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Reactivity of Thiyl Radicals Generated from Thiomethoxide and Dimethyldisulfide in Microheterogeneous Media

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Abstract: Sodium thiomethoxide and dimethyldisulfide will be shown to be convenient sources of methyl thiyl radicals for effecting the *cis-trans* double bond isomerization of oleic acid to elaidic acid residues arranged in phospholipid bilayers of the microheterogeneous aqueous environment of liposomes. The methods used for methyl thiyl radical generation encompass radiolytic and photochemical methods. The convenience of these methyl thiyl radical sources will be interpreted in terms of the turn-over numbers in the radical chain *cis-trans* isomerization reaction of unsaturated lipids.

Keywords: Methyl thiyl radicals, Sodium thiomethoxide, Dimethyldisulfide, *cis-trans* isomerization.

INTRODUCTION

Thiyl radicals have been extensively studied in the context of free radical double bond isomerization. Fig. (**1**) shows the reaction mechanism of free thiyl radical RS• double bond isomerization that consists of a reversible addition of radical RS• to the double bond. The regeneration of the double bond is obtained by β-elimination of RS• and the result is in favor of the *trans* geometry, the most thermodynamically favorable arrangement. The energy difference between the two geometrical isomers of parent 2-butene is 1.0 kcal/mol. Many studies have shown that the radical RS• acts as a catalyst for *cis–trans* isomerization, and that positional isomers (those occurring from double bond transposition) cannot be formed as reaction products because the mechanism does not allow a double bond shift [1,2]. Large quantities of thiols present in the reaction medium, precursors of thiyl radicals, can lead to addition products instead. The rate constants for the four processes are shown in Fig. (**1**). Considering polyunsaturated substrates, the isomerization mechanism occurs as a step-by-step process depicted in Fig. (**2**) for linoleate moiety, *i.e*., each isolated double bond behaves independently as discussed above [3].

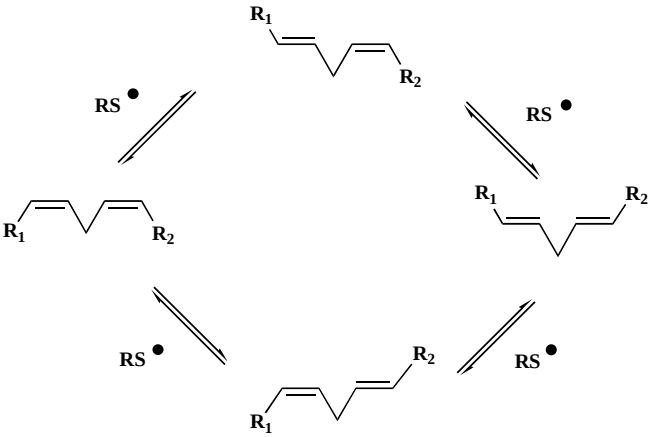
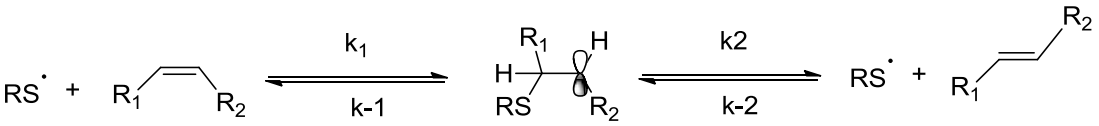


Fig. (2). Stepwise mechanism for the *cis—trans* isomerization of linoleate residues.



k_1= 1.6 x 10^5 $dm^3mol^{-1}s^{-1}$ k_{-1}= 1.7 x 10^7 s^{-1}

k_2= 1.6 x 10^8 s^{-1} k_{-2}= 2.9 x 10^5 dm^3 $mol^{-1}s^{-1}$

Fig. (1). The thiyl radical RS• acts as a catalyst for *cis–trans* isomerization by addition/elimination steps.

Other types of free radicals (e.g., RSe•, RSO_2•, NO_2•, R_3Sn•, $(Me_3Si)_3Si$•, etc.) and atoms (*e.g*., Br•, I•, etc.) are also known to induce *cis–trans* isomerization of double bonds by addition–elimination steps [4]. However, the efficiency of the isomerization process strongly depends on the characteristics of the attacking radicals, and although another biologically important radical is NO_2•, it has been shown that thiols are known as the dominant 'sink' for NO_2• in cell/tissues (Equation 1) [5]. The rate constant for reaction of NO_2• with thiols RSH is close to 2 x 10^7 $dm^3mol^{-1}s^{-1}$ with generation of thiyl radicals, therefore in the biological environment thiyl radicals are likely to be the most relevant isomerizing species [6]. Consequently, being CH_3SH a known metabolite [7a] found in human blood in concentration of ca. 5.7 μmol dm^{-3} [7a,b], we deemed appropriate the study of this source of thiyl radicals in connection with biological processes as well as the related $(CH_3S)_2$ metabolite.

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$$NO_2^{\bullet} + RSH \longrightarrow NO_2^- + RS^{\bullet} + H^+ \quad (1)$$

The studied sources of thiyl radicals known capable of inducing *cis-trans* double bond isomerization are chemical sources such as alkanylthiols, 2-thioethanol [8], thioethers, thioesters, and disulfides [8], and others. Compounds such as glutathione [9], and hydrogen sulfide [10], constitute known sources of thiyl radicals. Thiols, disulfides, and thioether functions present in aminoacid residues such as cystine [11], cysteine, and methionine [12], upon tandem radical damage release suitable sources of thiyl radicals.

The *in-vitro* methods used for generation of thiyl radicals encompass: photochemical methods, radiolytic methods, and thermal methods. In this account we shall describe other two sources of methyl thiyl radicals such as sodium thiomethoxide and dimethyldisulfide, probing their radical reactivity on the *cis-trans* isomerization of unsaturated fatty acid residues contained in the microheterogeneous environment of liposomes.

EXPERIMENTAL PART

Materials $NaSCH_3$, $(CH_3S)_2$, KOH, methyl palmitate, methyl oleate and methyl elaidate were purchased from Sigma Aldrich and used without further purification. Lyophilized 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was obtained from Avanti Polar Lipis Inc. and used as received. *tert*-Butyl alcohol, chloroform and hexane were purchased from Merck (HPLC grade) and used without further purification.

General Methods. GC analyses for the determination of the isomeric ratio of the unsaturated fatty acids were performed by using a Hewlett Packard 5890 gas cromatograph equipped with a flame ionization detector. An Rtx-2330 column (60 m x 0.25 mm of 10% cyanopropylphenyl and 90% biscyanopropylpolysiloxane) was used with nitrogen as carrier gas. The heating was carried out at a temperature of 150 ºC for 60 min followed by an increase of 5 ºC/min up to 195 ºC. The methyl esters were identified by comparison with the retention times of authentic samples. Continuous radiolysis were performed at room temperature (22 ± 2 ºC) on 4 mL samples using a ^{60}Co Gammacell at different dose rates. The samples were deareated using an Ar stream and if needed saturated with N_2O. The exact radiation dose absorbed was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.61\ \mu mol\ J^{-1}$ [13]. Photolysis was carried out under an Ar stream in a quartz photochemical reactor (Sigma Aldrich) equipped with a 5.5 W low-pressure mercury lamp. The temperature was maintained at (22 ± 1) ºC.

Isomerization of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine in LUVETs. A chloroform solution of POPC (1mL; 90 mmol dm^{-3}) was evaporated to a thin film in a test tube using an argon stream and then placing the sample under vacuum for 30 min. Degassed Milli-Q water (1 mL) was added, and the sample was vortex stirring for 5 min. To obtain LUVETs, the lipid emulsion was transferred into a LiposoFast extruder (Avestin, Inc.) and extruded 20 times through two polycarbonate membranes with a pore diameter of 100 nm. The suspension was then transferred to a vial and kept under Ar. The final suspensions were prepared in a vial equipped with an open top screw-cap and a teflon-faced septum by diluting the 90 mmol dm^{-3} POPC suspension to 2 mmol dm^{-3} and adding $NaSCH_3$ (0.2 mmol dm^{-3}) or $(CH_3S)_2$ (75 µmol dm^{-3}) and *tert*-Butyl alcohol (0.2 mol dm^{-3}) when needed. Aliquots of 200 µL were withdrawn and processed at different irradiation doses or photolysis times by partitioning between 2/1chloroform/methanol and brine, extraction, collection of the organic phases and dried over anhydrous sodium sulfate followed by evaporation of the solvent under reduced pressure at room temperature. The residue containing the phospholipids was transesterified using 0.5 mol dm^{-3} KOH/MeOH, for 10 min at room temperature and then extracted with *n*-hexane and the organic layer washed with water and dried over anhydrous sodium sulfate. The organic layer containing the corresponding fatty acid methyl esters was analyzed by GC and compared with the retention times of authentic samples. The reported values represent the mean of three independent measurements; errors are ± 2 %.

RESULTS AND DISCUSSION

1. Methods of Thiyl Radical Generation: Photochemical, Thermal And Radiolytic Methods

Although the thermal-induced methods for generating thiyl radicals from sources such as 2-mercaptoethanol have been documented in the context of *cis-trans* double bond isomerization of mono- and polyunsaturated fatty acids [1], we shall focus on the photochemical and radiolytic methodologies. The photochemical generation of thiyl radicals from 2-mercaptoethanol and their *cis-trans* isomerization reaction of monounsaturated and polyunsaturated fatty acids in homogeneous media has been described [1]. Also, the radiolytic generation of thiyl radicals from 2-mercaptoethanol, and their *cis-trans* isomerization reaction of monounsaturated and polyunsaturated fatty acids in homogeneous media has also been documented [14]. The reactivity of thiyl radicals derived from 2-mercaptoethanol on double bond moieties constrained in supramolecular organizations such as liposomes, has been elegantly described either by radiolytic and photochemical methods [15].

We shall focus on the radiolytic and photochemical methods for thiyl radical generation from sodium thiomethoxide and dimethyldisulfide in the supramolecular organization of liposomes.

In order to evaluate the reactivity of thiyl radicals generated from sodium thiomethoxide and dimethyldisulfide, we used the *cis-trans* isomerization of mono-unsaturated fatty acids contained in phospholipid membranes of 1-palmitoyl-2-oleoylphosphatidylcholine POPC (Fig. **3**), with one chain of saturated fatty acid residues of palmitic acid 16:0, and the other chain of the monounsaturated *cis* fatty acid, oleic acid 9*cis*- 18:1, the saturated one not participating into the free radical transformation, but having the role of internal standard for the quantitative analysis of the reaction outcome (Fig. **3**).

The supramolecular environment of these phospholipids, unsaturated lipid vesicles, is regarded as a good biomimetic model for the *in-vivo* double-bond isomerization. In our experiments large unilamellar vesicles are obtained by extrusion technique (LUVETs, Fig. **1**) with polycarbonate filter of 100 nm diameter, that form an almost transparent suspension, which is also suitable for studies under photolytic conditions [16]. The aqueous and lipid phases are the two distinct compartments of this non-homogeneous system.

This system constitutes a microheterogeneous environment, where the diffusible thiyl radicals effect the *cis-trans* isomerization of oleic into elaidic residues.

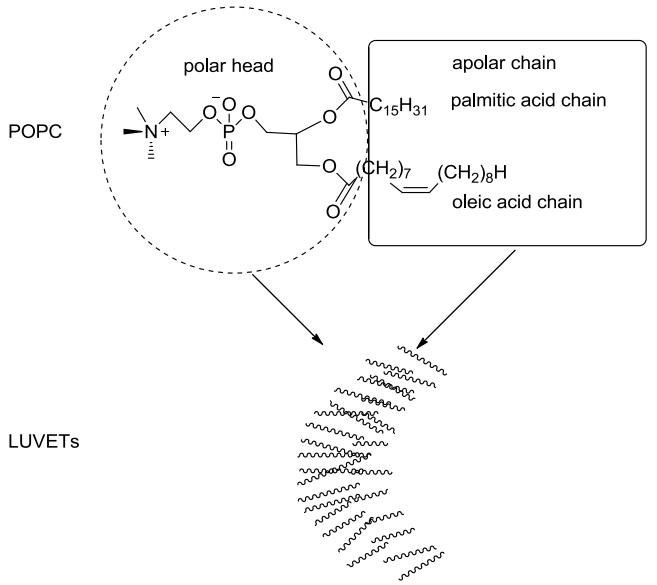


Fig. (3). Structure of POPC and LUVETs showing the lipid bilayer with the polar residues and apolar chains of oleic acid and palmitic acid residues.

2. $CH_3S^{\bullet}$ RADICALS GENERATED RADIOLYTICALLY FROM $NASCH_3$ (0.2 MMOL DM^{-3}) AND LUVETS OF POPC (2 MMOL DM^{-3}). LIPID ISOMERIZATION STUDY

We performed a radiolysis study of aqueous solutions of $NaSCH_3$ (0.2 mmol dm^{-3}, UV-vis spectrum in Supporting Information) and LUVETs of POPC (2 mmol dm^{-3}), under different gamma radiation doses and conditions (Table **1**). We monitored the *cis-trans* isomerization of the monounsaturated fatty acid residues. The fatty acid composition was determined by workup of the liposome, extraction of the lipids, transesterification to fatty acid methyl esters and gas chromatographic (GC) analysis [17].

Table 1. Methyl Elaidate and Methyl Oleate Percentages Obtained upon Gamma Irradiation of POPC Vesicles (2 mmol dm^{-3}) in the Presence of $NaSCH_3$ (0.2 mmol dm^{-3}) in an N_2O-Saturated Aqueous Solution at pH 6.5 at Different Irradiation Doses

Dose (Gy)	Methyl Elaidate (%)	Methyl Oleate (%)
0	0.1	99.9
25	42.8	57.2
50	70.2	29.8
100	89.6	10.4
200	90.1	9.9
450	90.2	9.8

Upon radiolysis of a water molecule, the major reactive species formed are depicted in equation 2; in parenthesis being the radiation yields. Hydroxyl radicals and solvated electrons are the dominant reactive species, with radiation yields 0.28 and 0.27, respectively. Using nitrogen protoxide, N_2O (0.02 mol dm^{-3}), solvated electrons are converted to HO• radicals, with a rate constant 9.1 x 10^9 $dm^3mol^{-1}s^{-1}$ (eq 3) [18]. Both HO• and H• radicals react with CH_3SH, formed this latter upon hydrolysis of CH_3SNa (eq 4,5; pKa CH_3SH = 10.7; pH = 6.5), to generate methyl thiyl radicals (eq 6 and 7). Combining the radiation yields of HO• radicals arising from

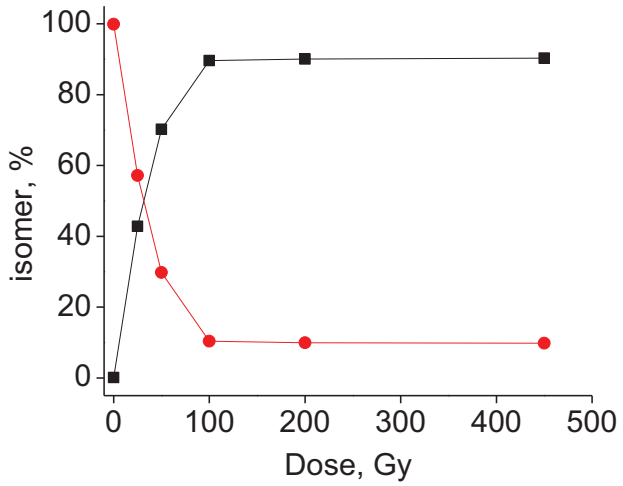
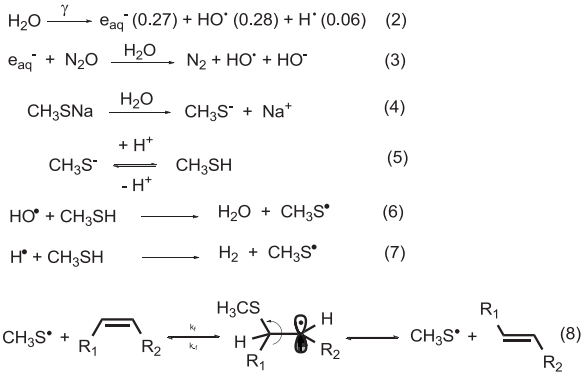
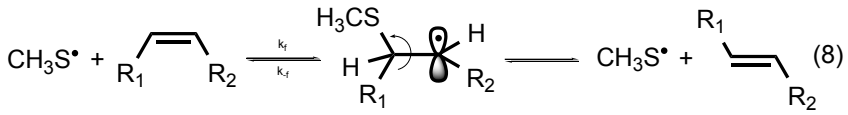


Fig. (4). Dose profile for the disappearance of oleate (●) and formation of elaidate (■) residues upon gamma irradiation of POPC vesicles (2 mmol dm^{-3}) in the presence of $NaSCH_3$ (0.2 mmol dm^{-3}) in an N_2O-saturated aqueous solution.

the direct radiolysis of the water molecule (0.28) and the yield of HO• radicals coming from the aqueous electron quenching by N_2O (0.27), account for more than 90% of the total radical reactivity, leaving the remainder to the reactivity of H• radicals (eq 7). The rate constant for reaction of HO• radicals with CH_3SH (eq 6) has been reported to be *ca*. 2 x $10^{10}dm^3$ $mol^{-1}s^{-1}$ [19]. Therefore, more than 90% of the resulting isomerization must come from CH_3S• radicals generated from primary HO• radicals on CH_3SH molecules. These CH_3S• radicals isomerize the double bond of the oleic acid residues from the LUVETs to elaidic acid residues (eq 8).



The percentages of fatty acid methyl esters are plotted against the irradiation dose (Fig. **4**). It is observed from Fig. (**4**) that upon increasing the gamma irradiation dose, a notable increase of the elaidate residues is observed, concomitant with the disappearance of oleic residues. A reasonable quantitative mass balance is obtained. The plateau region of Fig. (**4**), *i.e.*: 87%, denotes the thermodynamic stationary *trans-cis* isomer ratio. Of note is that this same isomer ratio is obtained in homogeneous media, purporting that thiyl radical diffusibility is not being impeded by the microheterogeneous environment. Also, the plateau for the *trans* isomer (Fig. **4**) is arrived at 100 Gy dose, demonstrating that the thermodynamic stationary ratio for the *trans:cis* isomerization is achieved very fast with the radiolytic methodology. The necessary total radical concentration (100-150 µmol dm^{-3}) to achieve equimolecular concentrations of *cis* and *trans* isomers occurs very early in the



gamma irradiation, at around 30 Gy dose. At 500 Gy, the total HO• radical concentration can be estimated to be *ca.* 250-300 μmol dm^{-3}. From the radical generation rate *Ri* and the known thiyl radical self-termination rate constant (*2kt* = 3 x 10^9 $dm^3mol^{-1}s^{-1}$[14,20]), the steady state concentration of thiyl radicals ([RS•] = $(R_i/2k_t)^{1/2}$ [14]) can be estimated at around 10^{-8} mol dm^{-3}. Taking into account that the molar concentration of elaidic acid at 500 Gy is 1.8 x 10^{-3} mol dm^{-3}, this may indicate that the radical chain reaction is efficient. In order to evaluate the number of cycles of isomerization involved in the chain reaction, *i.e.*: *the turn over number*, we embarked on constructing a plot of the radiation isomerization yield *versus* irradiation dose.

A plot of the radiation isomerization yield *G* (oleic to elaidic residues of POPC) *versus* dose irradiation is shown in Fig. (**5**). Extrapolation to dose zero brings about the radiation chemical isomerization yield *G*(0), which accounts for the efficiency of the propagation step. Considering that the initiation radiation yield (efficiency of the radical initiation step) can be taken as the sum of the primary reactive species, *i.e.*: $G(e^-)$ + *G*(HO•) + *G*(H•) = 0.61 (the primary initiating reactive species that produce CH_3S• radicals), then, the turnover number or number of cycles (under our reaction conditions) can be derived from the ratio of these two numbers, and taken as 67. It has been reported that for thiyl radicals derived from H_2S, the *cis-trans* isomerization of oleic acid to elaidic acid residues proceed with a number of chains *ca*. 28 [21].

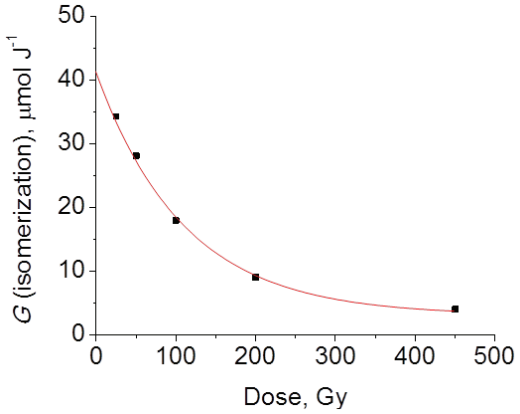


Fig. (5). Radiation chemical yields (*G*) of elaidic acid residues (■), *vs* dose (the line is the exponential fit to the data) obtained from the experiment reported in Fig. **2**; *G*(0) *(*elaidic acid) 41 μmol J^{-1} are obtained when the line is extrapolated to zero dose.

These results (lipid isomerization) supports the notion that thiomethanol arising from sodium thiomethoxide is a good methyl thiyl radical precursor, as has been demonstrated for other methyl thiyl radical precursors.

In order to study the sole effect of H• atoms in the formation of methyl thiyl radicals and the ulterior *cis-trans* isomerization of unsaturated fatty acids residues, we performed the radiolysis study in the presence of *t*-BuOH, known for trapping HO• radicals efficiently, with a rate constant k= 6 x 10^8 $dm^3mol^{-1}s^{-1}$ (eq 9) [22]. The rate constant of H• atoms with *t*-BuOH, has been estimated as 1.7 x 10^5 dm^3 $mol^{-1}s^{-1}$, a three-order of magnitude slower than that with HO• radicals. As mentioned above, the reaction of HO• radicals with CH_3SH (eq 6) has a rate constant of *ca.* 10^{10} $dm^3mol^{-1}s^{-1}$. Under these conditions, only primary H• atoms react with thiomethanol to yield methyl thiyl radicals, with a rate constant of 2.2 x 10^{10} dm^3 $mol^{-1}s^{-1}$(eq 7) [19,22].

Another source of methyl thiyl radicals is accounted for by equation 10, where $(CH_3)_2C(OH)CH_2$ • radical abstracts H• atoms from CH_3SH to render CH_3S• radicals.

Table **2** shows the reaction conditions for isomers under different irradiation doses, accompanied by Fig. (**6**). In Fig. (**6**), a similar profile to that obtained in the absence of *t*-BuOH is observed, showing that both H• atoms and carbon-centered radicals, $(CH_3)_2C(OH)CH_2$ •, are efficient sources at yielding CH_3S• radicals capable of performing the *cis-trans* isomerization of oleic-to-elaidic acid residues. Again, the thermodymamic equilibrium of isomers is established early in the gamma-irradiation (50 Gy), with very scarce radical concentration (10^{-8} mol dm^{-3}).

We estimate that rate constant for equation 10 to be *ca.* 1 x 10^7 $dm^3mol^{-1}s^{-1}$[14]. As the rate constant for equation 7 is 2.2 x 10^{10} dm^3 $mol^{-1}s^{-1}$, [19] and that H• and HO• radicals are produced in 10:90 ratio, it is assumed that the major source of CH_3S• radicals should arise from H abstraction of CH_3SH from alkyl radical $(CH_3)_2C(OH)CH_2$ •.

Table 2. Methyl Elaidate and Methyl Oleate Percentages Obtained upon Gamma Irradiation of POPC Vesicles (2 mmol dm^{-3}) in the Presence of $NaSCH_3$ (0.2 mmol dm^{-3}) and *t*BuOH (0.2 mol dm^{-3}) at pH = 6.5, in an N_2O-Saturated Aqueous Solution at Different Irradiation Doses

Dose (Gy)	Methyl Elaidate (%)	Methyl Oleate (%)
0	0.1	99.9
25	59.6	40.4
50	88.1	11.9
100	90.2	9.8
200	90.4	9.6
450	90.3	9.7

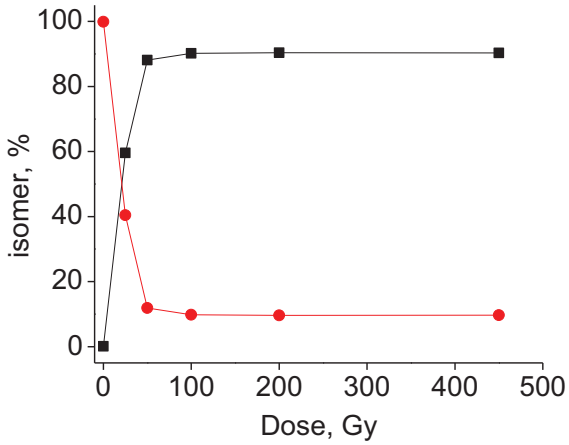


Fig. (6). Dose profile for the disappearance of oleate (●) and formation of elaidate (■) residues upon gamma irradiation of POPC vesicles (2 mmol dm^{-3}) in the presence of $NaSCH_3$ (0.2 mmol dm^{-3}) and *t*BuOH (0.2 mol dm^{-3}) in an N_2O-saturated aqueous solution.

$$HO^{\bullet} + {}^{t}BuOH \xrightarrow{k_9} (CH_3)_2C(OH)CH_2^{\bullet} + H_2O \quad (9)$$

k_9=6.0 x 10^8 mol dm^{-3} s^{-1}

$$(CH_3)_2C(OH)CH_2^{\bullet} + CH_3SH \xrightarrow{k_{10}} {}^{t}BuOH + CH_3S^{\bullet} \quad (10)$$

k_{10}= 1.0 x 10^7 mol $dm^{-3}s^{-1}$ [19]

3. $CH_3S^{\bullet}$ RADICALS GENERATED PHOTOCHEMICALLY FROM $NASCH_3$ (0.2 MMOL DM^{-3}) AND LUVETS OF POPC (2 MMOL DM^{-3}). LIPID ISOMERIZATION STUDY

Irradiation (250 nm,**15 watts**) of an Ar-saturated mixture of $NaSCH_3$ (0.2 mmol dm^{-3}) and LUVETs of POPC (2 mmol dm^{-3}) afforded isomerization of the oleic acid residues of the POPC (Table **3**, Fig. **7**). It is observed that the thermodynamic *cis-trans* stationary ratio is achieved early in the irradiation (5 min, 85% trans isomer). UV-irradiation produces the S-H bond homolysis of CH_3SH, to render CH_3S• radicals, according to equation 11.

$$CH_3SH \xrightarrow{h\nu} CH_3S^{\bullet} + H^{\bullet} \quad (11)$$

These CH_3S• radicals effect the *cis-trans* isomerization according to equation 8.

Table 3. Methyl Elaidate and Methyl Oleate Percentages Obtained Upon Photolysis (UV 250-260) of POPC Vesicles (2 mmol dm^{-3}) in the Presence of $NaSCH_3$ (0.2 mmol dm^{-3}) in an Ar-Saturated Aqueous Solution at Different Photolysis times

Time (min)	Methyl Elaidate (%)	Methyl Oleate (%)
0	0.1	99.9
1	59.1	40.9
5	87.3	12.7
10	87.6	12.4
20	87.4	12.6
40	87.0	13.0

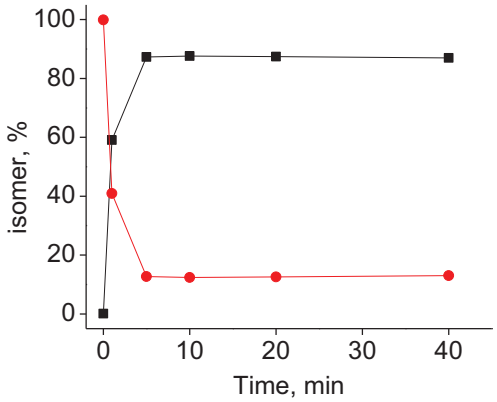


Fig. (7). Profile for the disappearance of oleate (●) and formation of elaidate (■) residues upon Photolysis (UV 250-260 nm) of POPC vesicles (2 mmol dm^{-3}) in the presence of $NaSCH_3$ (0.2 mmol dm^{-3}) in an Ar-saturated aqueous solution.

4. $CH_3S^{\bullet}$ RADICALS GENERATED RADIOLYTICALLY FROM CH_3SSCH_3, *T*BUOH AND LUVETS OF POPC. LIPID ISOMERIZATION STUDY

Gamma radiolysis of an Ar-degassed aqueous solution of POPC vesicles (2 mmol dm^{-3}) in the presence of CH_3SSCH_3 (75 µmol dm^{-3}) and *t*BuOH (0.2 mol dm^{-3}) affords isomerization of the oleic acid residues from the POPC to elaidic acid residues, according to Table **4**, and Fig. (**8**).

Table 4. Methyl Elaidate and Methyl Oleate Percentages Obtained upon Gamma Irradiation of POPC Vesicles (2 m mol dm^{-3}) in the Presence of CH_3SSCH_3 (75 µ mol dm^{-3})) and *t*BuOH (0.2 mol dm^{-3}) in an Ar-Saturated Aqueous Solution at Different Irradiation Doses.

Dose (Gy)	Methyl Elaidate (%)	Methyl Oleate (%)
0	0.1	99.9
42	52.5	47.5
84	82.2	17.8
126	90.9	9.1
252	89.9	10.1
336	89.4	10.6
504	90.8	9.2

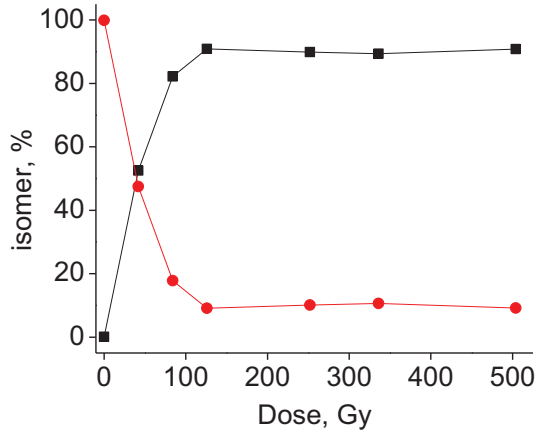


Fig. (8). Dose profile for the disappearance of oleate (●) and formation of elaidate (■) residues upon gamma irradiation of POPC vesicles (2 m mol dm^{-3}) in the presence of CH_3SSCH_3 (75 µ mol dm^{-3}) and *t*BuOH (0.2 mol dm^{-3}) in an Ar-saturated aqueous solution.

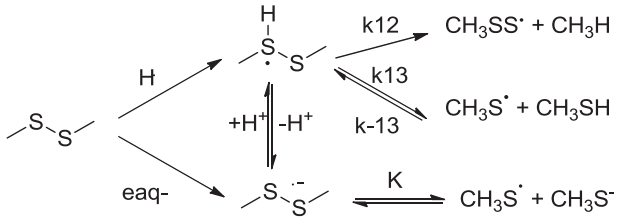


Fig. (9). Radiolytic production of CH_3S• radicals from dimethyl disulfide [11].

HO• radicals are suppressed by reaction with *t*BuOH, according to equation 9. H• radicals react with $(CH_3S)_2$ to yield a H-adduct radical (Fig. **9**), which equilibrates upon proton loss with the radical anion of $(CH_3S)_2$. This radical anion $(CH_3S)_2^{\bullet-}$ fragments into CH_3S• radicals and methylthiolate ion. In turn, the fate of the H-adduct radical $(CH_3S)_2H$• (Fig. **9**) can undergo fragmentation into CH_3SH and CH_3S• radicals by k_{13}, and k_{-13} (Fig. **9**). A fragmentation and rearrangement step (k_{12}, Fig. **9**) can lead to CH_3SS• radicals and CH_4.

Both in Table **4**, and Fig. **8**, we observe that *cis-trans* isomerization of oleic to elaidic methyl esters residues is an efficient process when methyl thiyl radicals are generated from gamma radiolysis

of an Ar-saturated aqueous solution of dimethyldisulfide in the presence of *t*BuOH (0.2 mol dm^{-3}). H• atoms, $•CH_2C(CH_3)_2OH$ and aqueous electrons are responsible for producing $CH_3S•$ radicals in sufficient yields to effect isomerization (eq 9,10, 12, 13) .

It is also known that $CH_3SS•$ radicals isomerize double bonds [23-25]. The rate constant for the reaction of dimethyldisulfide with H• atoms has been reported to be 4.81 x 10^9 $dm^3mol^{-1}s^{-1}$ [19]. The participation of $CH_3SS•$ radicals in the isomerization process cannot be underestimated.

It becomes apparent, that under these reaction conditions, the main source of $CH_3S•$ radicals come from the reaction of $•CH_2C(CH_3)_2OH$ radicals onto CH_3SH, and that of solvated electrons with $(CH_3S)_2$.

In the gas phase, the reaction of $(CH_3S)_2$ with H• has been reported to have a second order rate constant of *ca*. 5.7 x 10^9 $dm^3mol^{-1}s^{-1}$, according to equation 14 [23].

In order to visualize the efficiency of the propagation step of the isomerization reaction employing $(CH_3S)_2$ as the thiyl radical source, we proceeded as in section 1.- A plot of the radiation isomerization yield *G* (oleic to elaidic residues of POPC) *versus* dose irradiation is shown in Fig. (**10**). Extrapolation to dose zero brings about the radiation chemical isomerization yield *G*(0) of 34, which accounts for the efficiency of the propagation step.

One must consider that if the mechanism in Fig. (**9**) is correct, isomerization of the oleic acid residues from POPC under the present radiolytic conditions could arise from sources other than $CH_3S•$, such as $CH_3SS•$ radicals. Then, the turn over number would not reflect the sole result of $CH_3S•$ radicals, but the concomitant isomerization resulting from both $CH_3SS•$ and $CH_3S•$ radicals.

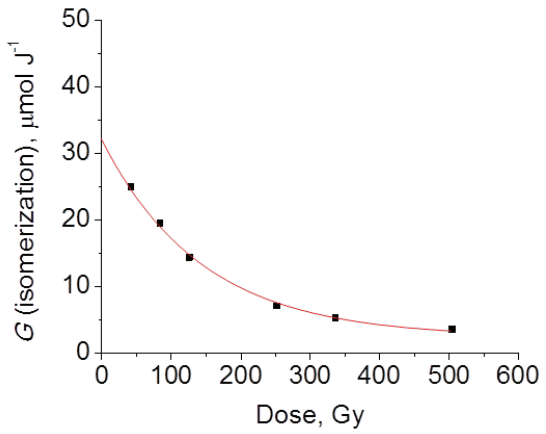


Fig. (10). Radiation chemical yields (*G*) of elaidic acid residues (•), *vs* dose obtained from the experiment reported in Fig. 7; *G*(0) (elaidic acid) 34 μmol J^{-1} are obtained when the lines are extrapolated to zero dose.

One must emphasize the characteristic supramolecular arrangement of the lipid assembly, with the fatty acid chains of phospholipid molecules (Fig. **3**) that form the hydrophobic core of the model membrane, and the polar heads that face the aqueous internal and external phases. Also, the partition coefficient of compounds that could be added to the system, which influences the distribution of the reactive species in the two compartments; lastly, and in particular, the location of the initiation step, that is, where the formation of an initial radical species, able to abstract the H-atom from the thiol group, occurs. As far as the lipid organization is concerned, there is a precise arrangement of the hydrophobic core, which can influence the position of the double bonds in the layer and the reactivity of the different fatty acids to the radical attack. This was found to be the case in the double bond isomerization, studied by Chatgilialoglu and Ferreri with an amphiphilic thiol, 2-mercaptoethanol, that is, a compound able to diffuse without restriction from the aqueous phase to the lipid bilayer, and *vice versa*. A regioselective process resulted where the double bonds are not involved at the same extent by the radical isomerization. The relatively high cycle numbers in the radical chain mechanism obtained with CH_3SH and $(CH_3S)_2$ radical sources attest to the excellent diffusibility of $CH_3S•$ radicals in the supramolecular environment.

CONCLUSIONS

We have shown that sodium thiomethoxide and dimethyldisulfide are excellent sources of methyl thiyl radicals by both radiolytic and photochemical methods, either through the *cis-trans* double bond isomerization efficiency in the supramolecular environment and by the turnover numbers. Our work contributes to the already known sources of thiyl radicals that effect double bond isomerization.

Work is in progress to assess the efficiency of thiyl radicals derived from sodium thiomethoxide and $(CH_3S)_2$ in the propagation step employing polyunsaturated fatty acid residues contained in supramolecular arrangements.

CONFLICT OF INTEREST

The author(s) confirm that this article has no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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