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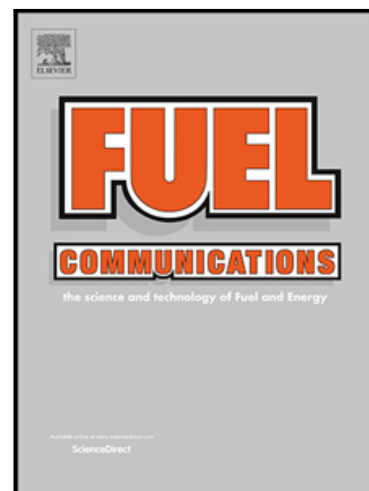
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# Preparation of a Novel Chromatographic Material and Its Application for Spectroscopic Characterization of Aromatics in Crude Oil

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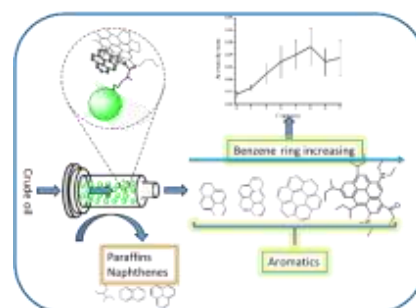
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## ABSTRACT

Petroleum analysis presents many unique challenges as a result of the chemical complexity of petroleum composition. In the present work a novel chromatographic material has been developed and utilized to separate crude oils. A total number of 13 fractions have been collected using different solvent mixtures with increasing proportions of chloroform. UV and IR spectroscopy has been employed to show that the size of the aromatic ring system increases and the extent of molecular branching decreases with elution gradient. This observation suggests that intermolecular non-covalent  $\pi$ - $\pi$  interactions between the material and the sample molecules are critical to the separation. This novel method for sequencing petroleum molecules based on the size of the aromatic ring systems has been applied to the 2D off-line analysis of crude oil samples. The 2D on-line separation (NPLC×NPLC) has been simulated by 2D off-line separation. A total number of 38 fractions have been obtained with two SPE columns based on the molecular branching and the aromatic ring system size. The chemical information obtained in this study of North Sea crude oil is complementary to standard SARA analysis. This material supports a potential solution to overcome the challenges of tandem NPLC and has demonstrated a new approach to the separation of crude oils into discrete compound classes.



## 1. INTRODUCTION

As one of the most complex materials on Earth, fossil fuel still remains the major source of energy due to its convenience, low cost, energy density and high flexibility. There are formidable analytical challenges associated with the characterization of petroleum due to its complexity. The complexity limits access to detailed structural information, not to mention the identification of each individual component.<sup>1</sup> However, a detailed characterization of the structures of compounds in such materials is mandatory, because it is necessary for optimization of its production, transportation and refining. Therefore, petroleum analysis has long been at the forefront for the development of novel analytical techniques.<sup>2</sup>

It is practically impossible to individually characterize each component of crude oil. A group-type analysis is a classic and general approach to the description of the properties of crude oil, such as PIONA analysis (Paraffins, Isoparaffins, Olefins, Naphthenes, and Aromatics) and SARA analysis (Saturates, Aromatics, Resin and Asphaltenes).<sup>3</sup> These two rough methods are, however, far from a description of the components of crude oil. As for the volatile components, such as paraffins, isoparaffins, olefins, naphthenes, saturates, aromatics, these compounds could be further separated by gas chromatography (GC). GC is ideally suited to the analysis of petroleum samples due to its high efficiency and its many theoretical plates.<sup>2,4</sup> Comprehensive two-dimensional gas chromatography (GC×GC) is another advanced analytical technique, which is one of the most powerful techniques for separating complex samples and has been developed for the analysis of petroleum. Recently, there have been several reviews published of the widespread application for the analysis of petroleum.<sup>2,4-5</sup> Two-dimensional LC×GC or the three-dimensional LC×GC×GC techniques

have also been applied to improve the chemical identification of components from crude oils.<sup>2, 4, 6</sup> Generally, LC separation is treated as an on-line fractionation step to separate some fractions from oil samples and the rest can then be transferred successively to GC. These fractions transferred to GC have to be volatile fractions and the non-volatile (polar) fractions are challenging for the petroleum analysis by GC.

The non-volatile fractions are still highly complex and mainly include compounds containing heteroatoms (O, N, S) or multiple benzene rings, such as resins and asphaltenes. These fractions are important for the production, transportation and refining of petroleum and can lead to precipitation and fouling. The non-volatile fractions are mainly characterized via LC-MS or fuel injection MS. There are few reports as of petroleum separation by on-line LC  $\times$  LC. Oro *et al.* evaluated several different normal phase liquid chromatography (NPLC) columns, and showed the potential for NPLC  $\times$  NPLC analysis of crude oil samples.<sup>7</sup> Bissada *et al.* reported a novel automated multi-dimensional high performance liquid chromatography (AMD-HPLC) approach to enhance group-type characterization of crude oil or bitumen.<sup>8</sup> The separation system is based on two columns, a cyano column (alkyl nitrile) and a silica column (silica), which is capable of running either SARA or PIN separations automatically. Schabron *et al.* developed a new on-column precipitation and redissolution separation technique with a ground polytetrafluoroethylene (PTFE)-packed column, which involves precipitation of asphaltene components from residue on the column using a heptane mobile phase.<sup>9</sup> Youtcheff *et al.* further developed the on-column precipitation and redissolution separation technique and his separation system is equipped with a set of a ground PTFE columns, a glass beads packed column, an aminopropyl column and an activated silica column.<sup>10</sup> This system can separate the crude oil to SARA fractions via automated high-performance liquid chromatography (HPLC). These two methods could separate oil to four fractions, which doesn't show the advantages of 2D separation. As these methods are mainly based on the switching control, they have lost the character of 2D separation, like GC $\times$ GC. Snyder *et al.* developed an off-line integrated scheme for the separation of the oxygen and nitrogen containing compounds in high boiling petroleum fractions, generating large numbers of fractions (36 fractions).<sup>11</sup> Robson *et al.* developed a three off-line trended SPE columns system, which approximated separation of the polar and non-polar components of petroleum. This method is able to fractionate crude oils into 11 discrete compound classes.<sup>12</sup>

There are few reports of petroleum separation by on-line LC  $\times$  LC, because there are few materials that meet the following criteria: 1) orthogonal selectivity and demonstration of a distinct retention profile 2) the components separated by any single separation must not be recombined in subsequent separation dimensions.<sup>4</sup> A lot of work has been performed successfully by off-line methods, however, these materials can't be used for on-line LC $\times$  LC, due to the solvent.<sup>12</sup> Recently, several novel materials have been prepared and shown the potential for application to analysis of petroleum, such as nitrophenyl columns,<sup>13</sup> PTFE columns,<sup>9</sup> Pd(II)-containing material,<sup>14</sup> graphitized carbons,<sup>15</sup> Pirkle type phases,<sup>16</sup> hypercrosslinked polystyrene columns,<sup>7</sup> molecularly imprinted polymers,<sup>17</sup> electron-donor-acceptor silica materials<sup>18</sup>. Oro *et al.* developed hypercrosslinked polystyrene columns to separate the nitrogen group-types (pyrrole and pyridine) and polycyclic aromatic hydrocarbons present in crude oils.<sup>7</sup> This work was performed with NPLC and the separation under gradient conditions takes less than 30 min, which showed the potential for NPLC  $\times$  NPLC analysis of crude oil samples. Tanaka *et al.* developed electron-donor- and electron-acceptor-bonded silica packing materials for analysis of hydrophobic environmental contaminants at reversed-phase conditions.<sup>18</sup> Non-covalent  $\pi$ - $\pi$  interactions could be a new approach for the separation of crude oils via specially designed stationary phases.

Given the compositional character of petroleum, the normal phase condition is more suitable for analysis of crude oil. The asphaltenes are characterized by being generally insoluble in n-alkanes (often n-heptane) and are typically precipitated from the oil samples prior to further characterization<sup>19</sup>. The island type molecular structure of asphaltenes and other structural features has been characterized by Atomic Force Microscopy (AFM) and spectroscopic methods.<sup>20</sup> Hence, compared to phenyl columns, the multiple benzene material columns are more suited to analysis of aromatics and asphaltenes.<sup>21</sup> In this work therefore, we report on the preparation of a material with a strong tendency to engage in non-covalent  $\pi$ - $\pi$  interactions, the quantification of separation at normal phase conditions and the application of the material for on-line NPLC $\times$ NPLC analysis of crude oil samples. The work focuses on lighter species than asphaltenes as these are too heavy to be successfully separated by these methods. The developed separation allows the collection of 38 separate fractions from crude oils samples, based on the molecular branching and the size of the aromatic ring systems.

## 2. EXPERIMENTAL SECTION

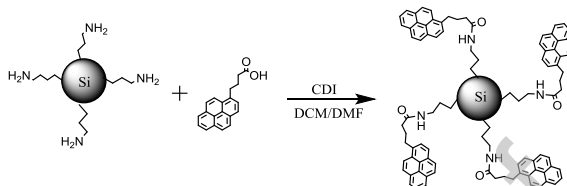
### 2.1 Materials and instrumentation

Naphthalene, coronene, pyrene, 1-pyrenebutyric acid, 3-aminopropyl-functionalized silica gel (Si-NH<sub>3</sub>), 1'-carbonyldiimidazole (CDI), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), all of these chemicals were from Sigma-Aldrich and used directly. Dichloromethane (DCM, A.R.), dimethylformamide (DMF, A.R.), hexane (A.R.), methanol (A.R.), toluene (A.R.), and acetonitrile (ACN, A.R.), all these solvents were from Sigma-Aldrich and used without further purification. Glass syringes and magnetic stir bars for synthesis with PTFE filter were from Sigma-Aldrich. Empty solid phase extraction (SPE, 3 mL) cartridges with filters were from phenomenex. 200 mg material was packed in the SPE columns and used for separation of crude oils. The sample concentrator (SBHCONC/1) was from Stuart. Uv-visible spectrophotometer (Genesys 180) was from ThermoFisher. Attenuated-total-reflection Fourier transform infrared spectroscopy (ATR-FTIR, Vertex 80v FTIR spectrometer) was from Bruker. The crude oil samples used

here were supplied from four different fields in the Danish North Sea. These oils are relatively light oils with densities around 850 mg/L, Total acid numbers around 0.2-0.6, aromatics fraction in the range 15-28% and very little asphaltene content.<sup>22</sup>

## 2.2 Preparation of material with enhanced $\pi$ - $\pi$ interactions

1-pyrenebutyric acid (Sigma Aldrich, C.P.) contains four benzene rings favoring non-covalent  $\pi$ - $\pi$  interactions. The pyrenebutyric acid was immobilized on 3-aminopropyl modified silica gel (Sigma Aldrich, C.P.). The chemical reaction was following the procedures as described in the work of DBU catalysis of N,N-carboxyldiimidazole-mediated amidations,<sup>23</sup> and this experimental procedure is shown in Scheme 1. 1-pyrenebutyric acid (154 mg, 0.535 mmol) was added to a solution of 1'-Carboxyldiimidazole (CDI) (259 mg, 1.60 mmol) in a dry Dichloromethane (DCM)/Dimethylformamide (DMF) mixture (V/V=5/5, 20 mL) at room temperature. The mixture was pre-active for 30 min in a glass bottle with a closed lid at room temperature (RT) and subsequently added to 3-aminopropyl modified silica gel (dry, 500 mg) in a three-neck glass flask. Subsequently, the heater and stirrer were turned on. After 0.5 h, DBU (111  $\mu$ L, 1.60 mmol) was added to the mixture. The reaction would continue reflux for 6 h with a magnet stirring. After the reaction, the material was washed with DCM/DMF ( $\times 3$ ) and Acetonitrile (ACN) ( $\times 3$ ). The material was kept in a vacuum oven at 60  $^{\circ}$ C overnight, and then stored in a vial for next use.



Scheme 1. Scheme for the preparation of the novel material.

200 mg of the material was packed into an empty SPE column (3 mL). The packed SPE column was cleaned with ACN ( $\times 3$ ) and Hexane ( $\times 6$ ) before use.

Table 1. The enhanced separation conditions

Solvent		Si SPE (Blank)	$\pi$ - $\pi$ SPE (Sample)
Dissolved		1 mL Hexane	1 mL Hexane
Elution	Hexane	2 mL	2 mL
	Hexane/Chloroform(9/1,v/v)	2 mL	2 mL
	Hexane/Chloroform(8/2,v/v)	2 mL	2 mL
	Hexane/Chloroform(6/4,v/v)	2 mL	2 mL
	Hexane/Chloroform(4/6,v/v)	2 mL	2 mL
	Hexane/Chloroform(2/8,v/v)	2 mL	2 mL
	Chloroform	2 mL	2 mL
	Toluene/Chloroform (5/5,v/v)	2 mL	2 mL
	Toluene/Chloroform(8/2,v/v)	2 mL	2 mL
	Toluene	2 mL	2 mL
	Toluene/Methanol(8/2,v/v)	2 mL	2 mL
	Toluene/Chloroform/Methanol (5:4:1)	2 mL	2 mL

## 2.3 Enhanced separation of crude oils

The enhanced  $\pi$ - $\pi$  interaction material was developed to reduce the complexity of crude oil samples and its application to separation of crude oil was comparable to the following procedures with a silica gel column as a control. The crude oil from the Danish North Sea (15  $\mu$ L) was diluted with hexane (1 mL) and loaded to a SPE column with a flow speed of 1 mL/min. It was washed with 2 mL hexane and the eluted fraction was collected as fraction 0. After this, a series of fractions were eluted with a 2.0 mL series of elution (Table 1) and the flow speed was set at 1 mL/min. After completion of the elution, the eluates were dried with a sample concentrator (Stuart@ SBHCONC/1) under a nitrogen stream at RT. The drying procedure was checked every 1 min and the vials were taken out if almost the solvent had evaporated. The obtained residue was redissolved in 1.0 mL of hexane or hexane/isopropanol (Table S11) for UV and IR analysis. All of the experiments were repeated once for reproducibility.

## 2.4 Spectroscopic characterization of crude oil fractions

A total number of 13 fractions were obtained after the enhanced separation of the crude oil. Half of each fraction was characterized with UV-vis spectroscopy (Thermo Scientific Genesys 180 spectrophotometer, delimit 1 nm, medium speed) and the other half was measured with attenuated-total-reflection Fourier transform infrared spectroscopy (ATR-FTIR) (Bruker Vertex 80v FTIR spectrometer).

From each fraction 20  $\mu\text{L}$  was pipetted off and diluted 100 times with corresponding solvents (Table S11). Then, the UV absorbance of the diluted fractions was measured from 200 nm to 800 nm with each scan repeated twice. These were diluted 10 times fractions and the diluted fractions were measured by UV absorbance from 200 nm to 800 nm and the scan repeated twice. After the measurement, the data was normalized by dividing by the maximum absorbance, due to the variation in the amount of absorbing molecules in the fractions.

The other halves of the fractions were dried with a sample concentrator (Stuart® SBHCONC/1) under a nitrogen stream at RT. The drying procedure was checked every 1 min and the vials were taken out and closed with lids if the solvent was almost evaporated. Each of the fractions was dissolved with a droplet of Toluene and transferred to the diamond crystal of the ATR accessory immediately. The solvent would evaporate immediately after the spectrometer vacuum ( $<2$  hPa) has been established and a series of ATR spectra ( $400\text{--}4500\text{ cm}^{-1}$ ) of the individual fractions was collected. Three blocks of 150 co-added scans with a spectral resolution of  $1\text{ cm}^{-1}$  were collected for each of the fractions. Background scans were collected of the cleaned ATR diamond crystal before and after the sample measurements. Subsequently, extended ATR corrections were applied to account for the wavelength-dependent penetration depth of the probe beam and smoothing procedures were carried out by Bruker's OPUS 7.2 software package prior to analysis. The data normalization and the peak deconvolution were performed by Origin 2018b.

## 2.5 Enhanced separation of crude oils with 2D off-line SPE separation

The prepared  $\pi\text{-}\pi$  interaction material could potentially be utilized for on-line NPLC-NPLC. In order to confirm this assumption, 2D off-line SPE separation was performed via Si SPE columns and enhanced  $\pi\text{-}\pi$  interaction SPE columns. This technique is suitable to transfer to on-line 2D separation methodology, because the elution conditions of 2 columns were performed at the same gradient of solvents. The off-line 2D separation procedures were conducted as follows. 30  $\mu\text{L}$  crude oil was diluted with 1 mL hexane and the diluted crude oil was loaded onto the Si-SPE column (NPLC 1) with a flow speed of 1 mL/min. It was subsequently washed with 2 mL hexane and the eluted fraction was collected as fraction 0. Then, fraction 1, fraction 2 and fraction 3 were eluted with 2 mL hexane, hexane/chloroform ( $V/V=6/4$ ), toluene/methanol ( $V/V=8/2$ ), respectively. Fraction 0 and fraction 1 were used for the next separation directly, which involved loading the sample onto the  $\pi\text{-}\pi$ -SPE column (NPLC 2). Fraction 2 was diluted with 6 mL hexane, and then loaded on the  $\pi\text{-}\pi$ -SPE column (NPLC 2). About fraction 3, the solvent was removed with a nitrogen stream at RT. When the volume was reduced to about 200  $\mu\text{L}$ , the nitrogen flow was stopped and the volume measured. Then, hexane was added up to a volume of 4 mL. After loading the fractions on the second dimension column, the second dimension separation was performed by a gradient concentration of chloroform (Table SI 2).

All the fractions from NPLC 2 were collected respectively. The concentrations of oil for these fractions were measured via UV spectroscopy. The experiment and measurement was both repeated once.

## 3. RESULTS AND DISCUSSION

### 3.1 Preparation of the chromatographic material

1-pyrenebutyric acid was chosen as the functionalized species to modify the 3-aminopropyl modified silica gel, due to its four benzene rings and butyric acid groups. The benzene ring is the basic unit of all  $\pi\text{-}\pi$ -conjugated systems, and the magnitude of the non-covalent  $\pi\text{-}\pi$  interactions is strongly size-dependent in aromatic systems<sup>24</sup> In addition, the intermolecular interaction energies could increase because of the matching between the pyrene groups and the island type molecular structure of aromatics or asphaltenes. The branching of petroleum molecules could influence the intermolecular  $\pi\text{-}\pi$  stacking between them and the SPE solid phase (see more information in section 3.4). Therefore, it would be helpful to increase the distance of pyrene groups and the stationary phase resulting in less steric hindrance. There are 7 carbons and one nitrogen atoms between the pyrene groups and the stationary phase (see Scheme 1), which also increase the flexibility of the pyrene groups, resulting in its suitability to interact with different kinds of aromatics or asphaltenes robustly. Potentially these are the two main reasons why the phenyl stationary phase is less advantageous for petroleum analysis.

The coupling between 1-pyrenebutyric acid and 3-aminopropyl modified silica gel was promoted by CDI catalyzer in DCM/DMF solvent. CDI has been an efficient reagent in the coupling of amino acids for peptide synthesis science since it was developed in 1960.<sup>25</sup> During the coupling,  $\text{CO}_2$  formation could potentially be a driving force of the reaction.<sup>25-26</sup> Bubbling of  $\text{N}_2$  would be helpful to remove  $\text{O}_2$  and  $\text{CO}_2$ <sup>26b</sup> but this procedure was not employed as the synthesis was run under reflux conditions. The  $\text{CO}_2$  can also be removed during the reflux and form a driving force of the reaction and the coupling was performed successfully via this approach. After coupling, the color of the silica gel was changed to dark yellow which is the color indicating the pyrene groups (Figure 1).

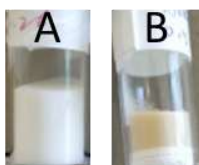


Figure 1. The color of silica gel before (A) and after (B) the modification.

### 3.2 Enhanced separation of crude oils

The separation of crude oil was performed via the novel  $\pi$ - $\pi$  interaction stationary phase. There are 12 kinds of elution solvent (Table 1) and 13 fractions obtained (Figure 2B). Progressing from solvent 1 to 10, there is a gradient of increasing solvent elution ability. Solvent 11 was utilized to elute the resin fractions. Solvent 12 was used for further elution. According to the results of Figure 2B and the characterization via UV and IR spectroscopy (Figure S11 and S12), solvents 8, 9 and 10 can't elute many fractions from the  $\pi$ - $\pi$  interaction stationary phase, because the molecules had been eluted already if they could elute at these conditions. There is a gradient of elution condition of increasing concentration of chloroform from solvent 1 to 7. Thereby, this could be achieved by NPLC with a gradient in elution condition with little difficulty.

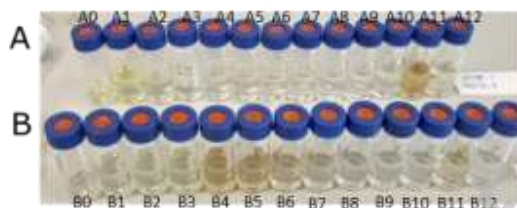


Figure 2. Analysis of crude oil from D well via prepared  $\pi$ - $\pi$  columns. A) Analysis with Si column (blank control). B) Analysis with the prepared  $\pi$ - $\pi$  columns.

In order to investigate the main interactions between petroleum molecules and the stationary phase, the silica gel SPE column was chosen as a control experiment. Both experiments were performed at the same conditions, but the elution fractions were different (Figure 2). There were more colored compounds visible in the fractions of A0 and A1 than fractions of B0 and B1. Similarly, there were more colored compounds in fractions of A11 and A12 than fractions of B11 and B12. On the other hand, there were more colored compounds in the fractions of B3, B4, B5, B6 and B7 than the fractions of A3, A4, A5, A6 and A7. A reason could be that there are more colored compounds captured by the pyrene groups. Hence, here were fewer colored compounds in the fractions of B0, B1 and B11; and there were more discrete compound classes in the group B compared with the Si group. This implies that the prepared  $\pi$ - $\pi$  column was more suitable to separate the crude oils and reduce the complexity of crude oils. This also elucidates that the main separation mechanisms were different.

SARA analysis is an established and very effective method of separation of crude oils. The proposed interactions between petroleum molecules and the stationary phase are between OH of silica gel and benzene rings, and N, O-containing groups of petroleum molecules. However, for the new material the  $\pi$ - $\pi$  interactions between benzene rings of the stationary phase and benzene rings and branches of the petroleum constituents are expected to dominate, which will be further elucidated by characterization with UV and IR spectroscopy.

### 3.3 Characterization by UV-Vis spectroscopy

UV-vis absorbance spectroscopy was utilized in order to compare the size of the aromatic systems of each fraction. The UV-vis absorption is influenced by mainly delocalized/unsaturated elements in the molecular structure, such as double bonds, aromatic rings, etc. As for the aromatics, the maximum absorption of the observed electronic  $n \rightarrow \pi^*$  or  $\pi \rightarrow \pi^*$  transitions would shift to longer wavelengths with increasing size of the aromatic ring system.

As there were different concentrations of molecules in each fraction, the UV spectra should be normalized before the comparison. Most of the fractions show the maximum absorption around 200 nm (Figure S11), thereby, the first ten points of absorption were averaged as the maximum absorption of each fraction. In each UV spectrum, every number of absorption was divided by the maximum absorption and the resulting data was plotted in Figure 3. Due to potential residues of absorbing toluene left in the samples, the comparison should be done above 300 nm. The UV absorption clearly increases at longer wavelengths in the region from 300 nm to 500 nm for the fractions from the  $\pi$ - $\pi$  column separation in contrast to the control group (Figure S13).

Figure 2B shows that there were a lot of compounds in fractions B0 and B1 although the exact colour is less evident. The absolute absorption of 100 times dilution of B0 and B1 was still stronger than some others (Figure S11). Hence, the data of fraction B0 and B1 was taken from 100 times dilution samples and the others were from 10 times dilution samples. However, this didn't influence the results due to the normalization procedure.



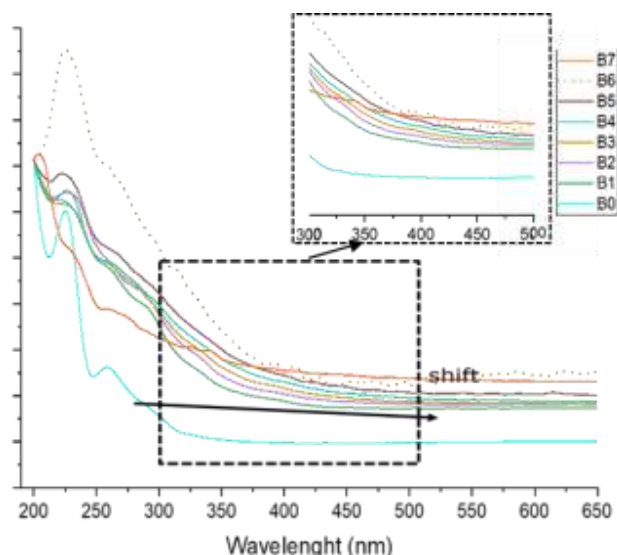


Figure 3. UV absorption spectra (200-650 nm) recorded for the different crude oil (D well) fractions

Table 2. The observed band origins and the vibrational assignments of IR spectra generated for the crude oil fractions.

Wavenumber (cm <sup>-1</sup> )	Mode of vibration	Functional group
3480	stretching vibration	hydrogen-bonded O-H (intramolecular)
3359	stretching vibration	-
3197	stretching vibration	phenols: O-H (Associated)
3050	stretching vibration	Aromatic C-H
2951	asymmetric stretching vibration	CH <sub>3</sub> : C-H
2921	asymmetric stretching vibration	CH <sub>2</sub> : C-H
2871	symmetric stretching vibration	CH <sub>3</sub> : C-H
2854	symmetric stretching vibration	CH <sub>2</sub> : C-H
1735	stretching vibration	open chain carboxylic acids: C=O
1701	stretching vibration	carboxylic acids: C=O
1658	stretching vibration	Conjugated carbonyl and/or amide: C=O
1632	Bending	Heterocyclic structures: C-N
1603	stretching vibration	Aromatic structures: C=C
1456	symmetric vibration	CH <sub>2</sub> and/or CH: C-H
1376	symmetric vibration	CH <sub>3</sub> : C-H
869	out-of-plane bending	5 substitution aromatics: C-H
810	out-of-plane bending	4 substitution aromatics: C-H
755	out-of-plane bending	3 substitution aromatics: C-H
743	out-of-plane bending	2 substitution aromatics: C-H
722	Rocking vibration	CH <sub>2</sub> groups in long aliphatic alkyl side chains (CH <sub>2</sub> ) <sub>n-4</sub>
698	out-of-plane bending	1 substitution aromatics: C-H

### 3.4 Characterization by ATR-FTIR spectroscopy

The attenuated-total-reflectance Fourier transform (ATR-FTIR) spectroscopy technique provides reliable information regarding the chemical composition and it has been widely used for the identification and characterization of complex samples.<sup>27</sup> Several works on analysis of petroleum fluids has been successfully performed via ATR-FTIR<sup>27a, 27b</sup>, where the aliphatic index, the aromatic index,



the ratio of CH<sub>2</sub>/CH<sub>3</sub> groups and the condensation degree of polyaromatics has been determined. Compared with UV-vis characterization, the ATR-FTIR spectroscopy technique provides more chemical information of the petroleum constituents.

One of the IR spectra is shown in Figure 4, and its range was cut out from 650-3800 cm<sup>-1</sup> and more IR spectra are shown in Figure SI2 and Figure SI4. The general features of the IR spectra don't vary significantly between fractions (Figure SI 2). After the IR spectra were normalized, the main variation is shown and this is mainly concerned with the relative intensity of the observed bands (Figure SI4). The band assignment for the IR spectra is shown in Table 2 and the main variation is discussed in details in the following.

In the region of 3800-3000 cm<sup>-1</sup>, the IR absorption is assigned to O-H and N-H stretching vibrations. With increasing concentration of chloroform (Table SI 2), the relative intensities of the bands observed at 3359 cm<sup>-1</sup> and 3317 cm<sup>-1</sup> increase from fraction B5 (Figure SI 5). Compared with the previous fractions, there are more polar OH and NH groups in the benzene ring fractions. At 3359 cm<sup>-1</sup>, the assignment might be a secondary aliphatic amine, a secondary aromatic amine, carboxylic acid or alcohol. The region of 1800-1500 cm<sup>-1</sup>, is the carbonyl stretching region. The band observed at 1603 cm<sup>-1</sup> was assigned to the C=C stretching vibration of benzenoid structures while the bands observed at 1735 cm<sup>-1</sup>, 1701 cm<sup>-1</sup> and 1658 cm<sup>-1</sup> were assigned to C=O stretching vibrations. The intensity of the 1603 cm<sup>-1</sup> band increases with increasing elution capability (Figure SI4), which means the benzenoid structures become gradually larger in these fractions. The relative intensity of the carboxyl region absorption bands also increased. Given the region of 3800-3000 cm<sup>-1</sup> and 1800-1500 cm<sup>-1</sup>, the benzene ring abundance was seen to increase and the carbonyl groups also increased with increasing elution capability. In the region of 1500-1200 cm<sup>-1</sup>, the CH<sub>3</sub> and CH<sub>2</sub> bending vibrations are the main absorption bands. Normally, there are two strong bands at 1376 cm<sup>-1</sup> and 1456 cm<sup>-1</sup> in hydrocarbon analysis with IR, which are attributable to alkyl chains. The "fingerprint" region between 650 and 1000 cm<sup>-1</sup> contains mainly the aromatic C-H out-of-plane bending vibration bands providing information about the degree of substitution and condensation present in the aromatic structures.<sup>27b</sup> The band at 698 cm<sup>-1</sup> features aromatic C-H out-of-plane signature associated with benzene rings (5 H) containing only a single substituent. This band appears clearly in the IR spectra for the B0 and B1 fractions, whereas the IR spectra for the remaining fractions did not show this feature (Figure SI6). At the same time, the "rocking" vibrational band at 722 cm<sup>-1</sup> representing alkyl chain structures with more than 4 carbon atoms, appears evidently in the spectra generated for fraction B0 and B1 (Figure SI6). This suggests a larger abundance of aromatic molecules with one substitution involving a long alkyl chain in fractions B0 and B1. In addition, the observed bands in general were more narrow for these fractions (Figure SI6), indicating smaller molecular weights. The detailed band assignments are shown in Table 2.

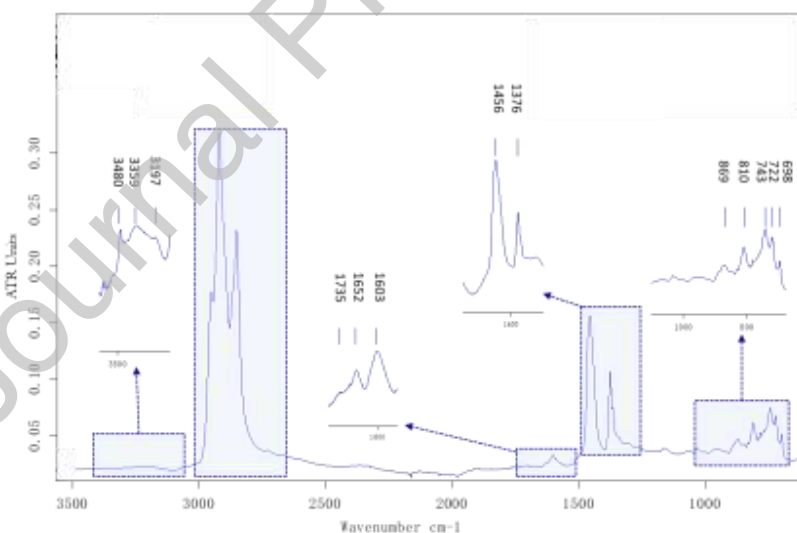


Figure 4. The ATR-FTIR spectrum collected for the fraction B1 of the crude oil from D well in the North Sea and the most important vibrational band assignments.

In order to investigate the relative abundances of molecular structures for the constituents of the different fractions, some spectroscopic indexes have been introduced following the procedure given below<sup>27a</sup>. The indexes were all calculated from deconvoluted band intensities where the band areas were measured from valley to valley. The sum of areas from all the selected deconvoluted bands of the IR spectrum is denoted  $\Sigma A$ .

$$\text{Aromaticity index: } A_{1600} / \Sigma A$$

$$\text{Aliphatic index: } (A_{1456} + A_{1376}) / \Sigma A$$

Branched index:  $A_{1376} / (A_{1456} + A_{1376})$

Long chain index:  $A_{721} / (A_{1456} + A_{1376})$

The aromaticity indices of the individual fractions are shown in Figure 5. Fraction B0 shows the smallest value, which represents the case with the smallest number of aromatic benzene rings in the molecular structures. The constituents of these samples have weak interactions with the stationary phase in the column and eluted directly. The aromaticity indexes of B1 to B5 increase due to the more dominant non-covalent  $\pi$ - $\pi$  interactions with the stationary phase. The elution needs higher concentration of chloroform to break the  $\pi$ - $\pi$  interactions. Fractions B6 and B7 show smaller aromaticity indices and potentially these constituents are involved in several classes of interactions between the molecules and the stationary phase. The bands in the spectra for fraction B7 are sharper than observed for the fractions B0 to B6. Furthermore, the fractions of B10, B11 and B 12 could help with the interpretation of this observation. The  $\pi$ - $\pi$  interactions would not be the dominant interactions for the constituents of the final three fractions. In the spectra for fraction B11 the relative intense bands observed at  $3359\text{ cm}^{-1}$ ,  $3193\text{ cm}^{-1}$  and  $1710\text{ cm}^{-1}$  suggest a high relative abundance of polar compounds with O and N atoms (Figure SI7). This could suggest that charge-charge interactions or intermolecular hydrogen bonding are the dominant interactions for the constituents in fraction B11.

Three model compounds were chose to validate the  $\pi$ - $\pi$  interactions with the prepared material (Figure SI10, SI11 and SI12). Naphthalene could be eluted out with hexane and not kept by the solid phase implying that the petroleum molecules with two benzene rings are hard to keep in the designed  $\pi$ - $\pi$  SPE column with hexane as a solvent. The four-benzene ring system, pyrene, shows a stronger non-covalent  $\pi$ - $\pi$  binding to the prepared material than the naphthalene molecules. There were about 50% (calculated via the UV absorbance at 334 nm) pyrene molecules eluted with hexane solvent directly and the other 50% of pyrene molecules could be eluted when washing by hexane. However, a significant delay was observed when pyrene was eluted under crude oil conditions. According to the UV absorbance at 334 nm, there were about 1/3 of pyrene molecules eluted with hexane directly and then 2/3 was delayed and eluted when washing by hexane (Figure SI12). The reason may be that the multiple benzene ring petroleum molecules help the pyrene to interact more strongly the column as an intermediate interaction layer. Coronene shows even more strong interactions with the  $\pi$ - $\pi$  material than pyrene due to larger aromatic ring system containing six benzene rings. There were not any coronene compounds eluted directly in hexane condition, neither in crude oil condition. Most of the coronene was eluted out at 10% chloroform. Even in crude oil conditions, the delay phenomenon is not observed (Figure SI11). In these three cases, the larger multiple benzene ring systems show stronger binding and elute later, which reveals the same trend as observed for the aromaticity of the crude oil fractions. The molecules with four benzene rings were at the limit of what could be retained on the prepared  $\pi$ - $\pi$  material. Thereby, most of the molecules in fraction 0 were with aromatic systems involving less than four rings.

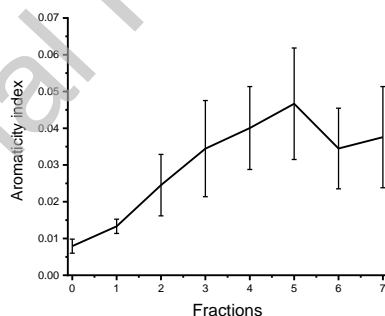


Figure 5. The aromaticity index for the different fractions of North Sea oil.

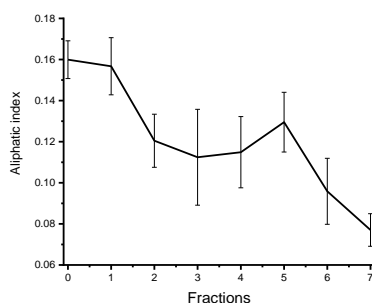


Figure 6. The aliphatic index for the different fractions of North Sea oil.

### 3.5 Application of 2D off-line separation

The interactions between oil constituents and silica gel are mainly hydrogen bonds or  $\sigma$ - $\pi$  interactions, which are different from  $\pi$ - $\pi$  interactions. If the Si SPE column is combined with the  $\pi$ - $\pi$  enhanced column, they can provide orthogonal selectivity for separation of crude oil molecules. In order to perform the on-line 2D separation, these two columns were therefore combined together for orthogonal selectivity. The Si SPE column was chosen as the first dimension column and the prepared  $\pi$ - $\pi$  column was chosen as the second dimension column (Figure 7). The transfer of this off-line 2D setup to the on-line setup is facilitated by this sequence. The crude oil can be separated into many fractions and all the fractions can be detected by UV spectroscopy without pre-concentration, thereby, 30  $\mu$ L of crude oil was used for the 2D separation. In Figure 2, it appears that there are low concentrations of the compounds observed in fraction A3 to A10, so all these fractions were collected in vials with 2 mL hexane/chloroform (V/V=6/4). There were four fractions obtained after the separation with a Si column (Figure 7).

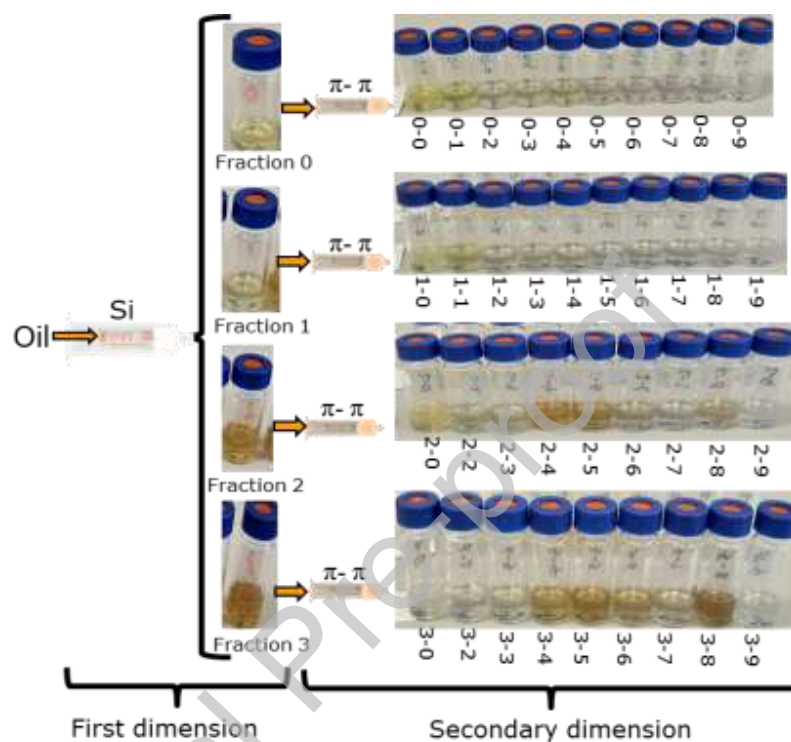


Figure 7. The 2D off-line separation of crude oil from D well.

Each of the four fractions from the first dimensional column continued to separate with the second dimensional  $\pi$ - $\pi$  enhanced column. In order to simulate the on-line 2D separation with off-line 2D separation, the elution solvent used for the second dimensional  $\pi$ - $\pi$  enhanced column was at a gradient condition (Table S12) and the loading samples were run with low concentration of toluene or chloroform. The fraction 0 and fraction 1 from the first dimensional column (NPLC1) was loaded onto the second dimensional  $\pi$ - $\pi$  enhanced column (the  $\pi$ - $\pi$ -SPE column NPLC 2). This is the same procedure as for the elution fractions which go through the first column and are loaded on to the second column, where the second column is run with a gradient condition in the on-line 2D separation system. Fraction 2 from the first dimensional column was diluted with 6 mL hexane, and there was about 10% Chloroform in the solvent of the loading samples. This procedure was analogous to increasing the flow speed of NPLC 2 in the on-line 2D separation system. Fraction 3 from the first dimensional column was concentrated to 200  $\mu$ L by  $N_2$ , and then diluted to 4 mL with hexane. The polar fractions were released with more polar solvent from the first dimensional column, and the gradient condition run on the second column. This step mimics applying 20 times flow speed on the second column in on-line system. By this approach, the off-line 2D system has been demonstrated, and it was feasible to transfer to the on-line 2D system, because the experiment was performed at similar elution conditions and could be performed with orthogonal selectivity, and any single elution fraction was not recombined again in next separation dimension.

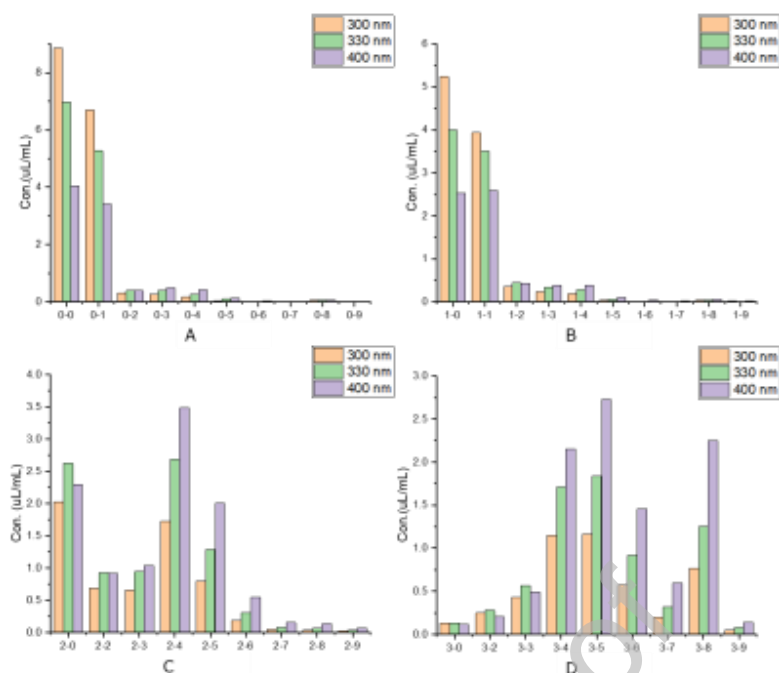


Figure 8. The oil concentration of each fraction in the secondary dimension. A, sample 0 from the first sample of Si separation; B, sample 1 from the elution by hexane from Si separation; C, sample 2 from the elution by Toluene/Methanol from Si separation; D, sample 3 from the elution by Toluene/Chloroform/Methanol from Si separation.

UV spectroscopic analysis was performed to measure the concentration of the 38 fractions. Before the measurement, the calibration curve of crude oil absorbance was measured (Table SI3). In case of the absorbance of the solvents (toluene and chloroform), the wavelengths were chosen at 300 nm, 330 nm and 400 nm. The separation changes the concentration of the components in the samples and the later the components elute, the higher the aromatic content. The three standard lines of absorbance were obtained from the same standard samples (crude oil). The UV absorbance in the spectra of the samples was compared with the calibration curve to measure the concentration and it was confirmed that the concentration varies. In Figures 8 A and B, the saturates are the main components, and the absorbance was higher at 300 nm in the fractions 0-0, 0-1, 1-0 and 1-1. The next fractions had higher absorbance at 400 nm, due to the increasing amounts of aromatics. There were more aromatic components in the spectra shown in Figures 8C and D. In Figure 8, the common trend was that the absorbance increased at 400 nm, which matches the trend of Figure 5. Furthermore, this off-line 2D separation shows more chemical information about crude oil molecules, compared with SARA analysis, which showed a less complete separation.

The concentrations were calculated at two different different wavelengths and the average of the measured concentration of oil was used to identify the concentration of each fraction. In order to see the trend of concentration clearly, the 2D off-line results were transferred to 3D spectrum format via OriginPro 2018b (Figure 9). In the Figure 9, the red spectrum and the cyan spectrum shows the saturates. The resin could be eluted from Si column with toluene/methanol (8/2, V/V), which is the spectrum shown with green color in Figure 9. The lighter components, such as saturates and aromatics, were the mainly fractions in this North Sea oil. The polar function groups were mainly located on the multiple benzene ring compounds. These conclusions can be confirmed by the results of the IR characterization of fractions (Figure SI2).

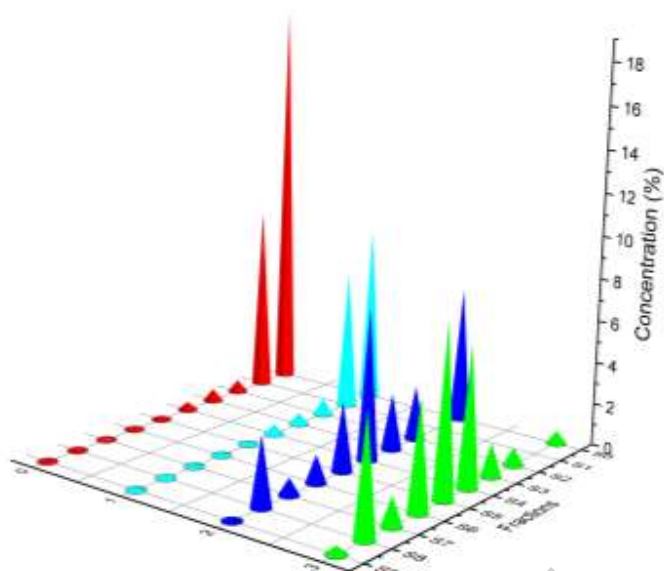


Figure 9. Conversion of the 2D off-line spectra to a 3D spectrum.

In order to investigate the chemical variation of North Sea crude oils, several crude oils (from the chalk fields D, H, K and V) were separated by this 2D offline separation. 38 fractions were obtained based on the difference in the aromatic ring size in each crude oil sample, and the concentration of each fraction was calculated via UV absorbance, which is shown in Figure 10. The total amount of fractions recoveries were as follows: D, 123% (37.35  $\mu\text{L}$ ); H, 95% (28.60  $\mu\text{L}$ ); K, 90% (27.06  $\mu\text{L}$ ); V, 90% (27.02  $\mu\text{L}$ ). Even though the sample set was limited to 38 fractions, the differences in the crude oils still were captured. There were higher concentrations of fraction 0-1 in the D oils, which implied there were more branched petroleum molecules in D oil. The petroleum molecules with large aromatic ring structures and polar functional groups are seen in higher concentration in D oils, and these correspond to fractions 2-8, 3-4, 3-5 and 3-6. As for the H oil, fractions 3-0 and 3-8 are observed in higher concentration than in the other three oils, especially fraction 3-0. Fraction 3-0 of H oil had a 9 times higher absorbance than these fractions in the V oil. Fractions 3-0 correspond to the compound class with high branching and low aromaticity. These compounds might be packed in the resin fraction as intermediate sized molecules. The observed fractions in the K oil sample were similar to those observed in H oil. This phenomenon was more marked in the aromatics fractions (from 2-0 to 2-9). All of the four crude oils show high concentration of fractions 2-0. Compared to the “polar” resin fraction, there are more intermediate sized molecules in the aromatics fractions, since the alkanes have a higher affinity to the non-polar fractions. As seen in the fractions from 2-2 to 3-8, there were more components with multiple benzene rings in the V oils (see additional detail in Table S14 and Figure S113). Compared to the SARA analysis, there is more detailed molecular information obtained. Furthermore, it is a new approach to investigate the petroleum molecules according to the molecular core size. It is a potential experimental method for identifying the Double Bond Equivalent (DBE) numbers of petroleum molecules together with mass spectrometry.

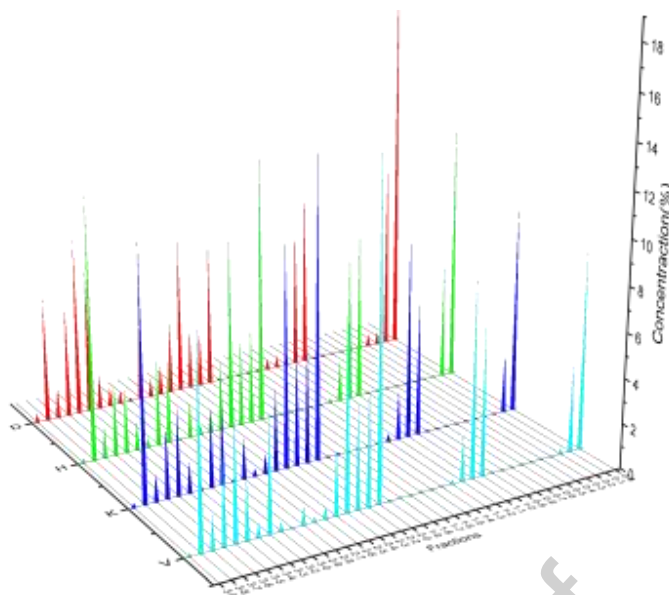


Figure 10. The variation in the fractions of the North Sea crude oil samples. There are four oil samples: D, H, K and V from different sources. Fractions 2-1 and 3-1 are omitted. The concentration = (the net oil volume of each fraction/ total volume of oil)  $\times$  100.

#### 4. CONCLUSIONS

In this work, a novel material favoring non-covalent  $\pi$ - $\pi$  interactions has been prepared successfully via modification of pyrene groups on silica gel. The pyrene groups enhance the strength of interaction between stationary phase and the aromatics at normal phase conditions. A pyrene group was immobilized on the stationary phase with a long arm, which increases the flexibility of the pyrene head. With a series of model compounds, we have found that compounds with four benzene rings are the size limit of compounds that can be captured by this material. This material has been applied to analysis of crude oil with a Si material column as a reference. It shows a more powerful separation than the Si material columns and is more suitable for reducing the complexity of crude oils. All the fractions eluted were analyzed quantitatively by UV and IR spectroscopy. After the UV spectra were normalized, the UV absorbance of each fraction shows spectral red shift with increasing concentration of chloroform of elution solvents, which implies that the electronic transition needs less energy and the number of the benzene rings increases. The degree of polyaromatic content and the branching of each fraction was investigated by IR spectroscopy. By increasing the fraction of chloroform in the solvents, the aromaticity index increases, which matches the conclusions in the UV analysis. Conversely, the length of the carbon chains, as shown by the aliphatic index, decreases. This material also has the orthogonal selectivity of the Si column material. Thereby, it is possible to use this in tandem with a Si column and set up a 2D off-line separation system. There were four crude oils used as models to investigate the chemical variation of North Sea crude oils. We found the light fractions were the main components in D oil and the compounds involving multiple benzene rings also had more polar groups. There were more aromatics in V oils. There was intermediate sized molecules in resin fraction in H and K oils, but these were not observed in D and V oils. Overall, the prepared material provided better separation ability compared with Si materials and more chemical information of crude oils. It is a complementary method to common approaches for analysis of crude oils. It can be used in LC $\times$ LC or LC $\times$ LC $\times$ LC on-line analysis of crude oils at normal phase conditions.

#### ASSOCIATED CONTENT

##### Supporting Information

Solvents tables for residue re-dissolved and gradient elution; spectroscopic characterization of aromatics in experiment fractions and blank control fractions; the analysis of model compounds elution from the enhanced  $\pi$ - $\pi$  interaction column; the table and figure for characterization of the variation in the fractions of the North Sea crude oil samples.

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## Declaration of interests

**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.**

## REFERENCES

1. Panda, S. K.; Andersson, J. T.; Schrader, W., Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: a challenge. *Anal Bioanal Chem* **2007**, *389* (5), 1329-1339.
2. Rodgers, R. P.; McKenna, A. M., Petroleum Analysis. *Analytical Chemistry* **2011**, *83* (12), 4665-4687.
3. (a) Standard Test Method for Determination of Individual Components in Spark Ignition Engine Fuels by 50-Metre Capillary High Resolution Gas Chromatography. 2016; (b) Fan, T.; Wang, J.; Buckley, J. S., Evaluating Crude Oils by SARA Analysis. In *SPE/DOE Improved Oil Recovery Symposium*, Society of Petroleum Engineers: Tulsa, Oklahoma, 2002; p 7; (c) Standard Guide for Characterizing Hydrocarbon Lubricant Base Oils. 2015; (d) Standard Test Method for Insolubles in Used Lubricating Oils. 2014.
4. Nizio, K. D.; Mginitie, T. M.; Harynuk, J. J., Comprehensive multidimensional separations for the analysis of petroleum. *Journal of Chromatography A* **2012**, *1255* (Supplement C), 12-23.
5. (a) Dallüge, J.; Beens, J.; Brinkman, U. A. T., Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool. *Journal of Chromatography A* **2003**, *1000* (1), 69-108; (b) Adahchour, M.; Beens, J.; Brinkman, U. A. T., Recent developments in the application of comprehensive two-dimensional gas chromatography. *Journal of Chromatography A* **2008**, *1186* (1), 67-108; (c) Murray, J. A., Qualitative and quantitative approaches in comprehensive two-dimensional gas chromatography. *Journal of Chromatography A* **2012**, *1261*, 58-68.
6. (a) Mao, D.; Van De Weghe, H.; Diels, L.; De Brucker, N.; Lookman, R.; Vanermen, G., High-performance liquid chromatography fractionation using a silver-modified column followed by two-dimensional comprehensive gas chromatography for detailed group-type characterization of oils and oil pollutions. *Journal of Chromatography A* **2008**, *1179* (1), 33-40; (b) Hyötyläinen, T.; Riekkola, M.-L., On-line coupled liquid chromatography–gas chromatography. *Journal of Chromatography A* **2003**, *1000* (1), 357-384.
7. Oro, N. E.; Lucy, C. A., Comparison of hypercrosslinked polystyrene columns for the separation of nitrogen group-types in petroleum using High Performance Liquid Chromatography. *Journal of Chromatography A* **2010**, *1217* (40), 6178-6185.
8. (a) Bissada, K. K.; Tan, J.; Szymczyk, E.; Darnell, M.; Mei, M., Group-type characterization of crude oil and bitumen. Part I: Enhanced separation and quantification of saturates, aromatics, resins and asphaltenes (SARA). *Organic Geochemistry* **2016**, *95*, 21-28; (b) Bissada, K. K.; Tan, J.; Szymczyk, E.; Darnell, M.; Mei, M., Group-type characterization of crude oil and bitumen. Part II: Efficient separation and quantification of normal-paraffins iso-paraffins and naphthenes (PIN). *Fuel* **2016**, *173*, 217-221.
9. Schabron, J. F.; Rovani, J. F.; Sanderson, M. M., Asphaltene Determinator Method for Automated On-Column Precipitation and Redissolution of Pericondensed Aromatic Asphaltene Components. *Energy & Fuels* **2010**, *24* (11), 5984-5996.
10. Youtcheff, J., Automated High-Performance Liquid Chromatography Saturate, Aromatic, Resin, and Asphaltene Separation. *FHWA Publication* **2016**.



11. Snyder, L. R.; Buell, B. E., Nitrogen and oxygen compound types in petroleum. A general separation scheme. *Analytical Chemistry* **1968**, *40* (8), 1295-1302.
12. Robson, W. J.; Sutton, P. A.; McCormack, P.; Chilcott, N. P.; Rowland, S. J., Class Type Separation of the Polar and Apolar Components of Petroleum. *Analytical Chemistry* **2017**, *89* (5), 2919-2927.
13. Herod, A. A.; Bartle, K. D.; Kandiyoti, R., Characterization of Heavy Hydrocarbons by Chromatographic and Mass Spectrometric Methods: An Overview. *Energy & Fuels* **2007**, *21* (4), 2176-2203.
14. Sripada, K.; Andersson, J. T., Liquid chromatographic properties of aromatic sulfur heterocycles on a Pd(II)-containing stationary phase for petroleum analysis. *Anal Bioanal Chem* **2005**, *382* (3), 735-741.
15. (a) Danumah, C.; Myles, A. J.; Fenniri, H., Graphitic Carbon Nanoparticles from Asphaltenes. *MRS Proceedings* **2011**, *1312*, mrsf10-1312-jj05-26; (b) Pfau, S. A.; La Rocca, A.; Haffner-Staton, E.; Rance, G. A.; Fay, M. W.; Brough, R. J.; Malizia, S., Comparative nanostructure analysis of gasoline turbocharged direct injection and diesel soot-in-oil with carbon black. *Carbon* **2018**, *139*, 342-352.
16. Bargmann-Leyder, N.; Truffert, J. C.; Tambuté, A.; Caude, M., Evaluation of Pirkle-type chiral stationary phases by liquid and supercritical fluid chromatography: Influence of the spacer length and the steric hindrance in the vicinity of the stereogenic centre. *Journal of Chromatography A* **1994**, *666* (1), 27-40.
17. Sun, Y.; Pang, Y.; Zhang, J.; Li, Z.; Liu, J.; Wang, B., Application of molecularly imprinted polymers for the analysis of polycyclic aromatic hydrocarbons in lipid matrix-based biological samples. *Anal Bioanal Chem* **2017**, *409* (29), 6851-6860.
18. (a) Kimata, K.; Hosoya, K.; Kuroki, H.; Tanaka, N.; Barr, J. R.; McClure, P. C.; Patterson, D. G.; Jakobsson, E.; Bergman, A., Selectivity of electron-donor- and electron-acceptor-bonded silica packing materials for hydrophobic environmental contaminants in polar and non-polar eluents. *Journal of Chromatography A* **1997**, *786* (2), 237-248; (b) Tanaka, N.; Tokuda, Y.; Iwaguchi, K.; Araki, M., Effect of stationary phase structure on retention and selectivity in reversed-phase liquid chromatography. *Journal of Chromatography A* **1982**, *239*, 761-772.
19. Andersen, S. I.; Keul, A.; Stenby, E., Variation in Composition of Subfractions of Petroleum Asphaltenes. *Petroleum Science and Technology* **1997**, *15* (7-8), 611-645.
20. (a) Schuler, B.; Fatayer, S.; Meyer, G.; Rogel, E.; Moir, M.; Zhang, Y.; Harper, M. R.; Pomerantz, A. E.; Bake, K. D.; Witt, M.; Peña, D.; Kushnerick, J. D.; Mullins, O. C.; Ovalles, C.; van den Berg, F. G. A.; Gross, L., Heavy Oil Based Mixtures of Different Origins and Treatments Studied by Atomic Force Microscopy. *Energy & Fuels* **2017**, *31* (7), 6856-6861; (b) Ostlund, J.-A.; Wattana, P.; Nydén, M.; Fogler, H. S., Characterization of fractionated asphaltenes by UV-vis and NMR self-diffusion spectroscopy. *Journal of colloid and interface science* **2004**, *271* 2, 372-80.
21. Schuler, B.; Meyer, G.; Peña, D.; Mullins, O. C.; Gross, L., Unraveling the Molecular Structures of Asphaltenes by Atomic Force Microscopy. *Journal of the American Chemical Society* **2015**, *137* (31), 9870-9876.
22. Sundberg, J.; Feilberg, K. L., Characterization of heteroatom distributions in the polar fraction of North Sea oils using high-resolution mass spectrometry. *Journal of Petroleum Science and Engineering* **2020**, *184*, 106563.
23. Larrivée-Aboussafy, C.; Jones, B. P.; Price, K. E.; Hardink, M. A.; McLaughlin, R. W.; Lillie, B. M.; Hawkins, J. M.; Vaidyanathan, R., DBU Catalysis of N,N'-Carbonyldiimidazole-Mediated Amidations. *Organic Letters* **2010**, *12* (2), 324-327.
24. Grimme, S., Do Special Noncovalent  $\pi$ - $\pi$  Stacking Interactions Really Exist? *Angewandte Chemie International Edition* **2008**, *47* (18), 3430-3434.
25. Paul, R.; Anderson, G. W., N,N'-Carbonyldiimidazole, a New Peptide Forming Reagent. *Journal of the American Chemical Society* **1960**, *82* (17), 4596-4600.

26. (a) Woodman, E. K.; Chaffey, J. G. K.; Hopes, P. A.; Hose, D. R. J.; Gilday, J. P., N,N' - Carbonyldiimidazole-Mediated Amide Coupling: Significant Rate Enhancement Achieved by Acid Catalysis with Imidazole • HCl. *Organic Process Research & Development* **2009**, *13* (1), 106-113; (b) Mevellec, L., Anne; FR; Pasquier, E., Thérèse, Jeanne; FR; Descamps, S. F.; Mercey, G., Jean, Maurice; FR; Wroblowski, B. B.; Vialard, J., Eduardo; BE; Meerpoel, L. B.; Jeanty, M., Ludovic; FR; Jousseume, T., François, Alain, Jean; CH Substituted 4,5,6,7-tetrahydro-pyrazolo[1,5-a]pyrazine derivatives and 5,6,7,8-tetrahydro-4h-pyrazolo[1,5-a][1,4] diazepine derivatives as rosi inhibitors 2014.
27. (a) Permanyer, A.; Douifi, L.; Lahcini, A.; Lamontagne, J.; Kister, J., FTIR and SUVF spectroscopy applied to reservoir compartmentalization: a comparative study with gas chromatography fingerprints results. *Fuel* **2002**, *81* (7), 861-866; (b) Sánchez-Lemus, M. C.; Schoeggl, F. F.; Taylor, S. D.; Andersen, S. I.; Mapolelo, M. M.; Mahavadi, S. C.; Yarranton, H. W., Characterization of Heavy Distillation Cuts Using Fourier Transform Infrared Spectrometry: Proof of Concept. *Energy & Fuels* **2016**, *30* (12), 10187-10199; (c) Snyder, R. G., Vibrational Study of the Chain Conformation of the Liquid n - Paraffins and Molten Polyethylene. *The Journal of Chemical Physics* **1967**, *47* (4), 1316-1360.

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