions combined with the addition of radioprotective excipients to lower the drug degradation would allow radiosterilization of aqueous solutions (the final purpose being the terminal sterilization).

In this work, metoclopramide, an anti-emetic drug, was chosen as a model since it is a small molecule with various chemical bonds, which allows to study the effects of irradiation on these bonds. Metoclopramide aqueous solutions with and without excipients were irradiated at various doses. Mannitol, which is known to react very fast with the hydroxyl radical (Buxton et al., 1988), was selected as a radioprotective excipient. The effects of nicotinamide (vitamin B3) and pyridoxine (vitamin B6), which react with both the hydroxyl radical and the aqueous

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electron (Buxton et al., 1988), were also assessed. The irradiations were performed either with gamma rays or high-energy electrons to compare the effects of both irradiation types. The irradiation dose should be determined according to the guidelines (ISO, 2006) and is a compromise between both the initial contamination of the drug (bioburden) and its radiosensitivity. Various irradiation doses have been applied so that the effect of the absorbed dose on the degradation of the drug may be assessed.

High performance liquid chromatography linked to a diode-array detector (HPLC-DAD) was used to quantify the loss of metoclopramide (chemical potency) as a function of the absorbed dose and the degradation products which were also analyzed by liquid chromatography tandem mass spectrometry (LC–MS-MS). The probable structures of the major degradation products were investigated as well as the reactive species of water and the routes that might be responsible for their formation. The results were compared to those previously obtained with solid state-irradiated metoclopramide (Maquille et al., 2006).

2. Materials and methods

2.1. Materials

Metoclopramide hydrochloride monohydrate (purity > 99%), nicotinamide hydrochloride (purity > 99%) and pyridoxine (purity > 99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and stored in the dark at room temperature. Ammonium acetate, D-mannitol and cerium sulfate were from UCB. HPLC supra-gradient acetonitrile was supplied from Biosolve (Amsterdam, the Netherlands). Deionized water was generated in our laboratory with a Milli-Q system from Millipore (Bedford, MA, USA). Nitrogen (N28 purity) was supplied by Air-Liquide (Liège, Belgium).

2.2. Samples for irradiation

Metoclopramide hydrochloride 5 mg ml^{-1} solutions (which corresponds to the concentration of the commercial drug) were prepared either without excipients or with 5% mannitol, 10 mg ml^{-1} pyridoxine hydrochloride or 10 mg ml^{-1} nicotinamide. The solutions were prepared with tri-distilled water (Spinks and Woods, 1990) put in glass vials protected from the light, saturated with nitrogen, sealed and irradiated at room temperature.

2.3. Irradiation sources

2.3.1. Gamma rays

Gamma irradiations were performed with a ^{60}Co Gammacell (Sterigenics, Fleurus). The dose rate was about 12.5 kGy h^{-1}. The absorbed doses were 0, 5, 10, 15 and 25 kGy.

2.3.2. High-energy electrons

For electron beam irradiations, a linear electron generator (Mölnlycke, Waremme, Belgium) was used. The beam power of the electron generator was about 16 kW and the electrons were delivered in pulses of 474 Hz. The desired dose was achieved by adjusting the speed of the conveyor belt. The absorbed doses were 0, 10, 15 and 25 kGy. The dose rate was about 6×10^7 Gy h^{-1}.

2.4. Dosimetry

The absorbed doses were measured with ceric sulfate dosimeters (Spinks and Woods, 1990) that were irradiated in similar glass vials at the same time as the samples.

2.5. HPLC-DAD system

The Merck-Hitachi (Darmstadt, Germany) HPLC system was composed of a L-6200 Intelligent Pump equipped with an AS-2000 autosampler with a 20 μL sample loop and a L-4500 diode array detector (DAD). The Merck-Hitachi D-7000 HPLC system manager software (HSM) version 4.0 was used. Chromatographic separation was achieved on a 250 mm × 4 mm Merck LiChrospher® 60 RP Select B column 5 μm particle size. The mobile phase consisted of 12% acetonitrile and 88% ammonium acetate 10 mM aqueous solution adjusted to pH 5 with glacial acetic acid. The flow rate was 1 ml min^{-1}. The analyses were performed in triplicate at room temperature. The absorbance was measured between 200 and 700 nm. The wavelength for quantification was 273 nm. For the quantification of radiolytic products, the solutions were diluted twice, prior analysis. For the assessment of chemical potency, the solutions were diluted 10-fold.

The percentage of metoclopramide recovery (chemical potency test) was calculated from the ratio of the areas under the curve (AUC) of the metoclopramide peak between irradiated and non-irradiated samples (EMEA, 1996; European Pharmacopoeia, 2005; USP, 2005). The concentrations of the degradation products were calculated from a calibration curve and expressed as percentages of the initial drug concentration. Given the absence of standards and according to pharmaceutical guidelines (EMEA, 1996), the response factors of all degradation products were assumed to be the same as metoclopramide. Metoclopramide solutions in concentrations ranging from 8×10^{-7} to 5×10^{-4} M were injected in order to establish the calibration curve, which was validated by a statistical analysis (XLStat; Addinsoft, Paris, France). Limits of detection and quantification were, respectively, 3 and 10 times the signal-to-noise ratio (European Pharmacopoeia, 2005; USP, 2005).

2.6. LC–MS analyses

A Thermo-Electron (Waltham, MA, USA) Advantage Ion Trap mass spectrometer with an atmospheric pressure chemical ionization (APCI) source was used. The chromatographic conditions were the same as in the HPLC-DAD method and the solutions were injected in triplicate. The following MS conditions were used: positive ion mode, capillary temperature, 200 °C; APCI vaporizer temperature, 500 °C; sheath gas flow,

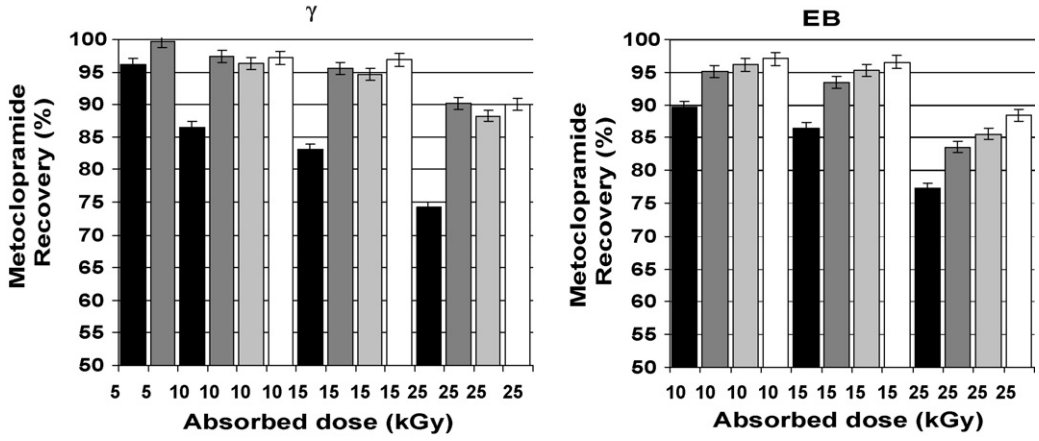

Fig. 1. Metoclopramide recovery as a function of the absorbed dose for gamma (γ) and electron beam (EB)-irradiated metoclopramide containing different excipients. Without excipients (■), mannitol (■), nicotinamide (■) and pyridoxine (□).

70 arbitrary units (A.U.); auxiliary gas, 20 A.U.; source voltage, 6 kV. Full MS scans of 50–700 units were performed.

3. Results

3.1. Color changes

Metoclopramide solutions became orange-yellow after irradiation. The color increased with the absorbed dose and, for similar absorbed doses, was less intense in electron beam than in gamma-irradiated solutions. Solutions containing either mannitol, nicotinamide or pyridoxine showed no color up to a 15 kGy dose.

3.2. Chemical potency

The percentage of metoclopramide recovery as a function of the absorbed dose for gamma and electron beam-irradiated solutions is presented in Fig. 1. For unirradiated reference samples, the recoveries were the same between solutions with and without excipients.

In the absence of excipients, metoclopramide degradation was significantly higher in gamma-irradiated samples ($P < 0.05$). All the tested excipients significantly reduced the drug degradation ($P < 0.05$). For samples containing mannitol, the recovery was higher for gamma than for electron beam-irradiated samples ($P < 0.05$). However, for samples with nicotinamide or pyridoxine, the protective effect was not significantly affected by the irradiation type ($P = 0.32$ and 0.58, respectively).

3.3. Quantification of degradation products

Fig. 2 shows the chromatograms at 273 nm zoomed on the degradation products for samples containing excipients superimposed to those of the samples without excipients for gamma (γ) and electron beam (EB) irradiations at 15 kGy. The quantification results for the degradation products are in Table 1. Only the results obtained for samples irradiated at 15 kGy are presented. The degradation peaks were numbered by order of their retention times.

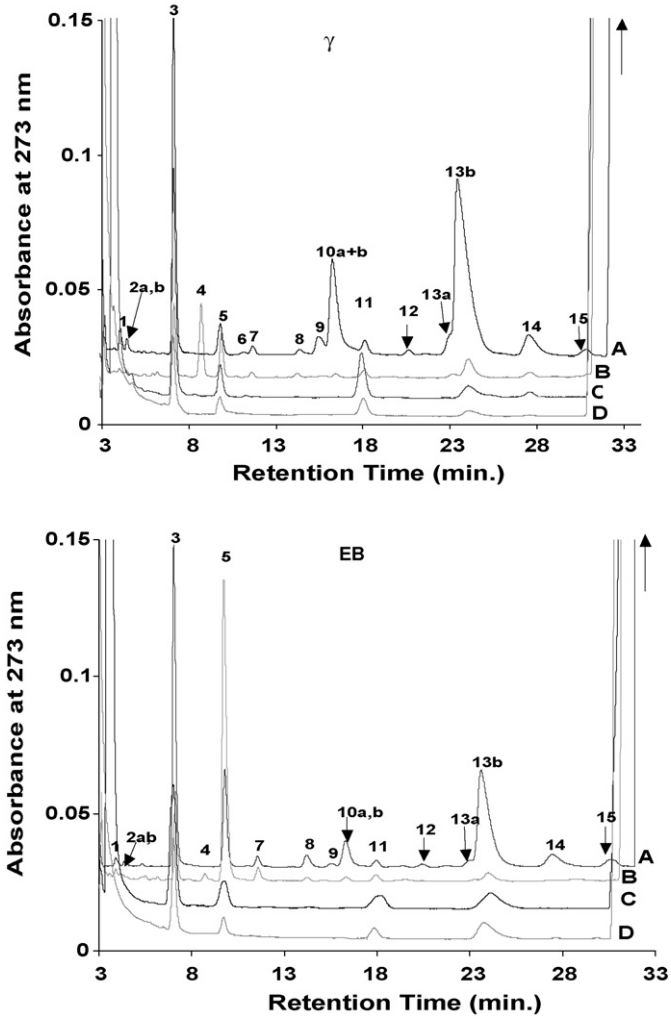

Fig. 2. Overlay of the chromatograms at 273 nm zoomed on the degradation products peaks for metoclopramide solutions without excipients (A) and containing mannitol (B), nicotinamide (C) or pyridoxine (D). (γ) 15 kGy gamma-irradiated; (EB) 15 kGy electron beam-irradiated.

Table 1
HPLC-DAD quantification of the degradation peaks from metoclopramide solutions with and without excipients irradiated at 15 kGy with gamma rays (γ) and high-energy electrons (EB) and Mass-to-charge ratios (*m*/*z*) of the corresponding products determined by LC–MS

Peak number	Mean percentages of impurity peaks relative to the initial drug concentration (%), $n = 6$										
	Peak RT (min)	*m*/*z* of $[M+H]^+$ ion	0 kGy	15 kGy γ	15 kGy γ with mannitol	15 kGy γ with vitamin B3	15 kGy γ with vitamin B6	15 kGy EB	15 kGy EB with mannitol	15 kGy EB with Vitamin B3	15 kGy EB with Vitamin B6
1	4.2	269	<LOD	0.09	<LOQ	–	–	0.04	<LOQ	–	–
2a,b	4.6	298; 316*	<LOD	0.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3	7.0	282	<LOD	1.58	0.38	1.31	0.40	1.34	0.38	1.03	0.54
4	8.8	462*	<LOD	<LOD	0.41	<LOD	<LOD	<LOD	0.04	<LOD	<LOD
5	9.8	266	<LOQ	0.32	0.42	0.28	0.13	0.54	1.60	0.34	0.15
6	11.3	316*	<LOD	0.03	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	11.8	267	<LOD	0.09	0.03	<LOD	<LOD	0.07	0.09	<LOD	<LOD
8	14.3	563*	<LOD	0.05	0.04	<LOD	<LOD	0.13	0.05	<LOD	<LOD
9	15.7	442*	<LOD	0.24	<LOD	<LOD	<LOD	0.04	<LOD	<LOD	<LOD
10a	16.4	451*	<LOD	1.06	<LOD	<LOD	<LOD	0.33	<LOD	<LOD	<LOD
10b	16.6	270*	<LOD		0.03	<LOD	<LOD		0.04	<LOD	<LOD
11	18.2	272*	0.03	0.11	0.08	0.52	0.21	0.07	0.06	0.38	0.13
12	20.5	316*	<LOD	0.05	<LOQ	<LOD	<LOD	0.04	<LOD	<LOD	<LOD
13a	23.0	286*	0.02	3.54	0.38	0.29	0.13	1.56	0.15	0.36	0.42
13b	23.7	316*	<LOD								
14	27.8	286*	<LOD	0.43	0.07	0.06	<LOD	0.28	<LOQ	<LOD	<LOD
15	30.7	301*	<LOD	0.10	0.03	<LOD	<LOD	0.11	<LOQ	<LOD	<LOD

The concentrations of the radiolysis products are expressed as percentages of the initial drug concentration. The maximum coefficient of variation observed was 5%.
* Presence of a chlorine atom.

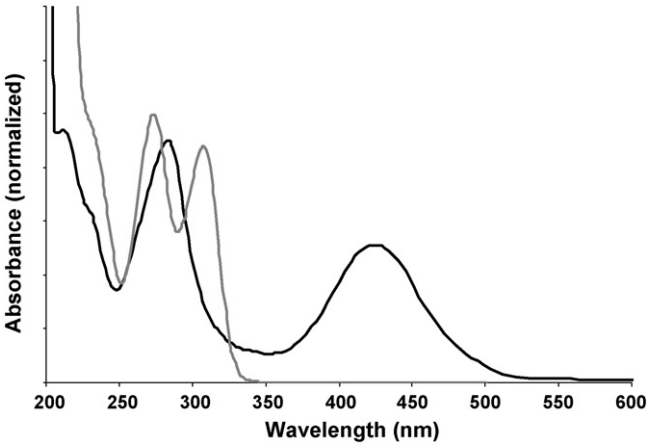

Fig. 3. Overlay of normalized DAD spectra of metoclopramide peak (lighter) and degradation peak 10 (darker).

3.3.1. Unirradiated solutions

Peaks 5, 11 and 13a were detected before irradiation. The amount of product 5 was below the limit of quantification (<0.02%) and the amounts of products 11 and 13a were, respectively, 0.02 and 0.03%. No difference was observed between solutions without excipients and solutions containing mannitol, nicotinamide or pyridoxine.

3.3.2. Irradiated metoclopramide solutions without excipients

The diode array detector (DAD) revealed that the majority of the degradation peaks had similar absorbance spectra than metoclopramide except peak 10a, which was characterized by a broad absorbance band in the visible. A normalized overlay of the DAD spectra of this product and of metoclopramide is shown in Fig. 3. According to pharmaceutical guidelines (EMEA, 1996; European Pharmacopoeia, 2005; USP, 2005), it was assumed for the quantification that all the degradation peaks had the same response factor as metoclopramide.

Different chromatographic profiles were observed between gamma and electron beam-irradiated solutions (see Fig. 2). Although the same peaks were detected, they were present in very different amounts. The three most abundant degradation peaks detected in gamma-irradiated solutions were, by decreasing order, peaks 13, 3 and 10. For electron beam-irradiated solutions, these were peaks 13, 3 and 5. Compared to gamma irradiations, the degradation peaks were detected in lower amounts in electron beam-irradiated samples except peaks 5 and 8 that are higher (e.g., at 15 kGy: 0.54% instead of 0.32% for peak 5 and 0.13% instead of 0.05% for peak 8).

3.3.3. Effects of excipients

For both gamma and electron beam-irradiated samples, the majority of the degradation peaks were present in lower amounts in the solutions containing mannitol, nicotinamide or pyridoxine. However, in samples containing mannitol, peak 5 was higher (especially in samples irradiated by high-energy electrons) and a new peak was detected (peak 4). In solutions containing pyridoxine or nicotinamide, only peak 11 was slightly higher. For irradiated solutions containing excipients, the DAD detector indicated that no chromatographic peak absorbed in the visible range.

3.4. Characterization of degradation products

In MS analyses with an APCI source, protonated molecules ($[M+H]^+$) are detected for each product. The mass-to-charge ratios (*m/z*) corresponding to each chromatographic peak are reported in Table 1. The MS-MS results for metoclopramide and some degradation products are in Table 2 and the structures proposed for the radiolysis products are shown in Fig. 3. The mass spectrum indicates the presence of a chlorine atom (presence of chlorine 37 isotope) as well as the number of nitrogen atom (an even mass corresponds to an even number of nitrogen, an odd mass to an odd number). The fragments observed in MS^2 may be correlated to the location of the modification. Altogether, these information may be used to gain insight on the structure

Table 2
m/z Values and corresponding major MS-MS fragments for metoclopramide and some degradation products

Product number	*m/z* of pseudomolecular ion	*m/z* of major MS-MS fragments					
1	269	196	153	143	117	100	
2a	298	225	182	143	117	100	
3	282	209	166	143	117	100	
5	266	193	150	100			
6	316	243	200	143	117	100	
7	267	194	151	143			
8	563	490	447	417	376	333	
9	442	369	326	253	143		
10a	451	378	342	335	415	420	299
10b	270	197	170	154	100		
11	272	227	184	156			
12	316	298	227	159			
13a	286	227	184	156			
13b	316	243	200	143	117	100	
14	286	213	117	143	170		
15	301	228	185	157	143		
Metoclopramide	300	227	184	156	143		

of a radiolytic product. The structures of the main fragments of metoclopramide may be found in the previous article (Maquille et al., 2006).

The mass detector revealed that peaks 2, 10 and 13 observed in UV were a co-elution of degradation products. Two products were detected within peak 2, one with a *m/z* of 298 and the other with a *m/z* of 316. Peak 10 also contained two products having *m/z* of 451 and 270 whilst products with *m/z* of 286 and 316 were detected within peak 13.

Products 1, 2a, 3, 5 and 7 had no chlorine. Their major fragments corresponded to the losses of 73 (diethylamino) and 116 *m/z* (*N*,*N*′-diethylethylene diamine). They also exhibited a *m/z* 143 fragment (HCO–NH–CH_2CH_2–$NH(C_2H_5)_2$). Such fragmentation indicated that no modification occurred on the lateral chain. Therefore, product 1 should have three hydroxyls instead of the amine (even mass), the chlorine and the methoxy groups. Product 2a could be due to the loss of the chlorine and the addition of two hydroxyls on the ring. Product 3 could correspond to the loss of the chlorine and the addition of a hydroxyl radical on the ring. Product 5 (due to the loss of the chlorine) has been identified in solid state-irradiated metoclopramide (Maquille et al., 2006). Product 7 might result from the loss of the chlorine and the aniline amino group (since it has an even mass) and the addition of a hydroxyl on the ring.

Product 8, *m/z* 563, possessed one chlorine and fragmented into *m/z* 490 (−73) and *m/z* 447 (−116) but also exhibited fragments with *m/z* 417 (−146 = 2 × diethyamino), *m/z* 376 (=diethyamino + *N*,*N*′-diethylethylene diamine) and *m/z* 333 (=−2 × *N*,*N*′-diethylethylene diamine), which indicated the presence of two unmodified lateral chains. This product might result from the loss of the chlorine from one metoclopramide molecule followed by coupling with another metoclopramide molecule.

Product 10a, with *m/z* 451, had a chlorine and a similar lateral chain as metoclopramide. A fragment corresponding to the loss of the chlorine (*m/z* 415) was also observed. Its very different UV–vis spectrum (see Fig. 3) might be explained by a deep modification of the ring. In irradiated aniline solutions, 2-aminophenoxazin-3-one that has a similar absorbance spectrum has been observed (Christensen, 1972). The formation of product 10a might have occurred through a similar way. Product 10a was not detected by MS in irradiated solutions containing mannitol, nicotinamide or pyridoxine.

Product 10b (*m/z* 270) had a chlorine and showed a fragmentation corresponding to an unmodified lateral chain. Therefore, it might be obtained after the loss of the methoxy from the benzamide ring. Product 11 (*m/z* 272) has been identified in solid state-irradiated metoclopramide (loss of ethyl from diethylamino) (Maquille et al., 2006) and was already detected before irradiation.

Products 2b, 6, 12 and 13b have *m/z* of 316, which is consistent with the addition of a hydroxyl radical. For products 6 and 13b, fragmentation showed that no modification occurred on the lateral chain and therefore, the hydroxyl was added on the ring. Fragmentation of product 12 showed that the modification was located on the diethylamino part. Product 2b was not present in sufficient amount to allow its fragmentation.

Both products 13a and 14 had *m/z* of 286 although they exhibited different MS^2 fragments. Product 13a has been identified previously as resulting from the loss of the methyl from the diethylamino group (Maquille et al., 2006). Contrary to product 13a, the fragmentation of product 14 indicated no modification of the lateral chain and its mass-to-charge ratio might correspond to the loss of the methoxy and the addition of a hydroxyl on the ring.

Product 15 had an unmodified lateral chain as shown by its major fragments. Its even mass could be explained by the loss of the amino group and the addition of a hydroxyl on the ring.

4. Discussion

4.1. Color change

The yellow color that appeared after irradiation of the solutions without excipients can be attributed to the formation of product 10a that has a maximum absorbance around 450 nm. For similar absorbed doses, this product is detected in higher amounts in gamma-irradiated solutions without excipients than in electron beam ones. Moreover, product 10a is not detected in irradiated solutions containing excipients, which explain the absence of coloration in these solutions, which is in accordance with Pharmacopoeial requirements (European Pharmacopoeia, 2005; USP, 2005).

4.2. Radiolysis of metoclopramide

The irradiated samples containing only metoclopramide meet the US Pharmacopeial requirements (USP, 2005) concerning metoclopramide content of metoclopramide solution for injection (90–110%) up to a 5 kGy gamma irradiation although this dose might not be sufficient to ensure sterility.

The determination of the structures of radiolytic products by LC–MS enables to determine some routes that may lead to the formation of these products. After the absorption of ionizing radiation, deoxygenated water decomposes following the overall reaction (Allen, 1961; Spinks and Woods, 1990): $H_2O \rightarrow H^{\bullet}$, $HO^{\bullet}$, H_2, H_2O_2, e_{aq}^{-}, H_3O^{+}. The attack of the solute is carried out by these primary species.

Fig. 4 presents some routes proposed for the formation of the degradation products. The formation of product 5 occurs through dissociative electron capture (Spinks and Woods, 1990; Quint et al., 1996). The aqueous electron, which has affinity for the chlorine adds itself to the ring. This addition is followed by dissociation of chloride and disproportionation of the resulting radical to give product 5. Product 8 might result from the attack of this radical on a metoclopramide molecule followed by dismutation of the resulting species.

Addition of the hydroxyl radical in ipso relative to each substituent of the ring is possible. For example, after an ipso attack, the chlorine dissociates and product 3 is obtained after disproportionation. In aromatic chlorine halides, the attack of the hydroxyl radical in the ipso position relative to the chlorine is favored (Naik and Mohan, 2005). Products 14 and 16

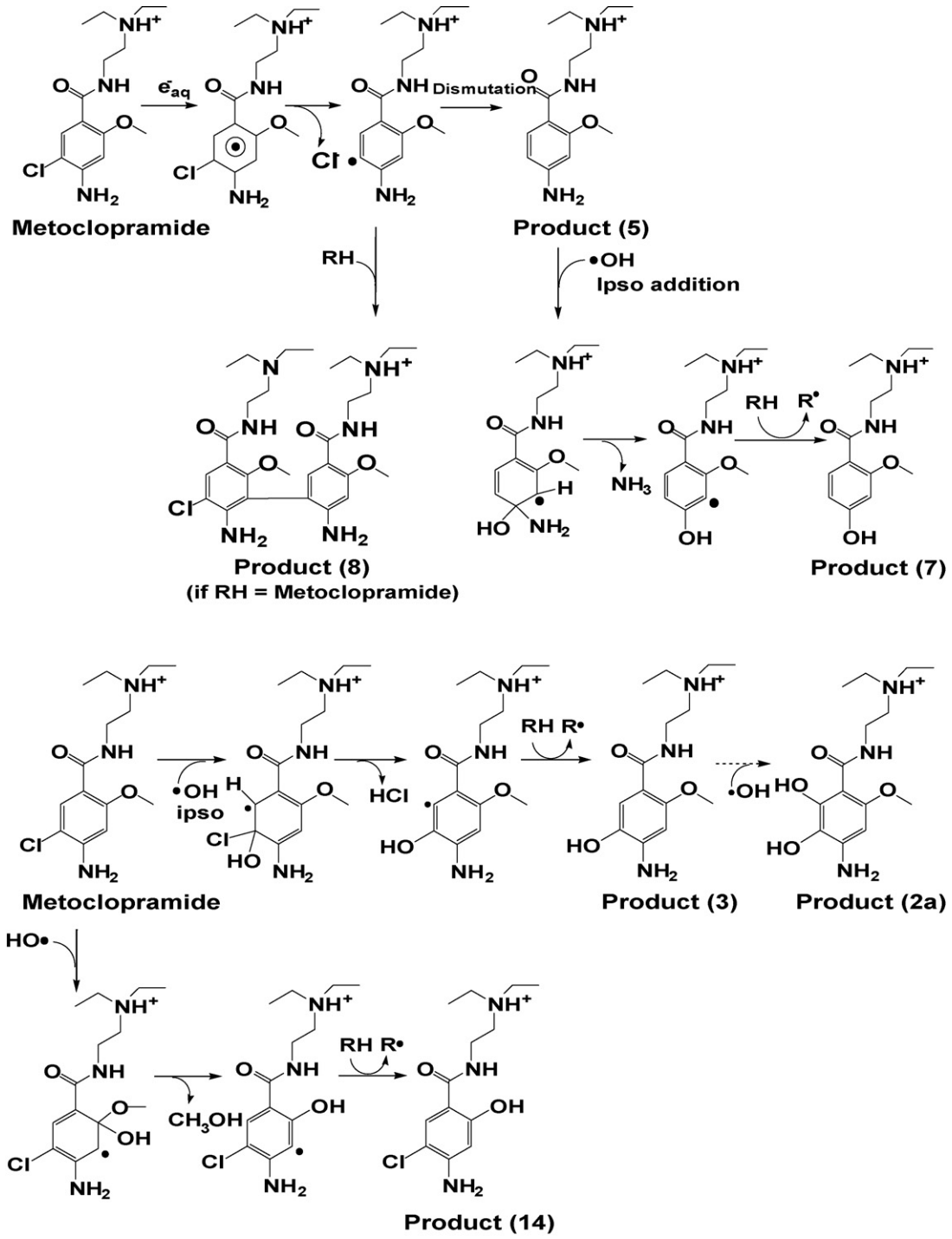

Fig. 4. Theoretical routes leading to some degradation products.

may be obtained following a similar reaction, which involves the attack of a hydroxyl in ipso relative to the methoxy and the amino groups, respectively. The formation of products 6 and 13b involves substitution by hydroxyl radicals on the ring (Sanchez et al., 2002; Slegers et al., 2006).

Considering only the yield of the hydroxyl radical without any competition with other reactions, one can calculate that 100% of metoclopramide should be destroyed at 25 kGy. However, the chemical potency test shows that a maximum of 25% of metoclopramide is destroyed after a 25 kGy irradiation (see Fig. 1). The difference is explained by the reaction between the radiolysis products and the reactive species of water so that the parent drug is protected from degradation. For example, according to the proposed structures for products 1, 2a and 7 (see Fig. 5), these products should result from the attack of primary radiolytic products by reactive species of water, e.g., 2a results from the attack of two hydroxyl radicals.

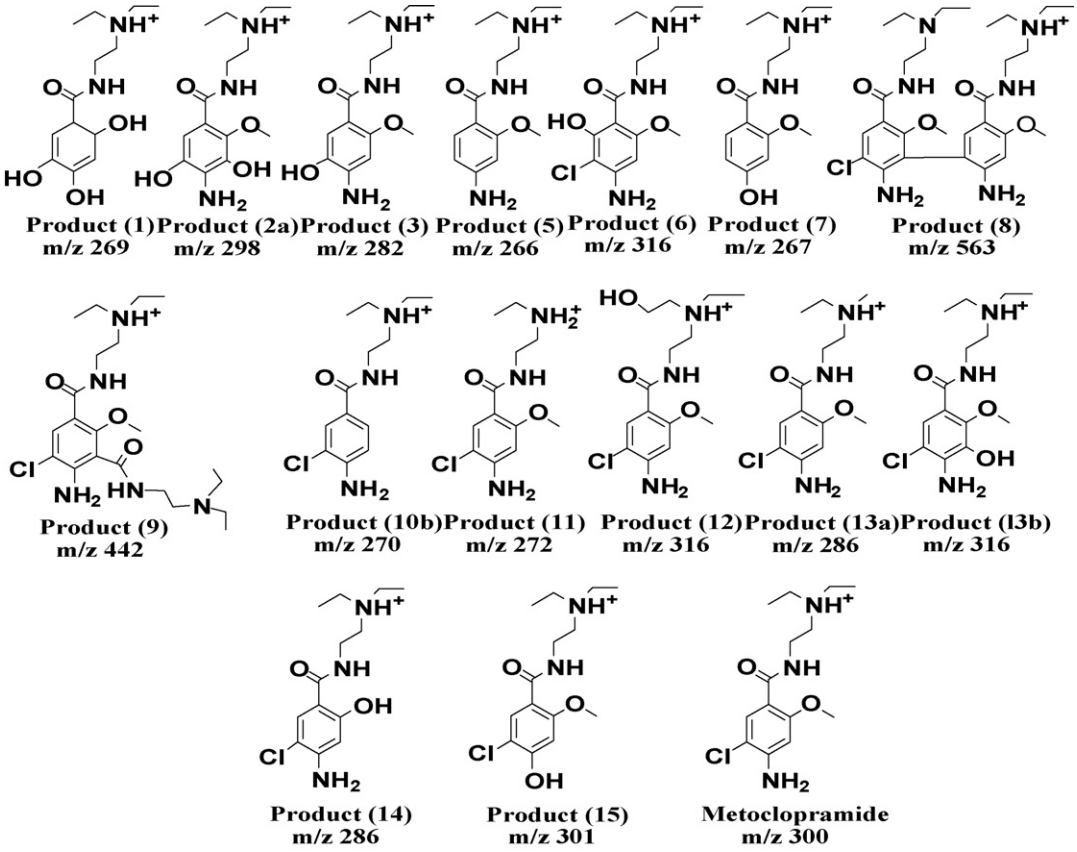

Fig. 5. Proposed structures (protonated) of main radiolysis products of metoclopramide.

4.3. Effects of excipients

As expected, all the tested excipients strongly reduced the loss in metoclopramide by scavenging reactive species (Buxton et al., 1988). The recoveries are far above 90% for solutions containing the excipients up to a 15 kGy irradiation. Samples containing either mannitol, nicotinamide or pyridoxine-irradiated up to 15 kGy with gamma rays or high-energy electrons meet the pharmacopoeial specifications (USP, 2005) concerning the chemical potency. As may be seen in Table 1, all the radiolytic products resulting from the reaction with the hydroxyl radical are present in lower amounts in irradiated solutions containing mannitol, nicotinamide or pyridoxine.

The attack on the chlorine may be carried out either by the aqueous electron or by the hydroxyl radical. It is thus conceivable that a competition between the aqueous electron and the hydroxyl radical may occur, which could explain the increase of product 5 in solutions containing mannitol. When mannitol is present within the solution, it scavenges hydroxyl radicals so that these no longer interfere with aqueous electrons for the attack on the chlorine. This hypothesis explains the higher yields observed for the dechlorinated product in solutions containing mannitol.

For example, product 13b that is due to the addition of a hydroxyl radical on the ring undergoes the highest decrease. In solutions containing nicotinamide or pyridoxine, product 11, due to the reaction with the hydrogen radical is detected in higher amounts. In solutions containing pyridoxine, peak 5 is also higher. The lowest amount of radiolysis products is observed in solutions containing pyridoxine. In solutions containing mannitol, product 4 might originate from a cross-reaction between mannitol and metoclopramide but this product is not present in high amounts.

4.4. Differences between gamma rays and electron beam irradiations

The difference in metoclopramide recovery between electron beam and gamma rays-irradiated samples suggests a difference in the radiolytic mechanisms between gamma and electron beam irradiations.

Differences between gamma and electron beam irradiations in terms of different distributions of radiolysis products or different effects of radioprotective excipients have been previously reported (Slegers and Tilquin, 2006). Computer simulations are currently unable to predict such differences (Slegers and Tilquin, 2005) since they focus on the destruction of the drug and not on the formation of radiolysis products. The results obtained for metoclopramide indicate that products resulting from the reaction of the drug with the aqueous electron are present in higher amounts in solutions irradiated by high-energy electrons.

Products 5 and 8, both resulting from the reaction with the aqueous electron, are detected in higher amounts in solutions irradiated with high-energy electrons, which indicates

that an electron beam irradiation seems to favor the routes involving the aqueous electron. Moreover, when radioprotective excipients that scavenge the aqueous electrons (pyridoxine or nicotinamide) are added, no significant difference concerning the chemical potency were noted for gamma irradiations, whereas for electron beam irradiations, the protection was higher with pyridoxine or nicotinamide than with mannitol.

4.5. Differences between solid state and aqueous solutions

In aqueous solutions, fragmentation by direct effect, which was the responsible for the formation of radiolysis products in solid state-irradiated metoclopramide, is replaced by the indirect effect (attack of reactive species from water radiolysis).

The formation of product 5 is based on dissociative electron capture, which is carried out by the aqueous electron. This reaction also occurred in solid state-irradiated metoclopramide since the electron can diffuse in solid matrix (Maquille et al., 2006). Products 11 and 13a that were present in solid state-irradiated metoclopramide and generated following fragmentation of excited molecules are not amongst the major radiolysis species observed in irradiated metoclopramide aqueous solutions.

5. Conclusion

From a chemical point of view, radiosterilization of metoclopramide aqueous solutions appears to be feasible although this would necessitate both a decrease of the irradiation dose (between 10 and 15 kGy) and the addition of radioprotective excipients. Previous studies have shown that sterility may be achieved at doses far lower than 25 kGy (Juanchi et al., 2000) so that lower doses may be validated if the product does not withstand the reference dose (ISO, 2006; EMEA, 1999).

Meeting the USP specifications alone does not allow the use of the irradiated product since its potential toxicity still needs to be evaluated. However, the elucidation of the structures of the radiolysis products may be useful to this regard.

All the tested excipients are potential radioprotectors that could be added to aqueous solutions of drug prior irradiation to limit the loss of the drug. However, a complete study on the radiolysis products from these excipients should be necessary. Although similar products are observed between electron beam and gamma-irradiated samples, the quantitative differences suggest that different routes might be favored by the different irradiation types.

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