



Mechanisms of sulfur introduction chemically controlled: $\delta^{34}\text{S}$ imprint

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Abstract

Organic sulfur in marine sediment is ^{34}S enriched relative to the co-existing pyrite. This phenomenon is still enigmatic. Timing of the sulfur incorporation, immobilization and different sulfur species involved are part of the explanations. The reduced sulfur species incorporation into organic matter (OM) is generally assumed to have negligible $\delta^{34}\text{S}$ fractionation. This assumption has never been confirmed by laboratory experimental data. The present study measures the $\delta^{34}\text{S}$ changes resulting from reduced sulfur species (sulfides and polysulfide anions) incorporation into organic model compounds in an aquatic and low temperature (25 °C) system that simulates diagenetic marine environment. In addition, we also investigate the $\delta^{34}\text{S}$ fractionation and the isotope chemical mixing in the formation of polysulfide anions produced from elemental sulfur and sulfide anions. The results showed total isotope mixing between the two species in the formation of polysulfides. Acidification of the polysulfides solution caused $\delta^{34}\text{S}$ fractionation between the released elemental sulfur and H_2S . The incorporation of polysulfides and sulfides into carbonyl groups, caused ^{34}S enrichment relative to the starting polysulfides and sulfide of 4–5‰. The ^{34}S enrichment of the sulfurized carbonyl groups showed a minimal effect by temperature (0–70 °C) and is not affected by salinity, polysulfides composition, reaction time or solubility in water. The incorporation of polysulfides and sulfides into brominated organic compounds was negligibly ^{34}S enriched. The chemical mechanisms controlling the polysulfides incorporation into OM depend mostly on the functional groups and determine the ^{34}S enrichment of the sulfurized OM. The results presented in this study can explain part of the difference between pyrite $\delta^{34}\text{S}$ and sulfurized OM $\delta^{34}\text{S}$ in natural marine sediments.
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1. Introduction

1.1. The incorporation of inorganic reduced sulfur species into organic matter

The incorporation of inorganic reduced sulfur species into organic matter (OM) during early diagenesis

stage has been demonstrated by chemical analysis of sedimentary sulfurized OM (Valisolalao et al., 1984; Brassel et al., 1986; Francios, 1987; Sinninghe Damsté et al., 1989; Kohnen et al., 1990; Wakeham et al., 1995; Lückge et al., 2002). Sulfide anion (HS^-) and polysulfides (S_x^{2-}) are the main sulfurizing agents in slightly basic environments (i.e. pH 7.5–9.0) that prevail in marine sediments (Krein, 1993). Polysulfides are much more reactive species as was previously demonstrated both theoretically and experimentally (Lalonde et al., 1987; Vairavamurthy and Mopper, 1989; Loch et al.,

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2002). Therefore, polysulfides are suggested to be the most important species involved in diagenetic secondary sulfur enrichment (Aizenshtat et al., 1983; Francios, 1987; Mossmann et al., 1991; Vairavamurthy et al., 1992). Laboratory sulfurization simulations with model compounds and polysulfide anions in bi-phase (aquatic-organic) systems were performed at relatively low temperatures (up to 50 °C) with different reaction mediums employed (Krein and Aizenshtat, 1993, 1994; Schouten et al., 1994). The main conclusions from these experiments were that electrophilic model compounds such as aldehydes, ketones and activated double bonds react rapidly (hours to days) with polysulfide anions and yield polysulfide cross-linked macromolecules (PCLM) similar to those found in marine sediments. Such reactions can also occur in aquatic systems and at very diluted polysulfides/sulfide concentration within a reasonable time scale (Amrani and Aizenshtat, 2004b; this paper). Photochemical sulfurization reactions were also suggested as an important pathway in the formation of organic sulfur (Adam and Albrecht, 1998; Amrani and Aizenshtat, 2004a). Recently, a growing number of studies suggest the formation of PCLM not only from the reaction of polysulfides with functionalized lipids but with carbohydrates as well (van Kaam-Peters et al., 1998; Sinnighe Damsté et al., 1998; Kok et al., 2000; Aycard et al., 2003; van Dongen et al., 2003). Carbohydrates are generally thought to be poorly preserved during early diagenesis (Arnosti et al., 1994). Scheme 1 describes the suggested pathways for the incorporation of polysulfides into sedimentary OM.

1.2. Sulfur stable isotopes

The main source for inorganic reduced sulfur species in marine sediments is the process of dissimilatory sulfate reduction performed by sulfate-reducing bacteria (SRB) (Kaplan et al., 1963; Goldhaber and Kaplan, 1974). The dissimilatory sulfate reduction by SRB to H₂S has a marked effect on the δ³⁴S, up to 72‰, though averaging around $-45 \pm 5\%$ (e.g. Kaplan et al., 1963; Kaplan and Rittenberg, 1964; Goldhaber and Kaplan, 1974; Fry, 1988; Habicht and Canfield, 1997; Peterson, 1999; Detmers et al., 2001; Worthmann et al., 2001). Since the formation of iron sulfides (mainly as pyrite) in the sedimentary environment is kinetically very fast and the fractionation associated with it is negligible (~1‰, Price and Shieh, 1979; Wilkin and Barnes, 1996; Bottcher et al., 1998) the dissimilatory reduced sulfur isotope imprint from SRB is recorded. Most marine sediments show the following pattern: pyrite which is always lighter (³⁴S depleted) relative to all other sulfur species, sulfate which is the most heavy (³⁴S enriched) species, and OM that is ³⁴S enriched relative to the pyrite but

³⁴S depleted in comparison to the coexisting sulfate (Anderson and Pratt, 1995 c.f.). Since the assimilatory sulfate reduction (biosynthesis) has a relatively small isotopic fractionation of about -2% (Goldhaber and Kaplan, 1974), the depletion of the sedimentary OM in ³⁴S compared to the coexisting sulfate is generally interpreted as evidence for the secondary enrichment of OM with dissimilatory reduced sulfur species (Dinur et al., 1980; Aizenshtat et al., 1983; Francios, 1987; Mossmann et al., 1991; Zaback and Pratt, 1992; Anderson and Pratt, 1995; Canfield et al., 1998; Passier et al., 1999; Werne et al., 2003). Organic sulfur in marine sediments is enriched in ³⁴S by up to 30‰ relative to co-existing pyrite in most of the modern marine sediments and sedimentary rocks with an average of about 10‰ (Anderson and Pratt, 1995 c.f.). This phenomenon is still enigmatic. Timing of the sulfur incorporation, immobilization and different sulfur species involved are some of the explanations (for reviews see Anderson and Pratt, 1995; Aizenshtat and Amrani, 2004). The reduced sulfur species incorporation into OM is generally assumed to have negligible δ³⁴S fractionation. This assumption as far as we know was never been confirmed by experimental data. The present study aims at understanding the δ³⁴S changes resulting from incorporation of reduced sulfur species (sulfide and polysulfide anions) into OM in an aquatic and low temperature (25 °C) model system that simulates a marine environment (Amrani and Aizenshtat, 2004b). We also investigated the δ³⁴S fractionation and the isotope chemical mixing in the formation and the degradation of polysulfide anions produced from elemental sulfur and sulfide anions. The complexity of the sulfur cycle at the diagenetic stages is discussed in light of these results.

2. Experimental

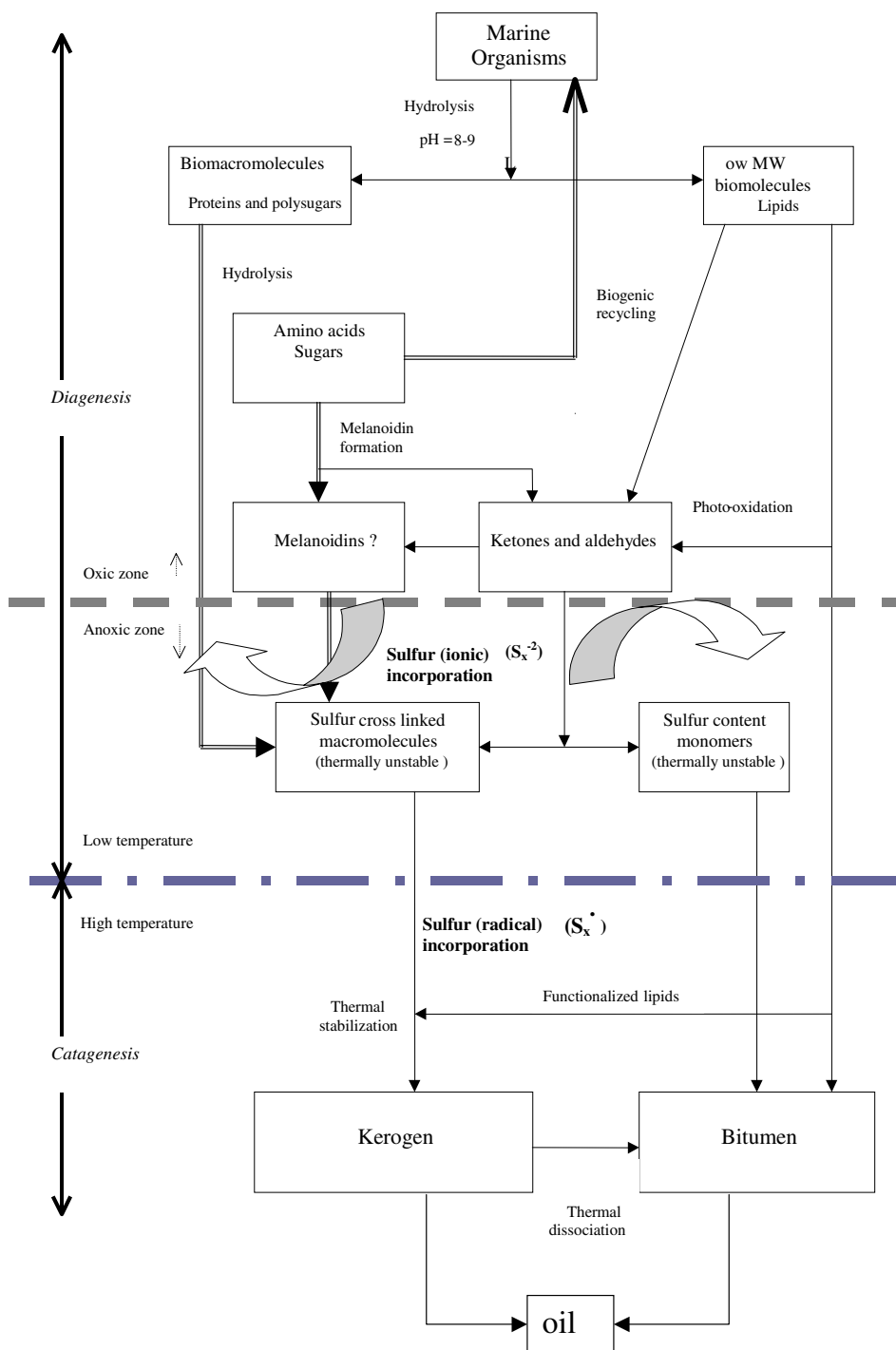
2.1. Substrates and standard compounds

Citral **1** (*cis* and *trans*, 97%) was commercially obtained (Fluka). Farnesal **2** and phytenal **3** were oxidized from farnesol (mixtures of isomers, 95%, Aldrich) and phytol (*cis* and *trans*, 97%, Aldrich) following the Singh et al. (1979) method, using pyridinium chromate absorbed on silica as the oxidation reagent.

2-undecanone **4** and *p*-tolualdehyde **6** (97%) were purchased from Aldrich.

Geranyl bromide **7** (*cis* and *trans*, 97%) was purchased from Fluka.

1,2 dibromohexane **10** was produced by reaction of Br₂ and 1-hexene (Aldrich). Bromination was performed by dropwise addition of bromine solution in CCl₄ (50 ml) to an ice bath cooled solution of an equivalent amount of the alkene in CCl₄ (50 ml) over



Scheme 1.

a period of 30 min. The solution was washed twice with water, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure.

Phytadiene **12** was produced by distillation (3 mmHg) of phytol (6.7 mmol) in the presence of 0.5 g phosphoric acid. The distilled product was extracted

with CH_2Cl_2 , dried over anhydrous MgSO_4 and evaporated under reduced pressure.

All substrates were confirmed for identity and purity (>95%) using ^1H NMR, GC and GC–MS analysis before use.

2.2. Chromatography and spectroscopy

GC analyses were carried out on a Chrompack 9001 gas chromatograph equipped with FI and FP detectors. A fused silica capillary column (30 m \times 0.25 mm ID coating CP-SIL 24CB) was used with He as the carrier gas. The GC was programmed at 4 $^\circ\text{C}/\text{min}$ between 50 and 300 $^\circ\text{C}$ with 20 min at the maximum temperature.

GC–MS analyses were carried out on a HP 5890 II gas chromatograph directly coupled to the source of a HP-G-1800B quadrupole mass-spectrometer. The mass-spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV, source temperature at 200 $^\circ\text{C}$, mass range 45–450 Da, a resolution of 800 and scan time of 1 s. GC conditions were as described above, except that the initial temperature was maintained for 5 min.

2.3. Preparation of polysulfide solutions

General procedure: 0.94 g (29 mmol) of elemental sulfur was dissolved in 10 ml of ammonium sulfide solution ($(\text{NH}_4)_2\text{S}$, 20% w/w in water, Merck) to give a stoichiometric ratio of sulfide to elemental sulfur of 1:1. Other $\text{S}^\circ/\text{S}^{2-}$ ratios were prepared by adjusting the quantity of elemental sulfur (see Table 1). The pH buffered by the ammonium solution to 8.5–9.0. Na_2S_x was prepared by dissolving 6.96 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (29 mmol, Merck) in 10 ml distilled water and adding of 0.94 g elemental sulfur (29 mmol). The pH was reduced to 8.5 by addition of diluted HCl.

2.4. Direct precipitation of polysulfide solution for $\delta^{34}\text{S}$ analysis

Direct precipitation of polysulfide solution by AgNO_3 solution was performed as an analytical tool for the $\delta^{34}\text{S}$ determination of unknown polysulfide solu-

tions. This method actually determines the $\delta^{34}\text{S}$ of total sulfur in the polysulfide solution. In order to examine the validity of this method we used standard polysulfide solutions with the same $\text{S}^\circ/(\text{NH}_4)_2\text{S}$ ratios as indicated in Table 1. The $\delta^{34}\text{S}$ of the standard polysulfide solutions were within $\pm 0.5\text{‰}$ of the stoichiometric isotopic values for the polysulfides that were calculated according to Eq. (2), using the measured $\delta^{34}\text{S}$ for S° and $(\text{NH}_4)_2\text{S}$. Procedure: AgNO_3 solution was added to 0.1 ml polysulfide solution, the precipitate Ag_2S washed several times with distilled water, and finally dried in an oven (120 $^\circ\text{C}$). The precipitate contained all sulfur species quantitatively.

2.5. Acidification of the polysulfides

Acidification of the polysulfide solution (different ratios of $\text{S}^\circ/(\text{NH}_4)_2\text{S}$, see Table 1) was performed in order to decompose the polysulfide solutions and release H_2S and elemental sulfur.

Procedure: into a three necked flask, 4 ml polysulfide solution (different ratios of $\text{S}^\circ/(\text{NH}_4)_2\text{S}$ each time, see Table 1) was mixed with 20 ml distilled water under nitrogen atmosphere. Diluted HCl (5%) was dropwise introduced till the polysulfides solution totally decomposed (colorless solution, pH = 0). The evolving H_2S was trapped by 3% AgNO_3 (10% NH_4OH) solution. The precipitate Ag_2S was filtered, washed several times with distilled water, and dried in an oven at 120 $^\circ\text{C}$. Elemental sulfur was filtered, washed with distilled water and carefully dried under vacuum. The precipitated Ag_2S and the recovered elemental sulfur were analyzed for their $\delta^{34}\text{S}$ values. In order to determine the accuracy of isotope measurement, this procedure was carried out in triplicate ($n = 3$) for each polysulfide ratio according to Table 1. Maximum standard deviation of 0.6‰ for the Ag_2S analyses and 0.2‰ for elemental sulfur is recorded.

2.6. General reaction of OM with polysulfides

Into a round bottom flask, 100 ml of simulated seawater (3% w/w NaCl) and polysulfide solution were mixed (to a total concentration of 0.3 Molar;

Table 1

Results of acidification of polysulfide solution prepared from different $\text{S}^\circ/(\text{NH}_4)_2\text{S}$ ratios. Initial ratios, calculated polysulfide values and the $\delta^{34}\text{S}_{\text{CDT}}$ of the released elemental sulfur ($\pm 0.2\text{‰}$, $n = 3$) and hydrogen sulfide ($\pm 0.6\text{‰}$, $n = 3$)

Amount of S° (mmol)	Amount of $(\text{NH}_4)_2\text{S}$ (mmol)	S° Mole fraction (%) from polysulfide solution	Polysulfide calculated $\delta^{34}\text{S}$ value (‰) ^a	Released S° (‰)	Released H_2S $\delta^{34}\text{S}$ (‰)
8.7	29	23	5.1	8.1	3.2
29	29	50	2.8	5.2	1.1
58	29	67	1.4	3.2	−2.4
102	29	78	0	1.5	−1.6

^a The stoichiometric isotope value of the S° and $(\text{NH}_4)_2\text{S}$ in the formation of the polysulfide solution. The initial $\delta^{34}\text{S}$ of $(\text{NH}_4)_2\text{S}$ is +7.0‰ and for S° is −1.4‰. See Sections 2 and 3 for more details.

pH = 8.5–9) and then 1.6 mmol organic substrate was dropwise introduced. The solution was magnetically stirred overnight under nitrogen atmosphere at 25 °C. The aquatic solution was extracted three times with dichloromethane (CH₂Cl₂). The organic solution was dried over anhydrous MgSO₄ and filtered. Elemental sulfur was removed from the organic extracts by reaction with activated copper curls (1–3 days) and subsequent filtration. The extracts were evaporated at ambient temperature, and weighed. The recovery yields were in the range of 70–95%. Conversions (to sulfurized matter) of the α,β unsaturated aldehydes were above 90%. The conversions of the other substrates are specified in the relevant tables.

2.7. Chemical degradation of the sulfurized OM

Selective cleavage of organic polysulfide (S–S) bonds was performed in order to characterize the sulfurized OM (Kohnen et al., 1991), based on the method of Eliel et al. (1976). To 50–200 mg of polysulfide oligo-polymer in diethyl ether, an excess of methyl lithium (MeLi) was introduced and the mixture stirred at ambient temperature. After 5 min, an excess of methyl iodide (MeI) was added and after an additional period of 15 min the reaction was quenched with water, extracted 3 times with hexane, the extract dried over anhydrous MgSO₄, and filtered. The products were analyzed by GC (FID and FPD), and GC–MS.

2.8. Stable isotopes analyses

The precipitated Ag₂S, elemental sulfur, and the organic samples were measured by a continuous flow elemental analyzer connected to a Finnigan Delta Plus stable isotope ratio monitoring mass spectrometer (EairmMS). Sulfur isotope compositions are expressed as permil (‰) deviations from CDT using the conventional delta notation with a standard deviation better than 0.3‰ ($n \geq 3$). The measurements were directly calibrated against sulfur isotopic standards IAEA-S-1 (Ag₂S, –0.3‰) and NBS-127 (BaSO₄, +20.3‰).

3. Results and discussion

3.1. Isotope chemical mixing in the formation of polysulfides

In order to understand the significance of sulfur isotope fractionation during sulfurization of OM, we need to examine factors influencing the sulfur isotope composition of each reactant, i.e. sulfides and polysulfides. The most important question to answer: can we consider the reduced sulfur species pool as isotopically homogeneous? This is crucial since polysulfides are produced by

reactions between various ratios of sulfide and elemental sulfur of different $\delta^{34}\text{S}$ values (Eq. (1)). In addition, the isotope mixing within the polysulfide structures had to be established.

Laboratory experiments with ³⁵S-labeled sulfur species showed rapid isotope mixing between H₂S, S⁰, S_x²⁻ and FeS at pH 7.6 and 20 °C (Fossing and Jorgenson, 1990). These authors conclude that the reduced sulfur species pool is homogeneous.

Here, we demonstrate rapid and total sulfur isotope mixing of stable sulfur isotopes ($\delta^{34}\text{S}$) according to Eq. (1) at pH 8.5–9.0 and 25 °C.

3.1.1. The chemical composition of polysulfide solution

There are three principal species of reduced sulfur: (i) Three forms of sulfide, H₂S, HS⁻(ADO⁻), S²⁻. At pH > 7 the sulfide anion (HS⁻) is quantitatively the dominant species (Schwarzenbach and Fisher, 1960; Boulegue and Michard, 1978; Boulegue, 1978). In order to simplify we will use the term sulfide for all three forms of sulfide, (ii) Elemental sulfur (S₈⁰) mainly in the crown-shaped ring structure (iii) polysulfides (S_x²⁻) produced by the reaction between HS⁻ and elemental sulfur.

The formation of polysulfides may be conceptualized through the following equilibrium:



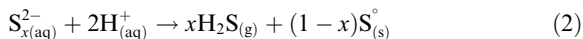
This reaction is not straightforward in terms of product since the produced polysulfide solution is complex and contains many species. However, Eq. (1) is a simplification of these species and serves only for the calculation of isotope mixing. Polysulfide solution is composed of four major components, i.e. H₂S, HS⁻, S_x²⁻ and S⁰. At the pH range that we work (8.5–9.0), practically all the elemental sulfur is in the polysulfide anions form, and most of the sulfide is in bisulfide form (HS⁻) (Schwarzenbach and Fisher, 1960; Boulegue and Michard, 1978; Boulegue, 1978).

At pH above 7 there is a small concentration of the mono-anion HS_x⁻ and the dominant species is the di-anion S_x²⁻ (Giggenbach, 1972; Licht et al., 1986; Licht and Davis, 1997). Thus in the pH 7.5–9.0 range that prevails in marine sediments, di-anions of polysulfides are instantly chemically produced, and stabilized. The polysulfides catenated forms are also influenced by the pH. As the pH increases the number of sulfur atoms in a polysulfides molecule decreases. In the pH range of 7.5–9.0 the most abundant species of polysulfides in aquatic media are S₄²⁻, S₅²⁻, and S₆²⁻ (Schwarzenbach and Fisher, 1960; Boulegue and Michard, 1978; Boulegue, 1978).

3.1.2. Isotope mixing in the polysulfide solution

The chemical and isotopic determination of the polysulfide anions component from the complex polysulfide

solution is very difficult. Therefore, we employed acidification of polysulfide solution in order to decompose it into hydrogen sulfide gas released, and precipitated elemental sulfur according to Eq. (2).



If there is an isotopic mixing between the different reduced species of sulfur, then we should get different $\delta^{34}\text{S}$ values compared with the initial measured S° and $(\text{NH}_4)_2\text{S}$ $\delta^{34}\text{S}$ values. To demonstrate this point we prepared several polysulfide solutions with different $\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ ratios.

The mole fraction (%) of sulfur/sulfide in the polysulfides was varied by adding different amounts of elemental sulfur (8.7–102 mmol) to a constant quantity of $(\text{NH}_4)_2\text{S}$ (29 mmol). Since the initial $\delta^{34}\text{S}$ values of the sulfide (+7‰) and the elemental sulfur (−1.4‰) have been determined, the stoichiometric isotope value (total sulfur) can be calculated according to the Rayleigh isotope mass-balance equation for a closed system:

$$\delta_{\text{T}} = x\delta_{\text{S}^{2-}} + (1-x)\delta_{\text{S}^{\circ}} \quad (3)$$

where δ_{T} represents the stoichiometric $\delta^{34}\text{S}$ value (i.e. total sulfur), x represents the mole fraction of the components (sulfide and elemental sulfur) in solution for the formation of polysulfide solution.

Addition of HCl (5% w/w) to the polysulfide solution releases $\text{H}_2\text{S}_{(\text{g})}$ and S_8° according to Eq. (2). The $\delta^{34}\text{S}$ of the released elemental sulfur and hydrogen sulfide (as Ag_2S) were measured and the results are summarized in Table 1. It is evident that the $\delta^{34}\text{S}$ values of the trapped H_2S is always ^{34}S depleted by about 4‰ compared with the elemental sulfur released, and about −1.5‰ relative to the calculated $\delta^{34}\text{S}$ of the polysulfide solution (i.e. total sulfur). Note the initial $\delta^{34}\text{S}$ value of the elemental sulfur is lighter than the initial sulfide ($(\text{NH}_4)_2\text{S}$). Thus, an isotopic mixing during the formation of polysulfides certainly occurred. Fig. 1 shows

the relatively constant fractionation (average $4.4 \pm 1\%$) between the $\delta^{34}\text{S}$ of the released elemental sulfur and hydrogen sulfide as a function of the calculated (Eq. (3)) total sulfur isotopic value. The deviation of the acid released sulfide and elemental sulfur from the calculated mix line is not even for both. With the increase of the S° stoichiometric ratio to sulfide it is clear that the released H_2S converged closer to the calculated mix line. The opposite trend is observed for the elemental sulfur. This observed trend could be explained according to isotope mass balance calculation (see Eq. (3)). Fig. 2 demonstrates the same trend as a function of S° mole fraction in the polysulfide solution. Obviously, the contribution of each species on the end products after acidification relates to the $\text{S}^{\circ}/\text{S}^{2-}$ ratio in the mixed polysulfide solution.

We could not find any isotopic data in the literature about the decomposition of polysulfides by acidification. This decomposition of polysulfides combines both H_2S volatilization and fractionation between H_2S and elemental sulfur. Hence it is important for the evaluation of acid volatilized sulfur (AVS) isotopic results in the analyses of marine sediments. It is evident that there is an isotope effect in the volatilization of $\text{H}_2\text{S}_{(\text{aq})}$ to $\text{H}_2\text{S}_{(\text{g})}$ of −0.5‰ (Fry et al., 1986). In our experiments, the volatilization reaction was complete and there were no sulfide species left in the solution. Thus, we would not expect any fractionation associated with the volatilization of H_2S . Theoretical calculations indicate enrichment in ^{34}S of elemental sulfur relative to H_2S by 3‰ at 25 °C but these calculations are based on chemical equilibrium between the species (Tudge and Thode, 1950). Since the polysulfide solution is a complex mixture of all species discussed above, it is possible that different species will have different $\delta^{34}\text{S}$ values. However, at this state of the art knowledge, there is no method of “freezing” the steady state profile hence any data on the species $\delta^{34}\text{S}$ differentiation.

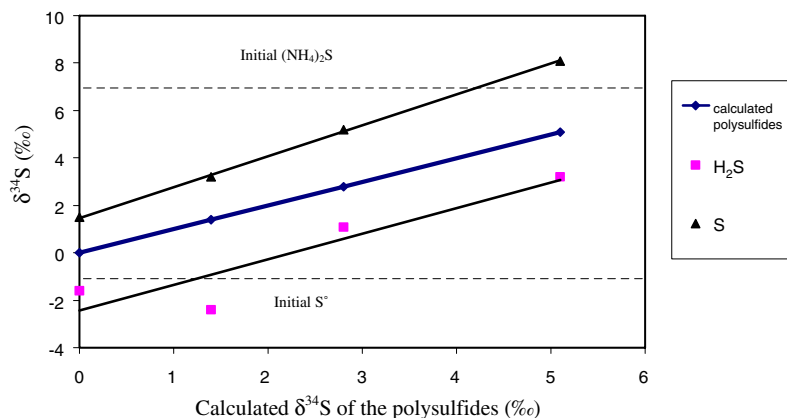


Fig. 1. Acidification products of polysulfide solutions prepared from different $\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ ratios. The released elemental sulfur ($\pm 0.2\%$, $n = 3$) and H_2S ($\pm 0.6\%$, $n = 3$) $\delta^{34}\text{S}_{\text{CDT}}$ results are plotted against the calculated polysulfide $\delta^{34}\text{S}$ for each ratio.

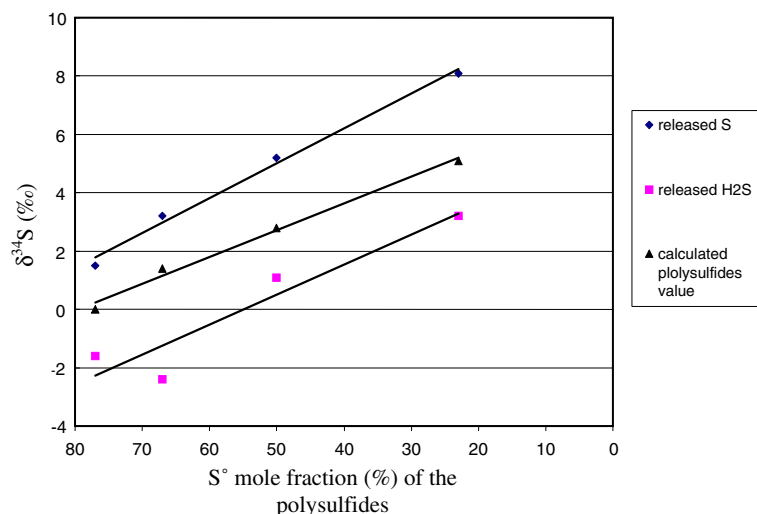


Fig. 2. Acidification products of polysulfide solution prepared from different $S^{\circ}/(NH_4)_2S$ ratios. The released elemental sulfur ($\pm 0.2\text{‰}$, $n = 3$) and H_2S ($\pm 0.6\text{‰}$, $n = 3$) $\delta^{34}S_{CDT}$ are plotted against the S° mole fraction (%) in the initial polysulfide solution for each ratio.

The process of acidification releases hydrogen sulfide gas and precipitates elemental sulfur, and is at least a two stage reaction. The first stage requires the cleavage of an S–S bond and the formation of HS^- and the second is the formation of the H_2S . Each of these two reactions could lead to fractionation. The S^{32} – S^{34} bond is stronger than the S^{32} – S^{32} bond therefore the cleavage of H_2S from the polysulfide will discriminate for the lighter isotope in the gas phase.

At this stage of the research, we cannot give an absolute mechanistic contribution to the observed difference in the isotope ratios. However, we will give an indirect answer in the organic section of this paper. It is clear from the data obtained in our experiments and in agreement with Fossing and Jorgenson (1990) that a total chemical isotopic mixing occurs between elemental sulfur and sulfide in the formation of polysulfides. This will be a key point in our experiment on the incorporation of polysulfides into OM.

3.2. Isotopic fractionation associated with reduced sulfur species incorporation into organic matter

The incorporation of inorganic reduced sulfur species into OM during early diagenesis stage has been demonstrated in sedimentary sulfurized OM (Aizenshtat et al., 1983; Valisolalao et al., 1984; Brassel et al., 1986; Francios, 1987; Sinnighe Damsté et al., 1989; Kohnen et al., 1990; Wakeham et al., 1995; Lückge et al., 2002; Werne et al., 2003). Elemental sulfur solubility and reactivity in water is very low at diagenetic temperatures, and therefore cannot be considered as a significant sulfurizing agent (Krein, 1993).

Polysulfides (S_x^{2-}) and sulfide anion (HS^-) are the main sulfurizing agents in slightly basic environments (i.e. pH 7.5–9.0) that prevail in marine sediments.

The incorporation of reduced sulfur species in OM is generally assumed to have negligible $\delta^{34}S$ fractionation (e.g. Mossmann et al., 1991; Werne et al., 2003). Bruchert and Pratt (1996) suggested a kinetic isotope effect during temporary sulfurization of OM, leading to ^{34}S depletion with respect to the reacted sulfide or polysulfides. In order to examine these hypotheses we chose citral (1) which is an α,β unsaturated aldehyde (C_{10}) as an initial OM model compound. α,β unsaturated aldehydes of the isoprenoid family such as citral react readily with polysulfides under phase transfer catalysis (PTC) conditions and yield sulfur containing molecules which are mainly PCLM (Krein and Aizenshtat, 1993; Schouten et al., 1994; Krein and Aizenshtat, 1994; Aizenshtat et al., 1995; Amrani and Aizenshtat, 2004a). Recently, we have demonstrated that PTC conditions or the presence of emulsifiers is not imperative for these reactions and that sulfurization can take place in salted water without the presence of an organic phase (Amrani and Aizenshtat, 2004b). The kinetics of these reactions is highly dependent on the immiscible–insoluble character of the substrate since the reaction is interface-controlled (organic substrate–water). However the products formed in both methods are similar and controlled by similar mechanisms (Amrani and Aizenshtat, 2004b). In this study, we use the aquatic method for the sulfurization reactions since it better simulates the conditions prevailing in marine sediments. We carried out several reactions with citral at different conditions and characterized the products by elemental analysis, chemical

degradation (MeLi/MeI) and stable sulfur isotope analyses.

3.2.1. The influence of the polysulfide composition on the $\delta^{34}\text{S}$ of OM

In order to understand if there is any correlation between the initial polysulfides ^{34}S value and the produced PCLM, we performed sulfurization experiments with citral with different ratios of $\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ in the polysulfide solution. In all cases the PCLM produced from citral were ^{34}S enriched relative to the calculated polysulfides (see Table 2). Very good correlation between the sulfurized citral and the polysulfides calculated values is shown in Fig. 3. The constant enrichment of $+4.5 \pm 0.4\text{‰}$ is shown schematically in Fig. 4 and plotted against the elemental sulfur mole fraction in the polysulfides used for the reaction with citral. It can be seen that as the elemental sulfur mole fraction decreases the $\delta^{34}\text{S}$ of the sulfurized citral increases, similar to the trend observed in Fig. 2 for the decomposition of polysulfides (see previous discussion). At this stage of the discussion we have to consider again whether the polysulfide solution is homogenous, i.e. polysulfides

and sulfides have the same $\delta^{34}\text{S}$. Total mixing suggests that the ^{34}S enrichment of the sulfurized citral derives solely from the polysulfide incorporation into citral (4–5‰). The second possibility is that the polysulfides were ^{34}S enriched relative to the coexisting sulfides and the polysulfide calculated value (see Fig. 2 and previous discussion), and thus, the $\delta^{34}\text{S}$ of the sulfurized citral reflected the polysulfides isotope value (i.e. released elemental sulfur) with small fractionation. If we consider the second option, then we have to assume that if the reactant is only sulfide $(\text{NH}_4)_2\text{S}$ (see Table 2) and not polysulfides $(\text{NH}_4)_2\text{S}_x$ then a smaller fractionation will occur. Nevertheless, Fig. 4 shows that even in the case of sulfide as the reactant, the same ^{34}S enrichment is recorded similar to the polysulfides, although the reaction of polysulfides is much faster than sulfides (Table 2). This ^{34}S enrichment is almost independent of the polysulfide composition. Thus, we assume that the polysulfides–sulfide–elemental sulfur pool is homogenous and its $\delta^{34}\text{S}$ value is the polysulfides calculated $\delta^{34}\text{S}$ (according to Eq. (2)). We therefore suggest that the incorporation of polysulfides or sulfide into citral causes $\delta^{34}\text{S}$ enrichment of 4–5‰ in salted aquatic system.

Table 2
 $\delta^{34}\text{S}_{\text{CDT}}$ ($\pm 0.2\text{‰}$) of Citral in reaction with polysulfides composed of different $\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ ratios

Precursor	$\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ mole ratio	Reaction time	Calculated polysulfides $\delta^{34}\text{S}$ (‰) ^a	$\delta^{34}\text{S}$ of the sulfurized OM (‰)	^{34}S enrichment (‰)
Citral 1	0:1 ^b	1 week	7 ^b	11.8	4.8
Citral 1	0.5:1	1 day	4.2	9.2	5
Citral 1	1:1	1 day	2.8	7.0	4.2
Citral 1	1.5:1	1 day	2	6.0	4
Citral 1	2.5:1	1 day	1.1	5.6	4.5

^a The stoichiometric isotope value of the S° and $(\text{NH}_4)_2\text{S}$ in the formation of the polysulfide solution. The initial $\delta^{34}\text{S}$ of $(\text{NH}_4)_2\text{S}$ is $+7.0\text{‰}$ and for S° is -1.4‰ . See Sections 2 and 3 for more details.

^b Reaction with $(\text{NH}_4)_2\text{S}$ only. In this case the “calculated polysulfides” is measured value of $(\text{NH}_4)_2\text{S}$ ($+7.0\text{‰}$).

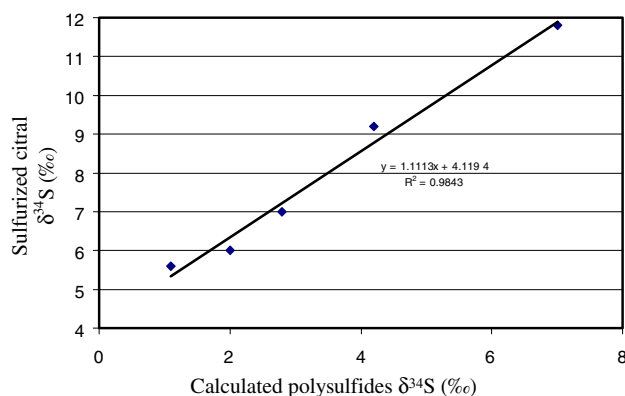


Fig. 3. Citral 1 reaction with polysulfides of different $\text{S}^{\circ}/(\text{NH}_4)_2\text{S}$ ratio, overnight in aquatic conditions. Products $\delta^{34}\text{S}_{\text{CDT}}$ ($\pm 0.2\text{‰}$) values of the sulfurized citral are plotted against the calculated polysulfide $\delta^{34}\text{S}$ value for each ratio.

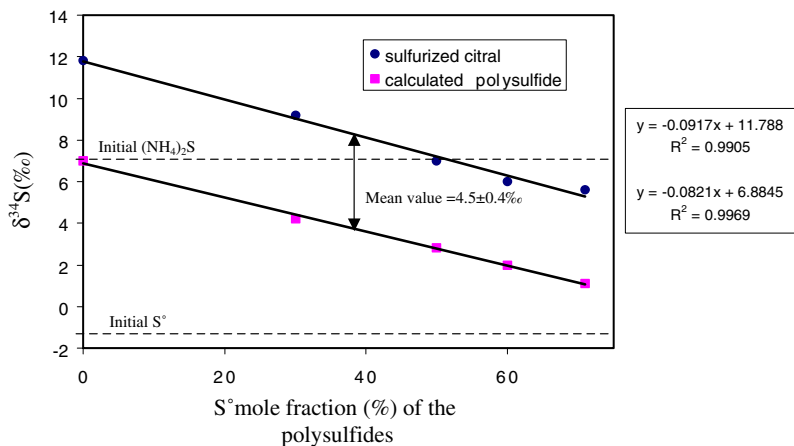


Fig. 4. Citral **1** reaction with polysulfides of different S°/(NH₄)₂S ratio, overnight in aquatic conditions. δ³⁴S_{CDT} (±0.2‰) of the sulfurized citral is plotted against the S° mole fraction of the polysulfide solution. Note that zero S° mole fraction is the reaction with (NH₄)₂S and citral. The calculated polysulfide value in this case is the measured δ³⁴S of (NH₄)₂S (+7‰).

3.2.2. The influence of reaction time, temperature, salinity and solubility on the δ³⁴S of sulfurized α,β unsaturated aldehydes

Kinetic rate controlled reactions show isotope fractionations that are mass dependent for non-equilibrium systems. The reaction with the lighter isotope will be favorable since it moves faster than the heavy isotope (Coplen, 1993 c.f.). Therefore, if the sulfurization mechanism is kinetically controlled we would expect depletion of the ³⁴S isotope for sulfurized citral, relative to the polysulfides δ³⁴S value.

Fig. 5 shows the very rapid reaction of the polysulfides with citral as a function of time and the δ³⁴S of the sulfurized citral. Note the S/C line rises for the sulfurized citral in comparison to the δ³⁴S line that remains invariable. After 5 min of reaction the ³⁴S enrichment sulfurized citral relative to the calculated polysulfides value were about 5‰. After 1 h, the reaction was complete and δ³⁴S value recorded showed negligible change. Prolonged reaction times yielded oligo-polymers that fluctuated between 4 and 5‰ ³⁴S enrichment. It is important to note that in these kinetic experiments the polysulfides (29 mmol) are in large excess compared with citral (1.6 mmol). Thus, the concentration of polysulfides and their δ³⁴S would remain almost constant during the reaction. Such a system can be considered as an open system for polysulfides.

Moreover, this reaction shows a small temperature isotopic dependence (see Table 3). The Δ_{sulfurized citral (70 °C) – sulfurized citral (0 °C)} is just 1.2‰.

These findings support the assumption that the mechanisms, which control the δ³⁴S of the polysulfide incorporation into citral, are not kinetically controlled, but rather depend on equilibrium or steady state conditions that will be discussed later.

Since the preservation of high concentrations of OM and reduced sulfur species is found to occur in hyper-saline conditions (Aizenshtat et al., 1999 c.f.), we examined the salinity effect on the δ³⁴S of the sulfurized citral. Salinity can affect the solubility of citral and hence, change its reactivity towards polysulfides. The δ³⁴S results obtained for 0%, 3% and 15% NaCl (w/w) were identical (Table 3). The solubility influence was checked by the reaction of polysulfides with α,β unsaturated aldehydes with longer carbon chain compared to citral (C₁₀), i.e. farnesal **2** (C₁₅) and phytenal **3** (C₂₀). The reactions of farnesal and phytenal with polysulfides are kinetically slower in aquatic conditions and yield the same principle product, that is, PCLM (Scheme 2) (Amrani and Aizenshtat, 2004b). Again, we recorded the same ³⁴S enrichment (Table 3). Thus, we can conclude that the enrichment in ³⁴S of the sulfurized α,β unsaturated aldehydes in the range of 4–5‰ is independent of reaction time, temperature, sulfur content, salinity, and solubility under aquatic conditions.

3.2.3. The influence of the functional group on the δ³⁴S of OM

Several sulfurization experiments on lipids of different functionalities were performed. The reaction of polysulfides with saturated ketones and aldehydes under PTC conditions was discussed previously (Krein and Aizenshtat, 1993, 1994; Schouten et al., 1994). The sulfurized products consist of polysulfide dimers and small amounts of thiols (replacing the oxygen). The reaction between 2-undecanone (**4**) and polysulfides yielded polysulfide dimers (**5**) showing ³⁴S enrichment relative to the calculated value of the polysulfides of 4.2‰. The same trend was observed

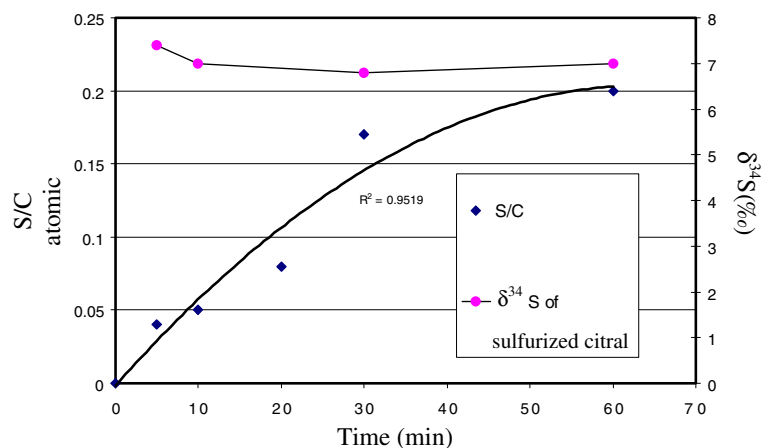


Fig. 5. Citral **1** reaction with polysulfides (0.3 M) in aquatic conditions. S/C and $\delta^{34}\text{S}_{\text{CDT}} (\pm 0.2\text{‰})$ of the sulfurized citral are plotted against time. Note the S/C rise compared with the steady $\delta^{34}\text{S}$ value of the sulfurized citral.

Table 3

$\delta^{34}\text{S}_{\text{CDT}} (\pm 0.2\text{‰})$ data for experiments of different temperature, salinity and solubility in the reaction of citral **1**, farnesal **2** and phytanal **3** with polysulfides

Precursor	Temperature (°C)	Carbon chain length	Reaction time	Salinity (% w.w.)	Calculated polysulfides $\delta^{34}\text{S}$ (‰) ^a	$\delta^{34}\text{S}$ of the sulfurized OM (‰)	^{34}S enrichment (‰)
Citral 1	25	10	1 day	3	2.8	7.2	4.4
Citral 1	0	10	1 day	3	2.8	7.6	4.8
Citral 1	70	10	1 day	3	2.8	6.4	3.6
Citral 1	25	10	1 day	15	2.8	7.2	4.4
Citral 1	25	10	1 day	0	2.8	7.3	4.5
Citral 1	25	10	1 week	3	2.8	7.0	4.2
Farnesal 2	25	15	1 week	3	2.4 ^b	7.2	4.8
Phytanal 3	25	20	1 week	3	2.4 ^b	6.8	4.4

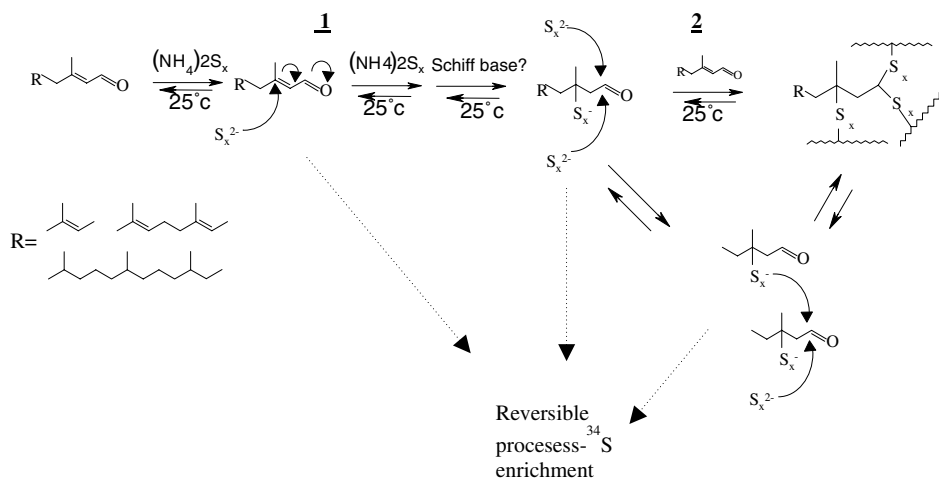
^a The stoichiometric isotope value of the S° and $(\text{NH}_4)_2\text{S}$ in the formation of the polysulfide solution. The initial $\delta^{34}\text{S}$ of $(\text{NH}_4)_2\text{S}$ is +7.0‰ and for S° is -1.4‰. See Sections 2 and 3 for more details.

^b The initial $(\text{NH}_4)_2\text{S}$ $\delta^{34}\text{S}$ in this case was 6.1.

for the reaction with *p*-tolualdehyde (**6**) (see Table 4). These results are similar to those obtained for the α,β unsaturated aldehydes. In order to understand these results we need to better understand the reactions mechanisms for saturated aldehydes and ketones as well as the α,β unsaturated aldehydes in the reaction with polysulfides. The Michael addition mechanism (see Scheme 2) is suggested as the first step for sulfurization of α,β unsaturated aldehydes, under aqueous and PTC conditions (Krein and Aizenshtat, 1994; Amrani and Aizenshtat, 2004b). The second step is a nucleophilic attack of polysulfide anions on the carbonyl (Scheme 2). This reaction transpires also for saturated aldehydes and ketones such as **4** and **6**. Both steps are reversible (1 and 2 see Scheme 2) and therefore are not kinetically controlled. The suggested mechanism of reductive sulfurization of carbonyls via

thiocarbonyls as intermediates and the formation of sulfide radical anions (Schneckenburger et al., 1998; Adam et al., 2000) is not the preferred pathway under our reaction conditions (Amrani and Aizenshtat, 2004b). The first stage in this mechanism is reversible; hence even if we adapt it, the reaction will still not be kinetically controlled.

In reversible mechanisms, C–S bonds are formed and cleaved and vice versa. If there is a large pool of polysulfides in the system (open system), the OM will be enriched by the heavier isotope since the C– S^{34} bond is stronger than the C– S^{32} bond and requires higher bond cleavage energy. Moreover, the formation of polysulfide dimers or oligo-polymers can cause further $\delta^{34}\text{S}$ fractionation during the rearrangement of these molecules and cleavage of S–S bonds in a similar manner to C–S bonds as described above.



Scheme 2.

Table 4
 $\delta^{34}\text{S}_{\text{CDT}} (\pm 0.2\text{‰})$ results showing the influence of functional group in the reaction with polysulfides

Precursor	Reaction time	Calculated polysulfides $\delta^{34}\text{S}$ (‰) ^a	$\delta^{34}\text{S}$ of the sulfurized OM (‰)	³⁴ S enrichment (‰)	Conversion (sulfurized matter)
2-Undecanone 4	1 week	2.8	7.0	4.2	60%
<i>p</i> -Tolualdehyde 6	1 day	2.8	7.4	4.6	100%
Geranyl bromide 7	1 day	2.8	3.9	1.1	55%
Geranyl bromide 7	1 week	2.8	4.6	1.8	100%
1,2 Dibromo hexane 10	1 day	2.8	2.9	0.1	60%
1,2 Dibromo hexane 10	1 week	2.8	3.5	0.7	100%
Phytadiene 12	6 weeks	2.4 ^b	4.5	2.1	10%

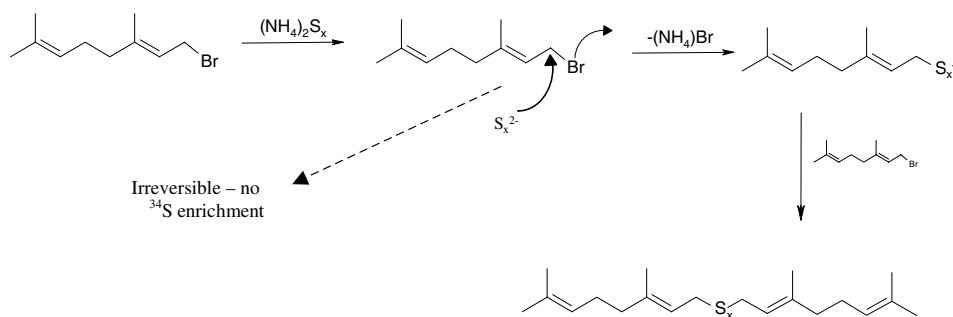
^a The stoichiometric isotope value of the S^0 and $(\text{NH}_4)_2\text{S}$ in the formation of the polysulfide solution. The initial $\delta^{34}\text{S}$ of $(\text{NH}_4)_2\text{S}$ is $+7.0\text{‰}$ and for S^0 is -1.4‰ . See Sections 2 and 3 for more details.

^b The initial $(\text{NH}_4)_2\text{S}$ $\delta^{34}\text{S}$ in this case was 6.1‰ .

In order to further understand these mechanisms we reacted polysulfides with brominated molecules that are known to react readily with polysulfides and sulfide according to the nucleophilic substitution mechanism (Krein and Aizenshtat, 1995; Loch et al., 2002). This reaction is not reversible, and thus we would not expect an isotopic enrichment. We reacted geranyl bromide (**7**), the brominated derivative of geraniol (**8**). This molecule has the same hydrocarbon skeleton as the citral, and the only difference is the bromine atom on carbon 1 (see Appendix A). The major products of geranyl bromide with polysulfides were the dimer **9**. The $\Delta_{\text{sulfurized geranyl bromid} - \text{calculated polysulfides}}$ was $+1.1\text{‰}$ and slightly higher after one week (see Table 4). Compared with sulfurized citral the ^{34}S enrichment is 3–4‰ less. In order to see if this phenomenon can be generalized, we reacted 1,2 dibromo hexane (**10**) under the same conditions, the major product was **11**. A similar trend was recorded, just 0.7‰ enrichment relative to the calculated polysulfides. Scheme 3 shows schemati-

cally the mechanism involved in the formation of **9**. We did not perform kinetic experiments for the brominated compounds reactions with polysulfides. However, from the results obtained (Table 4), such an effect may occur within shorter reaction times since the initial value for both sulfurized **7** and **10** were ^{34}S depleted relative to the final $\delta^{34}\text{S}$ of the sulfurized OM. After one week the reaction was completed and the products were slightly ^{34}S enriched. This small ^{34}S enrichment can be explained by the S–S cleavage during the formation of the polysulfide dimers and polymers as explained previously. This point will be further studied in the future.

The reaction of polysulfides with phytadiene (**12**) represents the addition of polysulfides into activated double bonds. The mechanism of this reaction under slightly basic aqueous conditions is not clear yet but the main sulfurized product is probably the dimer **13** as revealed by MeLi/MeI degradation (mass spectra compared with Grossi et al., 1998). Therefore, we can speculate that a nucleophilic attack on position 4 (1–4 addition) is the



Scheme 3.

first attack. The reaction rate was very slow, after 6 weeks, the conversion was just 10% (sulfurized matter). The isotopic composition showed ³⁴S enrichment of +2.1‰, which is lower in comparison to that for the sulfurized phytalen (Table 3).

The results presented so far and summarized in Tables 3 and 4 clearly demonstrate the $\delta^{34}\text{S}$ dependence on the sulfurization mechanisms. The sulfurization mechanisms are determined mainly by the type of the functionality group(s). These findings have significant geochemical implications that will be discussed in detail later.

3.2.4. The influence of the sulfurization method on the $\delta^{34}\text{S}$ of the sulfurized OM

The reactions presented in this paper were performed also in PTC systems for comparison. We will not detail these since the subject involves complex pathways that are out of the scope of the present discussion. Briefly, the PTC reactions showed in all cases +1.5–3‰ ³⁴S enrichment relative to the salted water method. For example, the citral in salted water method records ³⁴S enrichment of 4.3‰ relative to the polysulfides, whereas, when we apply the PTC method with the same composition of polysulfides, the ³⁴S enrichment was 6.1‰.

3.2.5. pH as a controlling parameter on the sulfurized OM $\delta^{34}\text{S}$

An important influence on the mechanisms that controls the incorporation of polysulfides and sulfide into OM is the reaction pH conditions which in turn determine the (poly)sulfide cation (Krein and Aizenshtat, 1993). The solution of sodium (poly)sulfide in water records pH ~ 12.0–13.5. Ammonium (poly)sulfide record pH ~ 8.5–9.0. Thus the mechanisms can change and affect the $\delta^{34}\text{S}$ of the sulfurized OM. We have conducted 3 reactions with citral that demonstrate the pH influence. All reactions were performed under the same concentrations of substrate and reactant, and compared to the reaction with citral and $(\text{NH}_4)_2\text{S}_x$ (see Table 5).

The reactions with Na_2S and Na_2S_x were very slow and yielded relatively low ³⁴S enrichment relative to the corresponding ammonium (poly)sulfide. The neu-

tralization by HCl of the Na_2S_x solution to a pH of 8.5 accelerated the rate of the reaction, and the sulfurized product distribution was different from the distribution at higher pH (Amrani and Aizenshtat, 2004b). The neutralization of the pH by an acid to the Na_2S_x solution released H_2S and hence changes the calculated $\delta^{34}\text{S}$ of the polysulfide solution. In order to determine the new $\delta^{34}\text{S}$ of the polysulfides solution we used the direct precipitation method. This method was found to have $\pm 0.5\%$ accuracy relative to the calculated stoichiometric value of standard polysulfides solutions (see Section 2). With this caveat, the ³⁴S enrichment of the sulfurized citral were similar to those obtain by the reaction with $(\text{NH}_4)_2\text{S}_x$.

These results show again the importance of the sulfurization mechanism on the $\delta^{34}\text{S}$ of the sulfurized OM.

3.2.6. Substrate and reactant concentration effect on the sulfurized OM $\delta^{34}\text{S}$

In marine depositional environments the concentrations of the polysulfides are much lower than the optimal chemical concentrations chosen for this study (Goldhaber, 2004). Thus, we conducted experiments with lower polysulfide concentration of 3 mM for the reaction with citral. After one week 4% sulfur (w/w) was introduced into citral. The isotopic composition showed 5.1‰ ³⁴S enrichment relative to the calculated polysulfides. This result is 0.6‰ higher than the average value under normal reaction conditions. This can be explained by the relatively low sulfur introduction. However, when we performed the reaction using the normal method (0.3 M polysulfides) conditions and under excess amount of citral (320 mmol instead of 16 mmol) an interesting phenomenon occurred. The ³⁴S enrichment was reduced to only 1.5‰. We precipitated the residual polysulfide solution with AgNO_3 . The isotopic composition of the residual solution was $2.1 \pm 0.5\%$ ³⁴S depleted relative to the calculated polysulfides value. Since we determined the %S (w/w) in the sulfurized citral obtained (~25%) and we know the initial amount of sulfur in the solution we can apply Eq. (2) for sulfur isotope mass balance to confirm these

Table 5
 $\delta^{34}\text{S}_{\text{CDT}}$ ($\pm 0.3\%$) data showing the influence of pH or polysulfide cations (NH_4^+ , Na^+) in the reaction of citral with polysulfides

Precursor	pH of the (poly)sulfide solutions	(poly)sulfide form	Calculated polysulfide $\delta^{34}\text{S}$ ($\%$) ^a	$\delta^{34}\text{S}$ of the sulfurized OM ($\%$)	^{34}S enrichment ($\%$)
Citral 1	8.5–9	$(\text{NH}_4)_2\text{S}$	7.0	11.8	4.8
Citral 1	8.5–9	$(\text{NH}_4)_2\text{S}_x$	2.8	7.0	4.2
Citral 1	12–13.5	Na_2S	2.8	5.8	3
Citral 1	12–13.5	Na_2S_x	0.70	3	2.3
Citral 1	8.5 ^b	Na_2S_x	2.3 ± 0.5^c	6.7	4.4 ± 0.5

^a The stoichiometric isotope value of the S^0 and $(\text{NH}_4)_2\text{S}$ or Na_2S in the formation of the polysulfide solution. The initial $\delta^{34}\text{S}$ of $(\text{NH}_4)_2\text{S}$ is $+7.0\%$ and for Na_2S is 2.8% . The initial $\delta^{34}\text{S}$ of S^0 is -1.4% . See Sections 2 and 3 for more details.

^b The pH of Na_2S_x neutralized by the addition of diluted HCl solution.

^c This value measured directly, see Section 2.

results. The result from Eq. (2) agrees within 0.1% with the calculated stoichiometric isotopic value obtained for the initial polysulfides solution.

Both reactions were conducted with a relatively small excess of polysulfides relative to citral (see Section 2 for the “normal” ratio). The big difference between these two experiments is the amount of sulfur that was incorporated into citral. In the first reaction (low polysulfide concentration), just 5% of the sulfur pool in the polysulfide solution was introduced into citral, while in the second reaction (high citral concentration) 60% was introduced.

Thus, if we would let the first reaction go to completion, we would expect to get a much lower ^{34}S enrichment as was demonstrated in the second reaction.

When the sulfur pool is limited, such reactions can be considered as closed systems. The other reactions presented in this paper were practically considered as an open system since the amount of the sulfur pool was considerably bigger than the amount of the OM.

4. Geochemical significances

4.1. $\delta^{34}\text{S}$ variations in the reduced sulfur pool of sulfur rich marine sediments

The sulfur isotope composition of intermediate species (e.g. polysulfides) could be influenced by isotope mixing between different reduced sulfur species, as shown by ^{35}S labeled experiments (Fossing and Jorgenson, 1990; Fossing et al., 1992). In the present work, we demonstrated complete isotope mixing of stable sulfur isotopes between S^0 and $(\text{NH}_4)_2\text{S}$. Polysulfides served as mediator for this isotope mixing. Thus, we can consider the reduced sulfur species pool in marine sediments as isotopically homogeneous. There is uncertainty as to the reported concentrations and $\delta^{34}\text{S}$ of polysulfides and elemental sulfur due to chemical treatment (mainly acidification) that decompose the polysulfides prior to the extraction of the elemental sulfur (Mossmann et al., 1991; Rozan et al., 2000; Neretin et al., 2003). This

decomposition of polysulfides can cause ^{34}S fractionation between elemental sulfur and AVS as we showed in the present study. Moreover, the chemical decomposition of the sulfur-rich OM by acidification and/or Soxhlet extraction at high temperatures can release elemental sulfur (Mossmann et al., 1991) that has probably similar $\delta^{34}\text{S}$ to the organic sulfur. Therefore, we suggest that the variations often recorded between AVS and elemental sulfur in field geological samples can be related to the methods employed for the separation of the different sulfur species. This suggestion undoubtedly needs further studies to support it.

4.2. The carbonyl functionality importance in sulfurization processes in marine sediments

The results presented in the present study show the importance of the functional group on the sulfurized OM $\delta^{34}\text{S}$. The most ^{34}S enriched functionalized group is the carbonyl. Carbonyl groups are the strongest acceptors for polysulfide addition as was shown theoretically (Lalonde et al., 1987), and experimentally (Krein and Aizenshtat, 1993; Schouten et al., 1994; Krein and Aizenshtat, 1994; Amrani and Aizenshtat, 2004a). Aldehydes and ketones-functionalized structures have been recorded in lipid fractions of some algae and bacteria species (e.g. Volkman et al., 1980; Capon et al., 1981; Paul and Fenical, 1984). Photo-oxidation of alcohols and other functionalized lipids that are abundant in the OM of marine sediments can be an important pathway in the formation of aldehydes, ketones and, $\alpha\beta$ -unsaturated aldehydes (e.g. phytanal) (Rontani and Giusti, 1988; Rontani et al., 1990; Rontani, 2001 c.f.). Biodegradation is another suggested pathway for the formation of aldehydes and ketones (Grossi et al., 1998; Rontani et al., 1999). Analysis of geo-macromolecules often revealed that their sub-units originate from lipids with carbonyl functionality (e.g. Kohnen et al., 1993; Schouten et al., 1995; Adam et al., 2000; Filley et al., 2002).

It is suggested that carbohydrates can react with polysulfides and produce sulfur rich OM (van Kaam-Peters et al., 1998; Sinninghe Damsté et al., 1998; Kok et al., 2000; Aycard et al., 2003). van Dongen et al. (2003) conducted laboratory sulfurization experiments with polysulfides and monosaccharides. They showed that the carbonyl functionality plays an important role in the polysulfide introduction into monosaccharides. There are no data about the $\delta^{34}\text{S}$ fractionation during this incorporation of reduced sulfur species into carbohydrates. Since we show in the present study that the functional group is the most important factor for $\delta^{34}\text{S}$ fractionation during incorporation of polysulfides into OM, we can assume that similar ^{34}S enrichment will occur in the sulfurization of carbohydrate. Scheme 1 summarizes the suggested pathways of carbonyl groups in the formation of sulfur-rich OM.

4.3. The differentiation between $\delta^{34}\text{S}$ of pyrite and sulfurized OM

As discussed in Section 1, in most marine sediments analyzed, organic sulfur is enriched in ^{34}S relative to co-existing pyrite (Anderson and Pratt, 1995 c.f.; Bruchert and Pratt, 1996; Canfield et al., 1998; Passier et al., 1999; Werne et al., 2003). There are three main controlling factors on sulfur stable isotope signature of OM:

- Timing of the sulfur incorporation – sulfate open versus closed systems;
- Assimilatory compared with dissimilatory sulfur incorporation into OM;
- Sulfur species that are incorporated into OM – diffusion factors and composition.

The above were discussed intensively and reviewed (Anderson and Pratt, 1995; Aizenshtat and Amrani, 2004 c.f.). Anderson and Pratt (1995), summarized $\delta^{34}\text{S}$ data of sulfur-rich marine sediments and found that organic sulfur is generally +5–15‰ heavier than the coexisting pyrite. The results presented in this study for the incorporation of polysulfides and sulfides into OM ($\sim+5\%$), can explain part of the difference between pyrite $\delta^{34}\text{S}$ and OM $\delta^{34}\text{S}$ in natural marine sediments. However, in natural environments, the concentrations of both, OM and polysulfides are lower, which was shown to increase the ^{34}S enrichment of the sulfurized OM. The presence of minerals and the inorganic matrix of shallow sediments might effect the ^{34}S enrichment, but we are not aware of any such studies. In some selected cases, this phenomenon (i.e. $\sim+5\%$ enrichment) is suggested to be the major explanation for this gap. For example, Werne et al. (2003) investigated sulfur-rich

sediments in Cariaco Basin (Venezuela) with high productivity of OM and a limited reactive iron pool. They recorded steady ^{34}S enrichment between kerogen sulfur and pyrite of +5–8‰. They concluded that most of the organic sulfur is diagenetically formed. Moreover, they suggested that sulfide oxidation in the sediment can lead to S^0 and polysulfide formation and successive reaction with OM. Thus, we suggest that in poor iron environments with high productivity of OM and SRB activity, the ^{34}S enrichment of OM during polysulfides incorporation is the major factor influencing $\Delta_{\text{OM-pyrite}}$. However, for most cases we suggest that the phenomenon described here, is part of the complex overall mechanisms and catalyses that influence the $\delta^{34}\text{S}$ of sulfurized OM.

5. Conclusions

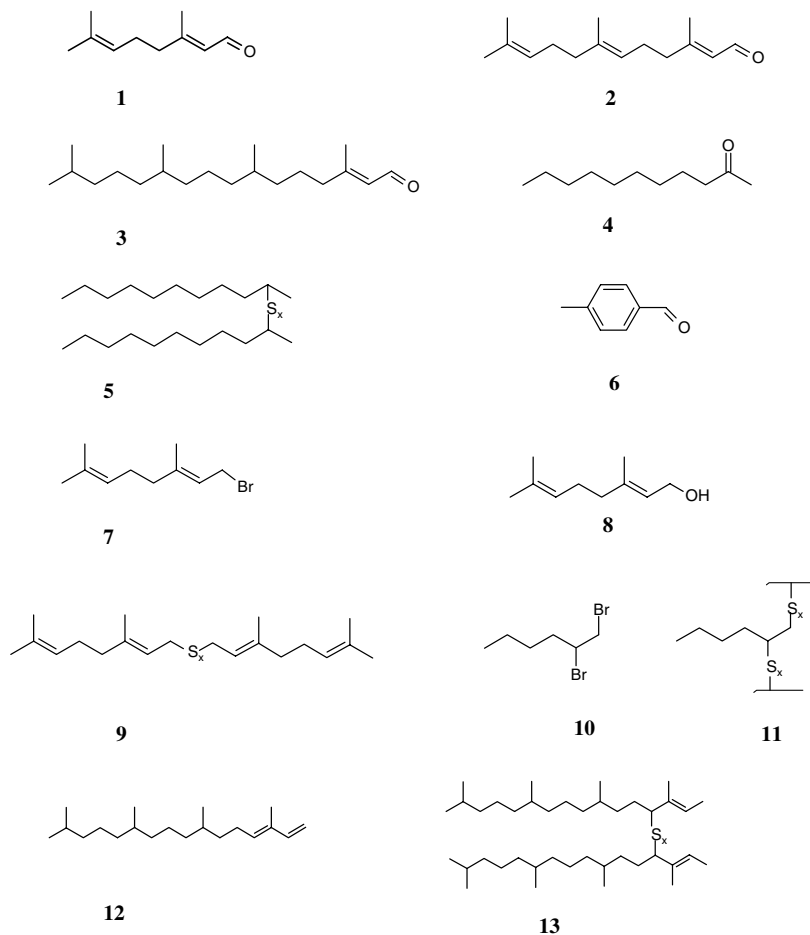
- Total chemical isotopic mixing occurs between elemental sulfur and sulfide during the formation of polysulfides.
- The acidification of polysulfide solution causes fractionation of 3–5‰ between the released H_2S and elemental sulfur.
- The incorporation of sulfide and polysulfides into carbonyls causes +4–5‰ ^{34}S enrichment relative to the calculated polysulfide isotopic value.
- The incorporation of sulfide and polysulfides into brominated compounds causes $\sim+1\%$ ^{34}S enrichment relative to the calculated polysulfide isotopic value.
- The ^{34}S enrichment of the sulfurized carbonyl groups showed small effect of temperature (0–70 °C) and is not effected by salinity, polysulfide composition, reaction time and solubility in water.
- The chemical mechanisms controlling the polysulfide incorporation into OM depend mostly on the functional groups and this determines the ^{34}S enrichment of the sulfurized OM.

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Appendix A



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