



Nondestructive investigation on hydrocarbons occluded in asphaltene matrix: The evidence from the dispersive solid-phase extraction

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ARTICLE INFO

Keywords:

Asphaltenes

Aggregates

Occluded hydrocarbons

Dispersive solid-phase extraction

ABSTRACT

Asphaltenes are the heaviest compound group in crude oil. The complex and porous macromolecular network of asphaltenes both adsorbs and occludes small hydrocarbon molecules, which are considered source-related petroleum biomarkers as they are shielded from secondary alterations. The current method to isolate the occluded content involves mild oxidative degradation of asphaltenes with $\text{CH}_3\text{COOH}/\text{H}_2\text{O}_2$, but it is generally not suitable for quantitative studies, as side reactions that chemically alter the bound hydrocarbons are difficult to mitigate. In the current study, we compared the performance of dispersive solid-phase extraction (DSPE) and mild oxidative degradation in isolating asphaltene-occluded hydrocarbons, using Lower Cambrian solid bitumen from northwestern Sichuan and Ordovician crude oil from the Tazhong area of Xinjiang, China. We demonstrated that DSPE was effective in extracting occluded hydrocarbons from asphaltene aggregates without undesirable chemical alterations, thereby allowing subsequent quantitative analysis. Further investigations revealed that the chemical composition of the occluded *n*-alkanes provided useful information with regard to the origin of the petroleum. Importantly, the enrichment of aromatic hydrocarbons inside the asphaltene aggregates, particularly the relative abundance of different methylphenanthrene isomers, provided crucial experimental evidence in support of our earlier hypothesis that the occluded hydrocarbons were protected from secondary alterations, and therefore had remained relatively stable over a long geologic period.

1. Introduction

Asphaltenes constitute the heaviest fraction of crude oil and source rock extracts, and are generally considered to result from the fragmentation of kerogen following the cleavage of its covalent bonds (Bissada et al., 2016). Because of this, asphaltenes retain the macromolecular network of kerogen, which can trap (adsorb or occlude) small molecules by non-covalent bonds (Bandurski, 1982; Pelet et al., 1986; Zhao et al., 2012). The asphaltene-adsorbed hydrocarbons are small organic molecules weakly bound by the peripheral side chains of the macromolecular structure, and the asphaltene-occluded hydrocarbons are formed in the inner space of asphaltene structure (Snowdon et al., 2016). The trapped (adsorbed or occluded) molecular species are protected from secondary alteration events and therefore often carry important geochemical information of the earlier oil (Cheng et al., 2017; Snowdon et al., 2016).

To isolate the sequestered hydrocarbons, asphaltenes are first dissolved in a polar solvent such as dichloromethane, and then re-

precipitated by adding excessive petroleum ether (Zhao et al., 2012). The asphaltene-adsorbed hydrocarbons can then be released by centrifugal elution (Fang et al., 2021; Liao et al., 2006c). The liberation of the covalently tethered molecules, on the other hand, usually requires a harsher separation method such as pyrolysis (Cassani and Eglinton, 1986; Jones et al., 1988; Oudot and Chaillan, 2010; Pan et al., 2017; Rubinstein et al., 1979). It has been demonstrated that mild oxidative degradation of asphaltenes by $\text{CH}_3\text{COOH}/\text{H}_2\text{O}_2$ can disrupt their cross-linking network and release the asphaltene-occluded hydrocarbons (Liao and Geng, 2002; Liao et al., 2005, 2006a, 2006b, 2006c). Despite the successful application of mild oxidative degradation in a number of previous studies to separate asphaltene-occluded hydrocarbons (Tian et al., 2012a, 2012b; Zhao et al., 2010), the strategy tends to generate a highly complex profile of hydrocarbon products that varies with the choice of oxidant, making quantitative analysis difficult.

Previous studies have established that asphaltenes often exist in varying tiers of supermolecular structures, such as aggregates (Rashid

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<https://doi.org/10.1016/j.petrol.2022.110890>

Received 11 August 2021; Received in revised form 12 July 2022; Accepted 16 July 2022

Available online 20 July 2022

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et al., 2019; Sjöblom et al., 2015). A number of physical models, including the peptization model proposed by Pfeiffer and Saal (1940), the Yen model of hierarchical aggregation (Yen, 1974, 1994; Yen et al., 1961), and the more recent Yen-Mullins model (Mullins, 2010, 2011; Mullins et al., 2012), have been developed to describe the macromolecular assembly of asphaltene molecules. Molecular simulation studies have confirmed the critical nanoaggregate and clustering concentrations of asphaltenes predicted by the Yen-Mullins model (Goual et al., 2011; Guan et al., 2019; Mullins, 2011; Zeng et al., 2009). Based on these concentration values, asphaltene aggregates can be reversed by dispersive solid-phase extraction (DSPE) to release the occluded small organic molecules (Anastassiades and Lehotay, 2003; Chisvert et al., 2019; Evdokimov and Fesan, 2016; He et al., 2018; Zhang et al., 2011).

In the current study, we evaluated the performances of DSPE and $\text{CH}_3\text{COOH}/\text{H}_2\text{O}_2$ -dependent oxidative degradation in isolating the hydrocarbons occluded in asphaltene aggregates. Following each separation, we analyzed the profile of common source-related biomarkers and interpreted their geochemical significance. This study sheds light on the geochemical behaviors of asphaltene-trapped hydrocarbons and on how they are influenced by the macromolecular structure of the asphaltene aggregates.

2. Experimental methods

2.1. Reagents and sample preparation

All experiments were conducted at the Key Laboratory of Petroleum Resources and Prospecting, China University of Petroleum (Beijing). Analytical-grade dichloromethane, petroleum ether (30–60 °C boiling range) and methanol were purchased from Jindongtiansheng company (Tianjin, China). The dichloromethane was distilled immediately prior to each use. Chromatography-grade silica gel was procured from Aladdin company (Shanghai, China), and chromatography-grade alumina (aluminum oxide) from Alfa Aesar Chemicals (Shanghai, China).

Solid bitumen sample S was collected from a Lower Cambrian bituminous vein from the Kuangshanliang area in the mid-northern section of Longmen Mountain, located in the northwestern part of Sichuan Basin, China (Huang and Wang, 2008). Previous researches have attributed the formation of the vein to the tectonic transformations (e.g. uplift, fracture, fault) and subsequent secondary alterations (water-washing, evaporation, biodegradation, abiotic oxidation) of Sinian Paleo-reservoirs (Charrié-Duhaut et al., 2000; Cheng et al., 2014; Wang et al., 2014). The Lower Cambrian bitumen is considered oxidized in nature based on its low degree of thermal evolution and enrichment of soluble organic matter (>90 wt%) (Liang et al., 2020; Wu et al., 2020).

Crude oil sample T was obtained from the Ordovician strata in the central Tarim Basin of China, where multiple sets of superimposed source rocks are present (Pan and Liu, 2009; Zhao et al., 2010). The formation of oil and gas reserves in the Tarim Basin is governed by a combination of geological factors, including multiple tectonic movements in the region, as well as the generation, transport, accumulation and secondary alteration of the crude oil (Wang and Xiao, 2004). Sample T had undergone considerable secondary alterations, leading to a loss of fluidity and a high asphaltene level that reached 30 wt% (Zhao et al., 2010).

2.2. Separation of occluded hydrocarbons by DSPE

Sample S was ground to around 100 mesh with an agate mortar and the fine powder was then extracted with dichloromethane in a Soxhlet apparatus at 65 °C for 72 h. The extracts were dried below 40 °C. Petroleum ether was added to the extracts, leading to the formation of asphaltene precipitate S0, which was then separated from the supernatant and dried below 40 °C. Around 0.1 g of S0 was dissolved in 6 mL of dichloromethane, and the resultant solution was divided into three

roughly equal aliquots. Each aliquot was transferred to a 100-mL glass centrifuge tube, to which 60 mL of petroleum ether was added. The tube was allowed to stand for 0.5 h, and then centrifuged at 3500 rpm for 20 min. The supernatant was carefully transferred to a clean flask, and the asphaltene precipitates were washed with petroleum ether and centrifuged again as above (Liao et al., 2006a, 2006b; Wu et al., 2020). This process was repeated until the supernatant after centrifugation became colorless. The purified asphaltene precipitates, designated as S1, were combined and dried. The asphaltene fraction of Sample T was obtained in a similar way, and was designated as T0 or T1. The pooled supernatants from all petroleum ether washes, containing adsorbed components, were concentrated and passed through an alumina-silica gel column to separate the saturates, aromatics, and resins (Wu et al., 2020).

Next, <50 mg of the purified asphaltenes were mixed with enough dichloromethane (ensuring that the concentration of asphaltenes was around 0.1 mg/mL) in a 100-mL beaker, sonicated until all solids were completely dissolved, and transferred to a separating funnel. In another 300-mL beaker, 50 g of silica gel (100–200 mesh) were added to 80–100 mL of dichloromethane. The resultant mixture was then vigorously stirred by a magnetic rotor to furnish a well-dispersed suspension, to which the aforementioned dichloromethane solution of asphaltenes was slowly added dropwise. The stirring continued for 30 min after all the asphaltene solution had been added (Fig. 1), and the resultant mixture was allowed to stand until all silica gel particles had deposited. The particles were collected by centrifugation and gravity filtration, and washed three times with dichloromethane. The filtrates of the washes were combined into a 500-mL flask, concentrated to a final volume of 2–3 mL in a rotary evaporator, and fractionated into saturated hydrocarbons, aromatic hydrocarbons and resin by column chromatography as described earlier.

2.3. Gas chromatography-mass spectrometry (GC-MS)

The saturated and aromatic hydrocarbons separated by column chromatography were analyzed on a 6890 GC - 5975i MS system (Agilent, USA) equipped with an HP-5MS fused-silica capillary chromatographic column (length, 60 m; inner diameter, 250 µm; film thickness, 0.25 µm). After splitless injection via the injection port, which was maintained at 300 °C, the sample was separated using helium as carrier gas at a constant flow rate of 1 mL/min. For the separation of saturated hydrocarbons, the column temperature was programmed as follows: 80 °C for 1 min, 80 °C to 120 °C at 20 °C/min, 120 °C for 1 min, 120 °C to 310 °C at 3 °C/min, and finally 310 °C for 20 min. The temperature program for the separation of aromatic hydrocarbons consisted of an initial isothermal phase at 80 °C for 1 min, a subsequent linear ramp

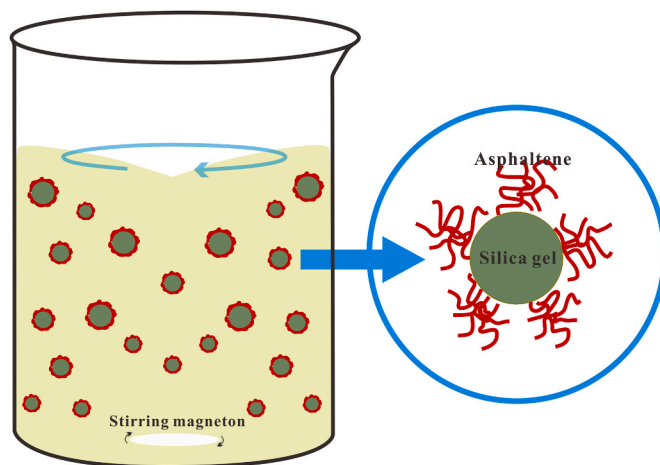


Fig. 1. Schematic diagram showing the separation of asphaltene-occluded hydrocarbons by DSPE.

from 80 °C to 310 °C at a rate of 3 °C/min, and a final hold at 310 °C for 18 min. In the MS system, the eluted products were fragmented by electron ionization (EI) at 70 eV, and then analyzed in the m/z range of 50–450. SCAN data and SIM data were collected simultaneously in a single analysis.

3. Results

It is generally accepted that hydrocarbon molecules adsorbed to asphaltenes can be isolated by centrifugation, but those that are occluded inside asphaltene aggregates require more vigorous separation methods, such as DSPE or oxidative degradation (Fang et al., 2021; Liao et al., 2005, 2006c). Our experimental analysis indicated that the S0 fraction contained around 6.3% ($\pm 2.7\%$) of the adsorbed hydrocarbons and 9.0% ($\pm 2.7\%$) of the occluded hydrocarbons by weight. In comparison, approximately 3.5% ($\pm 1.0\%$) of the hydrocarbons in T0 were asphaltene-adsorbed, and 10.3% ($\pm 1.0\%$) were aggregate-occluded (Table 1). Several biomarker parameters are provided in Table 2.

(1) Sample S

Aromatics were the most abundant type of asphaltene-adsorbed hydrocarbons in S0, and consisted mainly of bi- and tricyclics with some tetracyclic compounds. On the other hand, the proportions of saturates and resins were similar. The total ion chromatogram (TIC) of the isolated saturates showed a unimodal distribution of n -alkanes and a slight unresolved complex mixture (UCM) hump, with nC_{20} as the main peak and no odd-even predominance (Fig. 2a). The relative abundance of tricyclic terpanes was significantly lower than that of hopanes (Fig. 2b), and the levels of pregnane and homopregnan were substantially below that of regular steranes, with a clear V-shaped distribution of C_{27} – C_{29} α, α, α -R-steranes (Fig. 2c).

The hydrocarbons originally occluded inside the asphaltene aggregates contained similar levels of saturates and aromatics but a lower amount of resins. The TIC of the saturates exhibited a bimodal distribution of n -alkanes and also a slight UCM hump, with two main peaks corresponding to nC_{18} and nC_{22} , and no odd-even predominance (Fig. 2d). In addition, an n -alk-(1)-enes series of even carbon numbers was detected between C_{16} and C_{24} , with C_{18} as the main peak. Again, tricyclic terpanes were less abundant than hopanes, and the homohopane series was dominated by the C_{31} and C_{35} members (Fig. 2e). The distribution pattern for the steranes was similar to that in the adsorbed hydrocarbons, with a slightly higher level of C_{28} in the C_{27} – C_{29} α, α, α -R-steranes (Fig. 2f). The asphaltene-occluded aromatics comprised slightly fewer bicyclics and more tetracyclics compared to those isolated from the adsorbed hydrocarbons (Fig. 3a and b).

Table 1
Quantitative analysis of asphaltene-trapped hydrocarbons.

Sample		S		T	
		Weight (mg)	Yield (wt.%)	Weight (mg)	Yield (wt.%)
Asphaltenes before purification (S0 or T0)		11.1	/	29.2	/
Asphaltenes after purification (S1 or T1)		10.4	93.7	28.2	96.5
Adsorbed hydrocarbons	Saturates	0.1	0.9	0.4	1.5
	Aromatics	0.5	4.5	0.2	0.8
	Resins	0.1	0.9	0.4	1.5
Occluded hydrocarbons	Saturates	0.3	2.7	1.3	4.5
	Aromatics	0.5	4.5	1.2	4.1
	Resins	0.2	1.8	0.5	1.7

Note: The yield of each hydrocarbon fraction is calculated from the ratio of its weight to that of the asphaltenes prior to extraction. The detect limit and the precision is 0.1 mg.

(2) Sample T

The asphaltene-adsorbed hydrocarbons in T0 contained similar amounts of saturates and resins, and a comparatively lower level of aromatics (Fig. 3c). The TIC of the isolated saturates showed a large UCM hump that hindered the identification of *Pr*, *Ph* and n -alkane peaks (Fig. 4a). The saturated hydrocarbons were depleted in terpanes (Fig. 4b), and contained higher abundances of pregnane and homopregnan than regular steranes, with a mirrored L-shaped distribution of C_{27} – C_{29} α, α, α -R-steranes (Fig. 4c).

On the other hand, the occluded hydrocarbons consisted of similar levels of saturates and aromatics, both of which were more abundant than resins. Once again, the TIC of the isolated saturates registered a substantial UCM hump and slightly even-odd predominance, with nC_{22} as the main peak. In addition, an n -alk-(1)-enes series of even carbon numbers was detected in the range of C_{14} and C_{22} , with C_{18} as the main peak (Fig. 4d). Hopanes were found to be more abundant than tricyclic terpanes (Fig. 4e), whereas the levels of pregnane and homopregnan were far below that of regular steranes. The C_{27} – C_{29} α, α, α -R-steranes in the occluded hydrocarbons contained slightly more C_{28} and less C_{29} compared to the asphaltene-adsorbed counterparts (Fig. 4f). Bicyclics, tricyclics and tetracyclics were all detected in the aromatic hydrocarbons, with bicyclics being the most abundant (Fig. 3d).

4. Discussion

4.1. Geochemical significance of asphaltene-occluded n -alkanes in indicating the biological origin of petroleum

The free hydrocarbons in the Kuangshanliang bitumen samples underwent a series of secondary alterations, with the n -alkanes, terpanes and steranes impacted by biodegradation (Cheng et al., 2014; Huang and Wang, 2008; Wu et al., 2020). We have previously tested different elution methods for the isolation of asphaltene-adsorbed hydrocarbons, and discovered that the use of biodegradation led to significant alteration of biomarker parameters such as $Ts/(Ts + Tm)$ and $C_{29}S-20S/(20S + 20R)$. In our current study, the adsorbed and occluded hydrocarbons showed similar biomarker parameters but differed significantly in the distribution pattern of n -alkanes (Fig. 2a and d). Specifically, the asphaltene-occluded n -alkanes isolated by DSPE displayed a bimodal peak distribution in the TIC (Fig. 2d), with the back peak showing greater intensity than the front peak. The results suggested that these saturated hydrocarbons were not significantly affected by biodegradation, as their distribution profile was very similar to that of the n -alkanes extracted from Lower Paleozoic source rocks in the Upper Yangtze area of China. According to Liang et al., the bimodal distribution is unrelated to the degree of maturity of the source rocks; thus, the two peak groups most likely represented two types of algae (Liang et al., 2009). Wang and Han (2011) speculated that the Kuangshanliang bitumen could be derived from a low-maturity paleo-reservoir of the Sinian Dengying Formation. In our current study, the maturity parameters of the occluded hydrocarbons, such as $C_{31}H-22S/(22S + 22R)$ and $C_{29}S-20S/(20S + 20R)$, also confirmed that the bitumen sample was of low maturity. A recent study by Liang and coworkers indicated that the equivalent vitrinite reflectance of the Kuangshanliang bitumen ranged from 0.52% to 0.77%, suggesting that the deposits originated from Cambrian source rocks (Liang et al., 2020). Based on these literature references, the bimodal distribution of the asphaltene-occluded n -alkanes in S0 implied that the organic matter in the sample likely originated from two species of Cambrian algae. The fact that the two peak groups showed different intensities hinted at the different contributions of the two algae sources.

Although our geochemical analysis found no conclusive evidence for n -alkanes in the asphaltene-adsorbed saturates derived from T0, the TIC of the occluded fraction exhibited a bimodal n -alkane profile above the UCM hump (Fig. 4d), with nC_{22} as the main peak, even-odd

Table 2

Selected biomarker parameters from asphaltene-trapped hydrocarbons.

Biomarker parameters	Asphaltene S0		Asphaltene T0	
	Adsorbed hydrocarbons	Occluded hydrocarbons	Adsorbed hydrocarbons	Occluded hydrocarbons
Pr/Ph	1.10	0.76	/	1.01
C ₂₃ TT/C ₃₀ H	0.20	0.37	/	0.48
Ts/(Ts + Tm)	0.49	0.48	/	0.50
C ₃₁ H-22S/(22S + 22R)	0.61	0.60	/	0.62
(C ₂₁ P + C ₂₂ P)/(C ₂₇ S + C ₂₈ S + C ₂₉ S)	0.07	0.07	0.14	0.06
C ₂₉ S- 20S/(20S + 20R)	0.38	0.53	0.27	0.60
Relative proportions of regular steranes				
C ₂₇ $\alpha\alpha$ 20R (%)	27	21	24	25
C ₂₈ $\alpha\alpha$ 20R (%)	28	37	26	34
C ₂₉ $\alpha\alpha$ 20R (%)	45	42	50	41

Note: Pr, pristane; Ph, phytane; TT, tricyclic terpanes; C₃₀H, C₃₀ 17 α ,21 β (H)-hopane; Ts, C₂₇ 18 α (H)-trisnorhopane; Tm, C₂₇ 17 α (H)-trisnorhopane; C₃₁H, C₃₁ 17 α ,21 β (H) 22S- and 22R-homohopane; P, pregnane; S, regular sterane; C₂₉S, C₂₉ 5 α (H),14 α (H),17 α (H) 20S- and 20R-regular sterane.

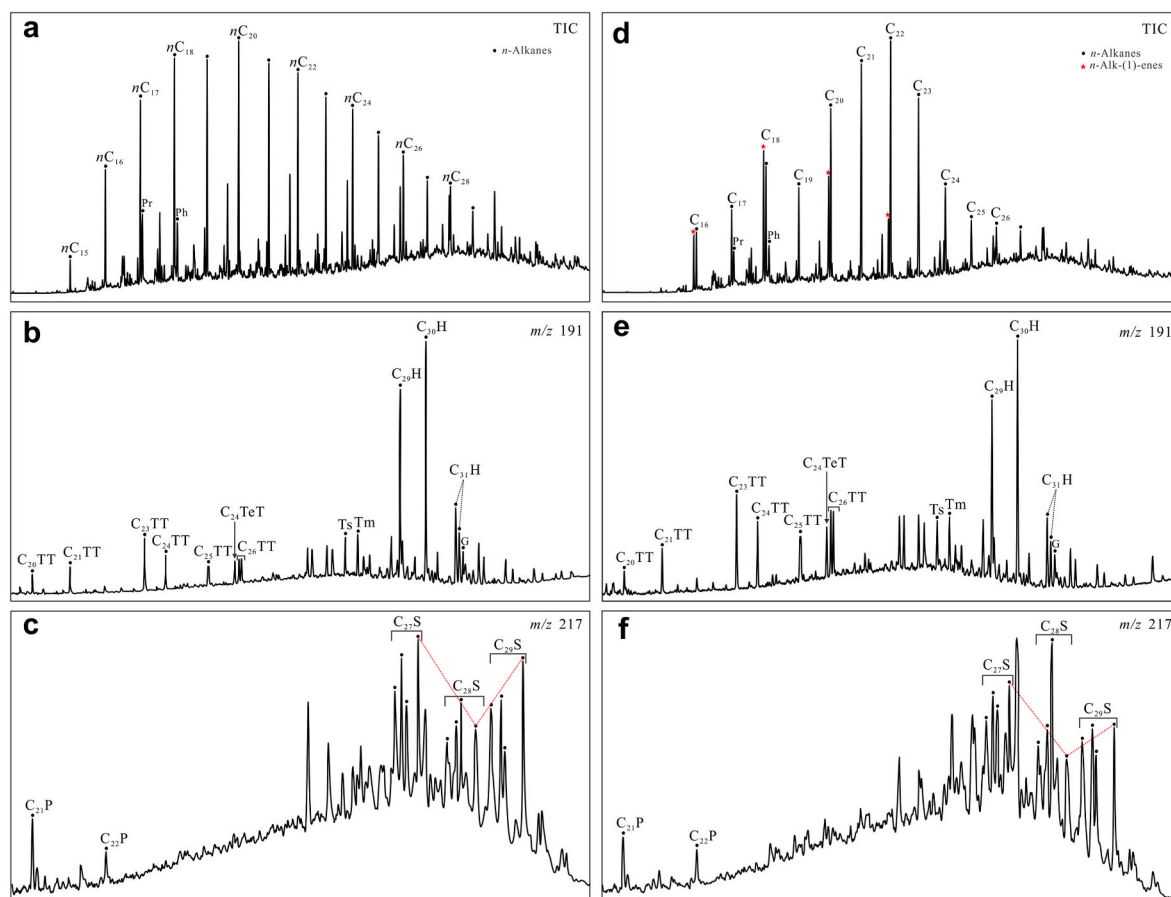


Fig. 2. GC-MS analysis of the asphaltene-trapped hydrocarbons isolated from S0. a) TIC, b) m/z 191, and c) m/z 217 chromatograms of the asphaltene-adsorbed hydrocarbons. d) TIC, e) m/z 191, and f) m/z 217 chromatograms of the asphaltene-occluded hydrocarbons. Pr, pristane; Ph, phytane; TT, tricyclic terpanes; TeT, tetracyclic terpanes; Ts, C₂₇ 18 α (H)-trisnorhopane; Tm, C₂₇ 17 α (H)-trisnorhopane; C₂₉H, C₂₉ 17 α ,21 β (H)-30-norhopane; C₃₀H, C₃₀ 17 α ,21 β (H)-hopane; C₃₁H, C₃₁ 17 α ,21 β (H) 22S- and 22R-homohopane; G, gammacerane; P, pregnane; S, regular sterane.

predominance below nC_{22} , and Pr/Ph around 1.0. Based on several previous studies, an n -alkane series characterized by a dominant nC_{18} or nC_{20} peak implies an evaporitic, hypersaline marine carbonate environment, which could serve as evidence that the organic matter was derived from chemosynthetic bacteria inhabiting the benthic zone or pelagic zooplankton (Mello et al., 1988). Previous studies have suggested that Cambrian-Lower Ordovician source rocks are mainly carbonate in nature, and are derived from sedimentary organic matter that deposited in an anaerobic, high-salinity environment over a long geological period (Pan and Liu, 2009). Therefore, our geochemical analyses of the occluded n -alkanes in T0 confirmed that the crude oil

sample was derived from Cambrian-Lower Ordovician source rocks. Furthermore, the free saturates in the Ordovician Tazhong crude oil showed high relative abundances of odd-carbon-numbered n -alkanes below C_{20} , particularly nC_{15} and nC_{19} , which is a geochemical hallmark of Middle-Upper Ordovician source rocks and indicative of an algal origin (Wang and Xiao, 2004; Zhao et al., 2010). In support of our findings, Luo et al. (2007) have argued that Lower-Paleozoic crude oils derived from the Upper Ordovician source rocks in Tazhong low-uplift area are enriched in n -alkanes of odd carbon numbers, whereas those from the Cambrian-Lower Ordovician source rocks are characterized by even-carbon-numbered n -alkanes. Thus, the geochemical characteristics

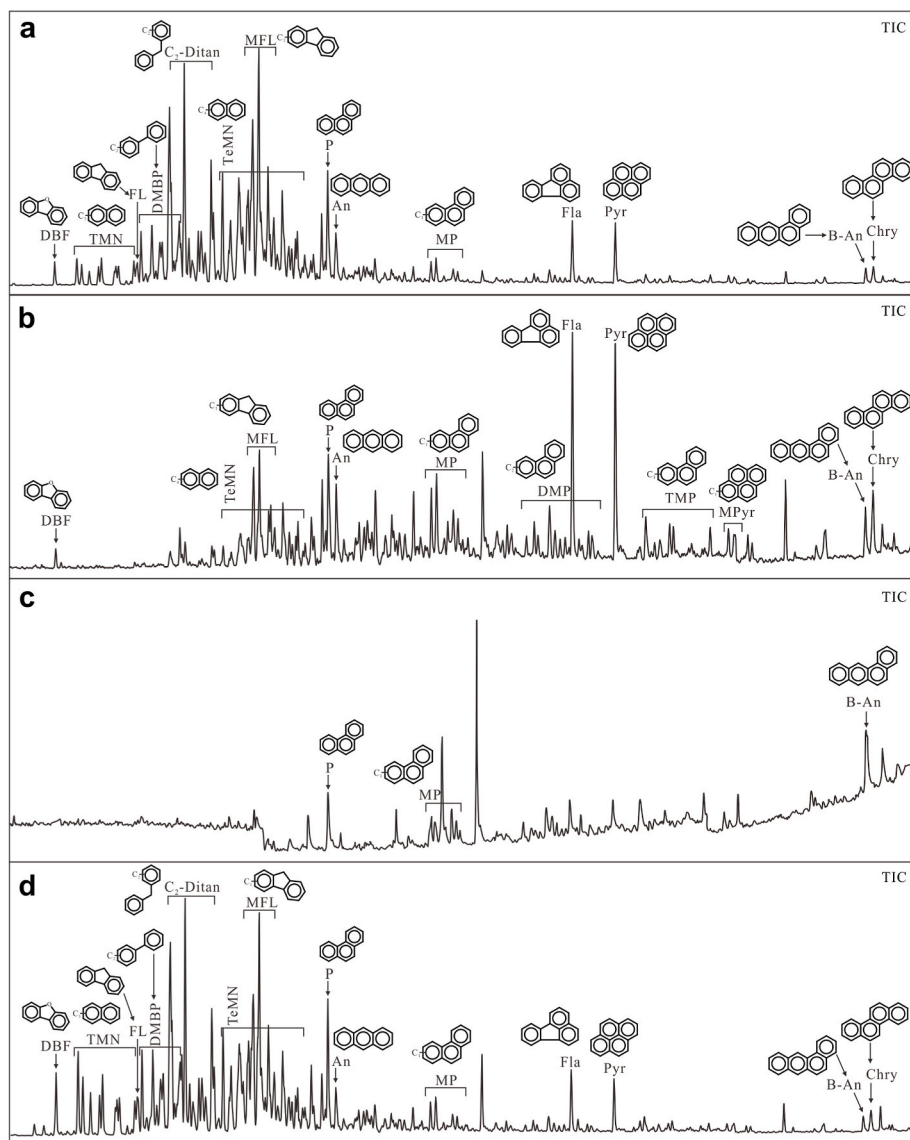


Fig. 3. Representative TICs of the a) adsorbed and b) occluded aromatics separated from the asphaltenes in S0, as well as of the c) adsorbed and d) occluded aromatics separated from the asphaltenes in T0. BF, dibenzofuran; TMN, trimethylnaphthalenes; FL, fluorene; TeMN, tetramethylnaphthalenes; DMBP, dimethylbiphenyl; P, phenanthrene; An, anthracene; MP, methylphenanthrenes; DMP, dimethylphenanthrenes; Fla, fluoranthene; Pyr, pyrene; TMP, trimethylphenanthrenes; MPyr, methylpyrene; B-An, benzo[a]anthracene; Chy, chrysene.

(such as odd-even/even-odd advantage) of asphaltene-occluded *n*-alkanes could be of great significance for studying complex petroleum reservoirs with mixed sources and multi-period charging in superimposed basins.

4.2. Ability of asphaltene aggregates to confine aromatic hydrocarbons

Based on our experimental data, the asphaltene-trapped aromatic hydrocarbons obtained by centrifugation and by DSPE showed noticeable differences in chemical composition (Fig. 3). It has been previously suggested that aromatics that can be isolated from asphaltenes by centrifugation are generally either free, residual molecules or weakly adsorbed to the outer surface of aggregates (Zhao et al., 2012). On the other hand, the aromatic hydrocarbons released by DSPE are believed to have been occluded inside the asphaltene aggregates. Mirroring these findings, we found that the asphaltene-adsorbed fraction of hydrocarbons contained very few aromatic molecules, whereas a rich array of polycyclic aromatics could be detected in the asphaltene-occluded hydrocarbon mixture (Fig. 3c and d). These results also support our previous hypothesis that the ability of small aromatic compounds to interact with the macromolecular network of asphaltenes via π - π stacking causes their better retention inside the aggregates compared to

saturate hydrocarbons (Mullins, 2011; Wu et al., 2020).

Despite significant differences in the physical properties, origins and maturation histories of S0 and T0, the relative abundances of methylphenanthrene isomers in the occluded fraction that we separated from both samples showed the same order of 2-MP > 3-MP > 9-MP > 1-MP (Fig. 5). Dick et al. (2013) calculated the thermodynamic parameters of different methylphenanthrene isomers via quantum chemistry when studying their stability in a hydrothermal system. Their results confirmed that β -isomers, including 2-MP and 3-MP, were significantly more thermostable than their α counterparts, 1-MP and 9-MP. Furthermore, Dick et al. (2013) proposed that the thermostability of the four isomers in sedimentary basins could be ranked in the decreasing order of 2-MP > 3-MP > 9-MP > 1-MP, a perfect match with our experimental data. The positive correlation between thermostability and relative abundance implied that the occluded methylphenanthrenes were well protected by the asphaltene aggregates from secondary alterations, and that they have reached a metastable equilibrium over a long period of geological history. In comparison, the methylphenanthrenes adsorbed to the surface of the asphaltene network are more susceptible to secondary alterations. This mirrors our observation of significantly more abundant 9-MP in the adsorbed hydrocarbons of T due to years of atmospheric oxidation during its storage (Fig. 5b) (Ahmed et al., 1999; Zhao et al.,

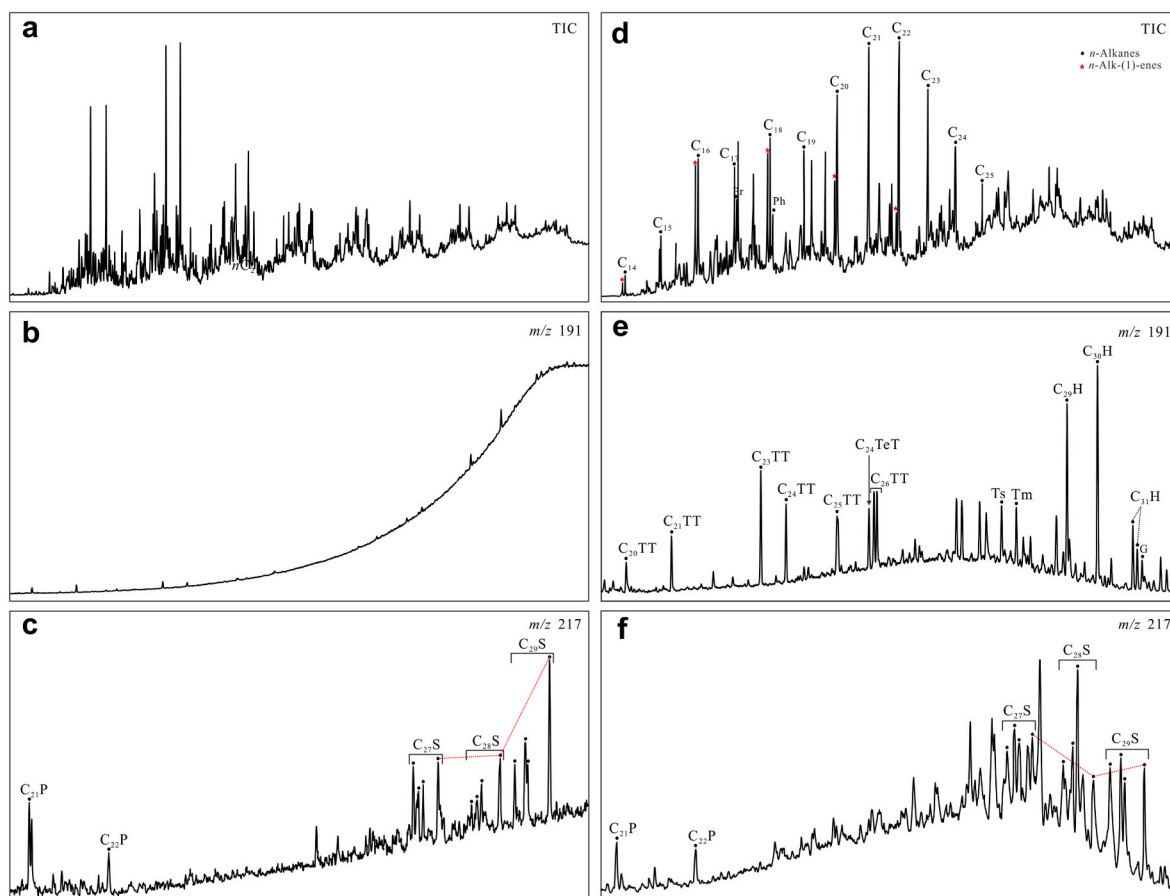


Fig. 4. GC-MS analysis of the asphaltene-trapped hydrocarbons from T0. a) TIC, b) m/z 191, and c) m/z 217 chromatograms of the asphaltene-adsorbed hydrocarbons. d) TIC, e) m/z 191, and f) m/z 217 chromatograms of the asphaltene-occluded hydrocarbons. Pr, pristane; Ph, phytane; TT, tricyclic terpanes; TeT, tetracyclic terpanes; Ts, C_{27} 18 α (H)-trisnorhopane; Tm, C_{27} 17 α (H)-trisnorhopane; $C_{29}H$, C_{29} 17 α ,21 β (H)-30-norhopane; $C_{30}H$, C_{30} 17 α ,21 β (H)-hopane; $C_{31}H$, C_{31} 17 α ,21 β (H) 22S- and 22R-homohopane; G, gammacerane; P, pregnane; S, regular sterane.

2010).

4.3. Comparison between mild oxidative degradation via CH_3COOH/H_2O_2 and DSPE

Cheng et al. (2014) reported the separation of occluded hydrocarbons from the Kuangshanliang bitumen by mild oxidative degradation with CH_3COOH/H_2O_2 . The isolated n -alkanes ranged from C_{15} to C_{28} , and exhibited a bimodal distribution pattern in the TIC with two main peaks that corresponded to nC_{16} and nC_{22} . Geochemical analysis of the saturated hydrocarbons showed Pr/Ph to be less than 1, tricyclic terpanes/hopanes in the range of 0.3–0.4, $C_{29}S-20S/(20S + 20R)$ no greater than 0.55, and $(C_{21}P + C_{22}P)/(C_{27}S + C_{28}S + C_{29}S)$ below 0.12. These data were largely consistent with what we obtained in our current study of S1-derived saturates from the same region (see Fig. 2d and Table 2). Notably, both oxidative degradation and DSPE led to the isolation of an n -alk-(1)-ene series with even carbon numbers between 16 and 22, and with C_{18} as the main peak (Cheng et al., 2014).

In another study, Zhao et al. (2010) investigated the geochemical characteristics of occluded hydrocarbons isolated by oxidative degradation from the Ordovician crude oil in the Tazhong area of Xinjiang, China. The n -alkanes that they isolated mainly consisted of a C_{16} – C_{28} series that showed clear even-odd predominance between C_{16} and C_{22} , with tricyclic terpanes/hopanes of 0.21–0.44, $C_{29}S-20S/(20S + 20R)$ of 0.50–0.54, and the relative abundance of C_{28} α,α,α -R-steranes in C_{27} – C_{29} α,α,α -R-steranes at 25.2%–28.9%. In our current study, we were able to obtain sufficient asphaltene-occluded hydrocarbons from T0 to determine their n -alkane distribution (Fig. 4d) and the related biomarker

parameters (Table 2), which were comparable to those reported by Zhao et al. (2010). Both of our separation methods allowed us to isolate even-carbon-numbered n -alk-(1)-enes, but those obtained via oxidative degradation exhibited a broader range of carbon numbers than those extracted by DSPE, and were found to be more abundant than the corresponding n -alkanes. These findings might be due to the extended air exposure of T0 prior to the extraction and analysis.

The results of our current study revealed that asphaltene-occluded hydrocarbons isolated by DSPE and by mild oxidative degradation are comparable to each other in chemical composition. In particular, both methods afforded even-carbon-numbered n -alk-(1)-enes, which are considered source-related petroleum biomarkers (Cheng et al., 2017). For example, even-chain fatty acids, commonly present in organic matter, can readily enter the porous macromolecular network of kerogen during diagenesis, and then undergo thermal decomposition via a pericyclic reaction mechanism to generate the corresponding n -alk-(1)-enes (Alexander et al., 1992, 1997). Therefore, the n -alk-(1)-enes occluded by geological macromolecules are generally considered to originate from kerogen. When asphaltene break off from kerogen macromolecules, they may also carry occluded small hydrocarbon molecules. The fact that the DSPE method yielded a series of n -alk-(1)-enes from two asphaltene samples served as evidence of its effectiveness in separating asphaltene-occluded hydrocarbons.

Of course, there are also key differences between the two separation methods. A number of earlier studies have indicated that terpenes and saturated fatty acid ethyl esters are generated from the oxidative degradation of asphaltene, which, unfortunately, were not detected in our current study when DSPE was employed (Cheng et al., 2017; Yang

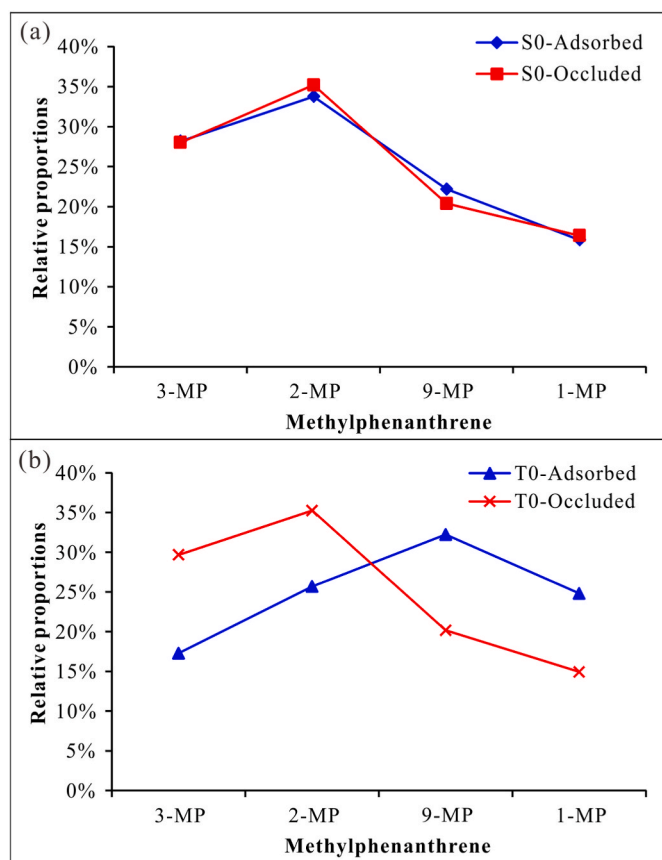


Fig. 5. Relative proportions of different methylphenanthrenes in asphaltene-trapped aromatic hydrocarbons extracted from a) S0, bitumen in Northwest Sichuan and b) T0, crude oil in Tazhong area crude oil. MP, methylphenanthrene.

et al., 2009). While sample differences certainly could be a contributing factor, the ability of $\text{CH}_3\text{COOH}/\text{H}_2\text{O}_2$ oxidation to disrupt the macromolecular network of asphaltenes and solid bitumen might have resulted in the release of more occluded hydrocarbons (Bandurski, 1982; Liao and Geng, 2002). However, it should be stressed that the use of excessive H_2O_2 can easily over-oxidize the alkene species. Therefore, oxidative degradation is generally unsuitable for quantitative analysis of asphaltene-occluded hydrocarbons. Furthermore, the chemical oxidant might not be able to penetrate into the asphaltene aggregates. On the other hand, DSPE is a physical technique that achieves separation by breaking down asphaltene aggregations without chemically consuming or altering the occluded hydrocarbons, thereby allowing more reliable measurement of their relative abundances and molecular marker parameters (Mullins, 2010, 2011; Mullins et al., 2012). Nevertheless, DSPE is ill-suited for isolating hydrocarbon molecules that are covalently bonded to macromolecular structures (Pan et al., 2017).

Taken together, DSPE is effective for the quantitative analysis of asphaltene-occluded hydrocarbons, whereas the main advantage of oxidative degradation method lies in its ability to isolate biomarkers chemically tethered to the asphaltene network. As a result, the two methods can complement each other to help researchers better understand the mechanisms that govern the interaction between the asphaltene network and the occluded small organic molecules, and interpret the geochemical information that the latter carry.

5. Conclusion

The current study evaluated the performances of mild oxidative degradation by $\text{CH}_3\text{COOH}/\text{H}_2\text{O}_2$ and DSPE in isolating asphaltene-

trapped hydrocarbons. The occluded hydrocarbons obtained with DSPE showed comparable geochemical characteristics to those isolated using the oxidative method in previous studies, with the exception that no saturated fatty acid ethyl esters or terpenes were detected, likely due to their sequestration inside the macromolecular cage of asphaltenes. Based on our experimental data, the asphaltene-occluded *n*-alkanes in the Kuangshanliang bitumen showed a bimodal distribution in the TIC, implying that the organic matter was derived from two species of algae. The distribution pattern of the asphaltene-occluded *n*-alkanes from the Ordovician crude oil in the Tazhong area of Xinjiang supported the hypothesis that the early-stage petroleum originated from Cambrian-Lower Ordovician source rocks. The use of DSPE also allowed us to obtain aromatic hydrocarbons trapped in asphaltene aggregates, and detailed chemical analysis indicated that the relative abundances of common methylphenanthrenes in the isolated mixture were consistent with their predicted thermal stability in sedimentary basins. These results provided convincing evidence that chemical conversion of the occluded aromatics reached metastable equilibrium over a long geological time period without being significantly affected by the external environment.

Credit author statement

Peng Fang: Investigation, Methodology, Writing - Original Draft; **Jia Wu:** Conceptualization, Methodology, Investigation, Writing - Original Draft. **Botian Li:** Conceptualization, Methodology, Investigation, Validation. **Bin Cheng:** Writing - Review & Editing, Resources. **Daofu Song:** Investigation, Resources. **Ningning Zhong:** Writing - Review & Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We would like to thank Shengbao Shi, Lei Zhu and Shuo Gao for their help with the organic geochemical experiments. We are also grateful to Dr. Guangli Wang for his advice on asphaltene pretreatment procedure. Stephen R. Larter and Haiping Huang are thanked for their help in promoting the process of this study. This paper was granted by State Key Laboratory of Petroleum Resources and Prospecting, China University of Petroleum (Beijing) (Grant No. PRP/indep-3-1715) and State Key Laboratory of Organic Geochemistry, GIGCAS (Grant No. SKLOG202123).

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