

Experiments on $\delta^{34}\text{S}$ mixing between organic and inorganic sulfur species during thermal maturation

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Abstract

Reduced sulfur species were studied to constrain isotopic exchange-mixing with synthetic polysulfide cross-linked macromolecules (PCLM), model sulfur containing molecules and natural sulfur-rich kerogen, asphalt and oil of the Dead Sea area. PCLM represents protokerogens that are rich in sulfur and thermally unstable. Mixing rates of PCLM with $\text{HS}^-_{(\text{aq})}$ (added as $(\text{NH}_4)_2\text{S}_{(\text{aq})}$) at low to moderate temperatures (50–200 °C) are rapid. Elemental sulfur and $\text{H}_2\text{S}_{(\text{gas})}$ fully mix isotopes with PCLM during pyrolysis conditions at 200 °C. During these reactions significant structural changes of the PCLM occur to form polysulfide dimers, thiolanes and thiophenes. As pyrolysis temperatures or reaction times increase, the PCLM thermal products are transformed to more aromatic sulfur compounds. Isotopic mixing rates increase with increasing pyrolysis temperature and time. Polysulfide bonds (S–S) in the PCLM are responsible for most of these structural and isotopic changes because of their low stability. Conversely, sulfur isotope mixing does not occur between dibenzothiophene (aromatic S) or hexadecanethiol (C–SH) and $\text{HS}^-_{(\text{aq})}$ at 200 °C after 48 h. This shows that rates of sulfur isotope mixing are strongly dependent on the functionality of the sulfur in the organic matter. The order of isotopic mixing rates for organic matter is kerogen > asphalt > oil, which is inverse to their sulfur thermal stability. Asphalt and oil with more refractory sulfur show significantly lower isotopes mixing rates than the kerogen with more labile sulfur. Based on the findings of the present study we suggest that sulfur isotopes mixing can occur from early diagenesis into catagenesis and result in isotopic homogenization of the inorganic and organic reduced sulfur pools.

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1. Introduction

Sulfur compounds in the geosphere are present in all fractions of organic matter (OM) in all stages of geochemical evolution (Krein, 1993). The isotopic signature of this organic sulfur shows that a significant part of it originates from secondary inorganic sulfur incorporation into OM during early stages of diagenesis (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; Aizenshtat and Amrani, 2004a). This process forms thermally unstable organic sulfur as polysulfide cross-linked polymeric structures, which later transform into Type-IIS kerogen (Aizenshtat and Amrani, 2004a). With increasing thermal maturation of the OM, structural and isotopic changes occur that coincide with massive release of sulfur, mainly as H_2S (e.g. Idiz et al., 1990; Koopmans et al., 1998; Putschew et al., 1998; Amrani et al., 2005b). This thermally cleaved sulfur is chemically reactive and can re-react with the OM (Amrani and Aizenshtat, 2004c). This may cause isotopic exchange that minimizes the isotopic discrimination among the sulfur species under closed-system conditions (Amrani et al., 2005a). An external sulfur source from bacterial or thermal sulfate reduction or exogenous H_2S from other reservoirs can react with the OM and result in mixed isotopes within the organic sulfur pool (Orr, 1974; Powell and MacQueen, 1984;

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Manzano et al., 1997; Worden and Smalley, 2001). Therefore, the isotopic signature of the mature organic sulfur may not only reflect the original diagenetic incorporated secondary sulfur but also external sulfur sources.

Limited data are available in the literature about isotopic exchange between organic sulfur and inorganic sulfur species during heating experiments. These studies report on a wide range of organic sulfur compounds that can exchange isotopes with inorganic reduced sulfur species (Edwards et al., 1948; Douglas et al., 1949; Guryanova et al., 1952; Obloentsev and Nikitin, 1964; Fedoseev, 1990; Zielinski and Kanska, 1993). These exchange data relate to the radioactive sulfur isotope (^{35}S) rather than stable isotopes (^{34}S and ^{32}S) and therefore, have a relatively large analytical error. A recent study (Amrani et al., 2005a) reports on preliminary stable isotope (^{34}S) exchange/mixing experiments that were conducted between synthetic polysulfide cross-linked macromolecules (PCLM) and aqueous ammonium sulfide solutions with distinct $\delta^{34}\text{S}$ values. The results showed that $\text{H}_2\text{S}_{(\text{gas})}-\text{S}_{(\text{organic})}-\text{HS}^-_{(\text{aq})}$ were isotopically mixed after only 4 h at 200 °C.

In the classic definition of isotope exchange, the relative abundance and composition of reactants remain unchanged but their isotopic composition changes with time. However, during pyrolysis experiments with PCLM, products are compositionally distinct from the reactant and manifest different sulfur functionalities. Therefore, our experiments represent both isotope exchange and changes in chemical species. In this study, the term isotope mixing is used to reflect changes of the $\delta^{34}\text{S}$ for both organic and inorganic sulfur species that occur with bond cleavage and formation during thermal maturation of OM. This study presents results from isotope mixing experiments between organic sulfur and inorganic sulfur species (S_8 , $\text{HS}^-_{(\text{aq})}$ and $\text{H}_2\text{S}_{(\text{gas})}$) with distinct $\delta^{34}\text{S}$ values between 50 and 200 °C. Structural and isotopic changes were monitored in order to discern feasible mechanisms for isotope mixing during thermal maturation.

2. Experimental

2.1. Starting compounds

Starting compounds were commercially obtained as follows: citral (*cis* and *trans* 3,7-dimethyl-2,6-octadienal, 97%, Fluka), dibenzothiophene (98%), hexadecanethiol (99%), 1-tetradecene (>98%, after re-distillation), and $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ were purchased from Aldrich. $(\text{NH}_4)_2\text{S}$ (20 wt% in aqueous solution) was purchased from Merck.

A detailed PCLM-synthesis procedure is described by Amrani and Aizenshtat (2004a). The only procedural difference is the amounts of the reactants. In the present work, PCLM was synthesized with 15 g of citral and a $(\text{NH}_4)_2\text{S}$ solution in three separate batches (5 g each). The resulting PCLMs were mixed in one vial. Two PCLMs were synthesized with different $\delta^{34}\text{S}$ values (19‰ and

4.4‰) by using solutions of ammonium polysulfide that had different $\delta^{34}\text{S}$ values (7.0‰ and 34.5‰).

Naturally occurring OM (kerogen, asphalt and oil) were collected from the Dead-Sea area; the exact location is described in the reference given for each substrate. The character of the kerogen used in the present study is described in Amrani et al. (2005b). The asphalt and oil are operative definitions. The asphalt (P-2) is solid at room temperature and was collected from the floating block in the Dead-Sea as described by Nissenbaum et al. (1980). The Gurim-4 oil is heavy asphaltic oil that is liquid at room temperature and described in more detail by Tannenbaum et al. (1987).

2.2. Chromatography and spectroscopy

GC-MS analyses were carried out on an 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 °C, mass range 45–450 Da, a resolution of 800 and scan time of 1 s. A fused capillary column (silica 30 m \times 0.32 mm, ID 0.25 μm , and CP-SIL 24CB coating) was used with He as the carrier gas. The GC was programmed at 4 °C/min between 50 and 300 °C. The initial and final temperatures were maintained for 5 and 20 min, respectively.

Nuclear magnetic resonance (NMR) spectroscopy analyses were carried out using a Bruker AMX-400 spectrometer. Samples were dissolved in CDCl_3 and transferred into 3 mm tubes. Tetramethylsilane (TMS) was used as the internal standard.

2.3. Experimental methods

For sulfur isotopic mixing experiments involving PCLM, model compounds and naturally accruing OM, 100–400 mg of the substrate was introduced into a 15 ml Pyrex ampoule. The quantity of organic S used for the present experiments was 1.5 mmol S, which consist of 0.2 g of PCLM, 0.3 g of dibenzothiophene, and 0.4 g of hexadecanethiol. 1.5 mmol of inorganic S were introduced into the ampoules which consisted of 0.5 ml of $(\text{NH}_4)_2\text{S}$ (20% solution in water), $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (0.36 g in 0.5 ml distilled water, pH reduced by addition of HCl), or 48 mg of S_8 . In hydrous pyrolysis experiments, 0.5–1 ml of distilled water was added. The mixture was purged with nitrogen and the ampoule sealed by flame. The ampoule was then heated for 1–168 h at temperatures from 50 to 200 °C. After pyrolysis was completed, the ampoule was cooled to ambient temperature and then opened. The evolving gas was displaced by a stream of N_2 through a Tygon tube and bubbled in an $\text{AgNO}_3/\text{NH}_4\text{OH}$ solution where the hydrogen sulfide was trapped. The residue was extracted with CH_2Cl_2 (DCM), dried over anhydrous MgSO_4 , and then filtered. Recovery yields were in the range of 70–80 wt% of the original OM sample weight.

For samples derived from hydrous pyrolysis, the residual water after DCM extraction was placed into a vial equipped with a rubber septum. Two milliliters of concentrated HCl (32 wt% in water) were injected by syringe with the evolving gas collected by another syringe and injected into $\text{AgNO}_3/\text{NH}_4\text{OH}$ solution. This portion of H_2S represents the water-soluble sulfides. For comparison, a sample of the residual water was introduced into $\text{AgNO}_3/\text{NH}_4\text{OH}$ solution for direct precipitation of sulfides. The precipitate Ag_2S was filtered, washed several times with distilled water, and dried in an oven at 120 °C.

In the isotopic mixing experiments with $\text{H}_2\text{S}_{(\text{gas})}$, a stainless steel reactor (20 ml Parr autoclave) rather than glass ampoule was used. The PCLM was dissolved in DCM and introduced into the reactor. The DCM was evaporated carefully under a stream of nitrogen. The reactor was then evacuated (4 mm Hg) and closed. The gasbag with the prepared $\text{H}_2\text{S}_{(\text{gas})}$ (see preparation method in the following section) was connected to the reactor. The reactor was immersed into liquid nitrogen and the gasbag was opened to the reactor. When the gas in the bag equilibrates with the reactor (about 40 min), the reactor was closed. After reaching room temperature, samples of the gas from the reactor and the gasbag were introduced into $\text{AgNO}_3/\text{NH}_4\text{OH}$ solution to precipitate Ag_2S to determine $\delta^{34}\text{S}$ of the initial value of $\text{H}_2\text{S}_{(\text{gas})}$ in the reactor. The gasbag was weighed before and after the gas was introduced to the reactor to determine the amount of $\text{H}_2\text{S}_{(\text{gas})}$ in the reactor.

2.4. Preparation of $\text{H}_2\text{S}_{(\text{gas})}$ for isotope mixing experiment

Into a three-necked round bottom flask, 20 ml of $(\text{NH}_4)_2\text{S}$ solution was introduced. A concentrate HCl solution was introduced while mixing the solution. The evolved $\text{H}_2\text{S}_{(\text{gas})}$ flowed through a Tygon tube into a water-ice trap, and then into a 1-liter gasbag equipped with a valve. When the bag was full, the valve was closed and gas sample was taken with a syringe and introduced into a $\text{AgNO}_3/\text{NH}_4\text{OH}$ solution to precipitate Ag_2S for $\delta^{34}\text{S}$ analysis. This procedure yielded $\text{H}_2\text{S}_{(\text{gas})}$ similar ($\pm 1\%$) to the original $(\text{NH}_4)_2\text{S}$ solution. The ^{34}S enrichment of $\text{H}_2\text{S}_{(\text{gas})}$ was achieved by using a CaCl_2 trap instead of ice trap. This procedure enriched the $\text{H}_2\text{S}_{(\text{gas})}$ by more than 30%.

2.5. Choice of ammonium sulfide as the comparative HS^- species

Aqueous Na_2S solutions have a pH of ~ 13 to 14. Accordingly, when pH has to be adjusted to lower values, it needs an additional large quantity of acid or buffer, which makes it difficult to maintain and accurately determine the desired concentration of the solution. Because we could not observe significant differences in isotopic mixing rates when we used sodium or ammonium sulfide (Section 3.1.3), we decided to use ammonium sulfide (pH ~ 9), which is easier to control. The in situ pH in

our experiments at 200 °C is estimated to be in the range of ~ 6.5 to 7.5 for $(\text{NH}_4)_2\text{S}$ solutions (calculations made with StreamAnalyzer 1.3 software, OLI Systems, Inc.). In this in situ pH range, the abundant sulfur species in the aqueous phase are divided almost equally between bisulfide (HS^-) and hydrogen sulfide (H_2S). For convenient, we use the symbol $\text{HS}^-_{(\text{aq})}$ to collectively describe both aqueous HS^- and H_2S . From a geochemical perspective, the pH range from 6 to 9.5 is within the range of waters from petroleum producing wells although pH values less than 8 are most common (Collins, 1975; Palandri and Reed, 2001). In recent sediments, the degradation of amino acids produces ammonia that in typical marine sediments at pH values of 6.5–8.5 is predominantly in the ammonium NH_4^+ form (see concentration of ammonium and sulfide at Schulz et al., 1994; Passier et al., 1998).

2.6. Chemical degradation of the residual OM by MeLi/MeI

Selective cleavage of organic polysulfide (S–S) bonds was performed in order to characterize sulfur in OM based on the method of Eliel et al. (1976) and Kohnen et al. (1991). Excess methyl lithium (MeLi) was added to 50–100 mg of OM in diethyl ether. The mixture was 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 and extracted three times with hexane. The extract was dried over anhydrous MgSO_4 , and filtered. The products were analyzed by GC–MS.

2.7. Elemental analyses and stable sulfur isotopes measurements

Elemental analysis was performed by the Microanalysis Laboratory at the Hebrew University of Jerusalem using a Perkin-Elmer Series II 2400 CHNS Analyzer (max of $\pm 0.3\%$ error range).

$\delta^{34}\text{S}$ of the precipitated Ag_2S and the organic samples was measured by a continuous-flow elemental analyzer connected to a Finnigan/Mat Delta Plus stable-isotope ratio monitoring mass spectrometer (EA-irmMS). Sulfur isotope compositions are expressed as per mil (‰) deviations from V-CDT (Vienna Canyon Diablo Troilite) standard using the conventional delta notation with a standard deviation better than 0.4‰ ($n \geq 2$). The measurements were directly calibrated against sulfur isotopic standards IAEA-S-1 (Ag_2S , -0.3%) and NBS-127 (BaSO_4 , $+20.3\%$) with a standard deviation better than 0.2‰ ($n \geq 3$).

3. Results

3.1. Aqueous sulfide and PCLM

In these experiments, PCLM sulfur and inorganic sulfur were introduced into the vessel in a 1:1 sulfur molar ratio.

The polysulfide cross-links are at C1 and C3 positions as described elsewhere (Amrani and Aizenshtat, 2004a).

3.1.1. Reaction time

Fig. 1a shows the rate of isotope mixing between $(\text{NH}_4)_2\text{S}_{(\text{aq})}$ and PCLM. After less than an hour, the large isotopic difference between all sulfur species is reduced considerably from 30‰ to only 2–3‰. The broken line is the isotope mass balance line for total binary isotopic mixing according to the following equation:

$$\delta_T = x\delta_{\text{IS}} + (1 - x)\delta_{\text{OS}}, \quad (1)$$

where δ_T represents the stoichiometric $\delta^{34}\text{S}$ value (i.e. total sulfur), x represents the mole fraction of the inorganic sulfur (IS) and OS represent the organic sulfur. The isotope values of all sulfur species at longer pyrolysis times converge on this line and suggest rapid isotope mixing. Fig. 1b presents the sulfur content in the PCLM after the experiments. The amount of sulfur in the polymer is higher than the starting PCLM for the first 4 h. This increase suggests introduction of inorganic sulfur into the polymer. After 4 h of heating, the amount of sulfur in the PCLM is gradually decreases to about 20%. However, this decrease in amount of S in the PCLM is lower than in the hydrous pyrolysis experiments (Fig. 1b). In addition, the

hydrous experiments do not show the initial increase in PCLM sulfur during the first 4 h.

Isotope mixing of heavy PCLM with light sulfides appears to be complete after 48 h, which is similar to the experiments with heavy sulfides (Table 1). The results showed less than 1‰ deviation from the calculated stoichiometric isotopic balance Eq. (1). The only difference is that the organic sulfur is slightly isotopically heavier than the inorganic sulfur.

The residual organic matter after each pyrolysis was treated with MeLi/MeI to selectively cleave S–S bonds and then analyzed by GC–MS and in some cases by ^1H NMR spectroscopy. The spectra are typical for polymeric matter with wide peaks hindering quantification. During the pyrolysis experiments, the PCLM undergoes significant structural changes. The main structural change is the decomposition of the polymeric structure to polysulfide dimers as shown (compound 1) in Scheme 1. These dimers have been identified by MS fragmentation patterns of their MeLi/MeI decomposition products (compound 2) as suggested by Krein (1994). Although compound 2 could be derived from the thiol structure (compound 3) as well, it is evident from direct injection into the GC that only a small amount of thiol is present prior to the MeLi/MeI treatment. This finding suggests that most of compound 2 is derived from the dimeric polysulfide 1 (Scheme 1). The unknown compounds are isomers of m/z 183 or 184 and after MeLi/MeI treatment are changed to m/z 198 suggesting methylation. These compounds are probably oxygen and sulfur containing, but at this time we cannot suggest

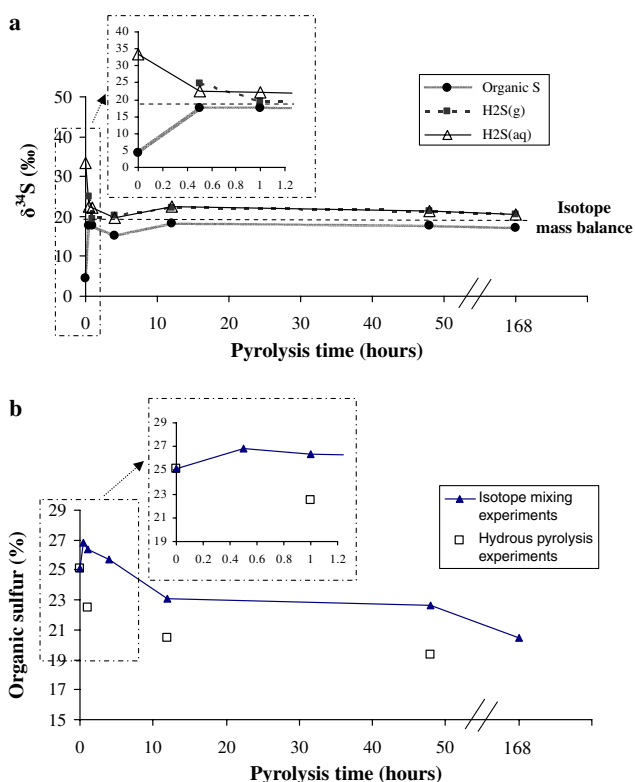
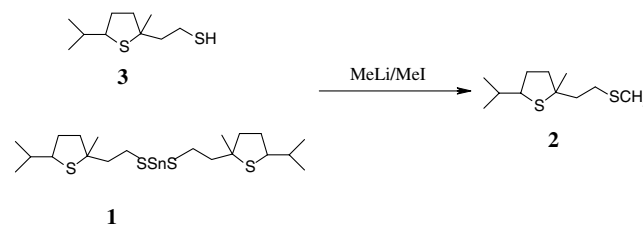


Fig. 1. The influence of reaction period on isotopes mixing experiments between polysulfide cross-linked polymer (25.1 wt% S) and $(\text{NH}_4)_2\text{S}$ at 200 °C. The amounts of reactants represent approximately 1:1 sulfur mole ratio. The binary isotope mass balance calculated according to Eq. (1). (a) $\delta^{34}\text{S}$ values. The graph in the box is enlargement of the experiments up to 1 h; (b) weight % of S in the residual polymer after each experiment.

Table 1
 $\delta^{34}\text{S}$ values of isotopes mixing (error < 0.4‰) for experiments between polysulfide cross-linked polymer (23.9 wt% S) and $(\text{NH}_4)_2\text{S}$ at 200 °C. The amounts of reactants represent approximately 1:1 sulfur mole ratio. The binary isotope mass balance calculated according to Eq. (1)

Time (h)	Organic S $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{aq})}$ $\delta^{34}\text{S}$ (‰)	Binary isotope mass balance (‰)
0	19.0		7.0	
4	13.5	11.5	12.2	13.0
48	12.8	11.9	12.5	13.0



Scheme 1. Two possible precursors for the MeLi/MeI degradation product of 5-isopropyl-2-methyl-2-(2-methylsulfanyl-ethyl)-tetrahydro-thiophene (compound 2). The precursors are: 2-(5-isopropyl-2-methyl-tetrahydro-thiophene-2-yl)-ethanethiol (compound 3) and the dimer form of this molecule, bis-2-(5-isopropyl-2-methyl-tetrahydro-thiophene-2-yl)-ethanpolysulfide (compound 1).

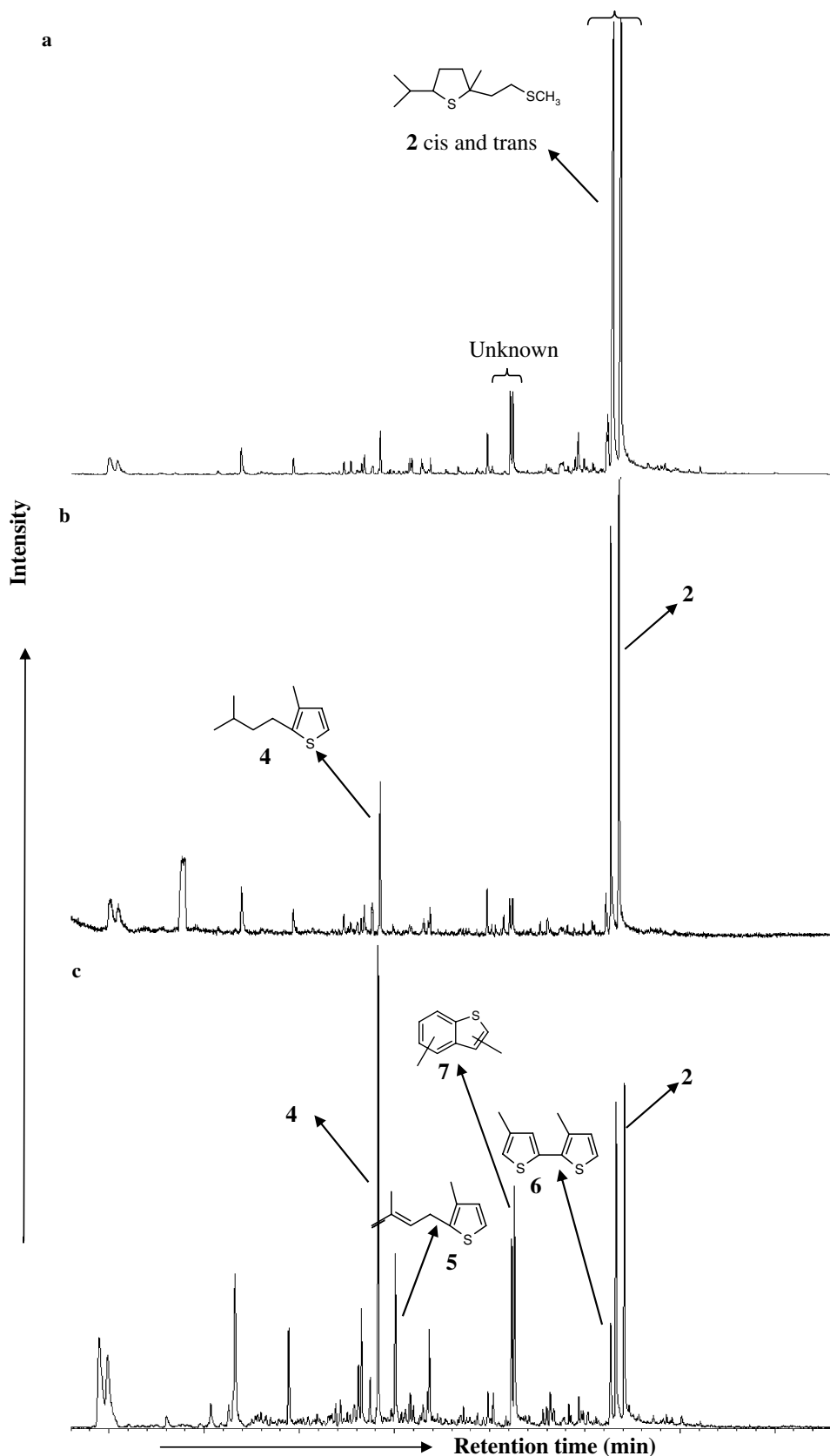


Fig. 2. Gas chromatograms (TIC) of the MeLi/MeI products of the residual organic matter resulting from the experiments described in Fig. 1. (a) One hour reaction, the main product is 5-isopropyl-2-methyl-2-(2-methylsulfanyl-ethyl)- tetrahydro-thiophene (compound 2). (b) Forty-eight hours reaction, generation of 3-methyl-2-(3-methyl-butyl)-thiophene (compound 4). (c) One hundred and sixty-eight hours reaction, increasing generation of aromatic sulfur compounds such as 3-methyl-2-(3-methyl-but-2-enyl)-thiophene (compound 5), 3,4'-dimethyl-[2,2']bithiophenyl (compound 6) and di-methylated benzothiophene (compound 7, the exact methyls locations is unknown) that is co-eluted with an unknown compound.

a molecular structure. PCLM transformations to monomers like thiophenes (compounds **4**, **5**, **6**, Fig. 2) and alkylated benzothiophene (compound **7**) increases with increasing pyrolysis time.

In order to further understand the link between the isotope mixing mechanism and the structure of the PCLM, we subjected our PCLM to an isotopic mixing experiment for 1 week at 200 °C (Table 2). The residual organic matter was extracted from the reaction mixture and its $\delta^{34}\text{S}$ value measured. This extracted OM was subjected to subsequent isotope mixing experiment at 200 °C for 48 h with distinct $\delta^{34}\text{S}$ values of the organic and inorganic sulfur pools (Table 2). The results showed a very small degree of isotopic mixing. This indicates that the structural changes during the pyrolysis experiments of the PCLM cause thermal stabilization that has significant influence on the isotope mixing results.

3.1.2. Temperature effect

The small isotopic change between ammonium sulfide and PCLM at ambient temperature suggests a very low rate of isotopic mixing. This coincides with small structural changes that were observed relative to the initial PCLM. Significant isotopic changes were observed in experiments at and higher than 50 °C (Fig. 4a). The isotopic mixing at relatively low temperatures (<100 °C) is not complete, as evident by the relatively large differences in all the sulfur species.

The sulfur content at the lower temperatures from 25 to 70 °C did not change significantly relative to the initial PCLM value (Fig. 4b). At the intermediate temperatures of 100–130 °C, we observed a slight increase in the amount of sulfur (Fig. 4b) and then a sharp decrease at 200 °C. The structural changes showed destruction of the polymeric structure as a function of increasing temperature with formation of dimers (compound **1**, Scheme 1). Fig. 5 presents the MeLi/MeI degradation products of the experiment at 50 °C. Reconstruction of these products shows that at low thermal maturation most of the organic sulfur is still in the polymeric structure because of the high abundance of the two and three polysulfides bonds (represented by MeS groups after MeLi/MeI treatment). At the 50 °C experiment, the products represent the transformation between the initial polymeric structure (compound **8**, Fig. 5) and the intermediate compounds **9**, **10**, **11** (Fig. 5, mass spectra for compounds **9**, **10** at Fig. 6) to form the dimeric (compound **1**) structure that dominates the higher temperature experiments. Insertion of methyl into carbon 1 (C1) is an artifact of the MeLi/MeI method and it may point to the residual unreacted carbonyl or imine group as discussed by Amrani and Aizenshtat (2004a). Examination of the temperature effect allows us to investigate the structural and isotopic changes from the initial polymer in steps that could not be observed in the isothermal experiments (200 °C). This is due to the high temperatures imposing rapid changes and more thermally stable products.

3.1.3. pH and sulfide cation influence

We examined the influence of the sulfide type (ammonium or sodium) in our experiments by performing an experiment for 48 h at 200 °C with Na_2S . The results showed no significant difference in comparison to the same experiment with $(\text{NH}_4)_2\text{S}$ (Table 3). Moreover, we did not observe nitrogen enrichment in the residual organic matter after the isotope-mixing experiment with $(\text{NH}_4)_2\text{S}$, which may suggest that ammonium does not significantly affect the structural changes under our experimental conditions.

The pH effect is not significant as demonstrated by experiments with Na_2S at pH values from 7 to 14 (Table 3). It is important to note that the pH range at 200 °C in our experiments was calculated to be 6–11 using the Stream Analyzer 1.3 software (OLI Systems, Inc.). In all cases, we observed full isotopic mixing between the organic and inorganic sulfur. The only important difference is in the amount of residual sulfur of the PCLM, which shows preferable preservation of sulfur in the lower pH experiments. In the experiment at a pH of 7, the reduction of pH by HCl caused a dramatic decrease in the concentration of the $\text{HS}^-_{(\text{aq})}$ due to massive release of $\text{H}_2\text{S}_{(\text{g})}$. Therefore, the organic S: inorganic S ratio in that case is not 1:1. However, the results show that full isotopic mixing also occurs, as demonstrated by the relatively similar $\delta^{34}\text{S}$ values of the pyrolysis products (Table 3).

3.2. S_8 or $\text{H}_2\text{S}_{(\text{gas})}$ and PCLM

These experiments were conducted to understand the mechanisms of isotope mixing between different phases of sulfur. The first set of experiments were conducted using isotopically distinct $\text{H}_2\text{S}_{(\text{gas})}$ or S_8 and PCLM at 200 °C for 48 h. The results of both sulfur phases were within 1‰ of one another (Table 4) and suggest full isotope mixing. The elemental analysis of the residual organic matter showed a large increase in the sulfur content from 25 to 38 wt%, which indicates significant introduction of sulfur into the PCLM. The reaction of PCLM with S_8 at 200 °C produces organic sulfur with a mixed isotopic signature and high amount of added sulfur (Table 4). The distribution of products for both $\text{H}_2\text{S}_{(\text{gas})}$ and S_8 thermal experiments were similar to those obtained in the experiment with $(\text{NH}_4)_2\text{S}_{(\text{aq})}$ after 48 h.

In some of the above experiments, the products have $\delta^{34}\text{S}$ values that are either slightly higher or lower than the binary isotopic mass balance. Such cases can be observed for the reaction with $\text{H}_2\text{S}_{(\text{gas})}$ 3:1 ratio (inorganic S:organic S) where all the products are heavier than the isotopic mass balance value. Conversely, the 1:1 ratio experiment showed the opposite trend (Table 4). A possible explanation for this lack of isotopic mass balance may be the formation of unidentified products with anomalous $\delta^{34}\text{S}$ values. These unidentified products may include elemental sulfur or volatile organic sulfur compounds that were not measured. Less probable is the formation of sig-

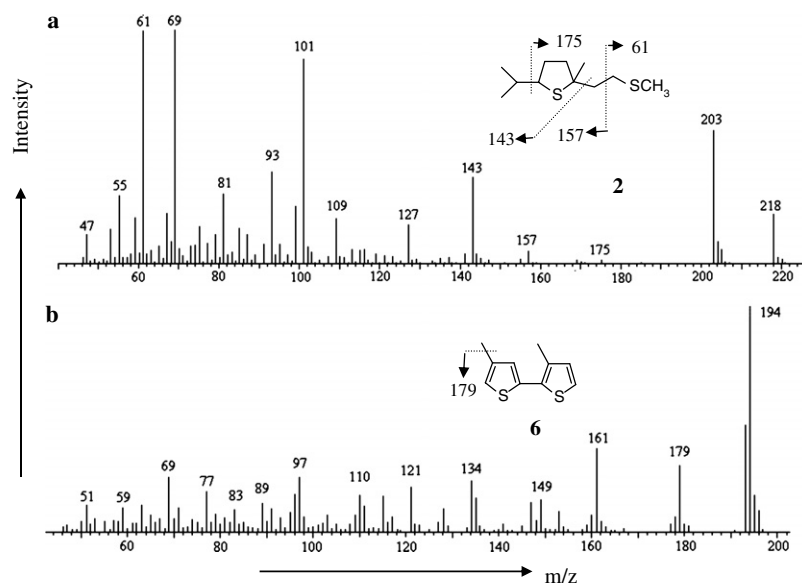


Fig. 3. Mass spectra for two of the MeLi/MeI treatment products of the residual organic matter resulting from the experiments described in Fig. 1. (a) 5-Isopropyl-2-methyl-2-(2-methylsulfanyl-ethyl)- tetrahydro-thiophene (compound 2). (b) 3,4'-dimethyl-[2,2']bithiophenyl (compound 6).

Table 2

$\delta^{34}\text{S}$ values of isotopes mixing experiments (error $< 0.4\text{‰}$) and wt% S of polysulfide cross-linked polymer after 1 week at 200 °C pyrolysis in the presence of $(\text{NH}_4)_2\text{S}$ with distinct $\delta^{34}\text{S}$ value. The residual organic matter extracted from the reaction mixture and subjected to another isotopic mixing experiment (48 h, 200 °C) under the same conditions

Precursor	Initial organic S $\delta^{34}\text{S}$ (‰)	Initial $(\text{NH}_4)_2\text{S}$	Residual organic S $\delta^{34}\text{S}$ (‰)	$\delta^{34}\text{S}$ (‰) enrichment	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{aq})}$ $\delta^{34}\text{S}$ (‰)	Binary isotope mass balance (‰)	Initial sulfur content (wt%)	Residual sulfur content (wt%)
Unheated PCLM	4.4	34.5	17.1	12.7	20.6	20.4	19.0	25.1	20.5
Heated PCLM	17.1	34.5	19.6	2.5	30.4	32.6	25.8	20.5	18.2

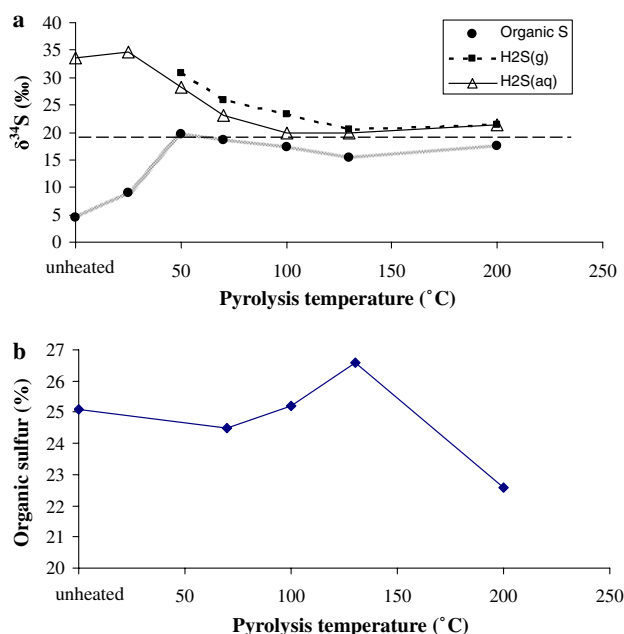


Fig. 4. The influence of reaction temperature on isotopes mixing experiments between polysulfide cross-linked polymer (25.1 wt% S) and $(\text{NH}_4)_2\text{S}$ for 48 h. (a) $\delta^{34}\text{S}$ values; (b) weight % of S in the residual polymer after each experiment.

nificant amounts of oxygenated sulfur compounds (such as thiosulfate) under our extremely reducing pyrolysis conditions, although we cannot rule out this possibility.

3.3. $(\text{NH}_4)_2\text{S}_{(\text{aq})}$ and model compounds

The selected model monomeric compounds (dibenzothiophene and hexadecanthiol) represent different sulfur bond strengths, and are therefore susceptible to different isotopic mixing mechanisms and rates than PCLM. These experiments were conducted with ammonium sulfide at 200 °C and showed no change in their isotopic ratios and molecular structures (Table 5). In addition, the $\delta^{34}\text{S}$ values of the $(\text{NH}_4)_2\text{S}_{(\text{aq})}$ and the released $\text{H}_2\text{S}_{(\text{gas})}$ showed no significant changes. These results indicate that no isotope mixing occurred between these monomeric compounds and $(\text{NH}_4)_2\text{S}_{(\text{aq})}$.

3.4. Inorganic sulfur species and 1-tetradecene

These experiments were conducted to investigate whether $\text{H}_2\text{S}_{(\text{gas})}$, S_8 or $\text{HS}^-_{(\text{aq})}$ can react with alkenes (1-tetradecene) in significant yields and record the isotopic signature for these reactions. The reaction under 6 atm of

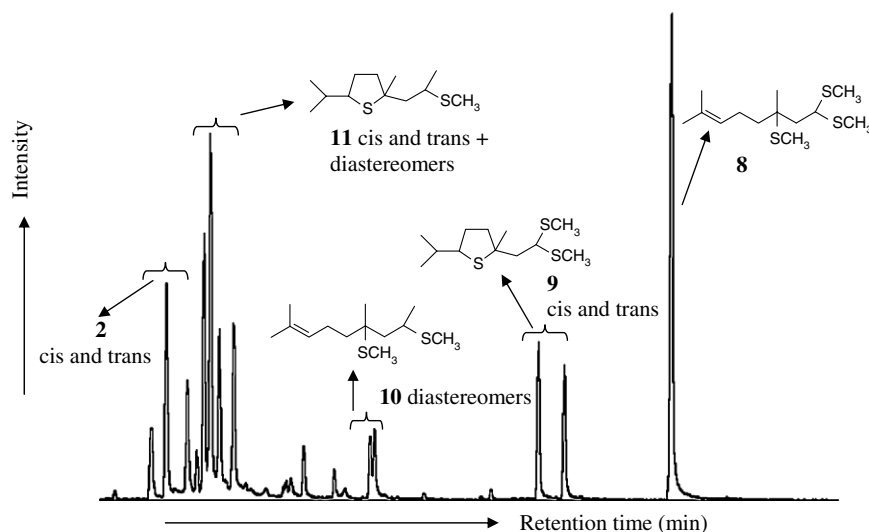


Fig. 5. Gas chromatograms (TIC) of the MeLi/MeI of the residual organic matter resulting from the experiment described in Fig. 4 at 50 °C. The main MeLi/MeI degradation products are: 5-isopropyl-2-methyl-2(2-methylsulfanyl-ethyl)-tetrahydro-thiophene (compound **2**), 2,6-dimethyl-6,8,8-tris-methylsulfanyl-oct-2-ene (compound **8**), 2-(2,2-bis-methylsulfanyl-ethyl)-5-isopropyl-2-methyl-tetrahydro- thiophene (compound **9**), 2,6-dimethyl-6,8-bis-methylsulfanyl-non-2-ene (compound **10**) and 5-Isopropyl-2-methyl-(2-methylsulfanyl-propyl)- tetrahydro-thiophene (compound **11**).

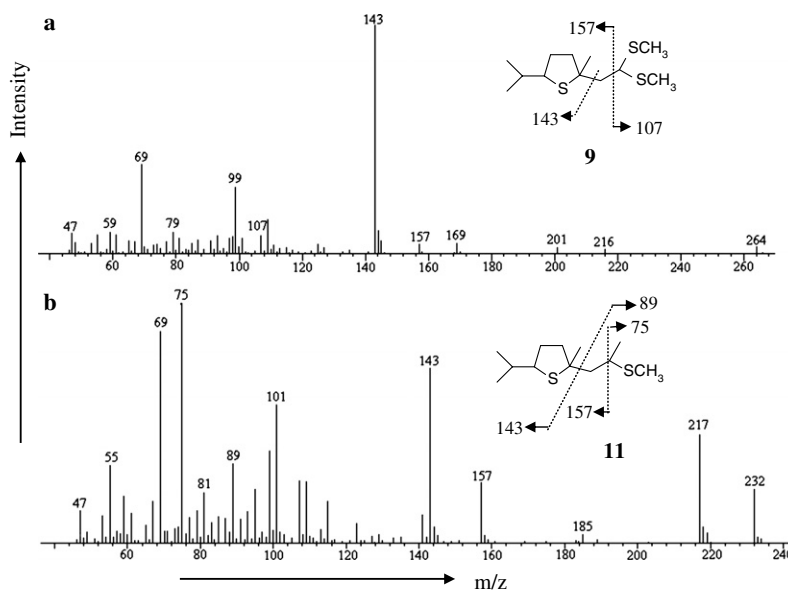


Fig. 6. Mass spectra for two of the MeLi/MeI products of the residual organic matter resulting from the experiments described in Fig. 4 at 50 °C. (a) 2-(2,2-Bis-methylsulfanyl-ethyl)-5-isopropyl-2-methyl-tetrahydro- thiophene (compound **9**). (b) 5-Isopropyl-2-methyl-2-(2-methylsulfanyl-propyl)-tetrahydro-thiophene (compound **11**).

Table 3

$\delta^{34}\text{S}$ values of isotopes mixing (error < 0.4‰) for experiments between polysulfide cross-linked polymer and Na_2S at 200 °C for 48 h at different pH values. The binary isotope mass balance calculated according to Eq. (1)

pH	Organic S: inorganic S molar ratio	Initial organic S $\delta^{34}\text{S}$ (‰)	Initial Na_2S	Residual organic S $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{aq})}$ $\delta^{34}\text{S}$ (‰)	Binary isotope mass balance (‰)	Initial sulfur content (wt%)	Residual sulfur content (wt%)
13–14	1:1	19.0	3.9	14.7	12.0	9.7	11.5	23.9	18.9
8.5	1:1	19.0	3.7	14.7	11.6	9.9	11.4	23.9	22.1
7.0	7.5: 1	15.7	3.5	14.4	14.3	14.1	14.2	25.0	24.5

Table 4
 $\delta^{34}\text{S}$ values (error < 0.4‰) and wt% S for isotopes mixing experiments between polysulfide cross-linked polymer (25.1 wt% S) and $\text{H}_2\text{S}_{(\text{gas})}$ or S_8 at 200 °C for 48 h

Reactant	Sulfur mole ratio reactant/organic S	Initial reactant $\delta^{34}\text{S}$ (‰)	Initial organic S $\delta^{34}\text{S}$ (‰)	Residual organic S $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	Binary isotope mass balance (‰)	Residual organic sulfur content (wt%)
$\text{H}_2\text{S}_{(\text{gas})}$	3:1	36.0	19.0	34	32.1	32.0	38.5
$\text{H}_2\text{S}_{(\text{gas})}$	1:1	60.0	4.0	29.1	30.4	32.0	34.3
S_8	1:1	-1.7	19.0	8.8	8.1	8.7	30 ± 2^a

^a Calculated by difference from other elements.

Table 5
 $\delta^{34}\text{S}$ values of isotopes mixing (error < 0.4‰) for experiments between dibenzothiophene or hexadecanethiol and $(\text{NH}_4)_2\text{S}$ at 200 °C for 48 h

Type of organic S	Initial $(\text{NH}_4)_2\text{S}$ $\delta^{34}\text{S}$ (‰)	Initial organic S $\delta^{34}\text{S}$ (‰)	Residual organic S $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{aq})}$ $\delta^{34}\text{S}$ (‰)
Dibenzothiophen	34.5	11.9	12.0	34.0	34.5
Hexadecylthiol	34.5	-3.5	-3.7	35.5	34.0

Table 6
 $\delta^{34}\text{S}$ values and wt% S for sulfur incorporation experiments between 1-tetradecene and inorganic sulfur species at 200 °C

Reactant	Time (h)	Sulfur mole ratio reactant/organic	Initial reactant $\delta^{34}\text{S}$ (‰)	Residual organic S $\delta^{34}\text{S}$ (‰)	Residual reactant $\delta^{34}\text{S}$ (‰)	wt% organic S
$\text{H}_2\text{S}_{(\text{gas})}$	48	3:1	35.6	35.1	37.1	21.7
S_8	48	1:1	-1.7	-0.7	n.m.	12.7
$(\text{NH}_4)_2\text{S}$	48	1:1	7.0	3.0	7.1	2.1

$\text{H}_2\text{S}_{(\text{gas})}$ was conducted at 200 °C for 48 h. The results show massive sulfur introduction (~22 wt%) and negligible isotopic difference between the organic and inorganic sulfur products (Table 6). The products were mostly mono-thiols, in all positions along the carbon chain. The most abundant product was 2-thiol-tetradecane. The reaction with elemental sulfur yielded similar products and showed high yield of elemental sulfur introduction into the organic structure (Table 6). Only trace amounts of elemental sulfur could be detected, and therefore, the isotopic value of the released S_8 could not be measured. The $\delta^{34}\text{S}$ values of the released H_2S and organic sulfur show small differences (Table 6). The reaction with $(\text{NH}_4)_2\text{S}_{(\text{aq})}$ at 200 °C for 48 h yielded relatively small amounts of organo-sulfur compounds (Table 6). The organic sulfur products (mainly thiols) showed about a 4‰ ^{34}S depletion as compared with the $(\text{NH}_4)_2\text{S}_{(\text{aq})}$. This is contrary to incorporation of sulfur into carbonyls at low temperatures that was reported to have ^{34}S enrichment for the organic sulfur as compared to the initial inorganic sulfur (Amrani and Aizenshtat, 2004b).

3.5. Kerogen, asphalt and oil

Immature kerogen was subjected to pyrolysis at 200 °C for 48 and 168 h in the presence of ammonium sulfide with a 1:4 sulfur ratio (wt%), respectively. The kerogen contains two types of sulfur: (1) organic sulfur (95 wt%) with a $\delta^{34}\text{S}$ of +0.6‰ and (2) pyrite (5 wt%) with a very light $\delta^{34}\text{S}$ of

-29.9‰. Pyrite at this temperature is thermally stable (Seewald et al., 1994; Amrani et al., 2005b) but its aqueous solubility may contribute inorganic sulfur to solution (Seewald, 2001). However, the pyrite in our kerogen is coated with organic matter and thus somewhat protected from the surrounding water (Amrani et al., 2005b). Moreover, isotope mass balance calculations of the pyrite potential to influence the results shows a value of less than -0.5‰ on the external inorganic sulfur pool and +2.3‰ on the organic sulfur. The experimental results show an intense isotope mixing after 48 h that suggest that the main isotopic mixing occurred between the organic sulfur and the external inorganic sulfur pool (Table 7). The $\delta^{34}\text{S}$ value of the kerogen increased from -0.9‰ to +10.3‰, while the inorganic sulfide decreased from 34.5‰ to 31.8‰ with a calculated isotope mass balance for total mixing of 26.6‰. This trend increased slightly after one week of reaction (Table 7). The amount of sulfur in the kerogen decreases by 2.3 wt% after 48 h and 1.7 wt% after 168 h of reaction.

Asphalt shows much smaller changes in its isotopic values with only a 3.2‰ change after 48 h. In the 168 h experiment, the asphalt changed by 7.1‰, however, the $\delta^{34}\text{S}$ of the inorganic sulfur did not change much, suggesting no isotopic mixing but rather sulfur introduction from the inorganic sulfur pool into the organic structure. This is supported by the elemental analyses that show a small increase in the amount of S in comparison to the unheated asphalt (Table 7).

Table 7

$\delta^{34}\text{S}$ values (error < 0.4‰) and wt% S for isotopes mixing experiments between Kerogen, asphalt or oil from the Dead-Sea area and $(\text{NH}_4)_2\text{S}$ at 200 °C for 48 or 168 h

Type of organic S	Time (h)	Initial $(\text{NH}_4)_2\text{S}$ $\delta^{34}\text{S}$ (‰)	Initial organic S $\delta^{34}\text{S}$ (‰)	Residual organic S $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{gas})}$ $\delta^{34}\text{S}$ (‰)	$\text{H}_2\text{S}_{(\text{aq})}$ $\delta^{34}\text{S}$ (‰)	Binary isotope mass balance (‰)	Initial Sulfur content (wt%)	Residual Sulfur content (wt%)
Kerogen	48	34.5	-0.9	10.3	31.6	31.8	26.6	12.6	10.3
kerogen	168	34.5	-0.9	11.3	32.7	32.0	26.6	12.6	11.0
Asphalt	48	34.5	7.3	10.5	35.1	35.4	29.7	10.0	11.2
Asphalt	168	34.5	7.3	14.4	35.4	34.7	29.7	10.0	10.5
Oil	48	34.5	3.8	4.6	n.d.	31.44	31.4	4.4	4.6
Oil	168	34.5	3.8	9 ± 0.6	37 ± 0.6	33 ± 0.6	31.4	4.4	4.0

The experiment with natural heavy oil showed an even smaller change in the isotopic value of the organic sulfur with only a 0.8‰ change that suggests very little isotopic mixing. This is supported by the increase in $\delta^{34}\text{S}$ of the aqueous sulfide. After one week, the $\delta^{34}\text{S}$ enrichment of the heavy oil increased to 5.2‰.

These results show that asphalt and oil have lower isotopic mixing rates than does the kerogen and the synthetic PCLM under the same conditions (Table 7).

4. Discussion

4.1. Sulfur activity during thermal alteration of PCLM

During pyrolysis experiments with PCLM, large amounts of H_2S are released due to the cleavage of the relatively weak sulfur bonds. Krein and Aizenshtat (1995) showed that sulfur elimination processes can be followed by addition of sulfur species into the PCLM. It may be presented by the simplified equation:



In hydrous and dry pyrolysis of PCLM, $k_1 > k_2$, therefore, we can observe overall decrease in the amount of S in the residual OM (Fig. 1b). However, when an additional source of sulfur is present, such as in our isotope mixing experiments, we observe an increase in sulfur content in the pyrolysis experiments after short durations (Fig. 1b) or at low temperatures (Fig. 4b) indicating sulfur addition is a competitive reaction with sulfur elimination. At long durations or high temperatures, this trend is reversed and the sulfur content is reduced. This reduction in sulfur content is smaller than the corresponding hydrous pyrolysis experiments (Fig. 1b) or dry pyrolysis experiments (Amrani et al., 2005a) that performed without external inorganic sulfur pools.

The cleavage and addition of sulfur during pyrolysis of PCLM in closed systems, result in mixing between organic and inorganic sulfur species that consequently lead to isotopic mixing. This serves as the foundation for the understanding of the sulfur isotopic mixing that is observed in our experiments.

4.2. Scenarios for sulfur isotopes mixing

Four likely scenarios are suggested to explain the observed interactions between organic and inorganic sulfur pools and their subsequent isotopic changes. These scenarios include no reaction, addition, elimination, and elimination/addition combination. Each scenario has a different influence on the isotopic partitioning of each sulfur species.

No interaction between the two sulfur pools results in no significant $\delta^{34}\text{S}$ changes in the organic or inorganic sulfur pools. This scenario is dominant for experiments with dibenzothiophene and hexadecanethiol, which showed no isotopic or structural changes (Table 5).

Addition of inorganic sulfur into organic matter [$k_2 \gg k_1$, Eq. (2)] results in the inorganic sulfur pool having a relatively small isotopic change, while the organic sulfur may have a significant isotopic change depending on the amount of introduced sulfur and its isotopic difference. This occurs when the organic sulfur is thermally stable and the release of sulfur occurs at a slow rate. However, functionalized positions such as double bonds along the carbon chain attract inorganic sulfur attack causing addition of sulfur into the organic matter (Krein and Aizenshtat, 1995). This can be demonstrated by the experiment with the asphalt that shows introduction of inorganic sulfur with isotopic change in the organic sulfur while minor change was observed in the inorganic sulfur pool due to no release of sulfur from the organic source (Table 7).

Elimination of sulfur from OM into the inorganic sulfur pool ($k_2 \ll k_1$) can be recorded by isotopic changes in the inorganic pool and relatively small isotope changes in the organic sulfur. However, this scenario is difficult to follow because cleavage of sulfur creates an active site (free radicals) that may promote chemical attack of sulfur from the mixed pool.

Combination of the elimination and addition scenarios may also occur. This combination can result in significant isotopic changes for both sulfur pools. This scenario is suggested to dominate the PCLM experiments as both organic and inorganic sulfur pools converge to the isotope mass balance value approaching total binary isotopic mixing (Fig. 1a).

4.3. Mechanism of sulfur isotope mixing as related to molecular structure

The present study shows that thiol (C–SH) and dibenzothiophene (aromatic C–S–C), do not mix isotopes at 200 °C with the inorganic sulfur pools (Table 5). Conversely, PCLM (C–S–S_x–C) mixes isotopes very rapidly at relatively low temperatures (Figs. 1a and Fig. 4a). These fundamental differences between the reacted model compounds suggest that the type of sulfur functionality has an important role in the availability of the bonded sulfur towards isotope exchange and mixing reactions.

Previous studies on isotope exchange using ³⁵S radiolabeled model compounds report on a wide variety of sulfur bonds that are isotopically exchangeable (Edwards et al., 1948; Douglas et al., 1949; Guryanova et al., 1952; Obloentsev and Nikitin, 1964; Fedoseev, 1990; Zielinski and Kanska, 1993). The data reported in these studies is difficult to compare to our results because of the variety of chemical conditions used such as organic solvents as the reaction medium. Therefore, these data can be misleading and caution should be used in evaluating their significance.

Complete isotopic exchange between aqueous Na₂³⁵S and Me₂S at ambient temperatures was reported by Zielinski and Kanska (1993). Isotopic exchange occurs between thiolane derivatives and Na₂³⁵S in ethanol, but not with thiophenic sulfur at temperatures above 98 °C (Obloentsev and Nikitin, 1964). Contrary to the exchange mechanism that involves cleavage of C–S bonds, Fedoseev (1990) suggested that the sulfur contained in C–SH, and C–S–C is more difficult to exchange because of the relatively strong C–S bond in comparison to the S–S bonds. He reported that organic polysulfides exchange sulfur isotopes very rapidly (2 h, 100 °C) and that the exchanged sulfur is only in the S–S bonds and not the C–S bonds.

Guryanova et al. (1952) suggested similar mechanism for the isotopic exchange between ³⁵S₈ and organic polysulfides at 100 °C. Similarly, Fava et al. (1957) found that mono-sulfides do not exchange isotopes even in extreme conditions, whereas the exchange with polysulfides and disulfides is rapid.

In order to understand the relation between isotopic mixing of sulfur and its functionality, we studied the structural changes that occurred in the experiments with PCLM. The results presented in Figs. 2 and 3, show that as thermal maturation increases PCLM structure changes to form more thermally stable structures like thiolanes and thiophenes. The amount of thiophenic sulfur produced increases with thermal maturation (Fig. 2). The bond dissociation energies of the C–S bond in thiophene and thiol are higher than the S–S bond in polysulfides by 30–50 kcal/mol and 5–30 kcal/mol, respectively (Aizenshtat et al., 1995). This difference may explain the lack of sulfur mixing in the cases of hexadecanthiol and dibenzothiophene (Table 5) as compared to PCLM. Reconstruction of the thermally modified PCLM using the MeLi/MeI products of the mixing reactions products is presented in Scheme 2. The gradual

change in the polymeric structure during thermal alteration from labile S–S structure to non-labile aromatic sulfur (such as thiophenic structures) is accompanied by H₂S release in each step. These structural changes are particularly apparent if we compare the structural changes as a function of temperature in the experiments from 50 °C (Fig. 5) to 200 °C (Fig. 2). As the temperature increases, the isotopic value becomes more homogenized. In the longer pyrolysis experiments conducted at 200 °C (Fig. 1a), full isotopic mixing appears to be reached. In contrast, pre-heated PCLM (200 °C) showed very small degree of isotopic mixing (see results and Table 2). This finding further suggests that the formation of thermally stable (mostly thiophenic) sulfur considerably reduces the isotopic mixing with the other sulfur species.

5. Geochemical implications

5.1. OM enrichment in ³⁴S during diagenetic processes

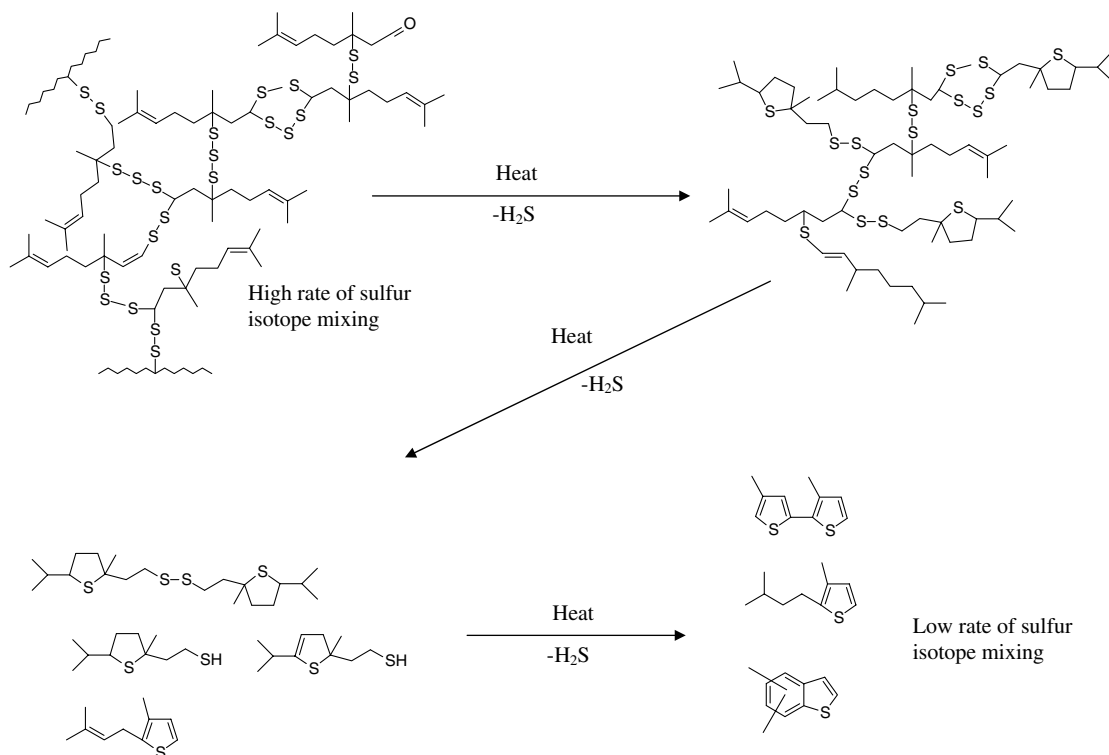
The ³⁴S enrichment of OM relative to the coexisting pyrite has been reviewed extensively (Anderson and Pratt, 1995; Aizenshtat and Amrani, 2004a). Pyrite is considered as the best marker for the “early” δ³⁴S value of biogenically produced sulfide. This is mainly because available iron reacts rapidly with reduced aqueous sulfide with negligible isotope discrimination (Price and Shieh, 1979). The phenomenon of ³⁴S enrichment of OM with burial (or maturation) is well documented in the literature (Mossmann et al., 1991; Anderson and Pratt, 1995; Werne et al., 2003; Aizenshtat and Amrani, 2004a; see references therein) and has been attributed to four major processes that occur individually or in combination:

(a) *Assimilatory compared with dissimilatory sulfur incorporation into OM*

The bacterial reduction of sulfate (BSR) follows two pathways: (1) Assimilatory sulfate reduction that biosynthesize the essential compounds for bacteria living (e.g. cysteine) with very small sulfur isotope fractionation. (2) Dissimilatory sulfate reduction coupled to oxidation of organic matter for gaining energy with a large ³⁴S depletion of the produced sulfide (Vairavamurthy et al., 1995). The δ³⁴S for the assimilatory sulfur reflects the associated sulfate δ³⁴S (i.e. ~20‰ for modern marine sulfate) that is incorporated mostly into sulfur containing amino acids. If a significant amount of the assimilatory sulfur fraction is preserved in the sedimentary OM then, the δ³⁴S of the OM will be ³⁴S enriched (Canfield et al., 1998; Passier et al., 1999). This explanation can not be applied to sulfur-rich OM were most of the S originates from dissimilatory source and therefore is ³⁴S depleted (Aizenshtat and Amrani, 2004a).

(b) *Timing of the sulfur incorporation*

With the increase of the extent of BSR in a system closed with respect to the sulfate supply, the isotope fractionation between the starting sulfate and the reduced sulfur species decreases (Anderson and Pratt, 1995). Incorporation of the



Scheme 2. Thermal transformations of the synthetic polysulfide cross-linked macromolecule (PCLM) during the isotopic mixing experiments.

later formed sulfide into OM will gradually enrich the OM in ^{34}S (Werne et al., 2003).

(c) *Type of sulfur species that are incorporated into OM*

Mossmann et al. (1991) suggested that polysulfides (and elemental sulfur) are isotopically heavier than pore water sulfide because they are formed by the oxidation of sulfide diffusing across the sediment–water interface that is isotopically heavier than pore water sulfides within surface sediment (Chanton et al., 1987). Recently, we have shown that aqueous polysulfide anions are isotopically enriched in ^{34}S compared with coexisting sulfide up to about 6‰ at equilibrium and ambient temperature (Amrani et al., 2006).

(d) *Fractionation during sulfur incorporation into OM*

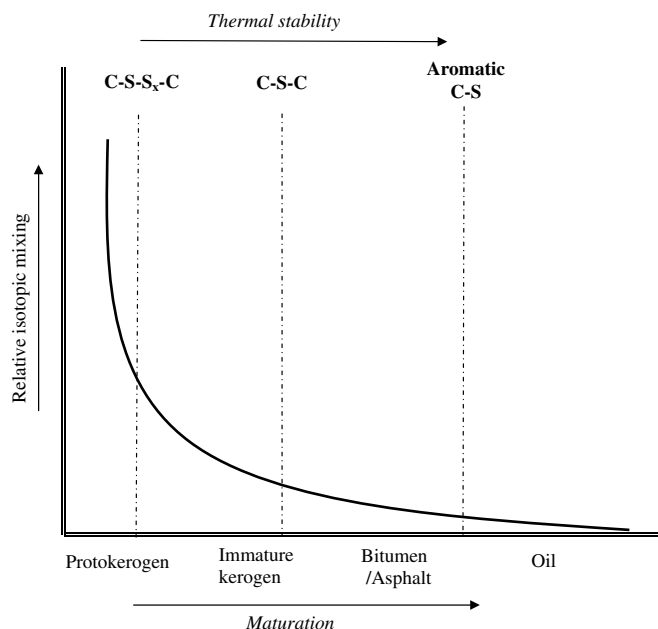
The incorporation of polysulfides into OM causes ^{34}S enrichment up to 5‰ relative to the initial value of the polysulfides solution at 25 °C. This has been shown by laboratory experiments simulating natural sulfur incorporation into carbonyl model compounds (Amrani and Aizenshtat, 2004b). This mechanism can explain part of the isotopic difference between pyrite and OM.

The experimental results presented here provide new insight on reaction mechanisms during mixing of sulfur isotopes in OM and external sulfur pools that lead to a gradual enrichment in ^{34}S . The pore water reduced sulfur species are ^{34}S enriched with depth because the system becomes closed to sulfate (Aizenshtat and Amrani, 2004a). The PCLM used in the present study may represent an analogue for thermally immature sulfur-rich proto-kerogens during early diagenesis. Protokerogen is thermally unstable and susceptible to significant structural changes under mild

thermal alteration (Aizenshtat et al., 1983; Adam et al., 2000; Luckge et al., 2002). The present study shows that at 50 °C, significant structural and isotopic changes occur. Polysulfide bonds in the OM are probably responsible for most of these structural and isotopic changes because of their low stability (Scheme 2). This indicates that reduced sulfur in both organic and inorganic pools may interact and mix sulfur isotopes throughout diagenesis that leads to isotopic homogenization of the reduced sulfur species, with the exception of pyritic sulfur. Therefore, the present study suggests that the early $\delta^{34}\text{S}$ imprint of the OM may gradually change *via* mixing with the inorganic reduced sulfur pool in the sediment. It is important to note that unlike the previously suggested explanations (e.g. mechanism b), this process does not necessarily involve a net increase in the sulfur content of the OM and may continue to occur as thermal maturity increases beyond the onset of kerogen formation.

5.2. *Structural changes as related to sulfur isotope mixing under catagenetic conditions*

The general view for oil formation through thermal alteration is the sequence: kerogen to bitumen and bitumen to oil (Tissot and Welte, 1978; Lewan, 1985; Baskin and Peters, 1992). This sequential maturation scheme coincides with the gradual loss of hetero-atoms (Tannenbaum and Aizenshtat, 1985; Orr, 1986; Koopmans et al., 1998; Putschew et al., 1998), a change in sulfur functionality from polysulfides in kerogen to sulfides in bitumen to aromatic sulfur in oil, and the release of H_2S (Krein, 1993; Nelson



Scheme 3. The suggested thermal maturity influence on isotopic mixing of organic with inorganic sulfur.

et al., 1995; Aizenshtat and Amrani, 2004b). The transformation of PCLM into smaller molecules through cleavage of the thermally unstable polysulfide bonds (Scheme 2) relates to the thermal maturity stage of kerogen transformation into bitumen.

Results of heating experiments involving kerogen, asphalt, and oil suggest that the rate of sulfur isotope mixing varies inversely as a function of thermal stability of organically bound sulfur. For example, kerogen containing the most labile organic sulfur showed the greatest extent of sulfur isotope mixing, while more thermally mature asphalt and oil showed lesser amounts of mixing (Table 7). This is further supported by the fact that while the kerogen loses sulfur during the experiments, asphalt and oil did not. Scheme 3, plots the conceptual connection between isotopic mixing and the sulfur functionality. Since the functionality changes with maturation, the extent of isotopic mixing may reflect the thermal maturity of a sample. This in turn, indicates connection between decreasing isotopic mixing rates and maturation. The most probable maturation stage of which isotopic mixing are likely to be intense is between protokerogen stabilization (end of diagenesis) and kerogen degradation to yield bitumen (beginning of catagenesis). Under these conditions, the relatively weak polysulfide bonds collapsed to form thermally more stable structures as demonstrated by our PCLM thermal and isotopic changes (Scheme 2). Nelson et al. (1995) showed similar result during laboratory heating of sulfur-rich kerogen from Monterey Shale.

The temperatures employed in this study were intentionally selected to allow for thermal activation of S–S bonds while minimizing C–C cleavage. However, under natural maturation conditions (slow heating over geologic time) both carbon and sulfur structural changes commonly occur

that could possibly lead to isotopic mixing involving more thermally stable compounds such as thiols and monosulfides. Moreover, other structural changes may occur simultaneously such as dehydration, decarboxylation, and deamination, that do not necessarily involve cleavage of sulfur bonds but are known to form active sites (such as double bonds). The availability of such reactive sites is directly related to the addition of sulfur at relatively high temperatures, which eventually enhances the isotopic mixing rate.

5.3. In reservoir secondary addition of sulfur into petroleum

Although oil does not manifest high rates of sulfur isotope mixing at the temperatures and time used in the present study, other processes can affect its $\delta^{34}\text{S}$ by reaction with external inorganic sulfur pools on a geological time scale. Such external reduced sulfur pools may be produced by a variety of processes, such as microbial or thermal sulfate reduction depending on the reservoir temperatures. If the system is closed, the various species of sulfur may equilibrate with minimal isotopic discrimination.

Recently, Méhay et al. (2005), reported addition of sulfur produced by sulfate reducing bacteria (SRB) during oil biodegradation in reservoir. This sulfur enrichment of the oil was accompanied by ^{34}S depletion of sulfur suggesting some introduction of light sulfur into the residual biodegraded oil.

However, secondary sulfur addition into oils is more likely to occur during thermal processes. There are many reports on addition of sulfur into oil at relatively high temperatures in reservoirs as revealed by their distinct $\delta^{34}\text{S}$ values (e.g. Orr, 1974; Powell and MacQueen, 1984; Manzano et al., 1997; Worden and Smalley, 2001). Cai et al. (2003), suggested that H_2S formed during thermal sulfate reduction (TSR) is incorporated into hydrocarbons to form thiols. This suggestion was supported by the finding of increased thiol concentration with increased H_2S concentration. A similar suggestion based on high thiol concentration in mature oils is also described by Ho et al. (1974). Enrichment of oil by evaporite-sourced H_2S were reported by Cai et al. (2005) and Zhang et al. (2005), although the process that produced this H_2S remains controversial (BSR or TSR, Worden and Cai, 2006; Zhang et al., 2006). This conclusion was based on the fact that H_2S and oil in this location have similar $\delta^{34}\text{S}$ values and the extremely high amount of sulfur in the oil (up to 14.7%).

We have demonstrated the reaction of 1-tetradecene with various sulfur species at 200 °C to produce sulfur containing compounds, mainly thiols with similar $\delta^{34}\text{S}$ values of the inorganic sulfur (Table 6). Saturated hydrocarbons can also thermally react with sulfur to produce organo-sulfur compounds at higher pyrolysis temperatures (Schmid et al., 1987; Stoler et al., 2003). Therefore, oil can gradually change its isotopic signature towards the $\delta^{34}\text{S}$ values of the reduced inorganic sulfur pool. This of course, will be at

much slower rates than immature organic matter (such as the kerogen in the present study). The magnitude of this process is likely to depend on reservoir conditions such as temperature, the sulfur ratio organic/inorganic and the nature of the hydrocarbons.

6. Conclusions

We have shown in the present study that isotopic mixing of organic and inorganic sulfur species from diagenesis through catagenesis is chemically feasible.

1. Significant structural changes of the PCLM occurs during mild thermal alteration ($\geq 50\text{ }^\circ\text{C}$) in the presence of $\text{HS}^-_{(\text{aq})}$, to form polysulfide dimers, thiolanes and thiophenes. As the pyrolysis temperature rises or reaction time is prolonged, PCLM pyrolysis products are transformed to more aromatic sulfur.
2. Isotopic mixing between PCLM and inorganic reduced sulfur species increases with increasing pyrolysis temperature and/or reaction time.
3. Isotope mixing between PCLM and $\text{HS}^-_{(\text{aq})}$ is an effective process at $50\text{ }^\circ\text{C}$ after 48 h, which suggests that it is likely to occur during early diagenetic formation of polysulfide-rich protokerogen in anoxic sediments. This process may also continue after the onset of kerogen formation, and therefore may represent a key mechanism to explain the enrichment of ^{34}S in natural OM with burial.
4. Isotopic mixing does not occur between dibenzothiophene (aromatic S) or hexadecanethiol (C-SH) and $\text{HS}^-_{(\text{aq})}$ at temperatures up to $200\text{ }^\circ\text{C}$ after 48 h. These results show that rates of sulfur isotope mixing are strongly depend on the functionality of the sulfur in the OM.
5. 1-Tetradecene thermally reacts with inorganic reduced sulfur species to form organic sulfur compounds that are isotopically homogenous with the inorganic sulfur at $200\text{ }^\circ\text{C}$ after 48 h. This result suggests that OM can gradually change its isotopic signature towards the $\delta^{34}\text{S}$ values of the reduced inorganic sulfur pool by secondary incorporation of inorganic sulfur into hydrocarbons.
6. The pH variations between 7 and 14 and sulfide cations (Na^+ or NH_4^+) show no significant effect on isotopic mixing.
7. A clear order of isotopic mixing rates and yields of OM observed for the samples used in the present study. The order of isotope-mixing susceptibility is kerogen > asphalt > oil, which correlates inversely with the thermal stability of sulfur. This suggests that the isotopic mixing rates are dependent on the level of thermal maturity, which determines sulfur functionality of the OM.

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Associate editor: Jeffrey Seewald

References

- Adam, P., Schneckenburger, P., Schaeffer, P., Albrecht, P., 2000. Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. *Geochim. Cosmochim. Acta* **64**, 3485–3503.
- Aizenshtat, Z., Stoler, A., Cohen, Y., Nielsen, H., 1983. The Geochemical sulphur enrichment of recent organic matter by polysulfides in the Solar Lake. In: Bjoroy, M. et al. (Eds.), *Advances in Organic Geochemistry 1981*. Wiley & Sons, Chichester, pp. 279–288.
- Aizenshtat, Z., Krein, E.B., Vairavamurthy M.A.A., Goldstein, T.P. 1995. Role of sulfur in the transformations of sedimentary organic matter: a mechanistic overview. In: Vairavamurthy, M.A.A., Schoonen, A. (Eds.), *Geochemical Transformation of Sedimentary Sulfur*. American Chemical Society Symposium Series 612, pp. 16–39.
- Aizenshtat, Z., Amrani, A. 2004a. Significance of $\delta^{34}\text{S}$ and evaluation of its imprint on sedimentary organic matter I. The role of reduced sulfur species in the diagenetic stage: a conceptual review. In: Hill, R.J. et al. (Eds.), *Geochemical Investigations: A tribute to Isaac R. Kaplan*, The Geochemical Society, Special Publication No. 8, pp 15–33.
- Aizenshtat, Z., Amrani, A. 2004b. Significance of $\delta^{34}\text{S}$ and evaluation of its imprint on sedimentary organic matter II. Thermal changes of Type II-S kerogens catagenetic stage controlled mechanisms. Study and conceptual overview. In: Hill, R.J. et al. (Eds.), *Geochemical Investigations: A tribute to Isaac R. Kaplan*, The Geochemical Society, Special Publication No. 8, pp. 35–50.
- Amrani, A., Aizenshtat, Z., 2004a. Reaction of polysulfide anions with α,β unsaturated isoprenoid aldehydes in aquatic media: simulation of oceanic conditions. *Org. Geochem.* **35**, 909–921.
- Amrani, A., Aizenshtat, Z., 2004b. Mechanisms of sulfur introduction chemically controlled: $\delta^{34}\text{S}$ imprint. *Org. Geochem.* **35**, 1319–1336.
- Amrani, A., Aizenshtat, Z., 2004c. Photosensitized oxidation of natural occurring isoprenoid allyl-alcohols as possible pathway for their transformation to thiophenes in sulfur rich depositional environments. *Org. Geochem.* **35**, 693–712.
- Amrani, A., Said Ahmad, W., Aizenshtat, Z., 2005a. The $\delta^{34}\text{S}$ values of the early-cleaved sulfur upon low temperature pyrolysis of a synthetic polysulfide cross-linked polymer. *Org. Geochem.* **36**, 971–974.
- Amrani, A., Lewan, M.D., Aizenshtat, Z., 2005b. Stable sulfur isotope partitioning during simulated petroleum formation as determined by hydrous pyrolyses of Ghareb Limestone, Israel. *Geochim. Cosmochim. Acta* **69**, 5317–5331.
- Amrani, A., Kamyshny Jr., A., Lev, O., Aizenshtat, Z., 2006. Sulfur stable isotopes distribution of polysulfide anions in $(\text{NH}_4)_2\text{S}_n$ aqueous solution. *Inorg. Chem.* **45**, 1427–1429.
- Anderson, T.F., Pratt, L.M. 1995. Isotopic evidence for the origin of organic sulfur and elemental sulfur in marine sediments. In: Vairavamurthy, M.A., Schoonen, M.A.A. (Eds.), *Geochemical Transformation of Sedimentary Sulfur*. ACS Symposium Series 612, pp. 378–396.
- Baskin, D.K., Peters, K.E., 1992. Early generation characteristics of a sulfur-rich Monterey kerogen. *Am. Assoc. Petrol. Geol.* **76**, 1–13.
- Cai, C., Worden, R.H., Bottrell, S.H., Wang, L., Yang, C., 2003. Thermochemical sulfate reduction and the generation of hydrogen sulfide and thiols (mercaptans) in Triassic carbonate reservoirs from the Sichuan Basin, China. *Chem. Geol.* **202**, 39–57.
- Cai, C., Worden, R.H., George, A.W., Bottrell, S.H., Wang, D., Xin, L., 2005. Origin of sulfur rich oils and H_2S in Tertiary lacustrine sections of the Jinxian Sag, Bohai Bay Basin, China. *Appl. Geochem.* **20**, 1427–1444.

- Canfield, D.E., Boudreau, B.P., Mucci, A., Gundersen, J.K., 1998. The early diagenetic formation of organic sulfur in the sediments of Mangrove Lake, Bermuda. *Geochim. Cosmochim. Acta* **62** (5), 767–781.
- Chanton, J.P., Martens, C.S., Goldhaber, M.B., 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 8.A sulfur isotopic budget balanced by differential diffusion across the sediment-water interface. *Geochim. Cosmochim. Acta* **51**, 1201–1208.
- Collins, A.G., 1975. *Geochemistry of Oilfield Waters*. Elsevier, New York.
- Dinur, D., Spiro, B., Aizenshtat, Z., 1980. The distribution and isotopic composition of sulfur in organic rich sedimentary rocks. *Chem. Geol.* **31**, 37–51.
- Douglas, D.L., Cooley, R.A., Yost, D.M., 1949. Non-exchange between carbon di-sulfide and hydrogen sulfide in benzene solution. *J. Am. Chem. Soc.* **71**, 3237.
- Edwards, R.R., Nesbitt, F., Solomon, A.K., 1948. Recoil-activated and thermal exchange reactions between sulfur 35 and carbon di-sulfide. *J. Am. Chem. Soc.* **70**, 1670.
- Eliel, E.L., Hutchins, R.D., Mebane, R., Willer, R.L., 1976. Endocyclic vs. exocyclic attack in nucleophilic displacement reactions on five and six-membered cyclic onium salts. *J. Org. Chem.* **41**, 1052–1057.
- Fava, A., Iliceto, A., Camera, E., 1957. Kinetics of the thiol-disulfide exchange. *J. Am. Chem. Soc.* **79**, 833–838.
- Fedoseev, V.M., 1990. Investigation of organic reactions by the use of radioactive sulfur. In: Belenkii, L.I. (Ed.), *Chemistry of Organosulfur Compounds General Problems*. Ellis Horwood, pp. 229–243.
- Francios, R., 1987. A study of sulphur enrichment in humic fraction of marine sediments during early diagenesis. *Geochim. Cosmochim. Acta* **51**, 17–27.
- Guryanova, E.N., Syrkin, F.K., Kuzina, L.S., 1952. Exchange reaction between sulfur atoms in organic polysulfides. *DAN SSSR* **86**, 107, in Russian.
- Ho, T.Y., Rogers, M.A., Drushel, H.V., Koons, C.B., 1974. Evaluation of sulfur compounds in crude oils. *Am. Assoc. Petrol. Geol.* **58**, 2338–2348.
- Idiz, E.F., Tannenbaum, E., Kaplan, I.R. 1990. Pyrolysis of high-sulfur Monterey kerogens - stable isotopes of sulfur, carbon, and hydrogen. In: Orr, W.L., White, C.M. (Eds.), *Geochemistry of Sulfur in Fossil Fuels*, ACS Symposium Series 429. pp. 575–591.
- Kohnen, M.E.L., Sinninghe Damsté, J.S., Kock-van Dalen, A.C., de Leeuw, J.W., 1991. Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. *Geochim. Cosmochim. Acta* **55**, 1375–1394.
- Koopmans, M.P., Rijijpstra, W.I.C., de Leeuw, J.W., Lewan, M.D., Sinninghe Damsé, J.S., 1998. Artificial maturation of immature sulfur- and organic matter-rich limestone from the Ghareb Formation, Jordan. *Org. Geochem.* **28**, 503–521.
- Krein, E.B., 1993. Organic sulfur in the geosphere: analysis, structures and chemical processes. In: Patai, S., Rappoport, Z. (Eds.), *The Chemistry of the Sulphur-containing Functional Groups. Supplement S*. Wiley, pp. 975–1032.
- Krein, E.B. 1994. *The Chemical Reactions between Polysulfide Anions and Organic Matter as a Model for Sulfur Enrichment of Organic Sediments*. Ph.D Thesis, The Hebrew university of Jerusalem. In Hebrew (English abstract).
- Krein, E.B., Aizenshtat, Z. 1995. Proposed thermal pathways for sulfur transformation in organic simulation macromolecules: laboratory simulation experiments. In: Vairavamurthy, M.A., Schoonen, M.A.A. (Eds.), *Geochemical Transformation of Sedimentary Sulfur*, ACS Symposium Series 612, Washington, DC, pp. 110–137.
- Luckge, A., Horsfield, B., Littke, R., Scheeder, G., 2002. Organic matter preservation and sulfur uptake in sediments from the continental margin off Pakistan. *Org. Geochem.* **33**, 477–488.
- Lewan, M.D., 1985. Evaluation of petroleum generation by hydroous pyrolysis experimentation. *Philos. Trans. R. Soc. Lond.* **315**, 123–134.
- Manzano, B.K., Fowler, M.G., Machel, H.G., 1997. The influence of thermochemical sulfate reduction on hydrocarbon composition in Nisku reservoirs, Brazeau river area, Alberta, Canada. *Org. Geochem.* **27**, 507–521.
- Méhay, S., Kowalewski, I., Fafet, A., Adam, P., de Penteadó, H., Albrecht, P. 2005. Role of anaerobic biodegradation on secondary incorporation of sulfur in the organic matter of petroleum reservoirs. *Org. Geochem.* 22st IMOG meeting (Seville, Spain) 2005. Extended abstracts book I, pp. 74–75.
- Mossmann, J.R., Aplin, A.C., Curtis, C.D., Coleman, M.L., 1991. Geochemistry of inorganic and organic sulphur in organic-rich sediments from the Peru Margin. *Geochim. Cosmochim. Acta* **55**, 3581–3595.
- Nelson, B.C., Eglinton, T.I., Seewald, J.F., Vairavamurthy, M.A., Miknis, F.P. 1995. Transformations in organic sulfur speciation during maturation of Monterey shale: constraints from laboratory experiments. In: Vairavamurthy, M.A., Schoonen, M.A.A. (Eds.) *Geochemical Transformation of Sedimentary Sulfur*. American Chemical Society, Symposium Series 612, Washington, DC, pp. 138–166.
- Nissenbaum, A., Aizenshtat, Z., Goldberg, M., 1980. The floating asphalt blocks of the Dead Sea. In: Douglas, A.G., Maxwell, J.R. (Eds.), *Advances in Organic Geochemistry 1979*. Pergamon press, Oxford, pp. 157–161.
- Obolentsev, R.D., Nikitin, Y.E., 1964. Isotopic exchange of sulfur between sulfur organic compounds. In: Obolentsev, R.D. (Ed.), *Chemistry of Organic Sulfur Compounds in Petroleum and Petroleum Products*. Academy of science of the USSR, Moscow, pp. 276–278.
- Orr, W.L., 1974. Changes in sulfur content and isotopic ratios of sulfur during petroleum maturation study of Big Horn Basin Paleozoic oils. *Am. Assoc. Petrol. Geol. Bull.* **50**, 2295–2318.
- Orr, W.L., 1986. Kerogen/asphaltene/sulfur relationships in sulfur-rich Monterey oils. *Adv. Org. Geochem. Org. Geochem.* **10**, 499–516.
- Price, F.T., Shieh, Y.N., 1979. Fractionation of sulfur isotopes during laboratory synthesis of pyrite at low temperatures. *Chem. Geol.* **27**, 245–253.
- Putschew, A., Schaeffer-Reiss, C., Schaeffer, P., Koopmans, M., de Leeuw, J.W., Lewan, M.D., Sinninghe Damsté, J.S., Maxwell, J.R., 1998. Release of sulfur- and oxygen-bound components from a sulfur-rich kerogen during simulated maturation by hydrous pyrolysis. *Org. Geochem.* **29**, 1875–1890.
- Powell, T.G., MacQueen, R.W., 1984. Precipitation of sulfide ores and organic matter reactions at Pine Point. *Science* **224**, 63–66.
- Palandri, J.L., Reed, M.H., 2001. Reconstruction of in situ composition of sedimentary formation waters. *Geochim. Cosmochim. Acta* **65**, 1741–1767.
- Passier, H.F., Dekkers, M.J., DeLange, G.J., 1998. Sediment chemistry and magnetic properties in an anomalously reducing core from the eastern Mediterranean Sea. *Chem. Geol.* **152**, 287–306.
- Passier, H.F., Böttcher, M.E., DeLange, G.J., 1999. Sulphur enrichment in organic matter of Eastern Mediterranean sapropel: a study of sulphur isotope partition. *Aqua. Geochem.* **5**, 99–118.
- Schmid, J.C., Conan, J., Albrecht, P., 1987. Occurrence and geological significance of long chain dialkylthiacyclopentanes. *Nature* **329**, 54–56.
- Schulz, H.D., Dahmke, A., Schinzel, U., Wallmann, K., Zabel, M., 1994. Early diagenetic processes, fluxes, and reaction rates in sediments of the South Atlantic. *Geochim. Cosmochim. Acta* **58**, 2041–2060.
- Seewald, J.S., Seyfried Jr., E., Shanks III, W.C., 1994. Variations in the chemical and stable isotope composition of carbon and sulfur species during organic-rich sediment alteration: an experimental and theoretical study of hydrothermal activity at Guaymas basin, Gulf of California. *Geochim. Cosmochim. Acta* **58**, 5065–5082.
- Seewald, J.S., 2001. Aqueous geochemistry of low molecular weight hydrocarbons at elevated temperatures and pressures: constrains from mineral buffered laboratory experiments. *Geochim. Cosmochim. Acta* **65**, 1641–1664.
- Stoler, A., Spiro, B., Amrani, A., Aizenshtat, Z. 2003. Evaluation of $\delta^{34}\text{S}$ changes during stepwise pyrolysis of bituminous rocks and Type II-S Kerogen. *Org. Geochem.* 21st IMOG meeting (Krakow, Poland) 2003 abstract book part I, pp. 103–104.

- Tannenbaum, E., Aizenshtat, Z., 1985. Formation of immature asphalt from organic-rich carbonate rocks -I. *Geochem. Correlation Org. Geochem.* **8**, 181–192.
- Tannenbaum, E., Starinsky, A., Aizenshtat, Z. 1987. Light oils transformation to heavy oils and asphalts. In: Meyer, R.F. (Ed.), *Exploration for Heavy Crude Oils and Natural Bitumen*. Studies in Geology Am. Assoc. Petroleum Geologists, Series 25, Tulsa, pp. 221–231.
- Tissot, B.P., Welte, D.H., 1978. *Petroleum Formation and Occurrence*. Springer-Verlag, Berlin.
- Vairavamurthy, M.A., Orr, W.L., Manowitz, B. 1995. Geochemical transformation of sedimentary sulfur: an introduction. In: Vairavamurthy, M.A., Schoonen, M.A.A. (Eds.), *Geochemical Transformation of Sedimentary Sulfur*, ACS Symposium Series 612, Washington, DC, pp. 1–14.
- Werne, J.P., Lyons, T.W., Hollander, D.J., Formolo, M.J., Sinninghe Damsté, J.S., 2003. Reduced sulfur in euxinic sediments of the Cariaco Basin: sulfur isotope constrains on organic sulfur formation. *Chem. Geol.* **27**, 245–253.
- Worden, R.h., Smalley, P.C. 2001. H_2S in North Sea oil fields: importance of thermochemical sulfate reduction in clastic reservoirs. In: Cidu, R. (Ed.), *The Proceedings of the 10th International Symposium on Water-Rock Interaction*, vol. 2. Balkema, Lisse, pp. 659–662.
- Worden, R.h., Cai, C. 2006. Discussion on “Geochemical characterization of the Zhaolanzhuang sour gas accumulation and thermochemical sulfate reduction in the Jixian Sag of Bohai Bay Basin” by Zhang et al. (*Org. Geochem.* 36, 1717-1730). *Org. Geochem.* **37**, 511–514.
- Zielinski, M., Kanska, M., 1993. Synthesis and the uses of isotopically labeled compounds with sulphur-containing functional groups. In: Patai, S., Rappoport, Z. (Eds.), *Supplement S: The Chemistry of the Sulphur-containing Functional Groups*. John Wiley & Sons, Chichester, pp. 495–597.
- Zaback, D.A., Pratt, L.M., 1992. Isotopic composition and speciation of sulfur in Miocene Monterey formation: reevaluation of sulfur reactions during early diagenesis in marine environments. *Geochem. Cosmochem. Acta* **56**, 763–774.
- Zhang, S.C., Zhu, G.Y., Liang, Y.B., Dai, J.X., Liang, H.B., Li, M.W., 2005. Geochemical characterization of the Zhaolanzhuang sour gas accumulation and thermochemical sulfate reduction in the Jixian Sag of Bohai Bay Basin. *Org. Geochem.* **36**, 1717–1730.
- Zhang, S.C., Zhu, G.Y., Liang, Y.B., Dai, J.X., Liang, H.B., Li, M.W. 2006. Replay to Comments by Worden and Cai (2006) on Zhang et al., (2005) [*Org. Geochem.* 36, 1717-1730]. *Org. Geochem.* **37**, 515-518.