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Characterization of gamma irradiated petrolatum eye ointment base by headspace–gas chromatography–mass spectrometry

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Abstract

The effects of gamma irradiation on petrolatum eye ointment base (EOB) and its ingredients (white petrolatum, liquid paraffin, and wool fat) were studied at different irradiation doses. Forty-one volatile radiolysis products were detected and identified by a combined system of headspace–gas chromatography–mass spectrometry (HS–GC–MS). The characteristics of the radiolysis products and the degradation pathway were discussed in each case, respectively. GC method demonstrates that the component distribution patterns of eye ointment as well as its individual ingredients have no differences before and after gamma irradiation. The influence of gamma treatment on EOB was quantitatively determined at 15, 25 and 50 kGy. The concentrations of the radiolysis products increase linearly with increasing doses. Both qualitative and quantitative data show that irradiated eye ointment is safe for human use.

Keywords: Headspace–GC–MS; Petrolatum eye ointment; Gamma irradiation

1. Introduction

Ophthalmic ointment products are among the most successful dosage forms to which radiation sterilization has been applied, mainly due to three factors. First, the most effective thermal sterilization cannot be applied to ointment products because of their low melting point. Second, the aseptic process, a traditional sterilization method for eye ointment products, involves the possibility of secondary contamination and excessive costs. Third, the intrinsic nature of strong penetration of gamma rays enables gamma sterilization to be the only method for terminal sterilization of eye ointment products.

Eye ointments are normally petrolatum-based and designed to have a melting point close to the human body temperature. The petrolatum base is used as an anhydrous medium to deliver moisture-sensitive drugs, like chloramphenicol. The base adopted as the excipient of chloramphenicol eye ointment consists of petrolatum, liquid

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paraffin, and wool fat. These ingredients are generally considered to be non-irritant and -toxic materials. Therefore, it is important to clarify whether or not the radiation sterilization process provokes the formation of unsafe radiolysis products for their use in pharmaceuticals.

Earliest investigation of radiated white petrolatum and liquid paraffin were reported during 1960–1963 [1–3]. However, a few cases concerned ointments or ointment ingredients, and these limited studies were focused on the changes in pH value, physical properties and active ingredients upon gamma irradiation [4–11]. There has been no report available on whether or not and how gamma irradiation causes chemical component changes in ointment base as well as its relevant ingredients although the information about potential decomposition products is required for the validation of gamma radiation sterilization.

Gas formation in petrolatum eye ointment products is a phenomenon unavoidable during gamma irradiation. Thus, the gas composition needs to be clarified and untoward activity or carcinogenic potential of these gases for human health must be assessed. Nevertheless, there has been no report concerning the composition of these gases, though the existence of hydrogen carbon dioxide, and methane in the gases was reported [11].

The present work pursued the chemical impact of gamma radiation on eye ointment base (EOB) as well as its ingredients (white petrolatum, liquid paraffin and wool fat). Organic volatile impurities produced from these materials during irradiation were clarified, with qualitative and quantitative data. The research findings enable the elucidation of radiolysis mechanism and the assessment of the toxic potential of the radiolysis products for human health.

2. Experimental

2.1. Reagent and materials

Eye ointment base (EOB, a petrolatum oily base without active ingredient), white petrolatum, liquid paraffin, and wool fat were supplied by CIBA Vision AG (Hettlingen, Switzerland). Reference compounds, solvents and reagents were of analytical reagent grade or better from Fluka Chemie GmbH (Buchs SG1, Switzerland).

Samples were irradiated with gamma rays from ^{60}Co at 15, 25, 50, and 100 kGy respectively, in a radiation sterilization plant, Studer Draht-und Kabelwerk AG (Däniken, Switzerland).

2.2. Apparatus

A BGB-Silaren column (10 m × 0.32 I.D., 0.12 μm, GBG Analytik AG, Adliswil, Switzerland) was used for gas chromatography analysis. A Rtx®-624 column (30 m × 0.32 I.D., 1.8 μm, Restek, Benner Circle, Bellefonte, PA, USA) was used for headspace–gas chromatography–mass spectrometry (HS–GC–MS) analysis. Operation conditions for gas chromatography (*Varian 3400cx*, Sugar Land, TX, USA) and HS–GC–

Table 1
Experimental conditions for GC and HS–GC–MS analyses

	GC analysis	HS–GC–MS analysis
Temperature	50 (2 min) to 180 °C at 30 °C min^{-1} to 280 °C at 5 °C min^{-1} (25 min)	45 (5 min) to 95 °C at 2 °C min^{-1} (25 min) to 125 °C at 6 °C min^{-1} (15 min)
Carrier gas	He (5.0 grade), 2.7 ml min^{-1}	55 kPa, He (5.0 grade), 1.1 ml min^{-1}
Detector	FID (350 °C)	MSD
Injection	SPI, 50 (0.5 min) to 280 °C at 200 °C min^{-1} (30 min), 1.0 μl	Headspace autosampler (Table 2)
Sample	4 mg ml^{-1} in cyclohexane	500 mg original sample per vial

Table 2
Operation conditions of the headspace autousampler

Varian Headspace Autosampler-Tekmar 7000			
Platen temperature	80 °C	Loop fill time	1.5 min
Sample equilibrium time	50 min	Loop equilibrium time	0.2 min
Mixing time	10 min	Inject time	0.5 min
Stabilising time	2 min	Valve temperature	200 °C
Vial pressure	8 psi	Transfer line temperature	200 °C
Pressurising time	1.5 min	Loop volume	1 ml
Pressurising equilibrium	0.20 min	Vial size	22.5 ml

MS (*Varian Headspace Autosampler-Tekmar 7000*, *Varian 3400cx*, *Saturn 4D/GC/MS/MS*, Sugar Land, TX, USA) are summarized in Table 1. Conditions for the Headspace Autosampler are shown in Table 2. Mass spectra were obtained at the electron impact of 70 eV and chemical ionization (with methane as reagent gas) at 15 eV. Chromatograms were recorded by monitoring the total ion current in the range of 30–400 amu. Transfer line and ion trap manifold were maintained at 220 and 170 °C, respectively.

The compound identification was carried out by matching mass spectra with those recorded in the NIST (National Institute of Standards and Technology) mass spectral search program as well as their molecular weights (obtained from chemical ionization process). Identification results were confirmed by the injection of authentic substance as reference, wherever possible (total 27 compounds), and by the regular cycle of retention time of the homologues.

2.3. Sample preparation

Five hundred milligrams of each ointment sample was weighed in a headspace vial and capped air-tightly for the chromatographic procedure.

For the preparation of calibration curves, reference compounds were dissolved in 1,3-dimethyl-2-imidazolidinone (DMI), then spiked in a small volume (10 μl) into EOB-0, which contained no detectable VOC compounds under the headspace conditions. Thus, the matrix of standard samples is created to be similar to that of eye ointment samples and the matrix effect [12] of EOB can be reduced.

3. Results and discussion

3.1. Degradation profiles of EOB and identification

The most obvious variation of irradiated eye ointment is manifested in the production of bubbles after irradiation, even at 15 kGy. This indicates the formation of volatile radiolysis products. HS–GC–MS was therefore employed to analyze these volatile products. A typical total ion chromatogram of an EOB sample irradiated at 50 kGy, along with the chromatogram of non-irradiated EOB, is presented in Fig. 1. Forty-one volatile compounds were detected and identified in the irradiated EOB, while only four small peaks were found in the non-irradiated sample. The size of the four peaks found in the non-irradiated EOB increases after irradiation, evidencing that all volatile compounds detected in the irradiated EOB are radiolysis products.

Hydrocarbons are the dominant radiolysis products, including 14 alkanes, 18 alkene, and five cycloparaffin. Alcoholate and ketone were also observed in radiolysis products. Because some alkene compounds have very similar mass spectra, the chemical structures of six alkenes (peaks 4, 11, 12, 22, 33 and 34) cannot be assigned with confidence. The main radiolysis products are *n*-alkanes (peaks 1, 3, 9, 20, 31, and 41), except peak 13 (Fig. 1). Hydrocarbon compounds in radiolysis products can be divided approximately into six groups sorted by their carbon numbers, i.e. hydrocarbon compounds in the same group have the same carbon numbers. Each *n*-alkane can be a mark of the group to

which it belongs. As Table 3 shows, the straight and branch chain hydrocarbons of radiolysis products appear in a regular order. Whenever the carbon number allows, certain homologues exist in each group.

3.2. Degradation profiles of individual ingredients

The production of bubbles was also observed in white petrolatum and wool fat, but not in the liquid paraffin. However, volatile radiolysis com-

1 n-Propane
2 Isobutane
3 n-Butane
4 Alkene, C_4H_8
5 2-Butene
6 3-Methyl-1-butene
7 2-Methyl butane
8 1-Pentene
9 n-Pentane
10 2-Pentene
11 Alkene, C_5H_{10}
12 Alkene, C_5H_{10}
13 Acetone
14 2-Propanol
15 4-Methyl-1-pentene,
16 2-Methyl pentane
17 Cyclopetane
18 3-Methyl pentane
19 1-Hexene
20 n-Hexane
21 2-Hexene
22 Alkene, C_6H_{12}
23 Unknown peak
24 Methylcyclopentane
25 2-Butanone
26 5-Methyl-1-hexene
27 2-Methyl hexane,
28 Cyclohexane
29 3-Methyl hexane,
30 1-Heptene
31 n-Heptane
32 2-Heptene
33 Alkene, C_7H_{14}
34 Alkene, C_7H_{14}
35 Methylcyclohexane
36 6-Methyl-1-heptene,
37 2-Methy heptane
38 Cycloheptane
39 3-Methyl heptane,
40 1-Octene
41 n-Octane

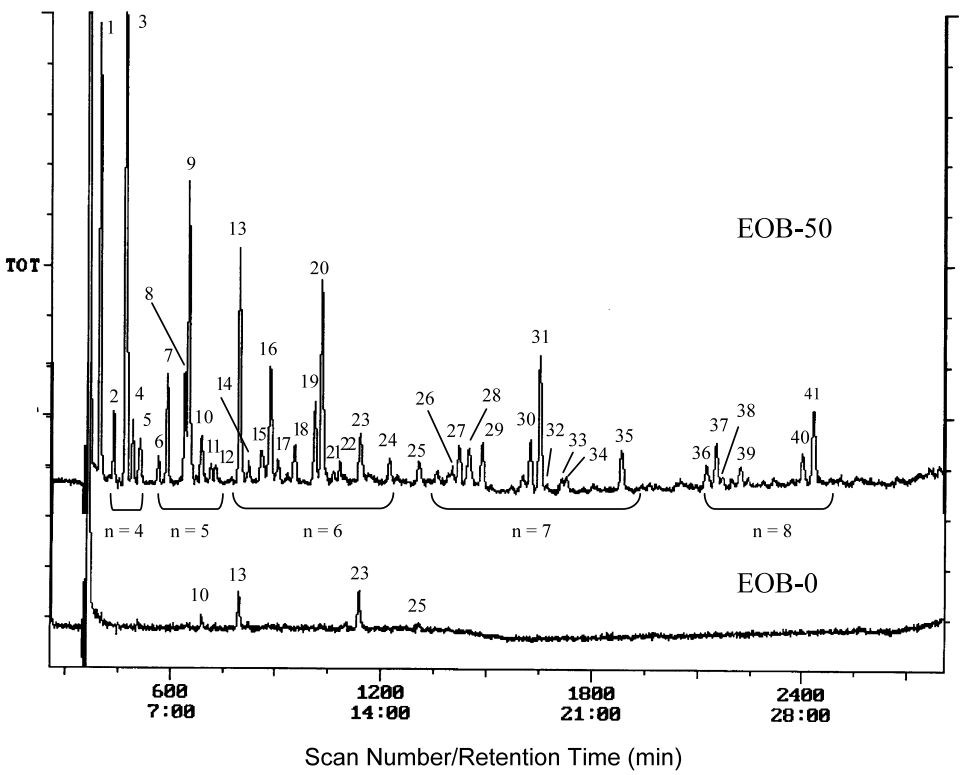


Fig. 1. Identification of the volatile radiolysis products of irradiated EOB by HS–GC–MS (EOB-50: irradiated at 50 kGy, EOB-0: non-irradiated EOB, n = carbon number).

Table 3
Molecule structures and the order of the straight and branch hydrocarbons of the radiolysis products in six groups, n = carbon numbers, the numbers are scan numbers (the same as in Fig. 1)

group n = 3	group n = 4	group n = 5
—	—	563 $H_2C{=}C(H)-C(H)(CH_3)-CH_3$
—	436 $H_3C-C(H)(CH_3)-CH_3$	589 $H_3C-C(H)(CH_3)-CH_2-CH_3$
—	—	639 $H_2C{=}C(H)-CH_2-CH_2-CH_3$
396 $H_3C-C(H_2)-CH_3$	469 $H_3C-CH_2-CH_2-CH_3$	650 $H_3C-CH_2-CH_2-CH_2-CH_3$
—	511 $H_3C-C(H){=}C(H)-CH_3$	686 $H_3C-C(H){=}C(H)-CH_2-CH_3$
group n = 6	**group n = 7**	**group n = 8**
859 $H_2C{=}C(H)-CH_2-C(H)(CH_3)-CH_3$	1356 $H_2C{=}C(H)-CH_2-CH_2-C(H)(CH_3)-CH_3$	2125 $H_2C{=}C(H)-CH_2-CH_2-CH_2-C(H)(CH_3)-CH_3$
883 $H_3C-C(H)(CH_3)-CH_2-CH_2-CH_3$	1420 $H_3C-C(H)(CH_3)-CH_2-CH_2-CH_2-CH_3$	2153 $H_3C-C(H)(CH_3)-CH_2-CH_2-CH_2-CH_2-CH_3$
953 $H_3C-C(H_2)-C(H)(CH_3)-CH_2-CH_3$	1486 $H_3C-C(H_2)-C(H)(CH_3)-CH_2-CH_2-CH_3$	2223 $H_3C-C(H_2)-C(H)(CH_3)-CH_2-CH_2-CH_2-CH_3$
1009 $H_2C{=}C(H)-CH_2-CH_2-CH_2-CH_3$	1623 $H_2C{=}C(H)-CH_2-CH_2-CH_2-CH_2-CH_3$	2400 $H_2C{=}C(H)-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$
1028 $H_3C-CH_2-CH_2-CH_2-CH_2-CH_3$	1650 $H_3C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$	2430 $H_3C-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$
1063 $H_3C-C(H){=}C(H)-CH_2-CH_2-CH_3$	1668 $H_3C-C(H){=}C(H)-CH_2-CH_2-CH_3$	—

pounds were detected in all the three materials using HS–GC–MS after irradiation (Fig. 2), while these volatile compounds were not found in the relevant non-irradiated samples. In addition, the concentration of these volatile radiolysis products in all samples was directly proportional to the irradiation dosage (unpublished data).

Volatile radiolysis products in liquid paraffin and white petrolatum are the same (Fig. 2B and C), but the radiolysis product profile of wool fat looks different from those of liquid paraffin and white petrolatum. In irradiated wool fat, straight chain alkanes (peaks 1, 3, 9. 20. 31, and 41) exist only in smaller amount (Fig. 2A). Acetone (peak 13) is the most predominant radiolysis product in

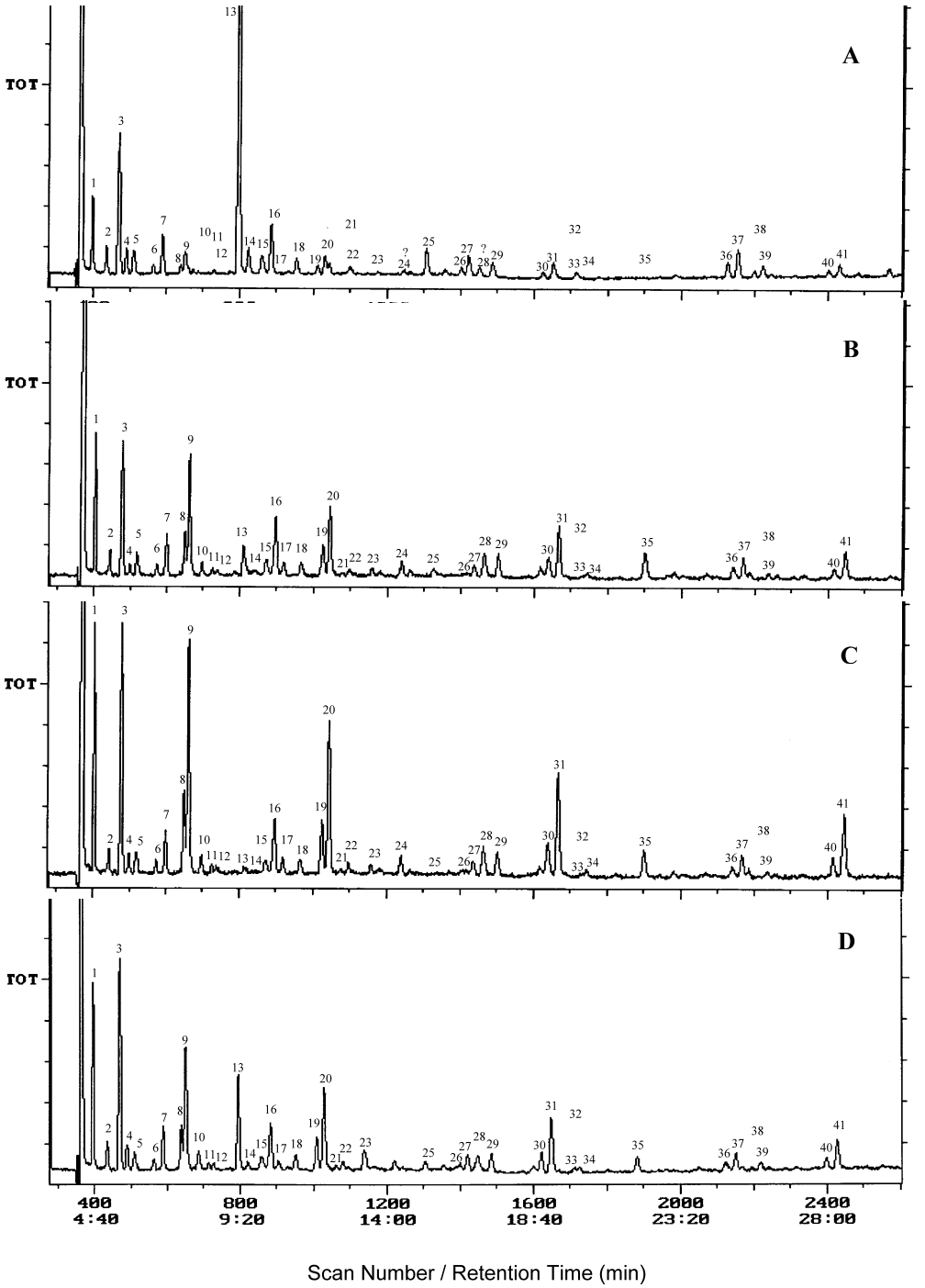


Fig. 2. Comparison of volatile radiolysis products at 50 kGy by HS–GC–MS: irradiated wool fat (A), liquid paraffin (B), white petrolatum (C), and EOB (D).

irradiated wool fat, whereas it is much less in irradiated liquid paraffin and almost disappears in irradiated white petrolatum. It is also true in the cases of 2-propanol (peak 14) and 2-butanone (peak 25). Cycloparaffin products (peaks 17 24, 28 and 35) disappear in irradiated wool fat. Although there are two peaks at the same retention time of peaks 24 and 28 in chromatogram of irradiated wool fat, their mass spectra prove that they are different from the radiolysis products in irradiated white petrolatum or liquid paraffin.

Nevertheless, white petrolatum, liquid paraffin and wool fat produce the same straight and branch chain hydrocarbons as radiolysis products, though their relative intensities are different.

All volatile radiolysis products that appear in white petrolatum, liquid paraffin and wool fat are present in irradiated EOB (Fig. 2D). Cycloparaffins are the unique radiolysis products of white petrolatum and liquid paraffin, and acetone, 2-propanol and 2-butanone are the only radiolysis products of wool fat. However, all of these compounds appear in irradiated EOB. This means that acetone, 2-propanol and 2-butanone in irradiated EOB originate from wool fat, while cycloparaffins come from white petrolatum and liquid paraffin.

3.3. Component distribution pattern

The distribution pattern of hydrocarbons was identifying by means of GC method (Fig. 3). The components of EOB, liquid paraffin and white petrolatum are so complex that the GC method with a direct injection does not achieve a complete separation even with a capillary column. However, the slow and drifted baseline suggests the presence of isoparaffins, cycloparaffins, etc. and the normal alkanes show appreciable peaks [13]. The gas chromatograms of non-irradiated and irradiated samples overlap perfectly in each case, even at very high irradiation dose of 100 kGy (Fig. 3). Although the GC method could not provide an enough separation capability, it hints that the amounts of the main composition of EOB changes little after irradiation.

Furthermore, normal alkanes in the GC chromatograms provide information on the distribution of carbon numbers. It was reported that for liquid or solid hydrocarbons, an important consequence of radiation effect was the formation of radiolysis products of higher weight molecules [14], and under prolonged irradiation, a sufficient amount of the molecule became linked together to form a gel extending throughout the system [15]. Present GC results show that the distribution patterns of *n*-alkanes remain unchanged after gamma processing and no peaks appear at the end part of the chromatogram. This provides strong evidences that there are no detectable products of condensation reaction in the petrolatum eye ointment.

In conclusion, the GC results demonstrate that there are no significant chemical changes in the main composition of the petrolatum ointment base as well as its individual ingredients after gamma irradiation. The distribution of hydrocarbons in EOB does not change significantly after irradiation although volatile radiolysis products are produced during irradiation.

3.4. Degradation pathways

3.4.1. Long carbon chain degradation

Paraffin differs from petrolatum in their carbon chains. Wool fat differs from paraffin and petrolatum in molecular structure. EOB differs from paraffin and petrolatum in composition. However, headspace chromatograms (Fig. 2) show that the radiolysis products of these different materials include the same straight and branch chain hydrocarbons. This indicates that the radiolysis products of white petrolatum, liquid paraffin, and wool fat are probably produced by the similar degradation pathway, possibly the same functional group in their molecules degrades in all these materials.

What all these materials have in common is the long carbon chains in their molecular structure. Since the C–C bonds in large straight molecules are of similar strength, and can be broken equally under irradiation [15], the initial ionization can occur virtually anywhere along the chain. Thus, the radiolysis products are independent of the original chain structure and its length after the initial ionization. Accordingly, petrolatum and

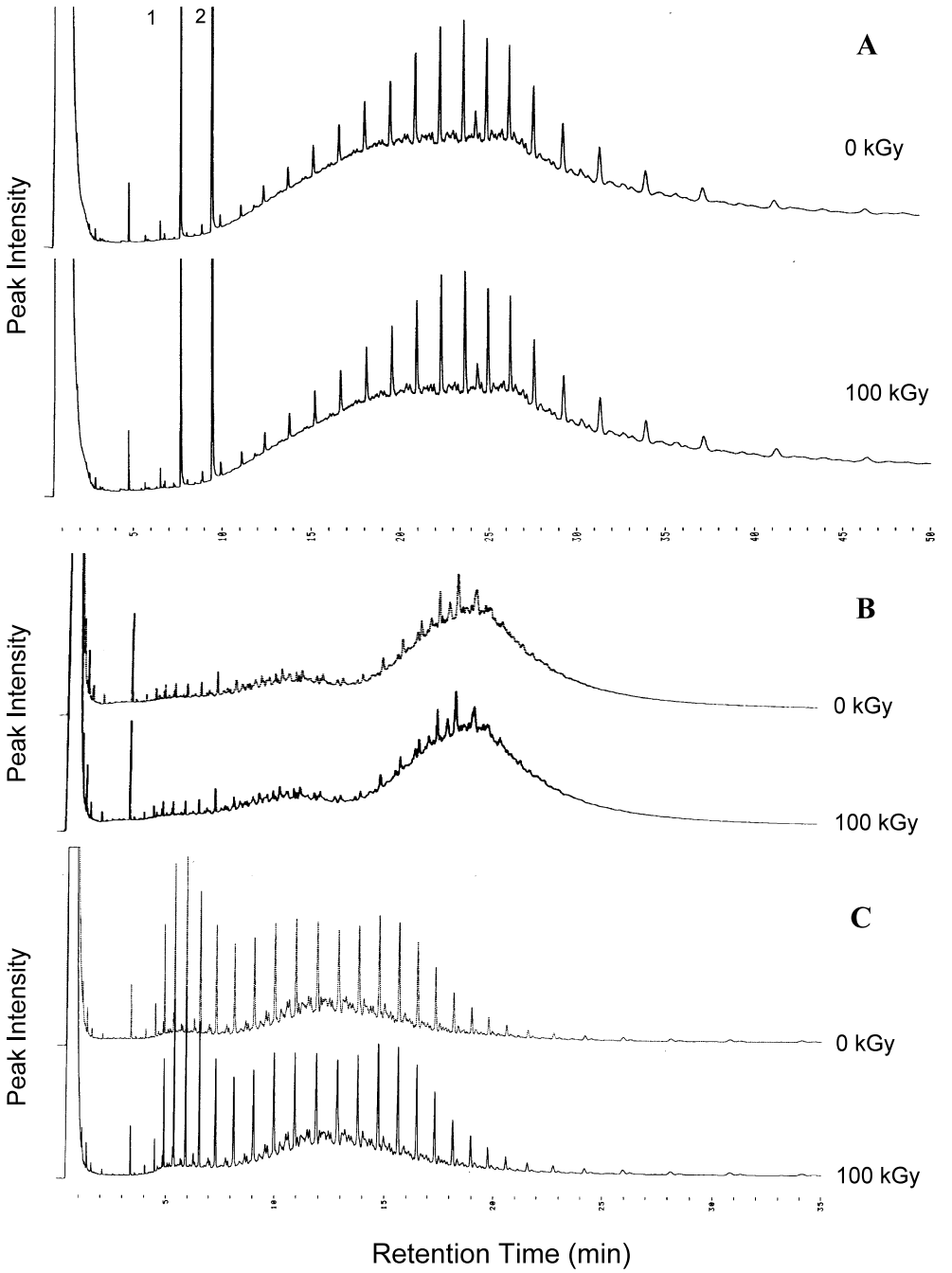


Fig. 3. Profiles by gas chromatograms: (A) irradiated and non-irradiated EOB (peak 1 = cetyl alcohol, peak 2 = stearyl alcohol, they are also the ingredients of the present EOB); (B) irradiated and non-irradiated liquid paraffin; (C) irradiated and non-irradiated petrolatum.

liquid paraffin give the same degradation products.

In the case of wool fat, as long as the carbonyl and long straight chain radicals are formed by bond rupture reactions, further bond scission of the C–C chains will proceed by the same pathways as in white petrolatum or liquid paraffin. Eventually wool fat has the radiolysis products similar to those of liquid paraffin and white petrolatum.

As unique radiolysis products of wool fat, on the other hands, the appearance of acetone, 2-propanol and 2-butanone reflects the chemical feature of wool fat. The presence of cycloparaffins in irradiated white petrolatum and liquid paraffin could be due to the existence of cycloparaffin groups in molecular structures of the original samples.

The radiolysis product profile of EOB shows that the radiolysis products of EOB are a total contribution of the radiolysis products of white petrolatum, liquid paraffin and wool fat. This indicates that the matrix changes have no influence upon the radiolysis pathway of these materials.

3.4.2. Mass spectrum degradation model

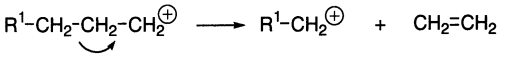
The earlier investigation evidenced that dissociation patterns obtained by mass spectrometry are essentially independent of the ionizing potential employed, so that mass spectral data could be applied to radiation–chemical systems irradiated with radiation of much higher energy. This means that the dissociation pattern of a compound in the mass spectrometer could be a useful source of information in establishing the radiolysis mechanism [16].

It was found in the present work that the radiolysis product profile of irradiated EOB detected by HS–GC was quite similar to a typical mass spectrum of long carbon chain *n*-alkane. The Fig. 4 shows that the radiolysis products of irradiated EOB exhibit the following characteristics:

- An abundance of low mass.
- Characteristic peaks of *n*-alkanes.
- Independent on the chain length.

These are features of mass spectra of long carbon chain *n*-alkane, i.e. *n*-octadecane. The characteristic ions in mass spectrum are $[(C_nH_{2n+2})-1]^+$ and $[(C_nH_{2n})-1]^+$, and the characteristic peaks in irradiated EOB profile are C_nH_{2n+2} and C_nH_{2n}. The series of intensive ions at m/z 43, 57, 71, 85, 99 and so forth are highly characteristic of saturated aliphatic hydrocarbons of *n*-propane (44), *n*-butane (58), *n*-pentane (72), *n*-hexane (86), *n*-heptane (100), etc.

This indicates that alkane chain in both mass spectrometer and radiolysis process degrades by a similar pathway. As the same in mass spectrometer, the secondary and especially tertiary C–C bonds are particularly liable to fission during ionization process with high energy [14], accordingly, the proportion of fragmentation products decreases in the order of *n*-alkane > *iso*-alkane ≫ *neo*-alkane, and *n*-alkanes become the featured radiolysis products. And the main rupture of C–C bond tends to continue from the chain end and results in secondary or further fragmentation in the following way:



The driving force for this fragmentation is the formation of the new π-bond in the extruded molecule of ethylene, which lowers the activation energy toward the secondary fragmentation of high mass ions [17]. As a result, intensive peaks of low mass radiolysis compounds were observed and material with different chain length gave similar radiolysis products.

This result suggests by means of molecule rapture pattern in mass spectrum a rupture model of radiolysis for long carbon chain hydrocarbons could be created or confirmed without a great deal of laborious work.

3.5. Quantitative analysis and evaluation

The quantitative determination of radiolysis products of the EOB is summarized in Table 4. The concentrations of all the radiolysis products of EOB increase linearly with increasing irradiation doses. Most of the correlation coefficients (r)

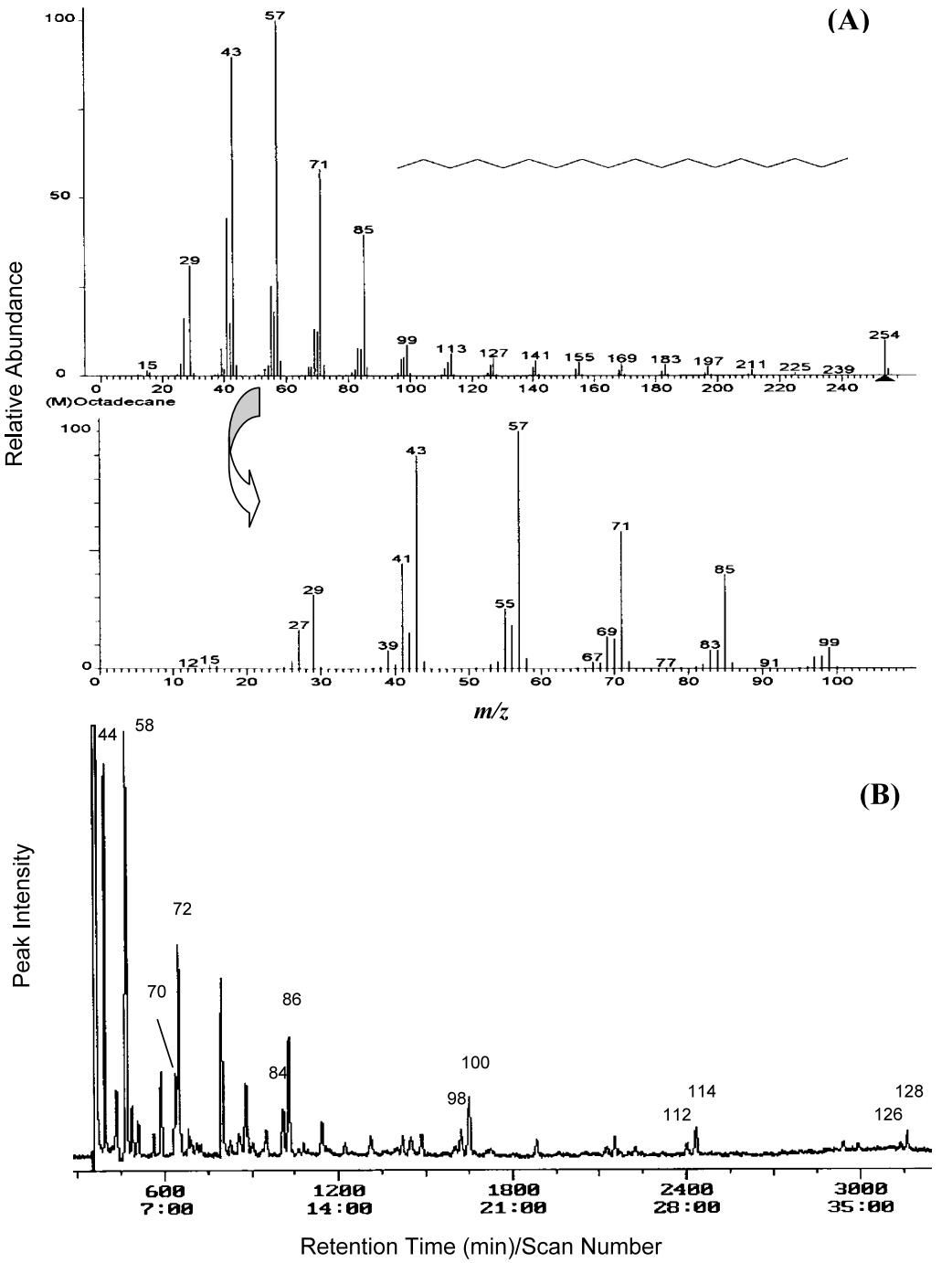


Fig. 4. Relationship between *n*-alkane degradations in MS process and gamma radiation process: (A) a mass spectrum of *n*-octadecane from NIST database, in which the numbers represent ion fragment m/z of $[M-1]^+$; (B) a total ion chromatogram of radiolysis products in irradiated eye ointment by HS–GC–MS, in which the numbers represent molecular weight of radiolysis products, *n*-propane (44), *n*-butane (58), 1-pentene (70), *n*-pentane (72), 1-hexene (84), *n*-hexane (86), 1-heptene (98), *n*-heptane (100), 1-octene (112), *n*-octane (114), 1-nonene (126) and *n*-nonane (128).

Table 4
Quantitative analysis of the radiolysis products of irradiated EOB

No.[a]	Scan no.	Concentration, ppm (RSD%)			r^b	No.	Scan no.	Concentration, ppm (RSD%)			r^b
		50 kGy	25 kGy	15 kGy				50 kGy	25 kGy	15 kGy	
1	396	26.4 (2.3)	15.5 (1.3)	7.0 (2.4)	0.985	22	1080	1.64 (2.4)	0.91 (6.9)	0.79 (5.2)	0.988
2	436	5.1 (6.8)	2.5 (0.6)	1.6 (3.1)	0.999	23	1138	7.3 (6.7)	6.6 (5.7)	6.4 (6.6)	0.995
3	469	40.3 (2.9)	21.6 (1.6)	13.3 (2.0)	0.999	24	1222	2.4 (6.7)	1.3 (2.4)	0.8 (3.5)	0.999
4	491	3.9 (3.4)	2.0 (3.6)	1.3 (3.3)	0.999	25	1303	7.8 (0.8)	5.1 (5.5)	3.5 (3.0)	0.995
5	511	3.4 (2.5)	2.1 (3.9)	1.6 (5.3)	0.999	26	1356	0.96 (4.4)	0.41 (5.6)	0.28 (8.7)	0.995
6	563	2.0 (6.3)	1.2 (1.6)	0.7 (5.3)	0.997	27	1420	3.8 (3.2)	2.1 (7.2)	1.2 (8.8)	0.997
7	589	7.7 (1.9)	3.9 (4.9)	2.3 (4.5)	1.000	28	1449	5.3 (1.5)	2.7 (1.6)	1.8 (2.0)	1.000
8	639	7.2 (4.5)	3.8 (1.6)	2.2 (6.3)	0.999	29	1486	4.6 (4.1)	2.3 (4.7)	1.5 (5.1)	0.999
9	650	21.5 (1.7)	10.9 (4.7)	6.6 (3.2)	1.000	30	1623	4.9 (7.2)	2.4 (2.6)	1.8 (2.5)	0.997
10	686	2.2 (6.2)	1.1 (16.4)	0.6 (11.2)	0.999	31	1650	12.0 (0.4)	5.8 (1.9)	3.9 (1.8)	0.998
11	713	1.05 (14.7)	0.55 (5.2)	0.48 (10.2)	0.985	32	1668	0.43 (5.6)	0.21 (2.4)	0.17 (4.2)	0.990
12	728	0.90 (0.5)	0.54 (3.0)	0.49 (5.1)	0.984	33	1715	3.0 (1.8)	1.6 (8.2)	1.1 (10.8)	1.000
13	795	12.1 (3.3)	8.2 (0.2)	6.3 (2.1)	0.999	34	1728	0.0	0.0	0.0	0.000
14	821	2.1 (3.4)	1.2 (6.9)	1.1 (4.5)	0.982	35	1884	3.9 (2.0)	2.1 (8.0)	1.4 (4.2)	1.000
15	859	3.2 (3.6)	1.6 (5.3)	1.3 (6.8)	0.992	36	2125	2.3 (4.1)	1.3 (1.5)	0.8 (2.6)	0.995
16	883	10.2 (1.0)	5.2 (3.2)	3.7 (2.1)	0.998	37	2153	3.9 (5.2)	2.0 (2.6)	1.1 (3.0)	0.999
17	904	2.3 (4.2)	1.2 (6.3)	0.5 (8.3)	0.994	38	2170	0.75 (5.7)	0.38 (13.5)	0.20 (8.8)	0.999
18	953	3.2 (5.5)	1.6 (9.8)	1.0 (5.5)	1.000	39	2223	2.0 (5.7)	1.0 (5.6)	0.6 (6.6)	0.999
19	1009	8.3 (1.2)	4.3 (1.5)	2.6 (1.8)	0.999	40	2400	3.1 (1.4)	1.6 (9.5)	0.9 (1.8)	0.998
20	1028	20.3 (2.8)	10.3 (2.1)	6.9 (2.0)	0.999	41	2430	6.8 (1.7)	3.7 (0.6)	2.2 (1.8)	0.999
21	1063	0.76 (8.0)	0.34 (10.5)	0.28 (8.7)	0.986						

The data in parentheses present relative standard deviation, $n = 3$.

[a] Peak number in Fig. 1.

[b] $r_{0.05,1} = 0.997$.

of linear regression between concentrations of the radiolysis products and the irradiation doses are higher than 0.997 ($r_{0.05,1}$, the critical value at 95% confidence interval), especially for the main radiolysis products. The main radiolysis products of the EOB irradiated at 25 kGy (Table 4) exist at the levels of about 10 ppm. An immediate question is whether or not these radiolysis products will cause unacceptable toxicity at such a concentration level.

All the radiolysis products of EOB detected in the present work are volatile organic compounds. They can be regarded as volatile residual solvents in pharmaceuticals. According to the *Impurities Guideline for Residual Solvents* developed by *International Conference on Harmonization* (*ICH*) [18], safety risks of residual solvents to human health are placed into three classes. Class 1 residual solvents should not be contained in pharmaceuticals, and classes 2 and 3 residual solvents in pharmaceuticals should be controlled at the levels lower than those required in the guideline. The limits of some compound, which are involved in the present work, are excerpted from the guideline in Table 5.

The present result shows that none of radiolysis products from EOB belongs to the compounds in classes 1 and 9 of them are listed in classes 2 and 3. Comparison of Tables 4 and 5 shows that the concentrations of the nine radiolysis compounds at 25 kGy (the reference dose for pharmaceutical sterilization), or even at a higher dose of 50 kGy, are much lower than their relevant thresholds in ICH guideline. This means that the nine radiolysis products are at safety levels for human health.

The rest 32 radiolysis products are hydrocarbon compounds, which not only belong to a lower toxicological class, but also are at safety concentration levels for pharmaceutical products. The highest concentration among the 32 radiolysis products is < 5 ppm at 25 kGy. According to ICH guideline, the concentration of 5 ppm is much lower than the safety limits for classes 2 and 3, even for class 1. For example, the very toxic compounds in class 1, benzene (carcinogen) and 1,2-dichloromethene (toxic and environmental hazard) have the concentration limits of 2 and 5 ppm, respectively [18].

Therefore, the both qualitative and quantitative data ensure that all these volatile radiolysis products have a low toxic potential, and are at the safety levels acceptable for human use at the reference radiation dose of 25 kGy.

4. Conclusion

HS–GC is an appropriate technique to determine trace volatile radiolysis products in irradiated eye ointment. Forty-one radiolysis products, 14 alkanes, 18 alkene, five cycloparaffin, and four others, are found. Acetone, 2-propanol and 2-butanone are the unique radiolysis products of wool fat, and cycloparaffins are the unique radiolysis products of white petrolatum and liquid paraffin. The common radiolysis products of the three

Table 5
Safety limits of residual solvents in pharmaceuticals for human use by *International Conference on Harmonization* (*ICH*) guideline

Peak no.	Name	Class	PDE[a] (mg/day)	Concentration limit (ppm)
9	*n*-Pentane	3	50	5000
13	Acetone	3	50	5000
14	2-Propanol	3	50	5000
20	*n*-Hexane	2	2.9	290
23	1-Propanol	3	50	5000
25	2-Butanone	3	50	5000
28	Cyclohexane	2	38.8	3880
31	*n*-Heptane	3	50	5000
35	Methylcyclohxane	2	11.8	1180

[a] Permitted daily exposure.

materials are straight and branch chain hydrocarbon, which appear regularly as homologues in six groups. The radiolysis products of EOB are a total contribution of the radiolysis products of its ingredients (white petrolatum, liquid paraffin and wool fat).

Ruptures of the long carbon chains are the main degradation pathways of EOB as well as its ingredients. Variation in molecular structure and matrix composition has little influence on the radiolysis behavior of these materials.

The radiolysis product profile of irradiated EOB determined by HS–GC is quite similar to a typical mass spectrum of long carbon chain *n*-alkane, suggesting that the radiolysis mechanism approximates the degradation mechanism in mass spectrometer. The fragment pattern of *n*-alkane in mass spectrometer can be used as a theoretical prediction for the radiolysis product study.

Gamma radiation treatment imposes no major influence on the composition of petrolatum eye ointment, and no significant condensation reaction can be observed. Both qualitative and quantitative data demonstrate that the volatile radiolysis products in irradiated petrolatum EOB are safe for human health at the standard irradiation dose of 25 kGy. Thus, it is feasible to perform gamma sterilization on petrolatum eye ointment, white petrolatum, liquid paraffin and wool fat.

References

[1] The Use of Gamma Radiation Source for the Sterilization of Pharmaceutical Products, Association of the British Pharmaceutical Industry, London, 1960.
[2] G. Schwenken, H. Vogt, Pharmazeutische Industrie 24 (1962) 163–167.
[3] G. Schwenken, Archiv der Pharmazie 66 (1961) 661–679.
[4] J. Controulis, C.A. Lawrence, L.E. Brownell, J. Am. Pharm. Assoc. 43 (1954) 65–69.
[5] T. Horne, 2nd UN Int. Cont. on the Peaceful Uses Atomic Energy, Geneva, 1958.
[6] Examination of Radiosterilization of Pharmaceuticals and Pharmaceutical Basic Materials, Final Report of the Agency Research Contract No. 351/RB, Pharmaceutical Institute of the Medical University of Budapest, 1968.
[7] G. Hangay, G. Hortobagyi, G. Muranyi, in: Proceedings of a Symposium, Budapest, 1967, pp. 55–61.
[8] H.L. Bhalla, M.R. Menon, N.G.S. Gopal, Indian J. Pharm. Sci. 45 (1983) 203–206.
[9] G. Hangay, in: E.R.L. Gaugham, A.J. Gaudie (Eds.), Sterilization of Medical Products by Ionizing Radiation, Multiscience Publication Limited, Montreal, Canada, 1978, pp. 247–263.
[10] G. Hangay, Acta Pharmaceutica Hungarica 40 (1970) 75–80.
[11] R.A. Nash, Bull. Parant. Drug Assoc. 28 (1974) 181–187.
[12] L. Hong, H. Altorfer, Pharmaceutica Acta Helvetiae 72 (1997) 95–104.
[13] H. Butler, Cosmetic raw material analysis and quality, in: Hydrocarbons, Glycerides, Waxes and Other Esters, vol. I, Micelle Press, England, 1994, p. 26.
[14] A.J. Swallow, Radiation Chemistry of Organic Compounds, Pergamon Press, London, 1960, p. 61.
[15] A. Charlesby, Proc. R. Soc. A222 (1954) 60–74.
[16] J.W.T. Spinks, R.J. Woods, An Introduction to Radiation Chemistry, John Wiley & Sons, Inc, New York, 1990, p. 142.
[17] R.M. Smith, Understanding Mass Spectra—A Basic Approach, John Wiley & Sons, Inc, New York, 1999, p. 96.
[18] ICH Steering Committee: Impurities Guideline for Residual Solvents, Q3C, Switzerland, 1997.