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Investigation and Characterization of Asphaltenes and Asphaltene Co-Precipitates and Their Role in Emulsion Formation/Stabilization

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INVESTIGATION AND CHARACTERIZATION OF ASPHALTENES AND ASPHALTENE CO-PRECIPITATES AND THEIR ROLE IN EMULSION FORMATION/STABILIZATION

By

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For my family….

past, present, and future.

Without their love and support I would not be able to accomplish all that I have.
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ABSTRACT

Asphaltenes are a solubility defined class of crude oil compounds that precipitate from solution upon normal alkane addition. They are experimentally investigated due to their tendencies to cause refinery issues. Their ability to aggregate (even at very low concentrations), flocculate, and precipitate cause refinery pipes to clog, foul, and (in some cases) corrode. Asphaltenes are a heterogeneous mixture of compounds; highly aromatic and rich in heteroatom content.

Nearly every aspect of asphaltene science is debated in literature. Varying opinions are presented in terms of asphaltene molecular weight, structure and composition. Molecular weight is highly controversial because of chemical aggregation tendencies. Molecular structure of asphaltenes is disputed in terms of molecular linkage. Whether asphaltenes are highly condensed molecular structures or highly alkylated is an intensely researched subject matter. Chemical composition is questioned in terms of heteroatom content and polarity. What role heteroatoms play in aggregation and molecular structure is also still largely unknown.

The aim of this document is to investigate asphaltenes using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) (see chapter two). The ultrahigh resolution, high resolving power capabilities, and high mass accuracy make the instrumentation ideal for probing a complex mixture, such as asphaltenes. Outlined in Chapter Three is an investigation into pre-fractionated asphaltenes by size-exclusion chromatography (SEC). The SEC technique is frequently used to aid in asphaltene molecular weight determination, but often times plagued by the asphaltene molecule’s inherent tendency to aggregate. The mass spectral analysis of these fractionated asphaltenes provides insight into asphaltene heteroatom content, aromaticity and alkylation. The observed decrease in alkylation trends point to a size-based separation. Class compositional profiles are provided as function of SEC eluents.

Chapter four presents a study that examines asphaltene molecular structure. Model compounds are probed by tandem mass spectral analysis to gain understanding into the fragmentation tendencies of catacondensed and pericondensed molecular structures. The observed patterns are correlated to the fragmentation results from a real asphaltene sample. Results offer new insight into asphaltene molecular structure.
Asphaltenes are often blamed for emulsion formation in crude oil because of their surface active characteristic and capabilities. Chapters five and six examine what role asphaltenes play in emulsion formation. A detailed crude oil compositional study is presented in chapter five. Generated results indicate that a sub-set of asphaltenes, known as asphaltene co-precipitates, with unique “transitional” characteristics may prove most responsible for the role of asphaltenes in emulsion formation.

Chapter six studies lab generated emulsions and their respective (isolated) interfacial layer. The isolated interfacial layer was depleted by successive emulsion formation. The implications from the successive depletion study point to distinct heteroatom functionalities that act as “primary” and “secondary” stabilizers. In addition, identical heteroatom classes and chemical functionalities are identified with similar “transitional” characteristics, just as those identified in chapter five (and previous studies).

Aggregation of asphaltenes causes erroneous molecular weight determination and/or leads to misinterpretations of molecular structure. In addition, aggregation and how it occurs may provide insight into crude oil bio-markers. Molecular polarity behavior of asphaltenes is also paramount because asphaltenes are a solubility defined fraction of crude oil. The data presented in chapter seven monitor asphaltene co-precipitate molecular progression as a function of Soxhlet extraction in normal alkane solvent. Asphaltene co-precipitates indicate that polarity may be a driving factor in asphaltene aggregation and solubility behavior.

Lastly, chapter eight details a massive collaborative series of experiments used to examine a real-world emulsion scenario. All efforts were made to provide a comprehensive explanation for why the observed field emulsion existed but the results and the mass spectral analysis showed no promising answer.
CHAPTER ONE

PETROLEOMICS

Introduction

Crude oil is one of nature’s most complex and interesting marvels. Conservative estimates have placed the total number of distinct chemical compounds found in a single crude oil in excess of 50,000.¹ Petroleomics has been defined as the “prediction of properties and behavior from composition, to aid in solving oil production and processing problems.”¹ Understanding the chemical properties of crude oil has implication on a vast area of upstream (production) and downstream (processing) issues. Property knowledge enables refiners to produce oil reserves more efficiently. Refining oil more effectively produces more product with less cost and less wasteful by-products.

Currently, there are several areas of intense research being conducted. All areas have the same goal in mind: how can we most effectively utilize the resource at hand, with the largest profit margin. The research behind crude oil refining is vast and storied; the remainder of this chapter will detail the basics of crude oil and crude oil analysis. Special focus will be given to two of the most intensely researched areas—-asphaltenes and emulsions. Subsequent chapters will detail asphaltene structure, heteroatom content, molecular weight, and investigate their role in stability of water-in-oil emulsions.

Basics

Crude oil exists on Earth in all three (basic) phases of matter. Liquid oil is the most representative form of crude and it is extracted from underground deposits at the earth’s surface by in-hole/deep sea drilling. Solid phase petroleum is known as bitumen (or oil sands). It is extracted in large mining operations as rocks or tar sands. The world’s largest bitumen mining operations take place in North America, more specifically, Canada. Gaseous petroleum exists as “natural gas”. Natural gas is extracted from the earth through pumping.
The chemical composition of each crude oil varies by location, age of the deposit and depth. The “average” crude oil composition is 84% carbon, 14% hydrogen, 1%-3% sulfur and less than 1% of each of nitrogen, oxygen, metals, and salts. But because there is large variation in crude oil composition, the American Petroleum Institute established an API gravity system that measures how “heavy” or “light” a petroleum is compared to water (H₂O)—density comparison. Heavy crudes are defined as crudes with an API gravity of 20° or less. The greatest commercial values are for crude oils that have an API of 40° - 45°. These crudes are rich in paraffins and usually produce a significant proportion of gasoline. Table 1.1 outlines the API ranges and the corresponding classification.

Table 1.1. American Petroleum Institute (API) Gravities.

<table>
<thead>
<tr>
<th>Crude Classification</th>
<th>API Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>31.1° or higher</td>
</tr>
<tr>
<td>Medium</td>
<td>22.3° – 31.1°</td>
</tr>
<tr>
<td>Heavy</td>
<td>below 22.3°</td>
</tr>
</tbody>
</table>

Crude oils with API gravities greater than 10° are less dense than water and float on top of it. Crude with API gravities lower than 10° are denser than water and sink. These crudes are commonly referred to as “extra heavy crudes.” Bitumen, typically, has an API gravity of 8°, but after upgrading, the synthetic crude oil generated yields an API gravity of 31°-33°.

Additional crude oil descriptors that are often used are based on location or origin of the crude oil reserve. Venezuelan crude oil may be used to describe a crude oil originating in or off the coast line of Venezuela, while Athabasca bitumen is used to describe bitumen mined from open pits in Athabasca, Alberta, CA.

**Heteroatoms**

As petroleum feedstocks become more and more depleted in lighter crude oil, the world and refineries have to move to production of heavier crude oils. These heavier crude oils are more chemically complex and contain more heteroatoms.
knowledge of type and structure of heteroatom-containing compounds in crude oil prior to refinery operations can help minimize or eliminate these species and their unwanted effects such as poisoning catalyst, corrosion, fouling, and or pollution. Heteroatom content in crude oil is typically a direct reflection of the geochemical environment in which the crude was formed. The presence of heteroatom-containing compounds is often times used to provide geological insight into biological building blocks that led to the final product.

**Sulfur.** Most crude oils contain between 0.1-3% sulfur. Sulfur is incorporated from sediment deposits (in both terrestrial and marine environments). Sulfur in crude is derived in two main forms, hydrogen sulfide and elemental sulfur. Elemental sulfur may originate from sulfur-containing amino acids, cysteine and methionine. Bio-organism generated hydrogen sulfide is incorporated with organic matter during early petroleum formation (e.g. kerogen formation). Sulfur functionalities in petroleum are typically limited to mercaptans (thiols), sulfides, disulfides, thiophenes, and sulfoxides. “Sour” crude oils are known as crude that contain significant amounts of reactive hydrogen sulfide (H$_2$S) and other reactive sulfur, while “sweet” crude oil contains little to no reactive sulfur.

The Environmental Protection Agency mandates that crude oil refineries limit the amount of sulfur in their final product in order to reduce harmful environmental pollution. Sulfur removal is often times done by hydrotreatment processes that convert sulfur into H$_2$S. The presence of nitrogen compounds may hinder this process by deactivating catalyst.

**Nitrogen.** The average crude oil contains about 0.1-2% nitrogen. Nitrogen content in crude oil is categorized in two ways, basic nitrogen and neutral nitrogen. Basic nitrogen (pyridinic homologs) makes up nearly two-thirds of the total nitrogen content of a crude oil. Neutral nitrogen (pyrrole homologs) compounds are weakly acidic species. Biological sources for nitrogen incorporation into crude are porphyrins and amino acids. The presence of nitrogen in crude oil can cause corrosion and/or inhibit the removal of other heteroatom-containing compounds.
**Oxygen.** The presence of oxygen in crude oil is generally concentrated in higher distillation fractions and/or heavier crude stocks. Immature and bio-degraded crude oils will also have elevated oxygen compositions. Oxygen species are targeted for removal due to their tendencies to cause corrosion of pipelines and refinery equipment. Chemical functionalities often represented are phenols, carboxylic acids, and ketones. Carboxylic acids concentrations have been shown to correlate well with the total acid number (TAN) of the crude oil. TAN is calculated as the number of milligrams of KOH (potassium hydroxide) needed to neutralize the acid content in one gram of crude oil. Oxygen content can also be used to study bio-origins of the crude material, as some acids may retain biomarker acids (e.g. hopanes).

**SARA Fractionation**

The combination of all geographic and geological information contributes to how the crude oil is in its ultimate state. All crudes are a mixture of saturated and unsaturated hydrocarbons, polar compounds of variable aromaticity and alkylation (with heteroatoms) and metals. Crude oil’s inherent complexity coupled with analytical instrumental constraints almost always necessitates the use of separation techniques prior to analysis. Industries most common form of fractionation is known as SARA (saturates/aromatics/resins/asphaltenes) fractionation. This solubility based separation technique allows for the investigation of crude oil components based on polarity.

Saturates are comprised of the paraffins—the nonpolar linear, branched and cyclic saturated hydrocarbons. They are separated from a crude oil after the maltenes (saturates/aromatic/resin mixture) are adsorbed to a packed alumina column and eluted with hexane. Saturates have, and still are, well characterized by gas chromatography methods. Aromatics are the aromatic hydrocarbons, such as benzene and naphthalene. The aromatics are eluted second from the packed alumina column with toluene. Characterization of the aromatics is often done through high-performance liquid chromatography (HPLC).

Resins are the transitional compounds between the aromatics and asphaltenes. They are comprised of small aromatic cores with some heteroatoms (N, S, and O). Isolation of the resin fraction occurs after washing an alumina column with 80:20
toluene:methanol. Characterization methods used to study the resin fraction is often very similar to that of asphaltenes. Previous studies have used HPLC and electrospray ionization (ESI) coupled with various mass spectrometry methods.$^{14-16}$

**Asphaltenes**

Asphaltenes are characterized as the most polar and most aromatic components in crude oil. Operationally defined as the $n$-heptane insoluble, toluene soluble components, they are removed first in SARA fractionation of the crude through precipitation and filtration. Asphaltenes are one of the most intensely studied and most argued areas in petroleum research. They are recognized as a significant part (0-20% or more) of heavy petroleum products.$^{17}$ Compositional analysis of asphaltenes has shown that the compounds precipitate, aggregate at concentrations as low as 0.1 mg/mL,$^{18}$ and are held responsible for flocculation and deposition that causes clogging/pipe flow disruption. Additionally, asphaltenes are surface active, meaning they accumulate at the interface between water and oil, and therefore, have been investigated at emulsion stabilizers.$^{19-21}$ These production and refinery issues reduce the amount of recoverable oil product, thus, asphaltenes have a huge economic impact in the petroleum industry.

Asphaltene analysis is extremely difficult due to their immense chemical complexity. The compounds are composed of condensed aromatic structures with variable (length and substitution) alkyl chains and contain heteroatoms (N, S, and O) & trace metals (e.g. V, Ni, and Fe).$^{22}$ Commonly, asphaltenes are characterized by their average (bulk) properties. Combustion elemental analysis of asphaltenes has determined atomic H/C ratios of 1.0-1.2 (wt %).$^{23}$ Results from various analytical techniques, such as field ionization mass spectrometry (FIMS),$^{6, 24}$ fluorescence correlation spectroscopy (FCS),$^{25}$ and time resolved florescence depolarization (TRFD)$^{26}$ experimentally conclude that asphaltene molecular weight distributions are in the 300-2000 Dalton (Da) range, with a mean mass between 700-800 Da.$^{18, 23, 26}$ Only recently, though, has the petroleum community become unified on the issue of asphaltene molecular weight.
**Emulsions**

Water aids in the removal of water-soluble salts and facilitates transport of the crude/bitumen through the operation pipelines by lowering viscosity. Emulsion formation (water-in-oil, oil-in-water and multiple emulsions) can occur at nearly any point throughout this process. However, downstream operations require the removal of water prior to refinery and petroleum product recovery. Production losses and the added cost associated with chemicals used to break water and oil emulsions are costly for the petroleum industry. Surface-active compounds in crude oil (e.g. sulfoxides, and carboxylic acids) exhibit surfactant-like characteristics that enable the formation and stabilization of water-in-crude oil emulsions. Characterizing the surface active compounds that lie at the interface, thus far, has provided significant insight to what components potentially act as stabilizers. In the past emulsion stability research has focused on asphaltene stabilization of the interfacial material.\textsuperscript{27-29} This dissertation addresses the possible role of asphaltenes in emulsion formation. More specifically, it points to a “transitional” sub-set of asphaltenes, known as co-precipitates. Details about asphaltenes and asphaltene co-precipitates will be provided in subsequent chapters.
CHAPTER TWO

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

History

Cyclotron resonance of ions was first reported by Ernest O. Lawrence and Stanley M. Livingston in 1932. Their fundamental experiments discovered that ion confinement in the presence of a magnetic field will allow for ions to spin in a cyclotron motion when placed between oppositely charged, opposing electrodes. Lawrence and Livingston were awarded the Nobel Prize in 1939 for their discovery. The first implementation of measuring cyclotron frequency was done by Sommer, Thomas, and Hipple. Their experiments were carried out in the first ion cyclotron resonance mass spectrometer, the “Omegatron” in the late 1940’s-early 1950’s. They used the mass spectrometer to experimentally determine the Faraday constant, by measuring the cyclotron frequency of electron ionization (EI) generated protons. In the mid-1960’s publications on resonant power absorption emerged as a means for detecting magnetic resonance. Published experiments applied a fixed frequency rf electric field while varying the magnetic field to achieve successive m/z ion resonance.

Ion cyclotron resonance (ICR) did not become what it is today until the 1970’s when Alan Marshall and Melvin Comisarow used a fixed magnetic field and excited ions into their cyclotron motion by applying a rf pulse to mounted electrodes (parallel to the magnetic field). In addition, the concept of Fourier transformation was incorporated as a means of cyclotron resonance detection. The combination of these two realizations revolutionized the mass spectrometry field and the use of ion cyclotron resonance as an analytical instrument. Their first paper demonstrated the use of broad-band detection, digitization of the (time-domain) transient response and Fourier transformation to produce the first (frequency-domain) absorption spectrum of CH$_4$\(^{+}\) (methane). Figure 2.1 shows the first FT-ICR mass spectrum of methane.
In a second publication by Marshall and Comisarow, they demonstrated frequency sweep excitation to bring into resonance a wide mass range of ions using an rf frequency that varies linearly over an irradiation time interval. This second paper demonstrated the technique’s advantages in experimental data acquisition time, data reduction time, and mass resolution. Since Marshall and Comisarow’s developments, FT-ICR mass spectrometry has underwent a whirlwind of advancements and development. Extremely diverse applications of the analytical technique are only beginning to be realized. From proteins, to peptide mixtures, to petroleum, FT-ICR mass spectrometry currently offers the highest analytical merits in mass resolving power, mass resolution, mass accuracy, and provides the highest data acquisition speed.

Figure 2.1 The first mass spectrum obtained by Fourier transform ion cyclotron resonance. The ionized molecule is methane.

Theory

A moving charged particle in a spatially uniform magnetic field, \( \mathbf{B} = -B_0 \mathbf{k} \), experiences a “Lorentz” force (given in S. I. units) by equation 2.1

\[
\text{Force} = \text{mass} \times \text{acceleration} = m \frac{dv}{dt} = q \mathbf{v} \times \mathbf{B} \tag{2.1}
\]

(ignoring electric field contribution) where \( q \) and \( \mathbf{v} \) are ionic charge and velocity, and the Lorentz force is perpendicular to the both the ion motion and the magnetic field. The magnetic field causes the ion (traveling at constant speed) to bend in a circular path, of
radius, $r$ (Figure 2.2). Because angular acceleration is $\frac{dv}{dt} = \frac{v_{xy}^2}{r}$, substitution into equation 2.1 affords equation 2.2

$$\frac{m v_{xy}^2}{r} = q v_{xy} B_0$$

(2.2)

and provides a relationship between mass, charge, and the magnetic field. Ion velocity in the xy plane is defined as $v_{xy} = \sqrt{v_x^2 + v_y^2}$. Angular velocity, $\omega$ (in rad/s), about the z-axis can be expressed as $\omega = \frac{v_{xy}}{r}$ and provides (with substitution) the conventional form of the cyclotron equation,

$$\omega_c = \frac{q B_0}{m}$$

(2.3)

where $\omega_c$ is the (unperturbed) cyclotron frequency. Stated in a second way,

$$v_c = \frac{\omega_c}{2\pi} = \frac{1.535611 \times 10^7 B_0}{m/z}$$

(2.4)

where $v_c$ is the cyclotron frequency in Hertz, $B_0$ is the magnetic field in Tesla, $m$ is the mass of the ion in Dalton, and $z$ in multiples of elementary charge.

**Figure 2.2** A charged particle in a magnetic field. The magnetic field bends the ion path into a circle, known as ion cyclotron motion. Reproduced with permission from reference 57.
Several useful attributes arise from equation 2.4. First, ion cyclotron frequency is inversely proportional to ion mass to charge, therefore, cyclotron frequency measurement can yield $m/z$ values directly. Second, all ions of a given mass-to-charge ratio have the same ICR frequency, independent of their velocity. And third, ICR frequencies for ions typically analyzed by today’s available magnetic fields and mass ranges ($\sim$7-15 T, $50 > m/z > 10,000$), span from a few kHz to a few MHz--a range well-situated to commercial electronics.\(^5^7\)

**Excitation and Detection**

The usefulness of ion cyclotron motion by itself is non-existent. Therefore, cyclotron motion requires **excitation**. Excitation in FT-ICR MS is useful for (a) accelerating ions coherently into a larger (detectable) orbital radius, (b) increasing ion kinetic energy above the threshold for ion dissociation, and (c) acceleration of ions to a cyclotron radius larger than the radius of the ion trap so that ions are removed from the instrument.\(^5^7\)

The post-excitation radius, $r$, is defined by equation 2.5 as

$$r = \frac{V_{p-p} T_{excite}}{2dB_0}$$

(2.5)

The post excitation radius is independent of $m/z$, therefore all ions of a defined $m/z$ range can be excited to the same ICR radius.\(^5^7\) This results in no mass discrimination from the excitation event. ICR signal also increases linearly with ion cyclotron post-excitation radius.\(^5^7\)

Broadband ICR excitation is accomplished experimentally with frequency-sweep ("chirp") excitation. This form of excitation is useful for excitation of a relatively flat magnitude over a broad frequency range and is accomplish by a relatively low excitation voltage.\(^5^7\)

Differential current generated by the coherent ion packet (after excitation to the defined orbital radius) is measured by two oppositely opposed detection plates. This signal is passed through a differential preamplifier and is used to generate the time domain signal of the ions. The time domain signal can be mathematically Fourier
transformed into the frequency domain which in turn can be calibrated using equation 2.6

\[ \frac{m}{z} = \frac{A}{v} + \frac{B}{v^2} \]  

(2.6)

into the mass spectrum as a function of \( m/z \) (mass to charge) ratio.

**Ionization Techniques**

**Electrospray ionization (ESI)**

Petroleum composition, as discussed in chapter one, is largely (non-polar) hydrocarbon, but the small percentage of polar compounds, e.g. those containing heteroatoms, are often times the ones most responsible of refinery complications. The application of the ESI technique to selectively ionize these compounds in crude was first pointed out by the ESI inventor himself, John Fenn, who noted the remarkable complexity of ESI generated ions detected by triple quadrupole mass spectrometry.\(^{58}\)

ESI is known as a “soft” ionization technique, meaning that it doesn’t fragment or alter the molecular compound during the ionization process. The technique easily transfers liquid analyte molecules into the gas phase at atmospheric pressure. Volatile solvent is used to dissolve the analyte molecules (at low concentrations).\(^{59}\) This solution is then pumped through a small diameter capillary aligned at the mass spectrometer inlet. At the end of the capillary a high voltage (positive or negative, depending on the charged analyte) is applied to produce an electric-field gradient that is necessary to create charge separation. Ions of similar charge to the applied voltage pass through to the end of the capillary. The solution is ejected from the tip of the capillary in the “Taylor cone” formation which creates ionic droplets.\(^{60}\) Desolvation occurs as they move through the atmosphere toward the mass spectrometer inlet. An oppositely charged electrode at the inlet aids in this movement. The desolvation process causes ions of similar charge to be brought in close proximity to one another. Once their Coulombic potential is reached, the droplet breaks apart into smaller droplets.\(^{60}\) This process repeats over and over until distinct individual ions enter the mass spectrometer ready for mass analysis. The ESI process is summarized in **Figure 2.3**.
The ESI generation of quasi-molecular ions ([M+H]⁺ or [M-H]⁻) is advantageous because it allows for multiple charging of the molecule (if molecular structure permits) thus making it possible to detect high molecular weight species at (potentially) much lower m/z (mass to charge ratio) than that of the molecular compound. Additionally, it enables production of ions at atmospheric pressure which eliminates the need to break instrumentation vacuum between sample analysis. This, in turn, leads to a higher throughput of samples.

Figure 2.3 Schematic of the positive mode electrospray ionization process. Reproduced with permission from reference 60.

Reversal of polarity on the ESI source voltages allows for the selective ionization of either acidic (negative mode ESI) or basic (positive mode ESI) species. Figure 2.4 demonstrates some of the most common functional groups ionized by the ESI method (for each mode). The functional groups also represent some of the most commonly ionized compounds in crude oils.
Derivatized Electrospray Ionization

Typical electrospray ionization conditions aid in “complete” solution phase deprotonation or protonation of analyte through the use of a weak base or acid. Under these normal operating conditions, ammonium hydroxide (NH₄OH) is used to deprotonate acidic compounds, while formic acid is used to protonate basic species. The use of weak base or weak acid limits ionization to only the MOST acidic or basic species in a given sample. Improved ionization efficiency can be accomplished if one considers the solution phase characteristics of the petroleum compounds.

Tetramethylammonium Hydroxide. If one considers solution phase pKₐ values for most petro-molecules (Table 2.1), it becomes clear that ammonium hydroxide is too weak to deprotonate most of these functionalities. Tetramethylammonium hydroxide (TMAH) is a very strong base. TMAH has excellent solubility in both aqueous and organic medias and therefore leads itself as a strong candidate as a solution modifier for use in ESI. Employing TMAH as a strong base for ESI solution deprotonation, has shown dramatic improvement in achievable compositional coverage for petroleum analysis. It is particularly effective when the molecule does not give up a proton easily (high pKₐ). Under normal ESI conditions the most acidic, those containing carboxylic acids

Figure 2.4. Representative polar species (from crude oil) ionizable by the defined ESI mode. All structures have alkyl side chains of variable length hanging off of the ring structures.
acid, almost universally outcompete all other compounds in the ESI process and therefore are usually the most abundant class of compounds observed in negative ion ESI. The use of TMAH “evens out” this competition and makes less acidic compounds more easily ionized. More uniform ionization is therefore achieved. TMAH used in the electrospray ionization of petroleum also allows for some non-polar compound speciation of sulfur compounds and hydrocarbons. In addition, studies have shown that ionized compounds are very similar among common classes in samples ionized by both negative mode ESI with NH$_4$OH and TMAH modifiers.

**Table 2.1. Acid Dissociation Constants for Some Common Organic Compounds Classes.**

<table>
<thead>
<tr>
<th>Compounds Class</th>
<th>Solution Phase pK$_a$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylic Acids</td>
<td>4-5</td>
</tr>
<tr>
<td>Thiophenols</td>
<td>6.5</td>
</tr>
<tr>
<td>Phenols</td>
<td>~9</td>
</tr>
<tr>
<td>Mercaptans</td>
<td>~11</td>
</tr>
<tr>
<td>Cyclopentadiene</td>
<td>15</td>
</tr>
<tr>
<td>Carbazole</td>
<td>17.6</td>
</tr>
<tr>
<td>Indene</td>
<td>21</td>
</tr>
<tr>
<td>Fluorene</td>
<td>23</td>
</tr>
</tbody>
</table>

Silver Triflate Cationization. The use of metal complexation reactions in positive mode ESI has proven helpful for obtaining broader chemical speciation of petroleum compounds. Simple addition of silver triflate to solution phase analyte samples at 3x the concentration of the analyte solution enables quick reaction of the silver (Ag) metal with various electron sources, generating a very stable metal complex [M + Ag]$^+$. The reaction is driven by the stability and non-nucleophilicity of triflate anion, enabling the electron deficient metal to react with available pi, sigma, and n electrons. In petroleum these electron sources are heteroatom containing compounds. The most stable complexes are formed with sulfur, nitrogen, and hydrocarbon compounds. The least stable complexes are formed with oxygen species. The use of this derivatization is beneficial in several ways, the least of which are the rapid reaction times, solution phase addition, and lack of need for prolonged sample prep or derivatization schemes.
Data Manipulation and Interpretation

Successful ionization, excitation, and detection of analyte ions through electrospray ionization (conventional or derivatized) coupled to mass spectrometry ultimately results in a mass spectrum for a given sample. A single FT-ICR mass spectrum of petroleum (at 9.4T) contains on average 25,000-30,000 peaks (Figure 2.5a). This extensive amount of information requires data sorting techniques and useful display graphics to aid in data interpretation. Internal mass calibration is carried out using homologous CH₂ series (see below). Kendrick analysis generates homologous series based on repeating mass units of 14.01565 Da (CH₂), by rescaling the mass spectrum from IUPAC mass to the “Kendrick” mass scale:\(^6\)

\[
\text{Kendrick mass} = \text{IUPAC mass} \times \left(\frac{14}{14.01565}\right) \quad (2.7)
\]

![Mass Spectrum](image)

**Figure 2.5.** Overview of the process of data processing. Assigned peaks from the acquired (a) mass spectrum are (b) Kendrick sorted for calibration and interpretation purposes. Comparative information is presented in the (c) class composition graph and each individual class generates an (d) iso-abundance color contour plot to aid in visual representation of the alkylation and aromaticity trends in the given class.

This calculation converts the mass of CH₂ from 14.01565 to exactly 14.00000, allowing for the visualizing a series of compounds of the same class (same heteroatom content)
and double bond equivalents (e.g. DBE; calculated as the number of rings plus double bonds), differing only by the extent of alkylation. All members of the same class and DBE will have the same Kendrick mass defect:

\[
\text{Kendrick mass defect (KMD)} = \left( \text{nominal Kendrick mass} - \text{exact Kendrick mass} \right)
\]

This sorting technique enables grouping of identified series into compound classes (Figure 2.5b). Generation of the “class graph” gives a quick overview of the sample’s chemical composition and allows for comparison of heteroatom content among samples (Figure 2.5c). Intra-class trends can be examined to understand compound characteristics. Double bond equivalents (DBE) is calculated as

\[
DBE = C - \frac{H}{2} + \frac{N}{2} + 1
\]

for a given formula of \( C_nH_mN_rO_sS_t \). Combining DBE and carbon number information allows for visualization of data in iso-abundance color contour plots. Plotted as DBE versus carbon number (a measure of the molecule’s alkylation) versus relative abundance of the detected mass spectral peak, the color contour plots are ideal for compact visualization and easily demonstrate compositional differences (Figure 2.5d).
CHAPTER THREE

CHARACTERIZATION OF SEC FRACTIONATED ATHABASCA BITUMEN ASPHALTENES BY ULTRAHIGH RESOLUTION MASS

Summary

Chapter three details Athabasca asphaltenes that have been separated by size exclusion chromatography (SEC) and mass analyzed by low- and high-resolution mass spectrometry methods. SEC pre-fractionation produces two distinct types of asphaltene compounds, graphitic and oily. The early eluting, graphitic, samples produce mass spectrometric results in low resolution instrumentation but not in high resolution instrumentation. Later eluting, oily, fractions produce mass spectra in both instruments. Additionally, the two fractions fragment/dissociate under tandem mass spectrometry conditions with very different results. The observed fragmentation/dissociation trends are discussed in terms of possible structural implications. Molecular weight distributions are consistent with the SEC separation method. Monomer molecular weight distributions decrease in molecular mass as a function of elution time. Alkylation and aromaticity trends show that nitrogen containing species increase in abundance as a function of increased elution time (decreasing molecular weight); while oxygen containing species decrease as a function of increased elution time (decreasing molecular weight).

Introduction

Size exclusion chromatography (SEC) has been used in this study as a method of fractionation of an Athabasca bitumen asphaltene. The SEC process separates molecules based on size (primarily). Large molecules are too big to partition into and out of the stationary phase of the SEC column and therefore these species elute relatively quick. Smaller molecules partake in the partitioning process and spend more time in the column as they travel into and out of the pore space, subsequently causing
later elusion times. The SEC technique produces a molecular weight profile of the compounds contained in the sample as a function of decreasing molecular weight.

SEC has long been a popular technique for obtaining crude oil, bitumen, coal, and asphaltene molecular weight. Yet, this technique is one that is often scrutinized for its functional and operation problems. Specifically, SEC data has reported broad molecular weight (3 to mega-Dalton) ranges and high monomeric asphaltenic compounds. These results are often attributed to solvents and elutes that cause asphaltene species to aggregate and flocculate. Critics contend that actual asphaltene species are not examined, but rather aggregates and flocs, data interpretations are often in error. Here we describe mass spectrometric results of the SEC eluents with both high and low resolution detection methods. The findings offer a unique examination of the fragmentation/dissociation, heteroatom composition, aromaticity, and alkylation trends not previously reported elsewhere.

**Experimental Methods**

**Sample Preparation**

Size exclusion separations were performed on isolated Athabasca asphaltenes with Bio-beads S-X1 (Bio-Rad) beads suspended in tetrahydrofuran. These beads are reported to have a molecular weight separation range from 600-14000 g/mol. Two series-connected 4 foot columns (volume of ~580 mL each) were connected in series and washed with three bed volumes of chloroform (void volume was _190 mL). The pump flow rate was set to 0.7 mL/min or all runs (SX-1 beads can withstand pressures up to 100 psi). It was found that the elution rate was not constant for all samples and so fraction volumes were measured at regular intervals to be able to standardize elution profiles by volume rather than by time. For each run, approximately 2 g of asphaltene sample was dissolved in 5 mL of chloroform and was sonicated in a bath sonicator for at least 1 h to homogenize. Fractions were collected in 20-mL test tubes using an LKB fraction collector. Fractions were dried under nitrogen in a TurboVap evaporator with water bath temperature at 45 °C. Fractions were then put in a vacuum oven at 45 °C overnight before final weights were measured. The sample were labeled alphabetically with “A” representing the first fraction eluting from the SEC column.
The dried samples were diluted to 1 mg/mL in 50:50 toluene:methanol and analyzed without additional purification. Samples selected for positive ESI were spiked with 1% formic acid (by volume), and samples used for negative ESI were spiked with 1% ammonium hydroxide (by volume). This procedure ensures efficient protonation/deprotonation of the ions, respectively. All solvents were HPLC-grade, obtained from Fisher Scientific (Pittsburgh, PA).

Instrumentation

Samples were analyzed with either a custom-built 9.4 T 22 cm diameter bore (Oxford Corp., Oxford Mead, UK) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer or a hybrid LTQ FT-ICR 14.5 T 104 mm bore (ThermoFisher, Bremen, Germany) mass spectrometer. Samples were introduced via a micro-electrospray source at a flow rate of 0.5 µL/min. A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data. Low-resolution mass spectrometry (linear ion trap) provided independent verification of molecular weight distributions and collision activated dissociation (CAD) tandem mass spectrometry of the samples. Time-domain data sets were co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit m/z separation between mass spectral peaks corresponding to the $^{12}\text{C}$ and $^{13}\text{C}^{12}\text{C}_{c-1}$ isotope for each elemental composition.

Results and Discussion

Negative Ion Electrospray Ionization for the Examination of Acidic Species

Visual examination of the eight dried SEC fractions yields two distinct crystal-like structures. The early eluting fractions, referred to as samples A-D throughout, are graphitic, and dull in appearance and are composed of large granular crystals. The latter eluting fractions, referred to as samples E-H, are oilier in appearance, with a glassier finish and form a fine powder. The two different types of samples produce two very different types of response to mass spectrometry results.
Electrospray ionization is a soft ionization technique that produces molecular ions thru protonation or deprotonation of the molecular compound. When coupled with mass spectrometry detection methods, the molecular ions are detected by their mass to charge ratios. The (-) ESI process probes the acidic species contained within a crude oil by deprotonating the molecular compound, enabling the detection of the deprotonated molecular ion, [M-H]. The low-resolution mass spectra of Samples A-D are shown in Figure 3.1. Data collection was challenged by poor solubility of the early eluting compounds. Upon addition of polar solvents, (methanol with either acetic acid or ammonium hydroxide) the asphaltenes instantaneously precipitate from solution. The use of other polar organic solvents, such as chloroform, also resulted in precipitation. As a result, prior to ionization the samples had to be centrifuged for removal of the precipitate from solution. The mass distributions for fractions A-D are broad and tail out to very high molecular weights (2 kDa in most cases). Extensively repeated efforts to collect spectra in high resolution instrumentation have proven futile. Ion lifetime may hamper ion detection in high resolution mode, and/or ion populations in the ICR cell may be too small for detectable signal.

![Figure 3.1. Low resolution ion trap mass spectra of the early eluting fractions obtained from the SEC separation. Variations in the molecular weight distributions are described in the text.](image-url)
Fractions A and B appear nearly identical with a molecular weight distribution (MWD) centered at m/z 700 (Figure 3.1). A small amount of that same distribution can be identified in fraction C although the dominant distribution of fraction C is centered on 1.7 kDa. The lower molecular weight distribution trails from sample to sample because these compounds apparently are not easily separated via the SEC mechanism. Previous publications have addressed the SEC technique, and while the primary separation mechanism is size, other structural and compositional elements of the sample, such as shape and polarity may affect the performance of the SEC technique.

![Mass spectra of SEC fraction A](image.png)

**Figure 3.2** Tandem mass analysis of SEC fraction A. The broadband mass spectra shows a MWD centered at 650-700 Da. Dissociation of compounds at both high (b) and low m/z (c) values produce secondary ions primarily adjacent to the mass isolation window with little to no elevation of the baseline in the lower mass regions of the mass spectra, suggesting that the dissociated compounds are monomeric in nature.

Samples C and D are composed of very high molecular weight compounds. The observed SEC (size) partitioning mechanism appears to begin within fraction C (Figure 1). From this point forward, monomeric MWDs decrease in molecular weight with increasing elution time (see explanation below). Although repeated effort was given, we were never able to obtain high resolution mass spectra of these early eluting (graphitic)
fractions. Therefore, we are unable to speak to any compositional differences that cause these observed similarities to occur from one SEC fraction to the next.

**Figure 3.2** shows the (-) ESI (a) broadband and (b) MS\(^2\) product ion spectra of sample A from precursors ions of \(m/z\) 1900 ± 50 Da and (c) \(m/z\) 800 ± 50 Da. In the MS\(^2\) experiments, precursor ions are isolated from broadband ion population and subjected to low-energy CAD at 15% NCE (~0.6 V). The isolated mass window is depicted with a rectangular shaped box. Smith, *et al.*\(^{77}\) demonstrated that collision energies in this range were insufficient at producing fragmentation. Thus, product ions produced at this collision energy arise from the breaking of non-covalent interactions or aggregation. Interestingly, sample A (Figure 2b-c) yields few product ions for both high and low mass isolation windows. The positioning of the product ions relative to the isolation window corresponds to small neutral mass (liable functional groups) loss from the precursor ions. The absence of fragmentation reveals monomeric compounds. This trend also holds true for fraction B.

![Dissociation Profile](image)

**Figure 3.3.** Selected mass spectra from the dissociation profile of SEC fraction D ionized by negative ion ESI. A mass isolation window of \(m/z\) 1400 ± 50 Da is subjected to incremental increases of the dissociation energy inside the ion trap. The higher energies produce fragments of lower \(m/z\) values that lie next to the mass isolation window, an artifact of small neutral mass loss.
Sample D appears to be the first fraction of SEC fractionated asphaltene to contain a single MWD that has been separated by the SEC mechanism. The ions between \( m/z \) 700-2000, centered at \( m/z \) 1300, were subject to MS\(^n\) experiments as previously described in the above paragraphs. Incremental increasing of the CAD energy permits the examination of what collision energies, if any; dissociate/fragment compounds contained in sample D. Figure 3.3 shows highlighted spectra from the dissociation profile. Precursor ions of \( m/z \) 1400 ± 50 Da are isolated from broadband ion populations and subjected to an incremental increase of collision energy over time. The onset of dissociation begins at 15% NCE (~0.6 V) (Figure 3.3b). Figure 3.3c shows the ions subjected to dissociation energies of 30% NCE (~1.2 V); the loss of compounds within the isolation window is significant at this point. Again, secondary ions formed are primarily found adjacent to the isolation window, an indicator of small neutral mass loss. Examination of lower \( m/z \) fragments yields an increase in the baseline levels but no apparent emergence of a lower molecular weight distribution. Increasing NCE levels to extreme collision conditions (NCE = 75% or ~2.7 V) continues to produce a large abundance of secondary ions adjacent to the isolation window (Figure 3.3d). The lower \( m/z \) baseline level continues to be elevated but the emergence of a lower molecular weight distribution is not observed. The absence of fragments at low-energy dissociation levels confirms monomeric molecular weight compounds. To date, these isolated monomeric species are the highest molecular weight monomeric asphaltenes detected by mass spectrometry.

The electrospray ionization method is very effective for the ionization of the later-eluting fractions E-H. Figure 3.4 shows the low resolution (-) ESI LTQ mass spectra for these samples. Multimodal distributions are observed in all samples in both modes. We suspected multimer formation and again subjected the oily fractions to CAD MS\(^n\) experiments.

Figure 3.5a shows the presence of trimers and dimers due to aggregation of lower molecular weight ions in sample H. The multimers are hetero- rather than homo-adducts, because a narrow isolated mass distribution of precursor ions leads to a broad distribution of product ion masses. Isolation of precursor ions (\( m/z \) 1650 ± 50 Da) produces two lower \( m/z \) MWD (Figure 3.5b). Subsequent isolation of the middle
distribution produced from Figure 5b (m/z 1100 ± 50 Da) also generates a lower m/z MWD centered at m/z ~550 (Figure 3.5c). A third MS² experiment on m/z 550 ± 50 Da does not yield product ions of lower m/z (Figure 3.5d), but only produces an area of fragments adjacent to the mass isolation window; equating to small neutral mass loss.

**Figure 3.4.** Negative ion ESI ion trap mass spectra of the later eluting SEC fractions. The observed multimodal distributions are caused by multimerization of the acidic compounds identified inside the samples.

MSⁿ at NCE of 15% (~0.6 V), a value previously shown to dissociate aggregates but not fragment compounds, has been conducted on each spectra in Figure 3.4. All spectra produced fragmentation patterns that reflect aggregation as the cause for the observation of these high molecular weight species. The observed fragmentation patterns of these later-eluting fractions is in stark contrast to those of the early eluting fractions (depicted in Figure 3.3), thus, it can be inferred that these compounds are structurally different. Additionally, the high molecular weights observed in these late eluting fractions arise from aggregation of low molecular weight monomeric species.
Figure 3.5. Broadband (a) and MS$^2$ (b-d) spectra obtained by negative ESI ionization of SEC fraction H. The tandem mass spectra show how isolation and subsequent dissociation of the compounds contained in the isolation window produce molecular weight distributions of lower m/z corresponding to multimeric aggregates.

Figure 3.6. High resolution FT-ICR MS spectra collected from the later eluting fractions of the SEC separation. The MWD of each sample aligns with the monomeric MWD obtained by MS$^n$ experiments of ion trap instrumentation. The high resolution and high mass accuracy capabilities allow for unique elemental assignment of the peaks observed in the mass spectra.
Low mass resolution limits the information obtainable from complex crude oil samples. Thus, high-resolution mass spectrometry methods can be employed to allow for alkylation, heteroatom content and aromaticity speciation. The high resolution and high mass accuracy abilities of the FT-ICR technique enable the assignment of unique elemental assignments. The high resolution mass spectra of the later-eluting fractions are shown in Figure 3.6. The molecular weight distributions of the spectra align with the monomer molecular weight distributions of the low resolution spectra in Figure 3.4. It is important to note at this point that the monomer molecular weights decrease as a function of increased elution time from the SEC column (a corresponding shift to lower molecular weight).

The assignment of each peak’s elemental composition based on mass accuracies of ≤ 1ppm yields the heteroatom content (such as nitrogen (N), oxygen (O), and sulfur (S)) of the compounds. Sorting the compounds by their heteroatom content produces a class distribution. Numerous heteroatom species are detected across the sample set, consequently, in order to simplify the presented data, a selected class graph is shown in Figure 3.7. These species were selected for presentation because they are some of the most common heteroatom classes detected in crude oil/bitumen analysis by FT-ICR MS. The class distributions are plotted as a function of % relative abundance of the heteroatom class contained within a sample versus that particular heteroatom class. The graph shows that nitrogen containing species increase in % relative abundance as a function of later-eluting fraction; while, oxygen containing species decrease in % relative abundance as a function of later-eluting fraction.

Alkylation and aromaticity trends within each heteroatom class can be examined to provide insight into the structural properties of the compounds. Figure 3.8 provides this analysis in the form of iso-abundance color contour plots. The contour plots are plotted at a function of DBE (double bond equivalents) versus carbon number. The DBE is a measure of aromaticity and is calculated as the number of rings and/or double bonds in a compound. The alkylation is measured as the number of carbons contained within the compound. The z-axis of the contour plots is color-coded to reflect the % relative abundance of the compound assigned. Figure 3.8 shows the trends of the acidic N\textsubscript{1} species across the later-eluting fractions. The compounds detect in all
Figure 3.7. Selected class distributions of the acidic compounds identified in each SEC fraction. The nitrogen containing species appear to increase in % relative abundance with increased elution time; while, oxygen containing species decrease in % relative abundance with increased elution time.

fractions are comprised of DBE values ≥ 10. The DBE range of the species identified does not shift from sample to sample. The differences in the plots arise in alkylation. The carbon number of the species detected decreases a function of later-eluting sample (as a function of lower molecular weight). The lack of change in DBE value and shift to lower carbon number suggest that the aromatic core of the asphaltene samples remains largely unchanged, but the alkyl substituents bound to this core decrease in length as a function of SEC fraction. Additionally, the % relative abundance (or color intensity) increases across later-elution samples. This result is expected as it is observed in the class distribution graph. All other N-containing classes observed in the SEC fractions also show this overall trend.

Figure 3.9 depicts the iso-abundance color contour plots for the O$_2$ class of compounds assigned in the spectra from Figure 3.6. The DBE values of the O$_2$ species range from 1 to 25 across all samples. There are minor shifts in DBE values of the most abundant species. Samples E & F are comprised of highly concentrated aromatic compounds at DBE = 4, while samples G & H are most abundant at DBE = 6. The
alkylation of the compounds decreases as a function of SEC fraction. Sample E contains C_{38-68}, while sample H is comprised of C_{25-45}. The % relative abundance of the oxygen species also decreases as a function of SEC fraction (depicted by the loss of color intensity within each plot).

**Positive-Ion Electrospray Ionization for the Examination of Basic Species**

*Figure 3.10* displays the (+) ESI low-resolution mass spectra for the later elution fractions. Basic species are ionized by protonation of the molecular compounds and detection of the protonated molecular ion, [M+H]^+. The (+) ESI spectra are hindered with a polymer contamination that arises from the SEC separation process itself. We conducted MS^n characterization of the compounds just as in the negative mode ESI experiments and again concluded that multimerization is observed in all species (results not shown). After obtaining the monomeric molecular weight distributions, high resolution spectra were also collected (not shown). Unique elemental compositions were assigned to peaks and sorted based on the assignments into classes to produce the heteroatom class graph in *Figure 3.11*. Selected heteroatom classes are again highlighted for simplicity and those classes represented are commonly observed in petroleum mass spectrometry analysis. The trends observed within the selected class graph of *Figure 3.11* are inconclusive. There is no overall underlying trend of the heteroatom composition as observed in the (-) ESI data. The percent relative abundances vary from sample to sample.

In order to examine alkylation and aromaticity trends, we again examine the iso-abundance color contour plots within a single heteroatom class. *Figure 3.12* shows these plots for the basic N₁ class. The same alkylation trends are observed across all classes, in that, the carbon number decreases as a function of elution time. The species also are comprised of approximately the same DBE (5 to 28 double bonds and/or rings) or aromatic core across all fractions, again suggesting a molecular compound comprised of a uniform aromatic core from which attached alkyl carbons decrease in length with increased elution time. These same alkylation and aromatic trends are observed across all basic classes observed by positive ESI FT-ICR MS.
Figure 3.8. Iso-abundance color contour plots for the acidic $N_1$ species identified in the SEC fractions. The aromaticity (DBE value) remains unchanged as a function of elution time, but the alkylation (carbon number) decreases with increased elution time. Thus, the SEC mechanism appears to successfully separate the Athabasca asphaltenes by size.

Figure 3.9. Iso-abundance color contour plots obtained from the acidic $O_2$ class of compounds identified in each SEC fraction. The DBE values (measure of how aromatic the compounds are) remain relatively unchanged as a function of elution time. Carbon number decreases as a function of increased elution time suggesting that the compounds in the SEC fractions are comprised of similar aromatic core structures that vary only in the degree of alkylation bonded to the core structure.
Figure 3.10. Low resolution positive ESI mass spectra collected for the later eluting samples of the SEC separation. The spectra exhibit the same multimodal mass distributions observed by negative ion ESI. Tandem mass spectrometry experiments show these compounds to be multimeric aggregates. The polymer contamination observed arises from the SEC separation.

Figure 3.11. Class distribution graph for the basic species detected by FT-ICR MS. There is no observable trend across the selected heteroatom classes with respect to the % relative abundance of the species detected.
Figure 3.12. Iso-abundance color contour plots for the basic $N_1$ species identified by FT-ICR MS. The compounds detected show an overall decrease in the number of carbon atoms per compound with increased elution time while the aromaticity of the compounds remains relatively unchanged across the separation process.

Conclusions

FT-ICR mass spectral results suggest that there are molecular weight dependent chemical differences in the SEC isolated oily asphaltenes. It is observed that aggregation and multimerization of the asphaltene compounds is prevalent at the analyzed concentrations. The oily asphaltenes demonstrate a very different response to the electrospray process and fragmentation studies, suggesting that the compounds contained in the graphitic fractions are compositionally and structurally different from those of the oily fractions.
CHAPTER FOUR

ASPHALTENE MOLECULAR STRUCTURE DETERMINATION
BY LOW- AND HIGH-RESOLUTION MASS SPECTROMETRY

Summary

Crude oil and bitumen asphaltenes have been characterized extensively by bulk properties. Even so, uncertainties about the molecular structures that comprise the asphaltene fraction of bitumen and crude oil still remain. In chapter four, two asphaltene structural models, archipelago vs. island, are examined through a comprehensive model compound study by tandem mass spectrometry ($MS^n$). Model compounds were selected on the basis of known functionalities that exist in bitumen and crude oil asphaltenes, as well as, aromatic ring geometry. Fragmentation patterns observed from tandem MS of model compound studies are compared to fragmentation patterns of size-exclusion chromatography (SEC) fractionated Athabasca asphaltenes. This report, to our best knowledge, provides some of the first evidence for asphaltene molecular structure determination via mass spectrometry.

Introduction

The chemical complexity of asphaltenes, the solubility defined fraction of petroleum that is $n$-heptane insoluble but toluene soluble, makes the compounds extremely difficult to analyze via conventional analytical methods. Their molecular structures are composed of condensed aromatic moieties with variable (length and substitution) alkyl chains and contain heteroatoms (N, S, and O) and sometimes, trace metals (e.g. V, Ni, and Fe). Undesirable refinery characteristics, such as, precipitation, aggregation (in toluene at concentrations as low as 0.1 mg/mL), and/or flocculation and deposition can cause clogging/pipe flow disruption, which in turn, reduces the amount of recoverable oil product. Asphaltenes are also surface active, meaning they accumulate at the interface between water and oil, and the compounds may contribute
to oil/water emulsion stability.\textsuperscript{20, 27-29, 80, 81} Thus, the asphaltene content of a crude oil can have a huge economic impact on a petroleum refinery operation’s bottom line.

Asphaltene characterization is often determined through studies based on average (bulk) properties. Asphaltene molecular weight distributions in the 300-2000 Dalton (Da) range, with a mean mass between 700-800 Da, have been experimentally determined by various analytical techniques, such as field ionization mass spectrometry (FIMS), fluorescence correlation spectroscopy (FCS), and time resolved fluorescence depolarization (TRFD).\textsuperscript{6, 18, 23, 25, 26, 82, 83} Direct measurements asphaltenic material by combustion elemental analysis of asphaltenes has determined atomic H/C ratios of 1.0-1.2 (wt %).\textsuperscript{23, 84}

Although research to date has provided much insightful information about asphaltene characterization, uncertainties remain in the area of asphaltene molecular structure. Two asphaltene structural models have been proposed: the archipelago model, where aromatic cores are bridged together by alkyl linkages; and the island model, that depicts asphaltene structure like that of your hand.\textsuperscript{18, 19} The aromatic core exists as a single fused ring system (like your palm) and alkyl side chains connect to the core (like your fingers).\textsuperscript{18, 19}

The archipelago model, first proposed by Speight and Strausz, using experimental observation from pyrolytic and selective oxidation studies.\textsuperscript{6, 25, 85-90} They argued that a structure like that depicted in Table 4.1A, could easily be refined to yield a wide range of petroleum products.\textsuperscript{83} In contrast, they also suggested that a molecular structure like that in Table 4.1E could not “account for the average amount of volatile product evolution under the pyrolysis conditions;”\textsuperscript{83} but instead would only produce light end petroleum (through cleavage of the small alkyl side chains).

The island model was first proposed by Yen.\textsuperscript{91} It depicts asphaltene molecules as highly condensed aromatic species. Although highly condensed, their solubility in toluene is explained by the addition of alkyl substituents around the aromatic ring periphery. Recently, there has been a large amount of evidence published that supports the island model.\textsuperscript{92} Primarily, spectroscopic analytical techniques have been used. Mullins frequently references two reasons for the existence of island structures. Initial observations of asphaltenes reveal that the molecules absorb light.\textsuperscript{19, 26, 93}
Asphaltenes are deeply colored in the visible and extend into the near IR (infrared) spectral regions. Small aromatic ring systems, even those with heteroatoms are non-absorptive or of very low absorptivity in the visible (e.g. benzene, naphthalene, anthracene). Hence, the color must arise from larger polyaromatic hydrocarbons (PAHs) that are found in the heavier crude oils and bitumens. The core structures that exist in asphaltenes are typically comprised of 4-10 PAHs.

The second factor in support of the island model, is that compounds contained in asphaltenes have had to exist on a geological time scale; and therefore must be comprised of very stable structures. In order to understand stability of the aromatic compounds, a few chemistry basics must be reviewed. There are two general classifications used to describe aromatic ring geometry: pericondensation and catacondensation. Pericondensation refers to a bridgehead carbon on an aromatic ring that is attached to 3 rings. Catacondensation refers to a bridgehead carbon that is attached to 2 or less aromatic rings. Pericondensed PAH structures are more stable than catacondensed PAH structures (comprised of the same number of rings). (An elaborate explanation on ring condensation can be reviewed in references 14 and 93).

Optical spectroscopy has also yielded electron density calculations of the molecular structure can also be used to assess (kinetic and thermodynamic) ring stability. The underlying rule is: the higher the ratio of aromatic sextet carbon to isolated double bond carbon, the more stable the aromatic ring system. Clar representations provide the best model of drawing molecular structures and depicting accurate ring electron density. A brief example entails the comparison of two simple, well known structures, benzene and naphthalene. Benzene is fully conjugated, so Clar representations allow for the structure to be drawn with an embedded circle to represent uniform electron density distribution. Naphthalene, on the other hand, shares one carbon-carbon bond between the two aromatic rings. Only one ring can be fully conjugated if this is the case, otherwise, octet rules would be violated. Thus, the correct electron density depiction is one ring with an embedded circle (like that of benzene) and the neighboring ring’s electrons that participate in double bonds are shown as isolated double bonds. Essentially, one ring in naphthalene is more stable
Table 4.1. Model compounds examined in this study. The chemical name, chemical formula, molar mass, asphaltene structural model for which they represent, and ionization mode of analysis is listed for each of the chemical structures.

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Chemical Formula</th>
<th>Exact Mass</th>
<th>Structural Model</th>
<th>Ionization Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Synthesized</td>
<td>$\text{C}<em>{46}\text{H}</em>{32}\text{N}_2$</td>
<td>612.26 Da</td>
<td>Archipelago</td>
<td>Positive Ion ESI</td>
</tr>
<tr>
<td>B: Benz[c]acridine</td>
<td>$\text{C}<em>{17}\text{H}</em>{11}\text{N}$</td>
<td>229.09 Da</td>
<td>Island</td>
<td>Positive Ion ESI</td>
</tr>
<tr>
<td>C: 7, 10 Dimethylbenz[c]acridine</td>
<td>$\text{C}<em>{19}\text{H}</em>{15}\text{N}$</td>
<td>257.12 Da</td>
<td>Island</td>
<td>Positive Ion ESI</td>
</tr>
<tr>
<td>D: 9-phenylanthracene</td>
<td>$\text{C}<em>{20}\text{H}</em>{14}$</td>
<td>254.11 Da</td>
<td>Archipelago</td>
<td>Positive Ion APPI</td>
</tr>
<tr>
<td>E: Benzo[e]pyrene</td>
<td>$\text{C}<em>{20}\text{H}</em>{12}$</td>
<td>252.09 Da</td>
<td>Island</td>
<td>Positive Ion APPI</td>
</tr>
</tbody>
</table>
Table 4.1 (continued)

F: Coronene
Chemical Formula: C_{24}H_{12}
Exact Mass: 300.09 Da
Island Model
Positive Ion APPI

G: Dibenz[a, c]anthracene
Chemical Formula: C_{22}H_{14}
Exact Mass: 278.11 Da
Island Model
Positive Ion APPI

H: Fluoroanthene
Chemical Formula: C_{16}H_{10}
Exact Mass: 202.08 Da
Island Model
Positive Ion APPI
than the other. With these two aromatic ring stability criteria in mind, the most stable PAHs are pericondensed with the highest ratio of aromatic sextet carbon to isolated double bond carbon.\textsuperscript{19}

As stated previously, all experimental measurements by all analytical techniques referenced above, rely on bulk chemical properties. Our aim is to test the proposed molecular structure models through mass spectrometry. In this paper we describe the results of tandem MS experiments on model compounds that depict the PAH systems of both the archipelago and island systems. The alkyl linkages that bridge the aromatic cores (archipelago model) should be vulnerable to collision-induced dissociation (CID). Thus, low resolution tandem mass spectrometry (MS\textsuperscript{n}) should suffice to test for the presence of archipelago structure. Island model compounds will also be examined via CID MS\textsuperscript{n} to test for possible fragmentation and ring stability. Island model compounds are highly stable and therefore, little fragmentation is expected.

High resolution mass spectrometry is employed for complex mixture analysis. Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) characteristics (resolution: \(m/\Delta m_{50\%} = 300,000\) (\(\Delta m_{50\%}\) denotes mass spectral peak width at half-height) and high mass accuracy (300 ppb for \(300 \leq m/z \leq 1000\)) make the technique capable of unambiguous elemental composition assignment (C\textsubscript{c}H\textsubscript{h}N\textsubscript{n}O\textsubscript{o}S\textsubscript{s}).\textsuperscript{79, 97, 98} FT-ICR MS allows for precursor ions present in an SEC (size exclusion chromatography)-fractioned Athabasca bitumen asphaltene to be chemically identified prior to fragmentation. Subsequent analysis of secondary ions (post-fragmentation) is compared to precursor ions to deduce fragmentation sites. Fragmentation patterns observed in the model compound study indicate structural motifs vulnerable to cleavage and we should be able to infer structural information through comparison mass spectra.

**Experimental Methods**

**Model Compounds**

Archipelago model compound (C\textsubscript{46}H\textsubscript{32}N\textsubscript{2}, 612.26 Da) was synthesized and obtained through collaboration with Dr. Murray Gray (University of Alberta; Alberta, Edmonton, Canada). Archipelago model compound was analyzed by positive ion electrospray (ESI). Island model compounds used for positive mode ESI were
synthesized and purchased from RT-Corporation (chrion.com) (Laramie, WY). Island model compounds analyzed via atmospheric pressure photoionization (APPI) were purchased from ThermoFisher (Pittsburgh, PA).

Positive mode ESI standards (archipelago and island) were serially diluted from stock to a final concentration of 0.1 mg/mL in 1:1 toluene:methanol. Formic acid was added at 0.1% (vol.) to the final solution to aid in protonation of the standards. APPI standards were serial diluted from stock to a concentration of 0.25 mg/mL in neat toluene. All solvents were HPLC-grade, obtained from ThermoFisher (Pittsburgh, PA).

**Athabasca Bitumen Asphaltenes**

Asphaltenes were first isolated from Athabasca bitumen oil sands by a modified Institute of Petroleum (IP) method. Further separation was carried out by size exclusion chromatography (SEC) as described elsewhere. SEC fractions were then diluted to a concentration of 1 mg/mL in 1:1 toluene:methanol and spiked with 0.1% formic acid to aid in protonation.

**Instrumentation**

Samples with sites for protonation were ionized by a micro-ESI source coupled to with a hybrid LTQ FT-ICR 14.5 T 104 mm bore (ThermoFisher, Bremen, Germany) mass spectrometer custom interfaced to a ThermoFisher LTQ (linear trap quadrupole) front end. Spray needle voltage was operated at 2.0-2.5 kV, provided spray stability was properly maintained. Solution flow rates were set at 0.5 µL/min.

Atmospheric pressure photoionization (APPI) was utilized for the ionization of aromatic hydrocarbon standards. The APPI source was supplied by ThermoFisher Scientific. The vaporized analyte gas stream flows orthogonally to the mass spectrometer inlet (heated metal capillary) and the krypton vacuum UV lamp. The source was mounted to the hybrid LTQ 14.5 T FT-ICR mass spectrometer. A Harvard Apparatus stainless steel syringe (8 mL) and syringe pump delivered solution to the heated nebulizer of the APPI source. Source conditions were set at: solvent flow rate 50 µL/min; the nebulizer heater was operated at 250°C with nitrogen operating as the sheath and auxiliary gases programmed to flow at 5 au and 64 au, respectively.
Dissociation profiles of all model compounds were collected by an in-house written experimental script that enabled collision-induced dissociation (CID) at dissociation energies ranging from 0-70% NCE® (normalized collision energy)\(^{103, 104}\) to yield the dissociation profiles for isolated mass segments of the broadband mass spectrum. The experimental scripts collected a single scan (comprised of 3 µscans) at each NCE® energy (increasing in units of 1) over a range of 0-70% NCE®. Ion peak area was integrated on the fly by the software. The optimum collision energy for fragmentation varies linearly with \(m/z\); therefore, ThermoFisher has devised a method that normalizes the collision energy applied to each \(m/z\) value selected for dissociation.\(^77\)

A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data.\(^{46, 48}\) Time-domain data sets were co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit \(m/z\) separation between mass spectral peaks corresponding to the \(^{12}\text{C}\) and \(^{13}\text{C}^{12}\text{C}_{c-1}\) isotope for each elemental composition. Singly charged ions identified in the mass spectra, with a relative abundance greater than 6 times the standard deviation of the baseline noise, were exported to an Excel spreadsheet. FT-ICR mass spectra were internally calibrated on double bond equivalents (DBE) series of ions containing one nitrogen atom. This heteroatom class was the most abundant class in each sample. Measured (IUPAC) masses were rescaled to Kendrick mass for data manipulation.\(^1, 57\)
Figure 4.1. Model compound dissociation profile mass spectra: (a) Positive ion ESI LTQ broadband spectrum of 0.1 mg/mL archipelago model compound in 1:1 toluene:methanol. (b) Isolation (50 Da window width) of the protonated parent by MS². Fragmentation of the alkyl linkages begins at ~15 NCE⁰ (c) and progressively increases with increased CID energy, producing the onset of fragments (m/z 215 and 398) (d-f).
Results and Discussion

The symmetrical archipelago model compound depicted in Table 4.1A is analyzed by positive ion ESI because the N-atom in the 6-membered (pyridinic) ring is easily protonated to form a positively charged ion. Although, the model compound contains two possible protonation sites, only singly charged species are observed in the mass spectra because the proton affinity of the second nitrogen atom drops significantly after the addition of the first proton. Figure 4.1 shows the spectral dissociation profile of the archipelago model compound. The broadband spectrum in Figure 4.1a reflects the presence of the singly charged ion at m/z 613.2, or the mass of the molecule plus one additional proton, [M + H]⁺.

Figure 4.1b shows the isolation of m/z 613 by tandem MS. A mass isolation window of 50 Da was arbitrarily chosen for the experiments. The mass spectrum is collected without any collisional gas addition to the ion trap. Figures 4.1c-f show fragmentation of the parent ion once collisional gas is added at various collisional energies. Two secondary ions were formed from bond cleavage in the archipelago model compound; masses 398 and 215. The onset of fragmentation begins at NCE® = 15 and progressively increases with increased NCE® energy. Isolated parent ion (m/z 613.2) is almost completely fragmented at a NCE® equal to 35%, while the presence to the two secondary ions is still prevalent.

The dissociation profile of the archipelago model compound can be summarized by Figure 4.2. The graph plots integrated peak area as a function of NCE®. As expected, the parent ion (red line) decreases in abundance with increased collision energy. Integrated areas of secondary ion (m/z 398 and 215) are also plotted as a function of NCE® (blue and green lines, respectively). The secondary ions abundance increases to a threshold limit with increased collision energy. This threshold is representative of the collision energy that not only causes fragmentation but also resonantly ejects the ions from the linear trap, thus the molecular ion is only observable up to this point. Parent ion fragmentation begins at NCE® = 15, while complete fragmentation (loss of parent ion) is achieved at ~40 NCE®. These plots prove useful because they establish the expected NCE® range for loss of alkyl-linked aromatic
structures. This range should also be applicable to molecules contained within real crude oil/bitumen asphaltene samples.

**Figure 4.3** shows the CID mass spectrum collected from the hybrid 14.5 Tesla LTQ FT-ICR MS at NCE$^{\circledast} = 25$. The tandem MS analysis was carried out as normal in the linear trap but instead of using the ion trap for detection of the secondary ions, the ions were transferred from the ion trap to the ICR cell by an additional octopole system.$^{105}$ The parent ion and fragments detected by ICR were assigned to within sub-ppm levels, allowing for unique elemental compositional assignment of each mass.$^{57, 78}$ The assignments reveal heterolytic cleavage between carbons 1 and 2 of the alkyl bridge linkage between the pyridinic nitrogen ring and the 4 fused aromatic ring (FAR) system.

**Figure 4.2.** Dissociation profiles for the archipelago model compound fragmentation over a range of 0-70 normalized collision energy (NCE$^{\circledast}$) units (incrementally examined at every 1 NCE$^{\circledast}$ unit). Each plotted value represents the integrated area over the stated mass range. As noted in Figure 4.1, dissociation begins at NCE$^{\circledast} = 15$, with complete parent loss at 40 NCE$^{\circledast}$. The dissociation profile for the archipelago model compound establishes the expected NCE$^{\circledast}$ range for loss of alkyl-linked aromatic structures.
Figure 4.3. Positive-ion LTQ FT-ICR MS spectrum acquired at 14.5 Tesla. The MS spectra were collected after ion trap isolation of an archipelago model compound (m/z 613.2). Sub-ppm mass accuracy enables explicit assignment of elemental compositions to the CID-generated fragments. The assigned elemental compositions correspond to fragmentation of the archipelago model compound along the alkyl bridge linkages.
Figure 4.4. Positive-ion ESI LTQ mass spectra of a bitumen asphaltene SEC fraction. At higher collision energies, the integrated signal magnitude of the ions immediately adjacent to the isolation mass window (i.e., 540-615 Da) corresponds to small neutral mass losses. Signal in the lower m/z region (i.e., 240-540) corresponds to large neutral mass loss (LNML).
As hypothesized, the alkyl linkages of archipelago compounds are susceptible to tandem MS\textsuperscript{n} experiment collision energies thus, fragmentation is achieved. Our focus now turned to the susceptibility of island model compounds to CID experiments. Island model compounds in Table 4.1B-C were chosen for analysis by positive mode ESI due to the available protonation sites on the pyridinic rings. The two structures only differ by alkyl side chains (two methyl units). The selection of these model compounds is also relevant for the investigation of the catacondensed ring structures under CID conditions. Additionally, the similarity of island model compounds was sought out in anticipation of possible alkyl side chain cleavage. Collision-induced dissociation tandem MS experiments were carried out just as before on the archipelago model compounds. Unexpectedly, both island model compounds exhibited fragmentation and small mass loss (results not shown). The fragmentation pattern occurred in mass units of 14 Da, or approximately the mass of a CH\textsubscript{2} unit (building blocks of organic compounds). Benzo[c]anthracene contains no alkyl substituents, as a result fragmentation and loss of CH\textsubscript{2} must be derived from bond cleavage and rearrangement. After ring opening subsequent CH\textsubscript{2} loss can be expected. The maximum fragmentation observed was loss of C\textsubscript{2}H\textsubscript{4} (28 Da). These unexpected results are attributed to the fact that both ESI island model compounds are catacondensed ring structures.

In order to gain a better understanding of ring stability of island model structures under CID MS\textsuperscript{n} conditions, the model compound series was expanded to include larger and more stable (pericondensed) structures (Table 4.1D-H). The hydrocarbon model structures are comprised of both catacondensed (Table 4.1D, G, & H) and pericondensed structures (Table 4.1E & F), as well as 5 and 6-membered ring systems. The hydrocarbon models all lack protonation sites that would allow for ion detection by conventional ESI. Therefore, in order to ionize the hydrocarbon compounds, atmospheric pressure photoionization (APPI) was employed.

Experiments conducted on the APPI catacondensed models (Table 4.1D, G, & H) showed the same small neutral mass loss fragmentation pattern (maximum loss of 28Da or the equivalent of a C\textsubscript{2}H\textsubscript{4} unit) observed in positive ion ESI for the catacondensed ring systems (Table 4.1B-C) (results not shown). The catacondensed structures produced two secondary ions that corresponded to successive loss of CH\textsubscript{2}.
after a ring opening. Benzo[e]pyrene and coronene (Table 4.1E-F), on the other hand, produced no stable or persistent secondary ions, thus these structures do not fragment under CID conditions. This experimental observation is attributed to the higher ring stability of the pericondensed structures (results not shown).

**Athabasca SEC Fractionated Asphaltenes**

Model compounds analyzed in this study were chosen to reflect known functionalities of components in asphaltenes. If this is truly the case collision-induced dissociation MS studies on real bitumen asphaltenes should produce fragmentation similar to those observed on model compounds. The observed results (significant fragmentation vs. small CH\(_2\) loss vs. no fragmentation) should allow for structural identification of components in crude oil.

Previous experiment on Athabasca bitumen has afforded us the luxury of having a SEC fractionated series of samples.\(^{106}\) This SEC experiment and our finding on both the basic and acidic species in the samples are detailed in chapter 3. From this sample set, SEC fraction F was selected as the sample for CID experiments because the protonated archipelago model compound’s (Table 4.1A) mass lies near the center of the monomer molecular weight distribution, which should ensure the best possible chance of subjecting archipelago type structure to CID. **Figure 4.4** shows the spectral dissociation profile of SEC fraction F examined by positive ion ESI LTQ MS. The broadband spectrum (**Figure 4.4a**) shows a monomer molecular weight distribution (MWD) of 450-1100 Da. The second MWD from 1100-1900 is comprised of dimers and has been verified spectrally through other studies (results not shown). The peaks that are spaced evenly throughout the monomer distribution are polymer contamination (suspected to arise from the SEC fractionation process itself). The mass isolation window (red box in **Figure 4.4b-f**) is centered at mass 640 and is 50 Da wide. The CID MS\(^n\) experiments were conducted in the same manner as all model compound studies. With minimal collision energy (**Figure 4.4c**) there is a rise in the baseline signal adjacent to the mass isolation window. As the energy progressively increases, the signal in the lower \(m/z\) region increases, but the majority of the signal (secondary ions) remains in the region directly adjacent to the mass isolation window (i.e., 540-615 Da)
(Figure 4.4c-f). This region is commonly referred to as small neutral mass loss and arises from the cleavage of liable functional groups and small mass substituents, such as CH₂ groups. The peaks that lie in the lower mass region (240-540 Da) are likely to be caused by cleavage of larger substituents originally attached to an aromatic core(s). This lower mass region is commonly called large neutral mass loss. A summary of the dissociation profile is provided in Figure 4.5.

![NCE Dissociation for SEC Fraction F](image)

**Figure 4.5.** Dissociation profile of SEC fraction F, constructed just as for Figure 5.2. The two fragment ion mass regions correspond to that of small neutral mass loss (540-615 Da) and large neutral mass loss (240-540 Da).

Elemental compositional assignment of asphaltene compounds isolated and fragmented by the CID MSⁿ experiments require the use of the FT-ICR technique. The mass analysis experiments were conducted in 4 segments; the isolation window (m/z 620-630), the region of small neutral mass loss (SNML) (m/z , and two (overlapping) regions of large neutral mass loss (LNML) (Figure 4.6). This technique provides improvement in the dynamic range of the experiment and exposes more components (peaks). All secondary ion regions were collected at NCE® = 35, in order to ensure complete fragmentation of the parent ions (if possible) as observed through the model compound studies.
The first experiment consisted of ICR data acquisition of peaks contained in the mass isolation window. Tandem MS analysis of this region at NCE\(^\circ\) = 0 should allow compound identification of the starting material prior to fragmentation. In order to avoid the polymer contamination observed in the broadband spectrum (Figure 4.4a) the mass isolation window was narrowed (10 Da instead of 50 Da) and centered at mass 625. The second experiment applied NCE\(^\circ\) = 35 to the isolated masses and detected fragments observed in the small neutral mass loss (SNML) region \((m/z\ 600-615)\). The third and fourth experiments were collected under the same conditions as the second, but the mass analysis window was shifted to observe fragments that lie in the large neutral mass loss (LNML) region \((m/z\ 470-600)\).

Determination of class, type and carbon number affords chemical insight into which species lie in the analyzed mass regions. Figure 4.7 summarizes the class distribution obtained for the compounds identified in all of the CID FT-ICR experiments.
The highest number of heteroatom-containing classes are contained within the mass isolation window containing only un-fragmented parent ions. Data trends within each heteroatom class offer more detailed analysis of the compounds contained in that particular class. Figure 4.8 shows the iso-abundance color-coded plots of DBE vs. carbon number for the N₁ class. The iso-abundance color-contour plots are graphed as DBE (double bond equivalents) vs. carbon number. DBE is calculated as the number of rings and/or double bonds contained within a given structure, and therefore, provides a measure of the molecules aromaticity. Carbon number affords a measure of the molecules alkylation. Points plotted in the same vertical line contain the same carbon number but different DBEs (number of H₂ units). The compounds detected within the mass isolation window (Figure 4.8 upper left) are comprised of 3-37 DBE and 44-47 carbons, with the highest concentration centered at carbon number 45 and DBE 13. This region of intense color is commonly referred to as the “hot spot.” Fragmentation of these compounds at NCE® = 35 produces a minor shift in carbon number (to lower values of 43-46 carbons) while the DBE remains largely unchanged (Figure 4.8 upper right). The (vertical) width of the contour plots also narrowed slightly in the SNML region. All of these observations are indicative that some fragmentation of the isolated masses occurred and was observable through this graphical method. In the LNML plots, a much broader carbon number distribution (33-44 carbons) is observed; due to the broader mass region over which the masses were collected. At the same time, DBE shifts downward and becomes most concentrated in the range of 11-15 DBE.

Surprisingly, observed changes in the plots all indicate shifts in carbon number, not DBE. Fragmentation of the archipelago structures (like that observed previously in Figure 4.1) should produce two concentrated regions of DBE and carbon number that are dramatically shifted to lower DBE and carbon number from the originally observed “hot spot” area. The lack in the presence of two “hot spots” disputes archipelago type fragmentation because cleavage of alkyl linkages between aromatic cores should produce two distinct regions. The observed fragmentation pattern most closely mimics that of catacondensed island model compounds observed by both ESI and APPI ionization methods. The DBE shift is less than 4 and the carbon number shift is less than 6 (the equivalent of a benzene (aromatic) ring). These observations lead to the
conclusion that archipelago type structures appear to be less abundant within this isolated mass region.

Figure 4.9 shows the summation of all of the data presented in Figure 4.8. Parent ions are highlighted in the light blue box. The “hot spot” centered between carbon numbers 44-47 and DBE 11-16 arise from the isolated masses (without fragmentation) and the SNML region. The large dark-gray to black region to the left of the “hot spot” region contains a smaller, less intense “hot spot” centered at carbon number 40-43 and DBE 10-15. These shifts again describe mass loss less than that of a benzene (aromatic) ring; while the presence of only one “secondary hot spot” disfavors the presence of archipelago type structures. Consequently, one must conclude that island type structures are dominant in this mass range of the asphaltene sample. Fragmentation that is observed closely mimics that of catacondensed island structures. Similar trends are also observed for all other classes analyzed (results not shown).

Figure 4.7. The class distribution of compounds identified by tandem FT-ICR MS. The most abundant class in all experiments was the $N_1$ class; thus it was used as the internal calibration series.
Figure 4.8. The iso-abundance color contour DBE vs. carbon number plots for the $N_1$ class of compounds. The fragmentation trends observed in the SNML and LNML regions closely resemble that of the catacondensed island model structures examined in the model compound portion of this study from both ionization methods.

Figure 4.9. The DBE vs. carbon number plot of all the data contained in Figure 4.8. The minor shifts to lower DBE and carbon number confirm fragmentation of the asphaltene structures; while the presence of only one secondary “hot spot” supports island model asphaltene structures.
Conclusions

This model compound based study provides evidence for archipelago model asphaltene compounds to fragment under CID MS\(^n\) conditions. Additionally, fragmentation of catacondensed island model compounds is also observed under tandem MS conditions. Pericondensed island model compounds do not fragment under tandem MS conditions. Spiking the model compounds into real bitumen asphaltenes produces fragmentation patterns most closely resembling that of catacondensed island models. For that reason, conclusions drawn from the experiments support the existence of asphaltene structures that are most similar to that of island structure, due to the fact that the overall fragmentation of bitumen asphaltenes failed to show archipelago model compound fragmentation.
CHAPTER FIVE

COMPOSITIONAL ANALYSIS OF ASPHALTENE CO-PRECIPITATES AND THEIR ROLE IN EMULSION FORMATION

Summary

It has been suggested that asphaltenes, the \textit{n}-heptane insoluble but toluene soluble fraction of crude oil/bitumen, absorb and accumulate at the emulsion water-oil interface and contribute to emulsion stability.\textsuperscript{27-29} However, straight solvent drop methods used to extract asphaltenes from the parent oil have been shown to include a small amount of maltene material, or co-precipitate.\textsuperscript{17} Chapter five details the experimental analysis of co-precipitant material, revealing that it is highly enriched in acidic species that contain sulfur and oxygen. Thus, it is thought that the co-precipitated material may also contribute to the stability of the water/oil emulsions because prior FT-ICR MS analysis of the acidic portion of interfacial material reveals that it is also enriched in specific Ox and SOx species relative to the parent crude.\textsuperscript{80} The similarity between the co-precipitate and isolated interfacial material suggest that naphthenic acids interact strongly with the asphaltenes and as a result co-precipitate with them even though naphthenic acids (by themselves) are soluble in \textit{n}-heptane.

Subsequent isolation and characterization of a pure co-precipitate fraction of the crude oil reveals significant Ox and SOx presence, similar to the observation of these species in the emulsion interfacial material. Mass spectral analysis of the co-precipitant material provides a direct comparison between the species identified in the interfacial material and those that interact with the asphaltene fraction of the crude oil.

Introduction

Upstream operational procedures introduce water into oil processing streams to increase product recovery. Water aids in the removal of water-soluble salts and facilitates transport of the crude/bitumen through the operation pipelines by lowering viscosity. Emulsion formation (water-in-oil, oil-in-water and multiple emulsions) can
occur at nearly any point throughout this process. However, downstream operations require the removal of water prior to refinery and petroleum product recovery. Production losses and the added cost associated with chemicals used to break water and oil emulsions are costly for the petroleum industry. Characterizing the surface active compounds that lie at the interface, thus far, has provided significant insight to what components potentially act as stabilizers.

Surface-active compounds exhibit surfactant-like characteristics that enable the formation and stabilization of water-in-crude oil emulsions. In the past, emulsion stability research, has focused on asphaltene stabilization of the interfacial material. Asphaltenes are a solubility-defined class of compounds that are \( n \)-heptane insoluble but toluene soluble. Asphaltenes are highly condensed aromatic compounds of high polarity (due to their heteroatom content (nitrogen (N), oxygen(O), and/or sulfur (S)). These characteristics contribute to their ability to flocculate and deposit, causing significant undesired refinery implication.

The emerging field of petroleomics aims to aid in the prediction of crude oil properties and their implications in refinery conditions. This goal is undermined by old analytical terminology and techniques. Several manuscripts have suggested that the solubility-based definition of asphaltenes (derived from SARA fractionation) is misleading and results in lots of ambiguity when defining what compounds “truly” comprise the asphaltene fraction. Klein et al. demonstrated that asphaltenes isolated by a \( n \)-heptane drop differ significantly from those asphaltenes isolated from “live” crude oil through depressurization. “Pressure-drop” asphaltenes were enriched in acidic sulfur- and oxygen- containing compounds. The \( C_7 \)-asphaltenes were enriched in more aromatic nitrogen-containing species.

Resins, the fraction of crude oil soluble in 80:20 toluene:methanol, may act as asphaltene stabilizers by absorbing at the surface of asphaltene molecules and aggregates. Neutral or basic processing water streams can cause resins to dissociate thereby, increasing their hydrophilic qualities. This increase results in the potential for these resin molecules to contribute to interfacial stability. Additionally it has been shown the emulsion stability is increased when the emulsion is derived from crude oils.
containing both the asphaltene fraction and resin fraction versus when the emulsion is formed from only the asphaltene fraction.\textsuperscript{110}

Recently the notion of asphaltene stabilization of emulsion was challenged by J. Czarnecki who suggested that “only a small fraction of the total asphaltene is involved in the emulsion stabilization.”\textsuperscript{20, 111} He and his collaborator’s research shows that emulsion interfacial materials, collected from a series of heptol diluted bitumen samples, yield mass spectral profiles that are dissimilar to that of asphaltenes, resins, or the parent bitumen.\textsuperscript{84, 111} The manuscript goes on to elaborate on observations (other than mass spectra) in support of this novel statement, and concludes that an exclusive asphaltene stabilization theory is a “gross oversimplification of the stability mechanism and the compounds involved.”

This paper details mass spectrometry data collected by ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)\textsuperscript{112} that builds on this new philosophy and provides more support for the existence of a smaller subset of compounds (not solely the asphaltene fraction) that are most responsible for the onset of emulsion formation and emulsion stability. We refer to this subset of compounds as co-precipitates, because they co-precipitate from the crude oil during the laboratory asphaltene drop (using an $n$-alkane), even though, the co-precipitates themselves are soluble in $n$-heptane.

In addition to compositional profiling, we present data on isolated interfacial layers generated from a crude oil emulsion and a pure co-precipitate (fraction) emulsion. If the co-precipitate fraction of the parent crude oil contains the compounds that are most responsible for emulsion stability, the two interfacial material compositions should produce compositional profiles detailing similar surface-active species.

**Experimental Methods**

**Crude Oil Fractionation**

We devised a modified asphaltene isolation procedure focused on the purification of the co-precipitates.\textsuperscript{99} A North American crude oil was diluted in 40:1 $n$-heptane to precipitate the asphaltene fraction. This mixture was refluxed for 1 hr. to ensure proper mixing. The flask was then left overnight (24 hr) in a dark cabinet to ensure complete
asphaltene precipitation. Following the settling period, the \( n \)-heptane mixture was filtered to collect all the precipitate, which we refer to throughout as the \textit{raw asphaltene} fraction. The \( n \)-heptane solvent (crude oil) was reduced to reconstitute the \textit{maltene} fraction (saturates, aromatics, and resins) and dried under nitrogen until constant weight was achieved.

Raw asphaltenes (co-precipitates + purified asphaltenes) remaining on the filter paper were refluxed in a 30 mL of clean \( n \)-heptane in order to collect the \textit{co-precipitate} fraction. Refluxing was performed until the solvent appeared colorless. Co-precipitates are compounds that precipitate during the \( n \)-heptane asphaltene drop, even though, they themselves are \( n \)-heptane soluble. The solvated co-precipitates were reduced (blown down) under a continuous stream of nitrogen until a constant sample weight was achieved.

The \textit{purified asphaltene} fraction remains precipitated on the filter paper during the \( n \)-heptane reflux of the co-precipitates. The filter paper (containing the purified asphaltenes) was refluxed in clean toluene (~40 mL) until the reflux solvent was colorless. All solvent was removed under a continuous stream of nitrogen until a constant sample weight was reached.

The parent crude oil emulsion interfacial layer was isolated using the experimental protocol outlined by Wu's heavy water method.\(^1\) In brief, crude oil was diluted in a 1:1 solution of heptane:toluene (heptol). A \( D_2O \)-in-(diluted) crude oil emulsion was prepared and placed on top of a deionized water (\( H_2O \)) layer. The solution was centrifuged and interfacial material located on the surface of the \( D_2O \) droplets were forced through the \( H_2O \) layer and trapped at the bottom of the centrifuge vial. The remaining crude oil (diluted in heptol), \( D_2O \), and \( H_2O \) layers were carefully removed by pipetting. The interfacial layer left on the bottom of the centrifuge vial in the form of a “wet cake,” was dried under a continuous stream of nitrogen until constant sample weight was achieved and all entrained water was removed.

**Sample Preparation and Instrumentation**

All crude oil fractions and the isolated interfacial layer were re-dissolved (after drying) for experimental analysis in toluene at 2 mg/mL. The 2 mg/mL solutions are

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diluted 1:1 with methanol to achieve a final sample concentration of 1 mg/mL in 1:1 toluene/methanol. Formic acid or ammonium hydroxide modifiers are added at 0.1 \% (volume) to solutions prepared for positive-ion/negative-ion electrospray ionization (ESI), respectively. Modifiers help achieve complete protonation/deprotonation of the bases/acids in solution. Samples at 1 mg/mL were delivered to the micro-electrospray source at a flow rate of 0.5 µL/min.\textsuperscript{44, 76}

FT-ICR MS experiments were conducted on a 9.4 T (22 cm bore diameter) custom-built magnet (Oxford Corp., Oxford Mead, UK).\textsuperscript{57} A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data.\textsuperscript{46} Time-domain data sets were co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit \textit{m/z} separation between mass spectral peaks corresponding to the $^{12}\text{C}$ and $^{13}\text{C}^{12}\text{C}_{\text{c-1}}$ isotope for each elemental composition. Isotope verification was also used to ensure proper elemental assignment.

Internal mass calibration was carried out using homologous CH\textsubscript{2} series (see below). The average root mean square (RMS) mass error for all assignments made was 0.13 ppm. Kendrick analysis generates homologous series based on repeating mass units of 14.015 65 Da (CH\textsubscript{2}), by rescaling the mass spectrum from IUPAC mass to the “Kendrick” mass scale.\textsuperscript{61}

\begin{equation}
\text{Kendrick mass} = \text{IUPAC mass} \times \frac{14}{14.01565} \quad (1)
\end{equation}

This calculation converts the mass of CH\textsubscript{2} from 14.01565 to exactly 14.00000, allowing for the visualizing a series of compounds of the same class (same heteroatom content) and type (e.g. number of rings plus double bonds), differing only by the extent of alkylation.\textsuperscript{62} All members of the same class and type will have the same Kendrick mass defect:

\begin{equation}
\text{Kendrick mass defect (KMD)} = (\text{nominal Kendrick mass} – \text{exact Kendrick mass}) \quad (2)
\end{equation}
Results and Discussion

The high mass resolving power \( m/\Delta m_{50\%} = 400,000 \), where \( m \) is molecular mass and \( \Delta m_{50\%} \) denotes mass spectral peak width at half-height) and high mass accuracy (\( \leq 300 \text{ ppb up to } \sim 800 \text{ Da} \)) characteristics of the FT-ICR technique provide unambiguous elemental composition (\( C_{c}H_{h}N_{n}O_{o}S_{s} \)) assignment for nearly all mass spectral peaks detected above the noise threshold.\(^{79, 97, 98, 113} \) Assigned elemental compositions sorted by Kendrick analysis allow for comparison of the relative abundances distributions for different heteroatom-containing classes and for the examination of alkylation and aromaticity trends contained within each heteroatom class.\(^{62} \) Figure 5.1 shows the class graph comparison for the basic species detected in the crude oil fractions, the parent crude oil, and the isolated interfacial material of the crude oil emulsion. The raw asphaltene fraction and purified asphaltene fraction were not successfully ionized by the positive-ion ESI process. The basic species detected (and % relative abundance) are very similar for each class highlighted. The co-precipitates show the greatest variation in % RA when compared to the other fractions. They contain less basic nitrogen and more basic O\(_{1}\)S\(_{1}\) when compared to the other ionized samples.

For each class presented in Figure 5.1, compositional comparison of alkylation and aromaticity trends can be detailed with DBE vs. carbon number iso-abundance color contour plots (for each given sample). DBE (also referred to as “type”) is the double bond equivalents or the number of rings and/or double bonds. It is a measure of the aromaticity of the compounds identified in each class. Carbon number is simply the number of carbons contained in the molecule, and provides a measure of alkylation. The % RA of the mass spectral peak identified is plotted on the z-axis and is pictorially presented by a color intensity scale, where red represents the most intense peak signal for that class.

Figure 5.2 shows the DBE vs. carbon number plots for the basic N\(_{1}\) class of compounds. The compositional similarity among each of the samples is easily depicted. The parent crude oil and co-precipitates contain the most aromatic (\( 4 \leq \text{DBE} \leq 25 \)) and most alkylated species (\( C_{15-60} \)), while the maltene and interfacial material contain compounds of similar aromaticity (\( 4 \leq \text{DBE} \leq 20 \)) with slightly lower alkylation
Figure 5.1. Basic heteroatom classes detected by (+) ESI FT-ICR MS. The \( N_1 \) species is the most abundant class in all samples analyzed. Raw asphaltenes and purified asphaltenes were not ionized efficiently and produced little signal, therefore, only four samples are shown. As previously demonstrated by Stanford et al., there is indiscriminate accumulation of basic nitrogen in the crude oil interfacial material. Thus, there is little to know variation in the class distributions between the parent crude and interfacial material.

(20-45 carbons per molecule). In previous work presented by Stanford, et al., indiscriminate pyridinic adsorption was observed in the emulsion interface when compared to the crude oil. This conclusion is still supported by our study, but we add that all compounds observed in the interfacial layer are observable in the co-precipitate layer as well.

The basic \( O_1S_1 \) class of compounds is presented in Figure 5.3. Compounds present in the interfacial layer are identified in each of the crude oil fractions. This observation again indicates an indiscriminate interfacial adsorption mechanism. The highest concentration of \( O_1S_1 \) species is found in the co-precipitate fraction (see figure 5.2). For all other basic heteroatom-containing class, the same trends are observed.

Stanford, et al., demonstrated that there is a specific heteroatom-containing class dependant adsorption mechanism that controls interfacial adsorption for the acidic species. Specifically, her work showed a significant enrichment of the \( O_x \) and \( SO_x \)
species when comparing a parent crude oil and the isolated interfacial material from its respective emulsion.\textsuperscript{80} Negative-ion ESI selectively ionizes the acidic species of the crude oil fractions.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.2.png}
\caption{Iso-abundance color contour plots of DBE vs. carbon number for the basic N\textsubscript{1} species detected by FT-ICR MS. All N\textsubscript{1} species have roughly the same DBE distribution and span similar carbon numbers. The similarity supports the proposed indiscriminate fractionation of the basic species in the experiment as previously noted in Figure 5.1. This trend is observed for all basic species detected in all samples.}
\end{figure}

We successfully ionized all crude oil fractions, except the purified asphaltenes, by the negative ESI mechanism. The class compositional comparison is presented in Figure 5.4. All classes presented are not detected in all samples. The raw asphaltenes (co-precipitates + purified asphaltenes) contain a very high % relative abundance of O\textsubscript{2} and O\textsubscript{4} species. After washing the raw asphaltene fraction to remove the co-precipitates and generate the purified asphaltenes, we observe a significant amount of O\textsubscript{2} compounds in the co-precipitate fraction. This indicates that these O\textsubscript{2} species act as co-precipitates, where they initially precipitate in the \textit{n}-heptane asphaltene drop but become soluble in the \textit{n}-heptane wash following precipitation! The O\textsubscript{2} class of compounds in the co-precipitate fraction is so incredibly responsive to the negative-ion
ESI ionization method that it potentially hinders the investigation of other heteroatom-containing classes not as easily ionized by the ESI process.

A second interesting observation of Figure 5.4 is the exceedingly high abundance of SO\(_4\) compounds observed in the interfacial material. The enrichment of these compounds relative to the parent crude oil is astounding. The SO\(_4\) species are not observed in the crude oil at \(\geq 1\)% RA, but they are observed in the interfacial material as the most abundant class of compounds at > 40% RA. This observation is consistent with Stanford, et al.\(^80\)

![Iso-abundance color contour plots of DBE vs. carbon number for the basic O\(_1\)S\(_1\) species detected by FT-ICR MS. The overall similarity among all plots supports indiscriminate accumulation of the basic O\(_1\)S\(_1\) species.](image)

Class alkylation and aromaticity trends for the acidic N\(_1\) class are shown in Figure 5.5. The acidic N\(_1\) class has been shown in the past to absorb proportionally to that of the parent crude oil, but less competitively than that of O\(_x\) and SO\(_x\) species.\(^80\) The crude oil, maltene, and emulsion interfacial material all occupy similar compositional space with a DBE range of 5-30 and 15 to 60 carbons per molecule. It should be noted that the interfacial material does contain a slightly less aromatic fraction.
with a lower degree of alkylation. The splotchy co-precipitate graph is attributed to the low relative abundance (~2%) of the compounds in the co-precipitate fraction.

Because of the relatively high abundance of the O\textsubscript{2} species contained in both the raw asphaltene and co-precipitate fraction, we present the DBE vs. carbon number plots of the acidic O\textsubscript{2} class in Figure 5.6. All images contain a significantly high concentration of O\textsubscript{2} compounds of very low (1-2) DBE. While, mass spectrometry results do not yield structural information, the molecular structure must relate to functional group. This compositional information is indicative of 1-2 carboxylic acid, carbonyl, or hydroxyl (or combo of) moieties contained on each compound.

Figure 5.4. Heteroatom classes for the acidic species detected by negative-ion ESI FT-ICR MS at 9.4 T. Raw asphaltenes are efficiently ionized in (-) ESI. Therefore, results from five samples are presented. The class trends agree with Stanford et al.\textsuperscript{80}, who previously noted, that O\textsubscript{x} and SO\textsubscript{x} species stabilize emulsions and preferentially accumulate in the interfacial material. We observe highly abundant O\textsubscript{x} species in all samples. The SO\textsubscript{x} species are highly enriched in the interfacial material relative to its parent crude oil.

The O\textsubscript{2} species observed in the interfacial material is approx. 10% lower in abundance than in the crude oil and the presence of the O\textsubscript{2} species at > 5% RA is consistent with resin participation in emulsion stabilization.\textsuperscript{80} In addition, these
observations are consistent with previous results, where the low DBE species indicate fatty and naphthenic acid participation in the emulsion stabilization mechanism. These fatty and naphthenic acids (which reside in the resin fraction of the crude oil) must precipitate with the asphaltene fraction (as co-precipitates) and artificially contribute to “asphaltene stabilized emulsions.” Here again, figure 5.5 shows that all interfacial O₂ species are also observed in the co-precipitate fraction.

Figure 5.7 details the iso-abundance contour plots for the SO₄ species. As observed in figure 5.4, the enrichment of this species relative to the parent crude oil is significant. It has been observed that SO₄ species “almost universally stabilized water-in-oil emulsions independent of parent oil specific gravity or geographic origin.” The compositional space occupied by the SO₄ compounds in the emulsion interfacial material is comprised of $1 \leq \text{DBE} \leq 15$ and $C_{15-50}$. This overlaps with both the compositional space occupied by the maltene and co-precipitate fraction, but the SO₄ are not a class observed in the raw asphaltene fraction of the crude oil (at >1% RA). This indicates that the SO₄ species exist at a concentration in the raw asphaltenes that is either not detectable at the analyzed concentration or the responsiveness of the compounds to the ESI ionization is not ideal. The latter explanation seems irrelevant due to their ability to be ionized and observed at equivalent concentrations in both the maltene and co-precipitate fraction.

Given all of the evidence above supporting the existence of a subset of compounds, consistent with co-precipitate fraction stabilization of the crude oil, we wondered what type of emulsion interfacial composition would be observed if only the co-precipitate fraction was used as the “oil” phase to form a water-in-“oil” emulsion (e.g. a “water-in-co-precipitate emulsion”). We purified a co-precipitate faction in large quantity using the procedure outlined in the experimental section and generated a heavy water emulsion from just the co-precipitate fraction (see experimental section and reference 21). The isolated co-precipitate emulsion interfacial layer was dried under a nitrogen stream and analyzed by negative-ion ESI FT-ICR MS.
Figure 5.5. Iso-abundance color contour plots of DBE vs. carbon number for the acidic \( N_1 \) species. Previous results have shown adsorption of these species (at the emulsion interface) that is proportional to their abundance in the parent crude oil, but at a relative rate that is less competitive than that of \( O_x \) or \( SO_x \) species.

Figure 5.6. Iso-abundance contour plots of DBE vs. carbon number for the acidic \( O_2 \) species in negative-ion ESI FT-ICR MS. The \( O_2 \) species in all samples are largely non-aromatic (DBE < 4). The higher DBE (aromatic) \( O_2 \) species appear to preferentially fractionate into the maltene fraction isolated in the SARA fractionation of the crude.
Figure 5.7. Iso-abundance contour plot of DBE vs. carbon number for the acidic SO₄ class in negative-ion ESI FT-ICR MS. The SO₄ class is not detected with a % relative abundance > 1% in the parent crude oil. Therefore, the high relative abundance of the SO₄ class in the emulsion interfacial material clearly indicates that SO₄ species preferentially accumulate at the interface.

Figure 5.8. Heteroatom classes for species detected by negative-ion ESI FT-ICR for an emulsion generated from a whole crude oil and the co-precipitate isolated fraction. The co-precipitate material is highly enriched in SOₓ species, similar in composition to the acidic species in the interfacial material. Thus, compounds that preferentially accumulate at the interface most likely originate from the co-precipitate material (a portion of the maltenes), rather than from purified asphaltenes.
**Figure 5.8** shows the results comparing the interfacial materials collected from a “conventional” crude oil emulsion interfacial layer and from a purified co-precipitate fraction emulsion interfacial layer. It becomes obvious that the co-precipitate interfacial layer is laden with Ox and SOx species when compared to the conventional crude oil emulsion, as a matter of fact the co-precipitate emulsion interfacial layer is comprised of **ONLY** Ox and SOx species. Many of the Ox and SOx classes are not initially observed in the parent crude oil or any fraction of the crude oil indicating that these Ox and SOx species preferentially partition into the co-precipitate fraction (in same quantities) and they become “more concentrated” (by virtue of other matrix being removed) due to the fractionation process, whereby they become more susceptible to ionization and subsequent detection. This novel finding supports the idea that the co-precipitate fraction is a major source for these species, and that these Ox and SOx species must initially precipitate with the asphaltenes in high quantities, as the co-precipitates are derived from the washings of the raw asphaltene fraction. The compounds do not identify closely with either “asphaltene” or “resin” but rather show properties of their...
own, with overlapping characteristics similar to that of asphaltenes and resins. Higher order \( \text{SO}_x \) and \( \text{S}_x\text{O}_y \) species are also prevalent in the co-precipitate interfacial layer, which may suggest that the co-precipitate class (comprised these fatty and naphthenic type compounds) compounds prefer partitioning into this fraction.

![Comparison of Interfacial Material Composition](image)

**Figure 5.10.** Acidic \( \text{SO}_4 \) species observed in the crude oil emulsion interfacial material (left) and the co-precipitate emulsion interfacial material (right). The crude oil interfacial material is comprised of species similar to that of the co-precipitate interface. The acidic \( \text{SO}_4 \) compounds have been shown to universally stabilize emulsions. It appears that most \( \text{SO}_x \) species reside in the maltenic material of the crude oil not the asphaltenes fraction.

We also examined the alkylation and aromaticity trends for the compounds observed at both interfacial layers. The acidic \( \text{O}_2 \) and \( \text{SO}_4 \) comparisons are presented in **Figures 5.9 & 5.10**, respectively. The acidic \( \text{O}_2 \) compounds in each interfacial layer are comprised of similarly alkylated compounds (C\(_{15-50}\)) and they also span similar aromatic ranges (1 ≤ DBE ≤ 10), with the crude oil emulsion interfacial material being only slightly more aromatic (DBE up to 15).

Figure 5.10 displays the iso-abundance contour plots for the \( \text{SO}_4 \) class. The interfacial layers are highly concentrated in low DBE (≤ 3, non-aromatic) compounds, indicating fatty and naphthenic acid participation in stabilization. The surfactant-like
characteristics of the SO$_4$ class potentially make it favorable for partitioning to the interface and appear to act as the primary acidic stabilizer at the emulsion interface (as shown by their high presence in interfacial material). The area of highest concentration differs for each interfacial layer and this observation is not completely understood. The species present in the co-precipitate-derived interface are comprised of a higher concentration of more alkylated compounds.

**Conclusions**

Resin stabilization (specifically fatty and naphthenic acid) of asphaltenes seems to be a significant contributor to emulsion stability. Our results show that asphaltene co-precipitates are comprised of the compounds known to contribute to emulsion stabilization and demonstrate chemical similarity to both asphaltenes and resins, while providing their own unique properties. These co-precipitates are highly enriched in both O$_x$ and SO$_x$ species.
CHAPTER SIX

CHARACTERIZATION OF SPECIFIC ACCUMULATION OF ACIDIC AND NON-POLAR SPECIES AT THE INTERFACE OF WATER-IN-OIL EMULSIONS BY FT-ICR MS

Summary

Chapter six characterizes the composition of a crude oil and its interfacial layer isolated by the heavy water method. Emulsion stability is linked to the type of components that exist at the interface therefore, it is of interest to investigate what role chemical composition plays in emulsion formation. In order to gain an understanding about what compounds may act as stabilizers, we attempt to regenerate an emulsion from a crude oil that has had the interfacial layer removed following emulsion formation. The differences and similarities that exist in the two interfacial layers collected successively from the same crude oil provides insight into which heteroatom classes specifically accumulate in the interfacial layer and which classes may act as primary stabilizers. Negative electrospray ionization high resolution FT-ICR mass spectrometry highlights the acidic species, while, a novel derivatization method followed by silver-triflate assisted positive ion ESI is used to investigate the nonpolar (aromatic) species in the parent crude oil, the interfacial depleted crude oil, and the isolated interfacial materials. The derivatized ESI process provides the first look into the individual nonpolar species (potentially accumulated asphaltenes) in the interfacial material. Additionally, we observe that non-polar species have a significant contribution to H/C ratios of the interfacial material.

Introduction

Global crude oil demand necessitates that crude oil production industries change pace and begin to refine heavier crudes (as defined by API gravities). Industry's ability to refine heavier crude oils potentially requires the use of more chemical additives and a higher need for addition of water. Water addition allows for lower viscosities, higher flow ability, and to aid the removal of unwanted salts and solids. Unfortunately, these
washing and diluting steps (desalting, etc.) often result in the formation of unwanted water-in-oil emulsions. Water-in-crude oil emulsions (along with oil-in-water and multiple emulsions) plaque many downstream processes and inhibit full recovery of the input petroleum product. In addition to decreased product recovery, refineries incur higher operation cost, revenue loss, and water pollution. It is therefore, advantageous to target the compounds that are most responsible for emulsion stability and determine if these compounds can either be removed or minimized (prior to refinery conditions) so that their negative and costly consequences do not impact the refining industry so dramatically.

Emulsion stability mechanisms have been shown to be largely controlled by a sub-portion of the compounds that exist in the crude oil. More specifically, asphaltenes, the \( n \)-heptane insoluble, but toluene soluble fraction, have significant impact on the overall stability. Resins, naphthenic acids, waxes, and solids have also been shown to contribute somewhat to the overall emulsion formation tendency. All of these examined compounds exhibit surface active characteristics.

Recent research by Stanford et al. probed the acidic and basic species that existed in nine geographically distinct light, medium and heavy crude oils by electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Their experiments concluded that there is a specific enrichment of acidic O\(_x\) and SO\(_x\) species, and that “O\(_4\)S species almost universally stabilize water-in-oil emulsions independent of parent oil specific gravity or geographic origin.” Additionally, they observed an absence of preferential enrichment among all basic heteroatom (nitrogen (N), oxygen(O), and sulfur(S)) classes.

Czarnecki examined interfacial layers by batch centrifugation and microslide isolation to test for molecular organization observable via microscopy. His observations concluded that molecules MOST responsible for emulsion formation were liquid crystal in nature, capable of molecular self-organization, and compositionally different from asphaltenes, resins, and the parent crude oil from which the emulsion was derived. In addition, he discussed the unknown role of non-polar molecules in emulsion stabilization.
Ehrmann et al.\textsuperscript{118} presented compositional findings on interfacial material by isolating emulsion interfacial layers and characterizing them with ultrahigh resolution FT-ICR MS. Results included compositional identification of a small sub-fraction of asphaltenes, namely co-precipitates, because they co-precipitate with asphaltenes during the $n$-heptane drop, even though, they are soluble in $n$-heptane, that may be most responsible for emulsion stabilization. Co-precipitates were significantly enriched in both acidic O$_x$ and SO$_x$ species, relative to the parent crude oil, just as observed by Stanford et al.

Here in this paper we aim to further characterize interfacial compounds by our so-called “depletion experiments.” A crude oil known to cause large emulsion deposition in the field was used to generate lab-emulsions by Wu’s\textsuperscript{81} heavy water method. Isolated interfacial layers were examined by FT-ICR MS. The parent crude oil was depleted in its “primary” interfacial layer and used to produce a “secondary” interfacial layer. This experimental design (as outlined below) is devised to identify which class of compounds (if any) act as stabilizers and examine whether their removal by subsequent emulsion formation, decreases the crude’s ability to produce emulsion interfacial layers while possibly identifying “secondary” molecular stabilizers.

**Interfacial Depletion Experimental Design**

The experimental scheme for the interfacial depletion experiment is shown in Figure 6.1. Parent crude oil is used to prepare a D$_2$O-in-oil emulsions as outlined in Wu’s heavy water method (HWM).\textsuperscript{81} The interfacial material is collected in the form of a wet cake and dried to constant weight in order to remove entrained water droplets. The solution phase of the emulsion (crude oil diluted in heptol with residual D$_2$O and H$_2$O) is then relocated into a Dean Stark apparatus. The distillation is carried out to remove all amounts of liquid from the crude oil. Once all solvents are removed a small aliquot is set aside for direct FT-ICR MS measurement. The bulk of the, now interfacial depleted crude oil was re-dissolved in a new heptol (1:1 heptane:toluene) solution and used just as before to generate a secondary D$_2$O-in-oil emulsion by Wu’s HWM\textsuperscript{21}. The new wet cake that is formed is removed and dried to constant weight. This schematic allows for elemental composition analysis of four samples, the parent crude oil, interfacial material
#1, the interfacial depleted crude oil, and interfacial material #2 (depicted by darkened gray boxes). From this data, comparisons can be made between the parent crude oil and the interfacial depleted crude oil, as well as, between the two interfacial materials.

![Diagram]

**Figure 6.1.** Experimental scheme for the interfacial depletion experiment. Four samples (gray boxes) were compositionally compared to investigate what species contribute most significantly to emulsion interface stabilization.

**Experimental Methods**

Parent crude oil, the interfacial depleted crude oil and both interfacial layers were prepared for mass spectrometry analysis by dissolving (dry and/or solvent free samples) in toluene at a concentration of 2 mg/mL. The sample was diluted further to a final concentration of 1 mg/mL in 1:1 toluene:methanol followed by spiking with 1% ammonium hydroxide. This procedure allows for more efficient deprotonation of all acidic species.

In silver triflate derivatization experiments, crudes and interfacial materials were prepared in toluene at 2 mg/mL. A stock solution of silver triflate was prepared at a concentration of ~6 mg/mL. Equal parts of each solution were then combined in a separate vial to achieve a final concentration of crude oil at 1 mg/mL (roughly 3× the
silver concentration is present). This sample was then used for mass spectrometry analysis. All solvents were HPLC-grade from VWR International. Samples were delivered to the ionization source via a syringe pump at a rate of 0.5 µL/min.\(^{119}\)

Samples were analyzed with a custom-built 9.4 T 22 cm diameter bore (Oxford Corp., Oxford Mead, UK) ESI\(^ {44}\) FT-ICR mass spectrometer.\(^ {120}\) A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data.\(^ {43, 48}\) Low-resolution mass spectrometry (linear ion trap) provided independent verification of molecular weight distribution. A total of 300 time-domain data sets was co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio by the quadrupolar electric potential approximation.\(^ {41, 121}\) All observed species were singly charged, as verified by unit \(m/z\) separation between mass spectral peaks corresponding to the \(^{12}\)C and \(^{13}\)C\(^ {12}\)C\(_{c-1}\) isotope for each elemental composition. Isotopic verification was used to ensure proper elemental assignments.

Multimer (both hetero- and homo-) detection commonly causes broad molecular weight distributions. In order to reduce the multimer formation and subsequent detection, FT-ICR mass spectra were detected after a 10 msec CO\(_2\) laser pulse inside the ICR cell. The CO\(_2\) laser pulse activates the multimers through heating and causes non-covalent interactions to collisionally dissociate prior to detection.

Internal mass calibration was carried out using homologous CH\(_2\) series (see below). The average root mean square (RMS) mass error for all assignments made was 0.14 ppm. Kendrick analysis generates homologous series based on repeating mass units of 14.015 65 Da (CH\(_2\)), by rescaling the mass spectrum from IUPAC mass to the “Kendrick” mass scale.\(^ {61}\)

\[
\text{Kendrick mass} = \text{IUPAC mass} \times \frac{14}{14.01565} \quad (6.1)
\]

This calculation converts the mass of CH\(_2\) from 14.01565 to exactly 14.00000, allowing for the visualizing a series of compounds of the same class (same heteroatom content) and type (e.g. number of rings plus double bonds), differing only by the extent of alkylation.\(^ {62}\) All members of the same class and type will have the same Kendrick mass defect:
Kendrick mass defect (KMD) =
(nominal Kendrick mass – exact Kendrick mass)  \hspace{1cm} (6.2)

Identification of this pattern enables quick sorting of similar compounds.

Results and Discussion

The ultrahigh resolution FT-ICR MS technique is ideal for obtaining the chemical identities of compounds that lie at the emulsion interface. The ESI process selectively ionizes the interfacial acids and bases (depending of source polarity) without fragmentation or matrix interference. While, the mass resolving power \( (m/\Delta m_{50\%} > 200,000 \) over the mass range of \( 200 \leq m/z \leq 1000 \) \) of the 9.4 T FT-ICR MS method allows for the differentiation of compounds of the same nominal mass but differing exact mass with an accuracy of \( \leq 300 \) ppb.[ref] The high mass accuracy enables unique elemental composition \( (C_{c}H_{h}N_{n}O_{o}S_{s}) \) assignment for each peak assigned above the allowable noise threshold. Kendrick analysis allows for the comparison of the relative abundance distributions for different heteroatom-containing classes. In addition, alkylation and aromaticity trends are easily identified for each given class of compounds. Presented in Figure 6.2 is the class graph for all identified acidic heteroatom classes in the parent crude oil and the interfacial depleted crude oil. There is much compositional overlap between the two crude oils, not only in heteroatom content, but also in relative abundance of a given heteroatom class. The \( N_{1} \) class of compounds is the most abundant class in each sample, followed closely in \% relative abundance by the \( O_{2} \) class. Differences include the loss of all \( N_{1}O_{2} \) and \( N_{2}O_{3} \) species in the parent crude oil vs. the interfacial depleted crude, and the detection of acidic \( O_{3}S_{1} \) and \( O_{4}S_{1} \) compounds after the removal of interfacial material #1. The onset of these new \( SO_{x} \) species indicates their presence in low quantities relative to that of other classes, but their presence has potentially significant impact.

Alkylation and aromaticity trends within a particular heteroatom class are most easily described by iso-abundance color contour plots. Double bond equivalents (DBE) is a measure of the molecular compounds aromaticity and is calculated as the number of rings and/or double bonds (relative to a carbon atom). Alkylation is simply the
number of carbon numbers contained in a determined molecular formula. Iso-abundance color contour plots are plotted with DBE on the y-axis and carbon number on the x-axis. Figure 6.3 shows a comparison of crude oil composition between the parent crude oil and the interfacial depleted crude oil. Three heteroatom classes are represented. The N₁ class of compounds in the parent crude oil spans a carbon number range of 18-60 and $6 \leq \text{DBE} \leq 26$. This indicates that all N₁ compounds are aromatic, most likely pyridinic in nature. The N₁ species of the interface depleted crude oil is only comprised of slightly less alkylated species of very similar DBE values.

The parent crude oil O₂ class of compounds is comprised primarily of long chain fatty acids. The compounds range from $1 \leq \text{DBE} \leq 16$ and C₁₅₋₅₂. The most abundant compounds lie at DBE = 1 of carbon number 18, or stearic acid. Stearic acid has long been observed in petroleum samples and dissolved organic matter studies. It has been shown to not only arise naturally in samples but also from impurities. Other fatty acids, such as palmitic acid (C₁₆) and oleic acid (C₂₀) are also known to cause impurity issues, which can lead to signal suppression. The long chain, fatty acid-type O₂ species

Figure 6.2. Heteroatom classes for acidic species detected by negative-ion ESI FT-ICR MS for the original crude oil interfacial material and the regenerated crude oil emulsion interfacial material. The original crude oil emulsion was dried with a Dean Stark trap to remove traces of water. The remaining dried crude depleted in the interfacial material was removed in previous steps and analyzed by FT-ICR MS.
are highly surface active and can contribute significantly to emulsion interface stability. Additionally, the relative abundance of the $O_2$ species increases in the interface depleted crude oil.

**Figure 6.3.** Selected iso-abundance color contour plots for the N1, O2 and O4 species observed in the parent crude oil and the interfacial depleted crude oil. There is significant compositional overlap between the two samples; variations are largely observed in the relative abundance of species detected.

The last color-contour plot images the O4 class of compounds. This species are presented to show that they are indeed, monomeric in nature, and not simply dimers of the O2 compounds in the samples. The O4 compounds range in DBE of 2-13 (mostly) and carbon number 15-50. The relative abundance of most O4 species decreases significantly in the interface depleted crude. Loss of the more alkylated species is also observed.

**Figure 6.4** presents the class graph of all identified compounds greater than 1% relative abundance for the interfacial material #1 and interfacial material #2. As observed in previous studies$^{80,84}$, the O4S1 species are highly abundant in the original interfacial layer, followed by the O2 class of compounds. The decrease in relative abundance of the O4S1 species is dramatic when examining interfacial material #2.
O₂ class becomes the primary class of compounds in the interfacial material #2. These O₂ compounds may act as secondary interfacial stabilizers, and promote interface stability even when all O₄S₁ compounds are removed. Additionally, even though they are of small abundance the detection of other SOₓ species is detected in interfacial material #2. Other classes vary (in relative abundance) only slightly between the two interfacial materials.

![Acidic Class Comparison Between Interfacial Materials](image)

**Figure 6.4.** Class graph for the observed heteroatom class at ≥1% relative abundance in interfacial material #1 and interfacial material #2. The graph indicates that the O₄S₁ species are significantly depleted relative to the original interfacial material, whereas the O₂ species are enriched in the second generation emulsion interfacial material. Oₓ and SOₓ species evidently stabilize the interface, as previously concluded, but with an order of preference for the interface. Simply, since O₄S₁ species are preferentially accumulated in the first generation emulsion interfacial material, there is higher proportion of O₂ species in the second generation.

Iso-abundance color contour plots for the N₁, O₂ and O₄S₁ species observed in interfacial material #1 and #2 are shown in **Figure 6.5.** There is significant compositional overlap between the two interfacial materials, compounds span approximately the same DBE values and carbon numbers. Compounds in the primary interfacial material are slightly more alkylated.
Given the similarities between the crude oil material and interfacial material, we feel that the depletion experiment (and the results that stem from it) are consistent with partitioning and molecular behavior as a result of exposure of the oil to aqueous material, and not the result of experimental manipulation. Therefore, classification of the molecular classes as interfacial stabilizers is a function of their inherent nature and surfactant-like characteristics.

**Figure 6.5.** Iso-abundance contour plots of DBE vs. carbon number for the acidic O$_2$S$_1$ species detected in the first and second generation crude oil emulsion interfacial materials.

**Silver Triflate Derivatization**

It is well known that ESI preferentially ionizes polar compounds of petroleum and is essentially blind to its non-polar constituents.$^{58}$ Recently though, derivatization of solution phase composition of the electrospray analyte solution has proven that it is possible to achieve some non-polar speciation of chemical compounds through ESI.$^{122}$ Through the use of metal complexation to readily available electron systems (primarily in the form or lone pairs on the heteroatom-containing species) non-polar species can be detected in FT-ICR MS as stable silver complex [M + Ag]$^+$ ions.
**Figure 6.6** presents the class composition of the non-polar species ionized with silver triflate assisted positive ion ESI FT-ICR MS. It becomes immediately apparent that sulfur plays a major role in emulsion interfacial material composition and may contribute significantly to overall emulsion stabilization. Sulfur is present at the interface in various forms including thiophenes, thiophenols, and sulfoxides.

Highlighted in **Figure 6.7** are the iso-abundance color contour plots of S₁, S₂ and S₃ species observed by silver triflate derivatization ESI. The observed species follow very similar (almost identical) aromaticity and alkylation trends for sulfur species observed by other ionization methods (e.g. atmospheric pressure photoionization (APPI)).¹²³,¹²⁴ As heteroatom content increases, so does aromaticity of the compounds.

![Non-polar Class Speciation of Crude Oil Interfacial Material with Silver Triflate Derivatization by Positive ESI FT-ICR MS](image)

**Figure 6.6.** Heteroatom classes for non-polar species from silver triflate assisted positive-ion ESI FT-ICR MS.

The role that these compounds play in emulsion stabilization is yet unknown, but it should not underscore their significance. Past data presented by Stanford et al.⁸⁴ reported an average H/C ratio for the interfacial material polar species at ~1.56 (calculated from negative ion ESI species). This number is significantly different from
Figure 6.7. Iso-abundance color contour plots of DBE vs. carbon number for the $S_X$ species from positive-ion ESI FT-ICR coupled with Ag$^+$ adduction. The observed aromaticity and alkylation trends in the sulfur species follow the same trends observed by other ionization methods used for non-polar speciation, such as Atmospheric Pressure PhotoIonization (APPI). Aromaticity increases as heteroatom content increases.

that of Wu’s H/C ratio of ~1.1 for the interfacial material (as determined by bulk measurements).\textsuperscript{21, 111} If asphaltenes are a major player in emulsion interfacial stabilization, then the discrepancies between these two numbers could be explained in part by the non-polar species observed through the derivatized electrospray ionization method. The ESI data (specific for only the acidic species; i.e. O$_2$ compounds in figure 6.5) suggests that the interfacial material is comprised of low DBE and low carbon number polar compounds, but figure 6.7 reveals that non-polar (PASH in this case) consist of highly aromatic and highly alkylated compounds. Inclusion of the non-polar species results in a lower overall H/C ratio for the emulsion interfacial material. In addition to the sulfur compounds, hydrocarbons and nitrogen-containing species surely will contribute to a lower H/C ratio for all species in the interfacial material to a value closer to that noted by Wu.
Conclusions

Successive emulsion generation from the same crude oil shows that there are functionalities of crude oil that exhibit a strong preference for partitioning into the interfacial material. $O_x$ and $SO_x$ species consistently show this preference, and their surfactant-like structural compositions lead us to believe that this is ideal. Data suggest that there is a preferential order for partitioning. In addition, the role of non-polar species should not be underscored. Their contribution to emulsion interfacial film formation and stability is not as well understood, but they incorporate easily, as evident by their relative abundance. More aromatic compounds in the non-polars contribute to the lowering of H/C ratios and could explain the discrepancies in previously reported H/C values of isolated interfacial materials.
CHAPTER SEVEN

ASPHALTENE CO-PRECIPITATE MOLECULAR PROGRESSION AS A FUNCTION OF SOXHLET EXTRACTION PERIOD: IMPLICATIONS FOR ASPHALTENE AGGREGATION

Summary

Data presented in chapter seven details changes in asphaltene co-precipitates observed over a three day Soxhlet extraction period. Aggregation of asphaltenes is one of the most debated aspects in terms of true monomeric molecular weight distributions. Aggregate formation is extremely concentration dependent and understanding its prevalence is paramount when determining conclusions about asphaltene structure, molecular weight, and heteroatom composition. n-heptane precipitated asphaltenes were Soxhlet extracted in n-heptane. Some asphaltenic compounds, namely asphaltene co-precipitates, reverse solubility behavior and collect in the Soxhlet solvent reservoir, even though they previously precipitated in n-heptane. The ultrahigh resolution, resolving power and mass accuracy capabilities of electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) were utilized to investigate the co-precipitates and monitor their composition. Analysis for all of the extraction periods reveals that the composition of the extracts exhibit dramatic shifts in aromaticity, alkylation, and heteroatom content. Global heteroatom classes trend toward more oxygenated species of increased aromaticity and alkylation. Interestingly, exceptions to this trend are noted at extensive extraction periods and may be linked to the release of occluded non-asphaltenic material or the onset of solubility for entrained resinous material. Class defined compositional images expose the unique signature of the suspected resinous material.

Introduction

Asphaltenes are operationally defined as n-heptane insoluble but toluene soluble compounds in crude oil. Their propensity to aggregate in solution at concentrations as low as 0.1 mg/mL\textsuperscript{85}, flocculate, and/or precipitate out of the crude oil creates
tremendous refinery issues. Literature suggests that aggregation tendencies are driven by asphaltene molecules inherent structural properties. Asphaltenes are comprised of fused aromatic rings containing an assortment of polar-heteroatom (nitrogen (N), oxygen (O) and sulfur (S)) functionalities (pyrroles, carbonyls, phenols, pyridine, and carboxylic acids, thiophenes). In addition, there have variable alkyl side chains attached to the aromatic core periphery. These molecular characteristics lend themselves to aggregation driven by hydrogen bonding, van der Waals forces, and \( \pi-\pi \) stacking of the aromatic rings, or a combination of.

Asphaltene aggregation is affected by solvent, temperature, and pressure. Yarranton, et al. and Smith, et al. have also shown that self-association increases with concentration. Asphaltene polydispersity and aggregation make it very difficult to accurately determine molar mass. Historical mass measurement techniques like vapor pressure osmometry (VPO) and size-exclusion chromatography (SEC) have experimentally determined a broad number-average molecular weight range for asphaltenes between 1000 Da and as large as tens of thousands Daltons. Mass spectrometry techniques and time-resolved fluorescence depolarization experiments have experimentally concluded an average molecular weight value of approximately 700-800 Da. The scientific community continues to work toward an agreeable value for asphaltene molecular weight.

What role other components play in asphaltene and asphaltene aggregate stability is still controversial, as well. Resins are a sub-portion of the maltenes that are isolated from adsorption chromatography by elution with 80:20 toluene:methanol. Resins compounds have been characterized as having properties that are related to both the aromatic and asphaltenic fractions of the crude oil. Previous research has shown that resins can decrease the size of the asphaltene aggregate and play a role in the stability of the asphaltenes causing more or less asphaltene to precipitate during gravimetric analysis. Compositional variations and even molecular weight distributions of both resins and asphaltenes vary based on the  \( n \)-alkane used to precipitate the asphaltenes. For example, asphaltenes precipitated with \( n \)-pentane typically contain some resinous material that is insoluble in \( n \)-pentane but soluble in longer chain alkanes, such as \( n \)-heptane. The “border region” across which
the asphaltenes and resins fractionate based on solubility depends on which \textit{n}-alkane used to separate the asphaltene fraction.\textsuperscript{136}

The resin role in asphaltene aggregation has been described by different mechanisms.\textsuperscript{136} The first depicts asphaltenes as colloidal aggregates with resins adsorbed in a thin layer on the exterior surface.\textsuperscript{137, 138} Resins on the surface of the asphaltene aggregate are thought to aid solubility of the asphaltenes in more aromatic solvents. Recent studies utilizing direct-current (DC) conductivity measurements\textsuperscript{136} of petroleum suggest that this model is inconsistent with asphaltene/resin aggregation behavior. At concentration equal to or below 1 mg/mL, Sedghi \textit{et al.}\textsuperscript{136} contend that aromaticity is aggregation’s driving force and not polarity of the molecules.

A second mechanism suggest that resinous material can become trapped inside the aggregate pore space (referred to as “cages” or “vesicles”).\textsuperscript{139-143} The trapped/occluded material may play a role in aggregate stability, potentially acting as a “glue” that allows for monomeric asphaltenic compounds to interact on a nearly permanent basis. The compounds captured in the pore space can be shielded from external conditions on a very long time scale and may provide insight to the original geological (kerogen) formation. This mechanism is currently being investigated through oxidation experiments used to release the trapped/occluded materials and test for crude oil bio-markers.\textsuperscript{144-148}

One of the most noteworthy citation for asphaltene aggregation/structural features was written in the early 1980’s by M. M. Boduszynski.\textsuperscript{149} Nearly 30 years in advance of the current day research findings, Boduszynski detailed asphaltene aggregation tendencies through a separation study using both VPO and mass spectrometric techniques to explain asphaltene structural properties and their behavior in solution. The paper concluded that asphaltenes were \textit{not high molecular weight species but rather, low molecular weight species of very high polarity}.\textsuperscript{149} In addition, the authors argued that precipitation of asphaltenes in normal alkane disrupts the solubility equilibrium and drives the most polar compounds to aggregate and become insoluble (thus precipitation occurs).

If asphaltenes are forced into precipitation as a result of loss of solubility equilibrium, asphaltenes could (if given enough time) become soluble in the same n-
alkane solvent used to precipitate them. Standard asphaltene preparation specifies the
dilution of crude oil by an \( n \)-alkane solvent (in this study, \( n \)-heptane) at a ratio of 1:40
(wt:vol). Filtration of the precipitate (asphaltenes) isolates the maltenes (comprised of
three fractions commonly termed saturates, aromatics, and resins). Purification of the
asphaltene fraction is achieved by simple \( n \)-heptane “washes” of the filter paper and is
followed by a brief Soxhlet extraction until “colorless” \( n \)-heptane is observed in the
extraction chamber. Research has shown\(^{150}\) that this brief Soxhlet extraction removes
maltene carryover from the filter paper and some precipitated asphaltenes. Thus, a
portion of compounds initially precipitated by \( n \)-heptane actually become soluble in \( n \)-
heptane and collect in the solvent reservoir.\(^{150}\) These co-precipitates we suspect play a
key role in asphaltene aggregation, precipitation, and stability. Here, we examine the
compositional trends of the co-precipitate that progresses as a function of continuous
Soxhlet extraction.

**Experimental Methods**

**Sample Preparation**

Asphaltenes were precipitated from California crude oil with a simplified SARA
crude fractionation procedure (IP-143).\(^99\) After \( n \)-heptane dilution of the crude oil, raw
asphaltenes were isolated by simple filtration through Whatman #1 filter paper. The
filter paper was transferred into an extraction thimble and placed inside the Soxhlet
chamber. The Soxhlet solvent reservoir was filled with \( n \)-heptane and set to cycle
approx. every 7 min. Compounds collected in the solvent reservoir (co-precipitate
fraction) were isolated after 1 h and again after 4, 8, 24, 48, and 72 h by removal of the
solvent reservoir and replacement with a new reservoir filled with fresh \( n \)-heptane.
Soxhlet washings were dried to constant weight and prepared for electrospray ionization
(ESI) coupled with FT-ICR MS.\(^{43,44}\) Samples were first dissolved to 1 mg/mL in toluene
to prepare ESI stock solutions. Dilution to a final concentration of 0.5 mg/mL in 1:1
toluene:methanol with 1% (by vol) tetramethylammonium hydroxide\(^{122}\) yielded the final
samples for negative electrospray (ESI) analysis. The use of TMAH is critical, as it
allows for deprotonation of less acidic chemical functionalities and therefore broader
chemical composition coverage for (-) ESI MS. All solvents were HPLC-grade from
Samples were delivered to the ESI source (held at ~2 kV) via a syringe pump at a rate of 0.5 µL/min.\textsuperscript{119}

**Mass Spectrometry**

Samples were analyzed with a custom-built 9.4 T 22 cm diameter bore (Oxford Corp., Oxford Mead, UK) ESI FT-ICR mass spectrometer with a modular ICR data acquisition system (PREDATOR).\textsuperscript{46, 151} 200 time-domain data sets were co-added and Hanning-apodized, followed by a single zero-fill, prior to fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit $m/z$ separation between mass spectral peaks corresponding to the $^{12}\text{C}$ and $^{13}\text{C}^{12}\text{C}_{c-1}$ isotopomers for each elemental composition.

**Results and Discussion**

Previous studies\textsuperscript{146, 148, 152} have used extended Soxhlet extractions (primarily with acetone) to help characterize asphaltenes. Agrawala\textsuperscript{152} studied the effects of asphaltene yields acquired from four different asphaltene cleaning procedures in $n$-heptane. He concluded that asphaltene yield decreased with additional washing, showing a behavior consistent with Boduszynski’s observations and theory. Asphaltene yields dropped from 15.3% for an unwashed asphaltene fraction to 12.6% for those Soxhlet extracted for an undefined time interval. In addition, Agrawala\textsuperscript{152} subjected the washed asphaltenes to solubility experiments and remarked that “the less asphaltenes were washed, the more heptane that is required to initiate precipitation.”

As stated previously, standard asphaltene precipitation experiments\textsuperscript{99} require purification of the asphaltene fraction by simple $n$-heptane “washing” of the filter paper and a brief Soxhlet extraction until “colorless” $n$-heptane is observed in the extraction chamber. This brief Soxhlet extraction removes maltene carryover from the filter paper and some precipitated material. These co-precipitated compounds\textsuperscript{20, 111} exhibit “transitional” characteristics, in that they display both asphaltenic and maltenic behavior\textsuperscript{20, 111} and are also observed in these asphaltene washings.
Representative FT-ICR mass spectra for samples collected after each of two different Soxhlet extraction periods are shown in Figure 7.1. The molecular weight range for the co-precipitants is lower (~500 Da) than that commonly cited for asphaltenes (~700-800 Da) but higher than that of the parent crude (results not shown). The molecular weight distribution from the 1-4 h time period (upper) is similar to that from 48-72 h (lower). However, closer inspection of the spectra at each nominal mass (zoom insets, upper right) reveals that the mass spectral peaks shift to lower mass defect (left) at longer extraction periods, a clear indication of increased aromaticity or heteroatom content of the later eluting species.

Co-precipitate washings observed in the crude oil are dominated by nitrogen containing compounds (Figure 7.2). N₁, N₂ and O₂ species decrease in relative abundance after prolonged Soxhlet extraction whereas the relative abundances of classes with additional heteroatoms increase or remain largely unchanged. Compositional trends, as a function of Soxhlet extraction period, indicate increased multi-heteroatom containing species. There is also an overwhelming presence of Oₓ and SOₓ species. Oₓ and SOₓ species are known to contribute to crude oil emulsion formation tendencies and provide emulsion stability.20, 80, 84, 111 Direct examination of class dependent compositional changes in double bond equivalents (DBE = rings plus double bonds to carbon) and carbon number (Figure 7.3) provides unique insight into n-heptane leachable asphaltenic species.
Figure 7.1. The molecular weight distribution from the 1-4 h time period (upper) is similar to that from 48-72 h (lower). However, closer inspection of the spectra at each nominal mass (zoom insets, upper right) reveals that the mass spectral peaks shift to lower mass defect (left) at longer extraction periods, a clear indication of increased aromaticity or heteroatom content of the later eluting species.
Figure 7.2. Heteroatom class distributions for compounds detected in the six co-precipitate washings over a three-day time period. Note the high prevalence of Oₓ and SOₓ containing classes. As extraction period increases, there is an increased presence of multi-heteroatom containing classes.

The first striking observation of figure 7.3 is that nearly all species detected border on the polycyclic aromatic hydrocarbon (PAH) "planar limit," namely, the maximum possible DBE for a defined number of carbons. A DBE value above the PAH planar limit would require buckyowl or buckyball (fullerene)-like structures that have not been found in crude oils. Thus, the diagonal boundary limits the available compositional space for petroleum species and is readily apparent for highly aromatic compounds, such as the present N₁ species. This finding is consistent with previous studies that outline the compositional region (aromaticity and alkylation) in which asphaltenes are known to exist.

As the experiment progresses in time (Figure 7.3, rows 2 to 7), molecules of a given carbon number become more aromatic (as observed by their shift in mass defect and shift in DBE (to the left) along the PAH planar limit). Surprisingly, for classes containing two or fewer heteroatoms (most notably O₂, N₁ and N₁O₁ classes), a second
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<th>Time Period</th>
<th>O₂</th>
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**Figure 7.3.** Combined iso-abundance color contour plots for selected heteroatom containing classes in the whole crude oil and six co-precipitate Soxhlet extraction periods.
group of compounds composed of lower DBE and carbon numbers appears. That material (most evident after 48 h) may result from the release of occluded maltenes freed by the progressive “stripping” of highly aromatic species removed during prior extraction periods or by a structural rearrangement of the fractal aggregate that results in solvent exposure of previously “protected”, solvent-inaccessible domains. The bi-modal distribution of compounds shows two distinct compositional regions, one consistent with asphaltenes (along the PAH boundary) and a second of lower carbon number and DBE values. The second region is consistent with maltenic material.

Examination of iso-abundance color contour plots for the $O_2$ and $N_1$ classes of the whole crude best exemplifies this observation. The whole crude images (Figure 7.3 row 1) are dominated by maltenic compounds. There is a complete lack of molecules along the PAH planar limit. Asphaltenic species are only observable once fractionated. But comparison of the bi-modal distributions in row 7 of the $O_2$ and $N_1$ class shows almost complete overlap with the whole crude oil image in row 1 (maltene dominated), plus the asphaltene region along the PAH planar limit.

Results suggest that maltenic material is definitely associated with the fractal asphaltene aggregates. We don’t yet understand the role (if any) of maltenic species in asphaltene stabilization or if they are simply “trapped” in the fractal aggregate during initially rapid precipitate formation. The release of similar material in the 0-1 hour period suggests that the appearance of those species in the 48-72 hour period is not due to slow dissolution kinetics. Rather, the asphaltene aggregate may undergo structural rearrangement to release the maltenic species to yield the class-specific bimodal distribution in DBE and carbon number compositional space. Carbon numbers observed in our bi-modal distribution (maltenic) are consistent with carbon numbers experimentally released from asphaltene aggregates through oxidation experiments and detected by gas chromatography (GC) and GC-MS analysis.$^{144}$

Examination of classes containing $\geq 3$ heteroatoms reveals very little change in the overall composition, as these compounds remain nearly constant in alkylation and aromaticity values. The presence of more heteroatoms translates into higher polarity compounds. These compounds may be held more tightly on the surface of or in the asphaltene aggregate. Prolonged washing over a 3 day time scale may be insufficient to
observe complete “stripping” of these compounds; making observation of the bi-modal distribution observed in compounds containing \( \leq 2 \) heteroatoms impossible.

**Hydrogen to Carbon Ratios**

Even though asphaltene molecular weight is still widely debated (along with nearly every other aspect of asphaltene chemistry), there is but one un-argued and highly agreed upon aspect of asphaltene science. The hydrogen to carbon ratio (H:C) of asphaltenes has been well defined as approximately 1.0 (by bulk analysis).\(^{23}\) Therefore, it makes complete sense to ground oneself in these measurements in order establish the argument that you are truly dealing with asphaltenic material.

Depiction of compositional space as DBE vs. carbon number has proven useful time and time again for outlining compositional trends for crude oil compounds. Asphaltenes have also been depicted in these iso-abundance color contour plots and show distinct compositional space (as presented here and in reference 153). DBE is a measure of aromaticity, just as H:C ratio. These measurements are interchangeable. **Figure 7.4** presents a new iso-abundance color contour graphical plotted as H:C ratio vs. carbon number for the N\(_1\) 0-1 h co-precipitates Soxhlet extraction interval (just as shown in **Figure 7.3** row 2). What is fundamentally different about this image when compared to DBE vs. carbon number images, is that aromaticity increases in the downward direction of the y-axis, whereas DBE (aromaticity) increases in the upward direction along the y-axis. A smaller graphic of DBE vs. carbon number (shown in **Figure 7.3** row 2) is provided in the upper left for visual orientation. This image shows that the majority of compounds lie at a value higher than 1.0. The calculated abundance weighted average H:C ratio is 1.32. This observation is consistent with maltenic material removed as carryover during the asphaltene filtration step.

**Figure 7.5** presents the data observed during the 1-4 h time interval. We observe a dramatic difference in the type of compounds, in that, nearly all species lie well below the asphaltene/malten transition region (H:C ratio \(~1.1\)). Compounds are much more aromatic. They exhibit an abundance weighted average H:C ratio of 0.9, a value clearly consistent with asphaltene properties. It is important to remind one at this point, that these species are isolated with \( n \)-heptane by simple Soxhlet extraction. These co-
Figure 7.4. Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 0-1 h co-precipitates. The abundance weighted average H:C of the compounds is 1.32, a value consistent with maltenic species. A smaller contour plot of DBE vs. carbon number is provided in the upper left for visual orientation. Compounds isolated and identified in this fraction are maltenic in nature with H:C ratios.

Figure 7.5. Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 1-4 h co-precipitates. The abundance weighted average H:C of the compounds is 0.9, a value consistent with asphaltenic compounds.
precipitate compounds originally possess asphaltene solubility properties and now become soluble in the same solvent used to precipitate them. H:C ratio calculations (and DBE values) show that they are highly aromatic with H:C ratios less than 1.

As we continue through the prolonged Soxhlet extraction time interval, from this point we observe only a slight decline in the abundance weighted H:C ratio of the compounds (0.87 for 4-8 h interval, 0.88 for 8-24 h interval and 0.83 for the 24-48 h interval (Figures 7.6-7.8)). H:C values of 0.9-0.8 are consistent with molecular structure that are almost bare PAH structures (such as coronene). There is progressive increase in the carbon number (as evident in the gradual shift to the right). Nearly all observed species lie well below the asphaltene/maltene transition region.

Iso-abundance color contour plots in Figure 7.3 of the N₁ species show that during the 24-48 h time period that a small amount of lower DBE, lower carbon number constituents begin to collect in the solvent reservoir. These compounds are also

**Figure 7.6.** Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 4-8 h co-precipitates. The abundance weighted average H:C of the compounds is 0.87, a value consistent with asphaltic compounds. Carbon number values of the isolated compounds increase when compared to the previous time interval but H:C ratios remain constant.
identifiable Figure 7.8 as they creep just above the H:C ~1.1 transition line (between C_{30-40}). **Figure 7.9** shows spectral peaks in terms of their H:C ratio and carbon number. We now observe a modest increase in the abundance weighted average H:C ratio of these compounds to 0.96. In addition, there is a higher prevalence of maltenic like compounds that exist above H:C of 1.1 between C_{20-40}. These are the same compounds that contribute to the bi-modal distribution of N_{1} compounds in Figure 7.3 row 7. The higher H:C values suggest that they are maltenic compounds. Again, we believe that these previously unobserved compounds result from the release of occluded maltenes freed by the progressive “stripping” of highly aromatic species removed during prior extraction periods or by a structural rearrangement of the fractal aggregate that results in solvent exposure of previously “protected”, solvent-inaccessible domains.

**Figure 7.7.** *Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 8-24 h co-precipitates. The abundance weighted average H:C of the compounds is 0.88, a value consistent with asphaltenic compounds.*
Figure 7.8. Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 24-48 h co-precipitates. The abundance weighted average H:C of the compounds is 0.83, a value consistent with asphaltenic compounds.

Figure 7.9. Iso-abundance color contour plot of H:C ratio vs. carbon number for compounds identified in 48-72 h co-precipitates. The abundance weighted average H:C of the compounds increases to 0.96. There is a bi-modal distribution of compounds, with the most abundant species observed higher H:C ratios.
Conclusions

The existence of appreciable $n$-heptane soluble material in asphaltenes after a 3 day extraction period is unexpected, given that asphaltenes are operationally defined as $n$-heptane insoluble material. Furthermore, it is counterintuitive that the heptane-soluble fractions of asphaltenes are the most aromatic and least sterically hindered; such behavior sheds considerable doubt on the most widely accepted mechanism for asphaltene aggregate formation ($\pi-\pi$ stacking) and begs the question of why asphaltenes are insoluble in heptane. Polarity (as evident by observations in this study and Boduszynski's\textsuperscript{149}) appears to play a major role in aggregation and precipitation of asphaltic compounds.

Utility of H:C ratios (as a measure of aromaticity) definitively shows that asphaltic compounds are reversing solubility behavior when given enough time. The release of occluded non-asphaltic material or the onset of solubility for entrained resinous material is still not well understood, but current results are consistent with some of the presented aggregation models. In future work, we seek to understand the fractal and “nano-” aggregate structural motifs suggested for asphaltenes and expand the compositional knowledge of the parent crude oil through chromatographic fractionation.
CHAPTER EIGHT

AN INVESTIGATION OF DOWNSTREAM OIL/WATER SEPARATION ISSUES CAUSED BY COMPOSITIONAL DIFFERENCES AMONG THREE DIFFERENT OIL WELL SAMPLES

Summary

Chapter eight describes a large series of experiments that were conducted on a sample set obtained through collaboration with Baker Petrolite. The chapter includes analysis of the samples by conventional electrospray ionization, derivatized electrospray ionization, and an ammonia based extraction method followed by conventional negative ion electrospray ionization. Extensive analysis on this sample set is aimed at understanding why one of the three samples causes an oil-water (emulsion) separation problem, while the other two do not. All samples are collected from a single sample drill site location, but each sample originates from a different well head. Results are outlined extensively, even though, little conclusion as to why oil-water separation issues are observed can be made.

Introduction

Collaboration between our petroleum research group and Baker Petrolite was initiated in an effort to characterize a series of samples collected from a test water separator. The sample set, comprised of three samples, originate from three different oil wells located within one drill site. One of the three samples, crude A, causes oil/water separator problems in downstream purification processes. Crudes B and C, do not generate any separation problems when subjected to downstream processes. It should be noted though, that Baker did mention that crude C had downstream issues in the past, but as drilling continued those issues have seemed to disappear.

It was theorized by those in the field that crude A’s problems may be due to possible surface active components that exist in the crude. Surface active components contribute to emulsion stability. We tested all samples via ESI FT-ICR MS to
probe the acid and base speciation contained in the samples to see what commonalities existed and if, indeed, there was a significant increase in previously identified surface active species, such as O$_x$ and SO$_x$ compounds.\textsuperscript{80, 84} Non-polar speciation was also conducted via solution derivatization with silver triflate and tetramethylammonium hydroxide and experimental ESI FT-ICR MS.\textsuperscript{122}

**Experimental Methods**

Crude oil samples were dissolved to 2 mg/mL in toluene for electrospray analysis. Dilution to a final concentration of 1 mg/mL in 1:1 toluene:methanol allowed for spiking with 1% formic acid or ammonium hydroxide for positive and negative electrospray (ESI), respectively. This procedure allows for efficient protonation/deprotonation. All solvents were HPLC-grade from VWR International. Samples were delivered to the ionization source via a syringe pump at a rate of 0.5 µL/min.

Silver triflate derivatization experiments, crudes were prepared in toluene at 2 mg/mL. A stock solution of silver triflate was prepared at a concentration of ~6 mg/mL. Equal parts of both solutions were then combined in a separate vial to achieve a final concentration of crude oil at 1 mg/mL (roughly 3× the silver concentration is present). Tetramethylammonium hydroxide (TMAH) experiments were conducted using a 2 mg/mL stock solution of crude oil in toluene. The crude was diluted in equal parts with methanol in order to achieve a final crude concentration of 1 mg/mL. TMAH was then spiked in at a concentration of 1% (by volume) and vortexed for complete mixing. Samples (at 1 mg/mL) were delivered to the ionization source via a syringe pump at a rate of 0.5 µL/min. All solvents were HPLC-grade from VWR International.

Experimental analysis was carried out on a custom-built 9.4 T 22 cm diameter bore (Oxford Corp., Oxford Mead, UK) ESI FT-ICR mass spectrometer. A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data. Low-resolution mass spectrometry (linear ion trap) provided independent verification of molecular weight distribution. A total of 150 to 300 (varying per sample conditions) time-domain data sets were co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to
mass-to-charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit \( m/z \) separation between mass spectral peaks corresponding to the \(^{12}\text{C} \) and \(^{13}\text{C}^{12}\text{C}_{\text{c-1}} \) isotope for each elemental composition.

**Results and Discussion**

Visual observation of the dilute crude oil samples at 1 mg/mL shows that crudes B and C are most similar in color, with a white wine-like, golden appearance. Crude A is darker and more amber in color. While these observations yield zero conclusions about the crude oil chemical similarities or differences it is noted because surface active compounds, like asphaltenes, have high absorptivity in the visible region.\(^{85}\)

**Negative Mode ESI**

All crude oil samples were first investigated by low resolution mass spectrometry methods. Low resolution mass spectrometry is ideal for pre-scanning petroleum samples to examine molecular weight distributions (MWDs) and check for possible multimerization. High resolution FT-ICR MS scans were then optimized to reflect the

![Graphs showing m/z distributions for Emulsion B](image)

**Figure 8.1.** Comparison of molecular weight distributions detected by negative mode ESI (a) low resolution ion trap mass spectrometry and (b) high resolution FT-ICR mass spectrometry for emulsion sample A.
MWDs detected by low resolution MS. Figure 8.1 displays the (a) low resolution ion trap mass spectrum with the (b) high resolution mass spectrum for crude B. Both spectra were collected for each sample in this study, but they are not shown for brevities sake. Signal to noise ratios are similar for all samples.

Low m/z peaks of high abundance are detected in all ion trap spectra. These peaks arise from noise that did not sum out due to low scan count. Multimers (both hetero- and homo-) were suspected as the cause of the broad MWDs observed in the ion trap spectra. Therefore, tandem MS experiments were conducted in order to fragment multimers and verify true monomer distributions (results not shown). For that same reason, FT-ICR mass spectra were detected after a 10 msec CO\textsubscript{2} laser pulse inside the ICR cell. The CO\textsubscript{2} laser pulse activates the multimers through heating and

![Class Comparison of Acidic Species Detected in Baker Emulsions (9.4 T)](image)

**Figure 8.2.** Class analysis for acidic species detected by negative ion ESI FT-ICR MS for emulsions A-C.

causes non-covalent interactions to collisionally dissociate prior to detection. Base peak signal to noise ratios of all spectra were roughly the same.

The high resolution MS results yield unique elemental composition for each of the m/z values detected. Analysis of acidic components by negative mode ESI FT-ICR
MS produces similarities between crudes A and C, with respect to the % relative abundances (% RA) observed for all heteroatom (nitrogen (N), oxygen (O), and sulfur (S)) classes greater than 1% (Figure 8.2). The most abundant class is N₁, ranging between 30-39% RA for each sample. Within most classes observed by negative mode electrospray, there appears to be simultaneous up-scaling or down-scaling in crudes A and C, with respect to crude B.

**Figure 8.3.** Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the acidic N₁ class for analyzed emulsion samples.

Visualize of heteroatom class trends within each class detected in Figure 8.2 are pictorially presented in iso-abundance color contour plots. These plots graph double bond equivalents (DBE) vs. carbon number. DBE is a measure of aromaticity and is calculated as the number of rings and/or double bonds contained within a particular molecular compound. **Figure 8.3** shows the iso-abundance color contour plot for the N₁ class. The compounds contained within each sample are extremely similar in both aromaticity and carbon number. Crude A does show a slightly narrower carbon number range (20-65 carbons), but this is believed to be caused more by the lack of high m/z
Figure 8.4. Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the acidic O₁ class for analyzed emulsion samples.

Figure 8.5. Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the acidic O₂ class for analyzed emulsion samples.
species detected by FT-ICR MS from crude A, when compared to the detected carbon numbers in crudes B and C.

For each heteroatom class detected at >1% RA in Figure 8.2, these same iso-abundance color contour plots were created. The same overall trends in aromaticity and alkylation are observed within other heteroatom class. There seems to be no compositional difference (DBE and carbon number) among the acidic species observed. Figure 8.4 examines the O₁ species detected in the crudes. All O₁ compounds are of DBE > 4, meaning they are all aromatic. Figure 8.5 shows the trends in the O₂ compounds. The highest concentration (indicated by deep coloration) of these species are non-aromatic (DBE < 4), or carboxylic type species.

Positive Mode ESI

Positive mode electrospray ionization probes the basic species contained within each crude oil. Just as for negative ESI MS, low resolution mass spectrometry was used to verify the monomer molecular weight distribution. Figures 8.6 depicts both the (a) low resolution and (b) high resolution mass spectra each crude B. This spectrum is

![Figure 8.6](image)

**Figure 8.6.** Comparison of molecular weight distributions detected by positive mode ESI (a) low resolution ion trap mass spectrometry and (b) high resolution FT-ICR mass spectrometry for emulsion sample B.
representative of the three samples analyzed. MS
experiments confirm that the broad MWDs arise from aggregation and multimerization of the monomer compound (results
not shown). High resolution FT-ICR mass spectra were detected after a 10 msec CO
laser pulse inside the ICR cell. The CO
laser pulse collisionally activates the multimers
through heating and causes non-covalent interactions to dissociate prior to detection.

The class analysis of the basic species detected is presented in Figure 8.7. The
most abundant species detected in all samples is the N
class. For the N
class of
compounds, the % RA for crudes A and C is slightly higher than for crude B. But, unlike
negative mode data, all other classes detected at > 1% RA in crudes B and C are more
compositionally alike than that of crude A.

Iso-abundance color contour plots generated from all basic classes show the
presence of similar aromaticity and alkylation trends in all classes detected (results not
shown). Results generated from both the negative and positive ESI FT-ICR MS data
are inconclusive. These results alone show no definitive reason for the observed
oil/water separation problems that arise in downstream processing.

Figure 8.7. Class analysis for acidic species detected by negative ion ESI FT-ICR MS
for emulsions A-C.

Bulk Elemental Analysis
As a follow-up to the ESI results discussed above, we examined each crude oil for bulk elemental properties via a flash elemental analyzer (C.E. Elantech) CHNS-O instrument. This instrument measures bulk CHNS-O weight percent properties by two methods. CHNS data is generated when a sample is combusted. The combustion process converts each of the elements into their oxidized forms. Elemental carbon is detected as CO$_2$, elemental hydrogen as H$_2$O and so forth. These vapors then pass from the combustion chamber into a packed chromatography column where they are separated based on their interaction with the column’s stationary phase. The gases elute from the chromatography column and signal intensity is measured as a form of resistance across an electrically heated wire on a thermal conductivity detector.

Each eluting gas causes an increase in the temperature around the TCD’s wires, this temperature increase results in an increase in resistance. The duration of the resistance event coupled with the intensity of the resistance yields a nice measure of the signal intensity. The results are generated visually in chromatographic fashion on a computer screen. Oxygen signals are detected after pyrolysis of the sample. The samples are pyrolyzed in an oxygen-free atmosphere and then the O$_2$ gas is measured after “separation” on a chromatography column by the TCD detector in the same manner outlined above.

Samples are analyzed in quadruplicates. Computer software uses a mathematical calculation to calibrate the instrument when prompted. Calibration values are computed from standards that are combusted or pyrolyzed just prior to unknown sample analysis. Ultimately, integration of the chromatographic peaks generates the % abundance of each element in the sample. Background noise is accounted for in sample preparation and subtracted out prior to generating results.

Experimental results for the nitrogen, oxygen and sulfur are shown in Figure 8.8. The carbon and hydrogen values detected were similar in all samples, at ~70% and 10%, respectively. Crude A contains nearly twice the % relative abundance of elemental sulfur when comparing all three crudes. This result invited exploration of non-polar speciation of the crudes using derivatization methods using silver triflate for positive mode ESI speciation and tetramethylammonium hydroxide (TMAH) for negative mode.
Figure 8.8. Elemental analysis results for heteroatoms nitrogen (N), oxygen (O) and sulfur (S). Carbon (C) and hydrogen (H) are not shown as they are roughly equivalent at ~70 and ~10%, respectively, in all crudes.

ESI. The procedure is outlined Chapter 2 of this document. In brief, silver triflate, and more specifically the silver cation (Ag+), complexes through coordination with the lone pair of electrons of sulfur atoms, as well as, pi electrons of aromatic ring systems. This enables the formation of a silver adduct [(M+Ag+)⁺] to be formed in the solution phase and detected, as such, in the gas phase by FT-ICR MS. Such complexation enables the observation of non-polar sulfur compounds and aromatic hydrocarbons that previously were not ionizable by conventional ESI methods. The results collected are very similar to that of other non-polar ionization methods, such as Atmospheric Pressure Photoionization (APPI).¹²⁴

The use of TMAH allows for more efficient deprotonation of the more acidic species of crude oil in the solution phase; enabling the detection of compounds not normally observed under conventional ESI methods. Non-polar sulfurs and hydrocarbons again are observed in these conditions. One additional benefit to the TMAH method, is that the normal domination of O₂ and O₄ species (easily deprotonated by ammonia hydroxide but not necessary highly abundant in all crudes) on electrospray
signal is significantly suppressed by the deprotonation of other species, therefore, detailing a more representative result of the acidic species that exist in the crude oil.

**Silver Triflate Cationization**

FT-ICR MS scans were optimized to reflect the molecular weight distributions (MWDs) detected by low resolution MS. Multimers (both hetero- and homo-) have been shown to cause the observance of broad MWDs. Therefore, prior to ICR, MS\textsuperscript{n} spectra were collected in order to fragment and verify true monomer distributions (results not shown). For that same reason, FT-ICR mass spectra were detected after a 10 msec CO\textsubscript{2} laser pulse inside the ICR cell. The CO\textsubscript{2} laser pulse collisionally activates the multimers through heating and causes non-covalent interactions to dissociate prior to detection. **Figure 8.9** displays the high resolution mass spectra of the three crude oils detected with silver triflate derivatization. The MWDs of the crude oil samples are all very similar. Minor variations in the detected signal to noise ratios are evident.

**Figure 8.9.** Mass spectra of the Baker crude oils as ionized by ESI with silver triflate metal complexation. The spectra are plotted on an absolute axis to depict the variation in the signal to noise ratios.
The high resolution results yield unique elemental composition of the \( m/z \) values detected. The classes detected at greater than 1% relative abundance (RA) are shown on a class graph in Figure 8.10. The results of Figure 8.10 seem to correspond well with that of the bulk elemental analysis, in that crude A shows a higher relative abundance of sulfur-containing compounds (such \( S_1 \)) as well as, the identification of several sulfur-containing classes that are not observed in both samples B and C. Additionally, samples B and C are much more similar in the % RA of each heteroatom class when compared to sample A. In all cases, crudes B and C show either a simultaneous increase/decrease in % RA with respect to sample A; one possible reason explaining why crudes B and C cause no oil/water separation problems in the field.

Figure 8.11 shows the iso-abundance color contour plot for the \( S_1 \) class. The images show similarity in all samples detected. The \( S_1 \) compounds of crude A are only slightly more aromatic (DBE 0-25) and have a higher degree of alkylation; ranging in carbon number 20-70, with the highest concentration between 30-50 carbons. The majority of \( S_1 \) species in sample A are of 5-7 DBE and ~40 carbons, while in samples B and C the samples are 5-7 DBE and ~36 carbons.

Figures 8.12 and 8.13 display the trends detected in the \( N_1 \) and hydrocarbon classes, respectively. The same underlying trend is observed within these heteroatom classes. The compounds in crude A are only slightly more aromatic with approximately the same alkylation trends when compared to crudes B and C. Due to lack of comparison, iso-abundance color contour plots for the other heteroatom classes (detected at >1% RA) are not shown here as most classes are only observed in one of the three samples.

**Tetramethylammonium Hydroxide**

Negative mode ESI analysis is known for its ability to efficiently ionize acidic species through the deprotonation of molecular compounds. The use of ammonia hydroxide is normally used to aid in the deprotonation process. The relatively low basicity of ammonia hydroxide enables this to only be done for the most acidic species in the crude oil. Therefore, an increase in the deprotonating base’s strength enables the less acidic species to become deprotonated and therefore observable by FT-ICR MS.
Class Speciation of Baker Crudes by ESI FT-ICR MS derivatized with Silver Triflate

<table>
<thead>
<tr>
<th>Class</th>
<th>Crude A</th>
<th>Crude B</th>
<th>Crude C</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁</td>
<td>25</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>N₁</td>
<td>15</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>HC</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>O₁S₁</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>N₂O₁</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>S₂</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>N₁S₁</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>O₁S₂</td>
<td>5</td>
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<td>5</td>
</tr>
<tr>
<td>O₂S₁</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>O₁</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Figure 8.10.** Class analysis for species detected by positive ion ESI FT-ICR MS for crude oils A-C.

**Figure 8.11.** Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the non-polar S₁ class detected in the analyzed crude samples.
**Figure 8.12.** Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the non-polar $N_1$ class detected in the analyzed crude samples.

**Figure 8.13.** Relative iso-abundance color contour plots of double bond equivalences vs. carbon number for the hydrocarbon class.
Our laboratory has chosen tetramethylammonium hydroxide as the “superbase” in these experiments. Figure 8.14 depicts the broadband mass spectra obtained in these experiments. Again, high resolution FT-ICR mass spectra were detected after a 10 msec CO$_2$ laser pulse inside the ICR cell. The CO$_2$ laser pulse collisionally activates the multimers through heating and causes non-covalent interactions to dissociate prior to detection.

The class analysis of all species observed is presented in Figure 8.15. The most abundant species detected in all samples is the N$_1$ class. All heteroatom classes show little to no variation in the % relative abundance observed in all samples. Although, the S$_1$ class is less than 1% RA in all samples, the S$_1$ compounds are presented in Figure 8.16 due to interest in non-polar sulfur compounds. Among all heteroatom classes examined, the compounds are equivalent in aromaticity and carbon number for the species detected. Therefore, no observable difference can be noted from the acidic species detected by FT-ICR MS.

Figure 8.14. Mass spectra of the Baker crude oils as ionized by ESI with tetramethylammonia hydroxide (TMAH). The spectra are plotted on an absolute y-axis.
All results presented up until this point have shown little to no differences in the aromaticity or alkylation of the compounds detected in all of the crude samples. No observation has definitively identified (or indicated) what may be the cause of the oil-water separation problems presented by these crude oils. Our last attempt at determining possible causes for the oil-water issues was the use of a newly patented technique, ammonia-based extraction.\textsuperscript{154}

**Ammonia-Based Extraction**

The novel ammonia base extraction method\textsuperscript{154} was used in laboratory settings to test crude oils for the presence of naphthenic acids. Naphthenic acids are a set of acids, commonly present in crude oil, that are targeted for removal because of their high prevalence to cause corrosion issues. Additionally, they are considered to be a cause of crude oil emulsion formations.\textsuperscript{116} In brief, the experiment is conducted by bubbling ammonia gas into a container of solvated crude oil. The ammoniated crude oil is then placed in the freezer overnight. Naphthenic acids are crystallized in the presence of the ammonia gas and they collect on the bottom of the cold container. The ammoniated crude is then removed by suction and the naphthenic acid crystals are washed to
Figure 8.16. Class compositional profile for heteroatom-containing classes identified in the ammoniated crude oils.

Figure 8.17. Class compositional profile for heteroatom-containing classes identified in the ammoniated crude extracts.
remove excess crude oil and solvated in methanol. Both the crystals and the crudes are retained and analyzed by FT-ICR MS for composition, under normal negative ion electrospray ionization conditions (e.g. ammonium hydroxide modifier).

After the allowable time period for crystal formation elapsed it was observed that only crude C contained naphthenic acids, but in very, very low quantities. Crystal formation was not observed in either crude A or crude B. Crude A, however, did have a sludge-like formation on the bottom of the vial after the experiment.

Figures 8.16 and 8.17 display the class graphs for the classes detected in the ammonia extraction experiments for the ammoniated crude and their corresponding extracts, respectively. The N₁ class is the most abundant class observed in all samples. The variation in % relative abundance is insignificant among all classes observed in the ammoniated crudes.

![Diagram](image)

**Figure 8.18.** Iso-abundance color contour plots for the N₁ compounds isolated in the ammoniated crude oils and their respective extracts.

There appears to be a significantly higher quantity of N₁ compounds in extract B when compared to samples A and C. Additionally, crude C’s extract shows much higher oxygenation across the classes detected. This result most likely corresponds with why
Figure 8.19. Iso-abundance color contour plots for the $N_1S_1$ compounds isolated in the ammoniated crude oils and their respective extracts.

Figure 8.20. Iso-abundance color contour plots for the $O_1$ compounds isolated in the ammoniated crude oils and their respective extracts.
the crude formed crystals during the ammonia extraction process. More specifically, those compounds in the O\textsubscript{2} class tend to indicate carboxylic acid functionality. Iso-abundance color contour plots for the N\textsubscript{1}, N\textsubscript{1}S\textsubscript{1}, and O\textsubscript{1} classes are shown in \textbf{Figures 8.18-8.20}. These figures show a comparison of alkylation and aromaticity trends for both the ammoniated crudes and their corresponding extracts. In all figures shown, there are only minor variations in alkylation ranges and aromaticity of the compounds.

One possible noteworthy observation as to why crude A may be the cause of an oil-water separator problem is seen in \textbf{Figure 8.21}. Extract A shows a high abundance of non-aromatic O\textsubscript{2} compounds, commonly referred to as fatty acids. The alkylation of these compounds is broad, spanning carbon numbers 15-70. High abundance of these acids is observed around carbon number 18. While, the O\textsubscript{2} class in extract A is not observed as the most abundant class of compounds and the ammonia extraction failed to produce crystals, it appears that the naphthenic acid functionality is highly prevalent.

\textbf{Figure 8.21.} Iso-abundance color contour plots for the O\textsubscript{2} compounds isolated in the ammoniated crude oils and their respective extracts.
Conclusions

Previous results from negative ESI FT-ICR MS and probing of surface active components have shown that there is an increase in the amount of O$_x$ and SO$_x$ species with respect to parent crude.$^{80}$ These results lack the detection of acidic SO$_x$ species. The observed trends from both positive and negative ESI have failed to detect vast difference in the species that may be causing emulsion A to cause oil/water separation downstream.

Elemental composition analysis shows that crude A has a slightly higher concentration of sulfur containing compounds. While supportive of this observation, derivatized electrospray ionization using silver cation complexation or a superbase, continues to show little difference in the crude oil composition. One possible solution for the oil-water separation problem is observed through the high presence of acidic O$_2$ compounds after ammonia-based extraction.
APPENDIX A

WATER-SOLUBLE ATMOSPHERIC ORGANIC MATTER IN FOG: EXACT MASSES AND CHEMICAL FORMULA IDENTIFICATION BY ULTRAHIGH-RESOLUTION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY


Summary

The detailed molecular composition of water-soluble atmospheric organic matter (AOM) contained in fog water was studied by use of electrospray ionization ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry. We identified 1368 individual molecular masses in the range of 100-400 Da from negative-ion spectra obtained after reversed-phase extraction with a hydrophilic solid phase sorbent. The detected organic anions are multifunctional with a variety of oxygenated functional groups. We observe organic nitrogen, organic sulfur, and organic nitrogen-sulfur compounds as well as many species with only C, H, and O elemental composition. Analysis of the double bond equivalents (DBE = the number of rings plus the number of double bonds to carbon) suggests that these compound structures range from highly aliphatic to aromatic with DBE values of 1-11. The compounds range in their extent of oxidation with oxygen to carbon ratios from 0.2 to 2 with an average value of 0.43. Several homologous series of compounds and multifunctional oligomers were identified in this AOM. The high extent of homologous series of compounds likely originates from primary components that have become oxidized. The multifunctional oligomers appear to represent atmospheric processing of primary and secondary compounds. The isolated water-soluble components identified here are amphiphilic, meaning that they contain both hydrophilic oxygenated functional groups and hydrophobic aliphatic and
aromatic structural moieties. Despite the high number of compounds with very high oxygen content, 60% of assigned chemical formulas have measured organic mass-to-organic carbon ratios \( \leq 2.25 \) (ranging from 1.25 to 3.5). Because the results reported here are not quantitative, an average ratio cannot be determined.

**Introduction**

Atmospheric organic matter (AOM) in the form of organic aerosol or dissolved organic species in suspended cloud or fog droplets is a complex mixture. Its components account for a significant fraction of the total mass of fine particulate matter in the atmosphere. Early work estimated atmospheric water-soluble organic compounds to comprise between 20 and 70% of the total organic aerosol. Identification of water-soluble organic compounds presents a very challenging task. Many have multiple polar functional groups (e.g., carboxyl, hydroxyl, carbonyl, nitro, nitrooxy, and sulfate) and are not well separated by traditional chromatographic techniques. Despite decades of effort water-soluble organic compounds are still not well characterized on a molecular level. It is desirable to characterize these compounds because they may play essential roles in fundamental environmental processes, such as chemical reduction/oxidation mediation, sorption, complexation, solubilization, and aerosol water-uptake.

Electrospray ionization coupled with Fourier transform ion cyclotron resonance (FT-ICR) MS provides detailed molecular characterization of organic matter due to its extremely high resolution and mass accuracy. ESI is a “soft” ionization technique that offers minimal fragmentation of the analytes, thus allowing for detection of intact molecules. ESI is especially effective for ionization of polar molecules since ions are typically formed by either protonation or deprotonation of polar moieties. The ultrahigh resolution of FT-ICR MS has revealed extremely complex mass spectra with up to 63 individual mass peaks per nominal mass. Furthermore, the high resolving power of the FT-ICR mass spectrometer provides sufficiently accurate mass measurement for unique assignment of molecular elemental composition of individual components. This very powerful technique has been used recently by many investigators for identification of complex organic species from a variety of
In this study, we present molecular level characterization based on FT-ICR MS of isolated water-soluble AOM from radiation fog collected in Fresno, California. Radiation fog droplets form in the surface boundary layer when the atmosphere in contact with the surface rapidly cools, forcing water vapor to condense from the gas phase onto suspended aerosol particles that act as CCN. Fog presents a combination of scavenged particles, water-soluble gases, and their reaction products and therefore defines a rich environment for the study of atmospheric organic matter. Radiation fog events occur during the winter in the central valley of California after several days of stagnant conditions. These stagnant conditions suggest that the aerosol in the valley is a mixture of aged and fresh emissions.

**Experimental Methods**

**Collection of Fog Samples**

Fog samples were collected in January 2006 in Fresno, CA. Fog samplers were set up at the California State University experimental farm in an open field in north Fresno. The site was chosen to represent polluted urban fog environment, influenced by emissions from residential, transportation, and industrial activities. Fog samples were collected with a large stainless steel version of the Caltech Active Strand Cloudwater Collector. The bulk collectors operate by pulling droplet-laden air across a bank of stainless steel strands where cloud/fog drops are collected by inertial impaction. The collected drops flow down into a stainless steel collection trough, through a stainless steel tube, and into a pre-baked glass bottle. The collector operated at a flow rate of ~40 m$^3$/min, allowing for the collection of large sample volumes. Sampling intervals typically ranged from 1-2 hours depending upon the fog liquid water content. A field blank was prepared in the field consisting of high purity water (UV treated and deionized). Samples were filtered immediately after collection with a pre-fired quartz filter. The sample filtrates were stored in pre-baked amber glass jars and refrigerated immediately after analytical aliquots were made. A sample collected on January 6th, 2006 between 12:30 am and 2 am at the beginning of a fog event was selected for the high resolution MS analysis presented here. An aliquot of this sample was analyzed for total and dissolved organic carbon concentrations by use of a Shimadzu TOC-500A analyzer.
calibrated with potassium hydrogen phthalate. Future publications will present additional samples in time series across single and multiple fog episodes.

**FT-ICR MS Sample Preparation**

Solid phase extraction (SPE) was used for sample preparation of fog water samples. This method was adapted from Kim et al.\textsuperscript{173} In this study, we substituted C\textsubscript{18} disks with a polymeric resin (Phenomenex Strata-X) SPE cartridge. Strata-X is a hydrophilic sorbent made of surface modified styrene-divinylbenzene polymer (similar to XAD-4 resin) with a specific surface area of ~800 m\textsuperscript{2}/g.\textsuperscript{174} The 1 g SPE cartridges were pre-conditioned with methanol (Fisher Optima grade) and then rinsed with 1% formic acid (Fluka LC/MS grade) in high purity water (UV treated and deionized). A sample volume of 100 mL of previously filtered fog water (with a dissolved organic carbon concentration of 12.7 ppm) was pH adjusted with formic acid to pH 4.5 and then applied to the SPE cartridge at a flow rate of 1 mL/min. After the sample had passed through the cartridge, the cartridge was washed with 1% formic acid in high purity water to remove inorganic compounds. 2 mL of alkaline (pH adjusted to 10.4 with ammonium hydroxide) high-purity water, methanol, and acetonitrile (10/45/45 vol/vol/vol) was used to elute the organic compounds from the SPE cartridge. A brown color band was observed moving down and off the SPE material into the sample vial.

**Instrumental Parameters**

Samples were analyzed with a hybrid 7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (LTQ FT Ultra, Thermo Scientific) equipped with a chip-based direct infusion nanoflow electrospray ionization (nESI) source (Triversa Nanomate, Advion Biosciences, Ithaca, NY). All mass spectra were acquired for negative ions produced by an electrospray source voltage of -1.35 kV and a N\textsubscript{2} gas pressure of 0.35psi. Selected-ion monitoring (wide SIM mode) allowed isolation of an m/z 600 window in the LTQ before FT-ICR analysis. We used two overlapping windows (50 < m/z < 650 and 400 < m/z < 1,000) to cover the full mass range. Mass resolving power, m/\Delta m\textsubscript{50\%}, in which \Delta m\textsubscript{50\%} is peak full width at half-maximum peak height, was set at 200,000 (at m/z 400) for all spectra. Automatic gain control was used to consistently fill the LTQ with the same number of ions for each acquisition and to avoid
space charge effects from over-filling the mass analyzer. The instrument was externally calibrated in negative ion mode with a standard solution of sodium dodecyl sulfate and taurocholic acid, and the resulting mass accuracy was better than 2 ppm. Hundreds of individual mass spectra were collected and stored as transients by use of Thermo Xcalibur software.

Data Processing and Assignment of Elemental Compositions

Approximately 370 transients recorded in the time domain were co-added with Predator v. 3.54.34. Co-addition of numerous time domain data sets prior to Fourier transformation enhances the signal-to-noise ratio. The empirical formula calculator (Sierra Analytics Composer) was used to assign chemical formulas to the masses of singly-charged ions of 100 < m/z < 400 and relative abundances (RA) ≥1%. The calculator uses the Kendrick mass defect to sort homologous ion series (species with a given double bond equivalents (DBE) and heteroatom content but differing by increments of –CH₂). The calculator was set to allow up to 30 carbon, 60 hydrogen, 20 oxygen, 3 nitrogen, and 1 sulfur atoms per elemental composition. Naturally present fatty acids were used as internal calibrants, thereby lowering the average mass error to < 0.1 ppm. Data filtering of the assigned formulas was done by applying rules and assumptions as described by Koch and colleagues. Further information is available in the corresponding supplemental information. DBE was calculated from Equation (A.1):

\[ \text{DBE} = c - \frac{h}{2} + \frac{n}{2} + 1 \]  

(A.1)

for elemental composition C\textsubscript{c}H\textsubscript{h}N\textsubscript{n}O\textsubscript{o}S\textsubscript{s}. Note that sulfur is divalent in Eq. (A.1). Additional bonds formed by tetravalent and hexavalent S are not included in DBE calculations.

Results and Discussion

Mass Spectra and Peak Assignments

With co-addition of ~370 ICR time-domain transients, more than 3000 individually measured monoisotopic masses were identified in the mass to charge ratio (m/z) range
of 100 < m/z < 400. Approximately 10 individual masses were observed within 0.3 Da of each nominal mass (Figure S-1). This great complexity observed in atmospheric fog water results from the several sources of AOM including primary components and secondary components formed in either gaseous and/or aqueous secondary reaction

![Kendrick mass defect vs. Kendrick nominal mass](chart.png)

**Figure A.1.** The Kendrick mass defect vs. Kendrick nominal mass for 1,368 assigned chemical formulas, excluding species containing lesser abundant stable isotopes (\(^{13}\)C and \(^{34}\)S). Kendrick mass is previously defined as the measured exact mass times the ratio of the nominal to exact mass of CH\(_2\).
pathways. We know from previous studies that even 2-3 component reaction systems can result in hundreds of individually measured masses. In this study, we analyzed a natural sample with unknown numbers of precursor compounds and a variety of potential reaction pathways. Likewise, Altieri and coworkers recently studied rainwater with a similar FT-ICR MS methodology (but without co-addition of transients); they reported elemental compositions for 552 individual masses in the range of 50-500 Da. Previously, Reemtsma et al. identified approximately 1000 compounds from 220 < m/z < 420 and Wozniak et al. identified more than 3000 peaks from 223 < m/z < 600 from aerosol filter samples. These large data sets provide a foundation for understanding the nature of atmospheric AOM, especially with respect to fundamental elemental composition and structural attributes and their changes due to atmospheric chemical transformations. Thus, we include with this paper a complete listing of the assigned chemical formulas and their corresponding measured masses (Tables S-1 and S-2).

Overall, 2,436 masses were assigned unique elemental compositions. Of those, 1,368 "monoisotopic" species contained only the most abundant isotopes ($^{12}$C, $^{1}$H, $^{14}$N, $^{16}$O, $^{32}$S) and the remaining 1,068 contained one or more $^{13}$C and/or $^{34}$S. All assigned ions were singly-charged (as evident from the unit m/z separation between the $^{12}$C$_{c}$ and $^{13}$C$_{c-1}$ isotopomers for each elemental composition) and even-electron, [M-H]$^-$ (due to electrospray ionization). We detected the $^{13}$C and $^{34}$S containing species for 66.5% of the assigned chemical formulas. Most of the remaining 33.5%, without detection of the corresponding $^{13}$C masses, were CHNO compounds. However, those compounds belong to Kendrick series (see below) for which at least one of the assigned formulas did include a heavy atom to confirm the assignment. Approximately 20% of the total observed mass spectral peaks remain unidentified. Possibly they contain additional elements or more nitrogen or sulfur atoms than permitted in the calculations. Further, because FT-ICR mass measurement precision and accuracy are directly proportional to peak height-to-noise ratio, it is not possible to obtain unique elemental compositional assignments for the many peaks of low signal-to-noise ratio, especially at the upper end of the mass range. Formula calculations were limited to peaks $\geq$ 1% RA; i.e., 15 times the peak height-to-noise ratio of 0.0667 % RA. A total of 238 masses
were assigned unique elemental compositions in our field blank, with a threshold of 6 times the peak height-to-noise ratio of 0.03 % RA. Of those 238 masses, 83 monoisotopic masses and 58 isotopomers were in common with the fog water sample. At present it is unclear if the common masses represent nESI-MS carryover from our samples or trace contaminants from laboratory handling. The common masses and chemical formulas are provided in Table S-1.

**General Characteristics**

Based on the chemical formula assignments, we observe low molecular weight AOM compounds with a range of 3-26 carbon atoms per molecule. The most common homologous series found in all natural OM is alkylation. The homologous series of CH$_2$ chains are easily revealed by Kendrick mass defect analysis, as in petroleum, for which several CH$_2$ series span the mass range up to 1500 Da. Both primary and secondary AOM also contain several homologous series in the range, 100 < m/z < 400, see the horizontal series of points in the Kendrick plot in Figure A.1. Note that these series exist for CHO, CHON, CHOS, and CHNOS compound families. The origin of the high number of homologous series is unknown; however this observation is consistent with other OM from other natural systems. DBE values determined from the neutral chemical formula range from 1 to 11 (Figure A.2A). In addition to the DBE vs. carbon number, we present the analyte RA (i.e., peak height relative to that of the base peak, m/z 183.0047). Note in ESI-MS analysis, relative abundance is the product of initial neutral concentration and ionization efficiency.

Although ionization efficiency can vary among different compounds, we do observe a few similar features between this method and GC/MS methods. For example, the fatty acid series, (namely, species containing two oxygen atoms and DBE = 1), extends from C$_3$ to C$_{25}$, with highest abundance corresponding to palmitic acid (C$_{16}$) and stearic acid (C$_{18}$). Long chain alkanoic acids are known to be prevalent in atmospheric aerosols. Other prominent components (C$_{10}$ and DBE = 2-3) may be monoterpene derivatives. Ions with C$_{6-8}$ and DBE = 5-6 likely correspond to previously observed benzoic acid and phenolic structures such as nitrophenols and nitrocresols. Figure A.2B displays the number of oxygen atoms vs. the number of carbon for all
formulas. It is important to note that DBE counts double bonds to carbon, and thus includes both C=C and C=O species. Decesari et al. suggested that a high percentage of the water-soluble organic compounds associated with aerosol/fog water are polyacidic. We find up to 10 oxygen atoms in some of our assigned molecular formulas, allowing up to 5 carboxylic groups. However, due to the observations of organic nitrogen and sulfur in this sample and in previous studies, it is likely that the AOM is multifunctional with a combination of hydroxyl, carbonyl, carboxyl, ester, nitrate, and sulfate functional groups.

Figure A.2. Atmospheric organic matter isoabundance contoured plots for 1,368 assigned elemental compositions, with color coding for analyte relative abundance (RA) % shown at right. A) Double bond equivalents versus carbon number, B) Oxygen number versus carbon number.

van Krevelen Analysis

The assigned molecular formulas of the AOM are presented in van Krevelen diagrams, shown in Figures A.3 and A.4. Each diagram is a plot of the atomic ratio of H:C vs. O:C for each assigned elemental composition. Aliphatic compounds have low O:C ratio (<0.5) and high H:C ratio (>1.5) (upper left). Likewise, aromatic compounds typically exhibit low O:C ratio (<0.5) and low H:C ratio (<1.0) (lower left). Further, van Krevelen plots are used to evaluate the degree of alkylation, hydrogenation, hydration and oxidation of AOM. However, it is important to note that although a typical compound family may occupy a defined area in a van Krevelen plot, the converse is not
true—i.e., location of an unknown compound in the same region does not prove that it belongs to that compound family.

As described by Kim et al.\textsuperscript{188}, distinctive lines in the plot can represent chemical trends within a compound group. Several lines (horizontal, vertical, and diagonal) similar to plots for organic matter from aquatic environments\textsuperscript{188} are shown in Figure A.3. These lines can reflect compound relationships such as oxidation/reduction (horizontal), hydrogenation (vertical), alkylation (diagonal-A) and condensation (diagonal-B). As expected from the number of homologous series in the Kendrick plot (Figure A.2), the data shown in Figure A.3 exhibit pronounced diagonal-A lines (relative to the box in the upper left portion of the plot). In addition, several diagonal-B lines are seen relative to the oval in the upper right portion of the plot. These compound relationships occur for many processes, and thus can provide clues regarding the nature of the primary and secondary organic aerosol simultaneously.

The chemical trends are further elucidated with abundance-weighted van Krevelen plots (Figure A.4). Figure A.4A includes all of the assigned chemical formulas whereas Figures A.4B, C, and D include those compositions containing only CHO, CHNO, and CHOS and/or CHNOS compounds. Although diagonal-A lines are prominent in Figs. A.4C and A.4D, diagonal-B lines are not easily distinguished in the abundance-weighted van Krevelen plots of Figure A.4. The prominence of the diagonal-A lines indicates an aliphatic predominance. The observed aliphatic character cannot be explained solely by the presence of alkanoic mono- and di-carboxylic acids, but must also be in the form of alkyl substituents, as indicated by the range of DBE values and other elements (N and S).

A total of 715 of the exact mass measurements could be assigned to elemental compositions containing only C, H, and O, presumably including carbonyl, carboxyl, and/or hydroxyl functional general, the compounds appear to be significantly oxidized with an average O:C ratio of \(~0.5\. The observed O:C and H:C ratios are higher than those from van Krevelen diagrams of dissolved organic matter (DOM) from surface water.\textsuperscript{189} In other studies of AOM\textsuperscript{169, 179}, the observed atomic ratio ranges are similar for CHO compounds, although Wozniak and co-workers\textsuperscript{169} found similar O:C ratios (0.1 - 0.8) in their aerosol samples than observed here for fogwater AOM or by Altieri et al\textsuperscript{179}. \textsuperscript{188}
Figure A.3. van Krevelen plot of h:c vs. o:c ratio for 1,368 assigned elemental compositions, $C_{c}H_{h}O_{o}N_{n}S_{s}$, without relative abundance coding. A) Aliphatic homologous series ($CH_{2}$ groups) are distinguished by diagonal lines relative to the upper left box (diagonal-A). A few selected homologous series are shown with the arrow pointing in the direction of chain elongation. B) Compounds related to each other (within a group) by multiples of $CH_{2}O$ are distinguished by the diagonal lines relative to the upper right oval (diagonal-B). A few selected condensation/dehydration series are shown with the arrow indicating the direction of chain elongation.

in rainwater AOM (approximately 0.1 – 2.0). That difference is not surprising, given the tendency of atmospheric water to be enriched in more polar compounds relative to atmospheric particulate matter. None of the observations of AOM (previous studies$^{166,179}$ and this study), report a particularly high number of aromatic compounds, determined by a low H:C ratio.
Aqueous secondary organic aerosol oligomer products of CHO compounds have been reported recently by Alteri et al.\textsuperscript{179}, The oligomers were reported to form by an esterification reaction resulting in a mass increase of $\text{C}_3\text{H}_4\text{O}_2$.\textsuperscript{165} In our study, we observe only a few of the same oligomer product masses (Table S-1). However, by re-scaling our Kendrick masses\textsuperscript{179} to identify homologous series differing by multiples of $\text{C}_3\text{H}_4\text{O}_2$,\textsuperscript{187, 189}

\begin{equation}
\text{Kendrick mass (C}_3\text{H}_4\text{O}_2) = \text{Observed Mass} \times \left(\frac{\text{Nominal Mass of C}_3\text{H}_4\text{O}_2}{\text{Exact Mass of C}_3\text{H}_4\text{O}_2}\right) \tag{C.4}\end{equation}

\begin{equation}
\text{Kendrick mass defect (C}_3\text{H}_4\text{O}_2) = \left|\text{Nominal Mass} - \text{Kendrick Mass (C}_3\text{H}_4\text{O}_2)\right| \tag{C.5}\end{equation}

\textit{Figure A.4.} van Krevelen plots with color-coded analyte relative abundance (right); A) all 1,368 $\text{C}_c\text{H}_h\text{O}_o\text{N}_n\text{S}_s$ compositions; B) 715 compounds containing only C, H and O atoms; C) 487 compounds containing only C, H, N and O atoms; and D) 166 compounds containing only C, H, O and S or C, H, N, O, and S atoms.
we did find many other compounds resulting from the esterification reaction mechanism reported in Altieri et al.\textsuperscript{179}

In this way, we identified 238 individual oligomer series of two to four compounds separated by multiples of C\textsubscript{3}H\textsubscript{4}O\textsubscript{2} within our CHO subset of assigned molecular formulas. This high number of oligomer series represents 88\% of the number of CHO assignments. The structural elucidations from that analysis will be presented in a future publication.

A high level of organic nitrogen in California fog waters\textsuperscript{190, 191} and in New Jersey rainwater\textsuperscript{192} has been reported previously. Here, we assigned 487 molecular formulas containing nitrogen. As shown in Figure A.4C, these compounds represent a substantial fraction of the integrated mass spectral signal. Most occur in the same O:C and H:C range as the CHO compounds, and appear to be aliphatic or include alkyl substituents as noted by the occurrence of diagonal-A lines. The CHNO group consists of 13 compound subgroups denoted by variation in nitrogen and oxygen atoms: NO\textsubscript{3} – NO\textsubscript{9}, N\textsubscript{2}O\textsubscript{5} – N\textsubscript{2}O\textsubscript{9}, and N\textsubscript{3}O\textsubscript{8}. The high number of oxygen and nitrogen atoms suggests that these compounds are multifunctional with up to three nitro and/or nitrooxy functional groups in addition to other polar functional groups mentioned previously. A high number of species with nitrooxy functional groups has been observed elsewhere.\textsuperscript{179} Evidence for secondary organic aerosol formation by NO\textsubscript{x} reactions has been presented previously.\textsuperscript{193} The mechanism was described as an addition of NO\textsubscript{3} to a carbon-carbon double bond with subsequent addition of O\textsubscript{2}. Thus it was suggested that a variety of nitrated organic compounds, including hydroxynitrates, carbonyl nitrates, dinitrates, hydroxydinitrates, and other highly nitrated compounds, could be formed.

The CHNO compounds span a wide DBE range (3 to 11), indicating a high prevalence of double bonds and/or ring structures. Examination of the DBE vs. carbon number indicates that the most abundant CHNO species exhibit DBE = 5, 6, 9, and 10 (Figure S-2), likely corresponding to mono- and di-nitro substituted phenols, cresols, benzoic acids, and naphthalenes. In a recent review\textsuperscript{184} of nitrophenols, concentrations of up to 40 µg/L for both 4-nitrophenol and 2,4-dinitrophenol were reported in fog. Within
the CHNO group, we found 145 oligomer series of 2-3 compounds, representing 71% of
the number of assigned CHNO chemical formulas.

We also assigned 168 different organic sulfur elemental compositions. The
CHOS and CHNOS compounds are shown in a van Krevelen diagram (Figure A.4D).
Most show $0.2 < O:C < 1.2$ and $1 < H:C < 2.1$, as for the CHO compounds: namely,
aliphatic with a low degree of oxidation. The four most prominent individual negative
ions in this group are $C_{16}H_{25}O_3S^-$, $C_{17}H_{27}O_3S^-$, $C_{18}H_{29}O_3S^-$, $C_{19}H_{31}O_3S^-$ corresponding to
$m/z$ 297.15298, 311.1587, 325.1844, and 339.2000. These compounds are 4 of 6 in a
Kendrick series of $SO_3$ compounds with DBE = 4, and $C_{7-20}$. Those four compounds
have been observed previously\textsuperscript{179} and are suspected to be synthetic linear
alkylbenzene sulfonates. The identical masses were also observed for the field blank
(Tables S-1 and S-2), although with much lower ion abundances.

The oxygen number for each of the remaining CHOS and CHNOS compounds
was 5 or more, suggesting that most of them are multifunctional with sulfate groups. In
2005, Romero and Oehme\textsuperscript{186} reported observations of organosulfates at $m/z$ 229, 265,
279, and 299 based on nominal mass accuracy. Our assigned chemical formulas are
consistent with organosulfates at several of these and other masses. For example, we
observe two species near $m/z$ 265 and 279, $C_{10}H_{17}O_6S^-$, $C_{11}H_{21}O_5S^-$, $C_{10}H_{15}O_7S^-$, and
$C_{11}H_{19}O_6S^-$ whose neutral precursors have DBE values of 2, 1, 3, and 2. Additional
observations of organosulfates have been made by Surratt et al.\textsuperscript{194}, in their study of
secondary organic aerosol formation via ozonolysis of biogenic hydrocarbons. We found
a few of the same masses ($C_{10}H_{15}O_7S^-$, $C_{10}H_{17}O_7S^-$, and $C_{10}H_{19}O_7S^-$) (Table S-1). We
have also identified several oligomers: 4 series in the CHNOS group and 18 series in
the CHOS group. Due to the higher mass of these molecules, the oligomerization series
range from 2-3 compounds each. The oligomers represent 16% and 32% of the number
of assigned molecular formulas of the CHNOS and CHOS families.

In general, the overall trend for the CHOS and CHNOS compounds is that of the
alkylation and de-alkylation lines (diagonal-A). It is unclear why the increasing or
decreasing chain length trends appear in each of the above described plots, especially
Figures A.4C and A.4D. It may be related to the random nature of oxidation reactions or
a high occurrence of aliphatic biopolymers in the atmosphere. In a recent paper,
Gómez-González and colleagues\textsuperscript{167} observed organosulfates that appear to originate from photooxidation of unsaturated fatty acids. Given the apparent trend of alkylation in the CHOS graph, we suspect that many of the presently identified organic sulfur compounds may also originate from oxidation of aliphatic compounds such as alkenes and alkenoic acids. Other CHOS and CHNOS compounds correspond to $\alpha$- and $\beta$-pinene oxidation products. Inuma and coworkers\textsuperscript{195} observed several nitroxy-organo-sulfates in nighttime (but not daytime) ambient samples collected in a forested site in northern Germany. One of the masses corresponds to nitro-organo-sulfates observed by Inuma \textit{et al.}\textsuperscript{195} and Surratt \textit{et al.}\textsuperscript{194} was observed at high relative abundance in our nighttime fog sample; m/z 342.05004 (DBE = 3) corresponding to C$_{10}$H$_{16}$NO$_{10}$S$. A few abundant members of this group are C$_{10}$H$_{16}$NO$_{9}$S$, C$_{9}$H$_{14}$NO$_{8}$S$, C$_{10}$H$_{18}$NO$_{8}$S$, C$_{10}$H$_{16}$NO$_{8}$S$, and C$_{12}$H$_{20}$NO$_{9}$S$, with neutral precursor DBE values of 3,3,2,3, and 3. Those compounds appear to be oxidized monoterpenes and may have formed in a manner similar to those observed in chamber studies.

\textbf{Atmospheric Implications}

The isolated water-soluble AOM identified in fog water was found to be quite complex with a range of O:C ratios from 0.2 to 2.0 and DBE values from 1 to 11. As expected, these components also have variable mass ratios for organic matter to organic carbon (also known as organic mass to organic carbon ratio (OM/OC)\textsuperscript{196}) ranging from 1.25 to 3.5 (Figure S-3). Consistent with previous studies\textsuperscript{166, 179}, we define OM/OC as the measured mass divided by the calculated mass of carbon in the assigned formulas. Despite the high number of compounds with very high oxygen content 60\% of assigned chemical formulas have OM/OC ratios $\leq$ 2.25. Since the results reported here are not quantitative, an average OM/OC ratio for mass closure\textsuperscript{196, 197} cannot be determined. It is important to note that fog scavenging of AOM and nESI-MS analysis are both likely to favor identification of hydrophilic and neutral polar compounds with higher than average OM/OC ratio. At the same time, the urban location (close to sources) and winter period (low oxidant concentrations) of the sampling period do not favor extensive oxidation of AOM which would typically increase the observed
OM/OC ratio. A histogram of the individually measured OM/OC values is provided in the supplemental information (Figure S-3).

The isolated water-soluble AOM identified in this study appears to result from oxidized variations of aliphatic and aromatic precursor compounds. The oxidized precursors undergo esterification reactions to form a high number of oligomer products. In this work, we found 406 individual oligomer series, accounting for a very high percentage of the CHO and CHNO assigned formulas. The origin of the highly prevalent CH$_2$ homologous series remains unknown. Because the oligomerization reactions$^{166}$ change the ratio of elements, the oligomers will have a different Kendrick (CH$_2$) mass defect. Note that each homologous series identified here varies in length from 5 to 18 carbon atoms. The high occurrence of homologous series observed here could result from the oxidation of primary aliphatic components from biopolymers as hypothesized by Poschl$^{198}$ and observed in Fresno fog water previously$^{191}$. Further work is needed to identify the primary reaction pathways involving additional types of primary aerosol and bio-aerosol.

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APPENDIX B

CHEMICAL ANALYSIS OF SURFACE ACTIVE ASPHALTENES

Introduction

Our hypothesis was that the most surface active components within the asphaltenes are also the components that first precipitate. These components may be chemically distinct from the majority of asphaltene components and they may have a significant role in asphaltene self-association, interfacial behavior, and phase behavior. We wish to determine the chemistry of these components in more detail.

We have collected the following precipitate samples from series of solubility experiments with heptane diluted Athabasca bitumen:

• “bulk” asphaltenes – the precipitate from a water-free bitumen at high dilution ratio (> 10% yield from bitumen)
• “least soluble” asphaltenes – the precipitate from a water-free bitumen at moderate dilution ratio (<2% yield from bitumen)
• “interfacial” asphaltenes – the precipitate from a bitumen containing emulsified water, at a dilution ratio below the normal onset of precipitation (these asphaltenes are likely adsorbed on the water-oil interface and separate from the bitumen with the emulsion)

The precipitates were dissolved in toluene to remove non-asphaltene solids and then dried.

Elemental analysis indicates that the “bulk” asphaltenes and the “least soluble” asphaltenes are nearly identical but the “interfacial” asphaltenes have significantly higher oxygen content. This result suggests that our hypothesis is incorrect but we still want to examine the chemical differences between interfacial and bulk asphaltenes.

The proposed analysis builds on previous work done at the National High Magnetic Field Laboratory. In this previous work, interfacial asphaltenes were isolated from D₂O-in-crude oil emulsions centrifuged through water. In our work, the interfacial asphaltenes are isolated from H₂O-in-bitumen emulsions settled through heptane-
diluted bitumen. Hence, we can corroborate the previous results with a slightly different technique. We are also sending samples to Heather Dettman at NCUT for NMR analysis and can develop a more comprehensive chemical description of the samples.

**Experimental Methods**

**Sample Preparation**

Samples were prepared from the three asphaltene samples precipitated and collected by Harvey Yarranton’s Lab at the University of Calgary. Samples were dissolved to 2 mg/mL in toluene for electrospray analysis. Dilution to a final concentration of 1 mg/mL in 1:1 toluene:methanol allowed for spiking with 1% ammonium hydroxide (NH₄OH) and analysis by negative electrospray (ESI). This procedure allows for efficient deprotonation of ions in the solution.

The addition of NH₄OH caused instantaneous precipitation of all asphaltene samples. For that reason, samples for negative mode ESI analysis were re-prepared in chloroform at 2 mg/mL. The 2 mg/mL sample was diluted with equal part methanol providing a final concentration of 1 mg/mL. The negative mode samples were then spiked with 1% NH₄OH and analyzed. All solvents were HPLC-grade from VWR International. Samples were delivered to the ionization source via a syringe pump at a rate of 0.5 µL/min.

All samples in this set are difficult to analyze by MS. Specifically, dramatic shifts in molecular weight distributions and complete loss of signal were observed on samples that had sat for 0.5 day or more. For that reason, samples were prepared at the time of analysis and used immediately following.

**Mass Spectrometry**

Samples were analyzed with a hybrid LTQ FT-ICR (linear trap quadrupole Fourier-transform ion cyclotron resonance) 14.5 T 104 mm bore (ThermoFisher, Bremen, Germany) mass spectrometer. A modular ICR data acquisition system (PREDATOR) was used to collect and analyze the data. Low-resolution mass spectrometry (linear ion trap) provided independent verification of molecular weight distribution. A 500 (negative ESI) time-domain data sets was co-added and Hanning-
apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit \( m/z \) separation between mass spectral peaks corresponding to the \(^{12}\text{C} \) and \(^{13}\text{C}^{12}\text{C}_{c-1} \) isotope for each elemental composition.

**Results and Discussion**

**Background**

This report outlines the observed results of three lab precipitated bitumen asphaltene samples obtained from H. Yarranton’s Laboratory. The hypothesis established by the collaborators suggested that the most surface active asphaltene components within the asphaltene fraction are also the components that first precipitate. They also have proposed that these first to precipitate asphaltenes may be chemically distinct from the majority of asphaltene components and they may have a significant role in asphaltenes self association, interfacial behavior, and phase behavior. They therefore, chose collaboration with our laboratory to gain an understanding of the chemistry of the components and their composition in more detail.

Prior to our acquisition of the samples, a series of solubility experiments were carried out on heptane diluted Athabasca bitumen. The samples produced included the “bulk asphaltenes,” that were precipitated from water-free bitumen at a high dilution ratio (> 10% yield from the bitumen). The second sample, the least soluble or “first to precipitate asphaltenes,” were precipitated from the water-free bitumen at a moderate dilution ratio (<2% yield from the bitumen). A final sample, the “interfacial asphaltenes,” were collected through precipitation from a bitumen containing emulsified water at a dilution ratio below the normal onset of precipitation. Asphaltenes from this sample are likely absorbed on the water-oil interface and separate from the bitumen with the emulsion. The precipitates were dissolved in toluene to enable the removal of non-asphaltene solids and then dried.

Additionally, the collaborators conducted bulk elemental analysis on the samples prior to mass spectrometry analysis and determined that the interfacial asphaltenes had significantly higher oxygen content when compared that of the bulk and first to
precipitate asphaltenes. Both the bulk and first to precipitate asphaltenes contained nearly identical oxygen content.

**Results**

As mentioned in the Methods section, molecular weight distributions (MWDs) were verified by low resolution mass spectrometry. **Figure B.1** compares the LTQ scans of the three samples. As evident, the MWDs are all very similar. Multimers (both hetero- and homo-) have been shown to cause broadening of the MWD observed in the ion trap spectra. MS\textsuperscript{n} spectra were collected in order to fragment and verify true monomer distributions (results not shown). The monomer MWD align well with those presented in **Figure B.1**.

**Figure B.1.** Low resolution LTQ mass spectra of the Athabasca bitumen asphaltene samples. The spectra are plotted on individual normalized y-axes.

FT-ICR MS scans were optimized to reflect the MWDs detected by low resolution MS (results not shown). The ultrahigh resolving power and mass accuracy of the ICR technique enables for the unique assignment of elemental composition for peaks detected at 6\(\sigma\) above the baseline noise level. **Figure B.2** shows a class graph representation of the data observed for the samples analyzed. Heteroatom classes are
classified by the number and type of heteroatoms (nitrogen (N), oxygen (O), and sulfur (S)). Classes making up greater than 1% of the ICR signal assignments are plotted with respect to % relative abundances (% RA) observed for all heteroatom classes in the sample. It is evident from the class analysis that the interfacial asphaltenes are comprised of much more oxygenated species than that of the first to precipitate asphaltenes.

![Class Analysis Graph](image)

**Figure B.2.** Class analysis for acidic species detected by negative ion ESI FT-ICR MS at 14.5 T for the asphaltene samples.

Additionally, it should be addressed at this point in the summary, that the bulk asphaltene sample was simply too difficult to conduct ICR on. The sample dissolved well in chloroform and methanol at the concentration discussed in the Methods section, but regardless of the spiking-base addition, precipitation was unavoidable. Therefore, alternate solvent systems were examined, such as 50% chloroform: 25% toluene: 25% methanol and still the addition of base caused precipitation. **Figure B.3** shows a poor quality photo of the sample after the precipitated solution was removed from the original solution vial and placed in a centrifuge tube to allow for the analysis of the supernant solution. But the signal acquired from the supernant was too low for ICR analysis. As a
last resort, the bulk asphaltenes were placed in a chloroform:methanol (1:1) solution and sprayed via electrospray without any modifying base. Again the signal from this solution was too low to acquire ICR signal for the necessary time frame of three seconds. For that reason the bulk asphaltenes have been removed from this data summary until further discussion with collaborator and advisors can be conducted.

Figure B.3. Poor quality photograph of the bulk asphaltene sample after centrifugation in an eppendorf tube to isolated precipitation caused by base addition. The dark black pellet at the bottom of the vial is the precipitated asphaltenes. The supernant was analyzed by LTQ but the signal was simply too poor for ICR collection.

In order to visualize trends within each heteroatom class detected in Figure B.2, iso-abundance color contour plots are created. The plots graph double bond equivalents (DBE) vs. carbon number, and provide a visual representation of the data. DBE is a measure of aromaticity and is calculated as the number of rings and/or double bonds contained within a particular molecular compound. Carbon number is simply a measure of the assigned molecules alkylation. Figure B.4 shows the iso-abundance color contour plot for the O₄ class. The majority of the acidic O₄ species are identical, but the first to precipitate asphaltenes are slightly more aromatic with DBE ranging between 1
Figure B.4. The iso-abundance color contour plots of the acidic O\textsubscript{4} class observed in the first to precipitate and interfacial asphaltenes.

Figure B.5. The iso-abundance color contour plots of the acidic O\textsubscript{3}S\textsubscript{1} class observed in the first to precipitate and interfacial asphaltenes.
Figure B.6. The iso-abundance color contour plots of the acidic $O_4S_1$ class observed in the first to precipitate and interfacial asphaltenes.

Figure B.7. The iso-abundance color contour plots of the acidic $O_5$ class observed in the first to precipitate and interfacial asphaltenes.
and 23 versus 1-20 DBE for the interfacial asphaltenes. It appears also in Figure B.4 that the interfacial asphaltene are slightly more alkylated than that of the first to precipitate. That is not true and simply is an artifact of the data collection. Optimization of the ICR conditions for the interfacial asphaltenes allowed a broader molecular weight distribution to be observed and therefore, more alkylated species (of higher m/z values) can be assigned. Similar trends can be observed for other common heteroatom classes such as the O$_5$S$_1$ and O$_4$S$_1$ (Figures B.5 and B.6).

![Asphaltene Comparison](image)

**Figure B.8.** The iso-abundance color contour plots of the acidic O$_5$S$_1$ class observed in the first to precipitate and interfacial asphaltenes.

**Figures B.7 and B.8** show the iso-abundance color contour plots for the acidic O$_5$ and O$_5$S$_1$ classes. They are includes in this summary in order to show aromatic and alkylation trends observed for the more oxygenated species detected in the interfacial asphaltenes, but due to the lack of comparison to other samples, little information can be concluded about them.

**Conclusions**

Given the difficulty of sample analysis for the bulk asphaltenes, our findings are inconclusive in either supporting or refuting the hypotheses of the Yarranton laboratory.
It does appear that the composition of the first to precipitate and interfacial asphaltenes are very similar. Indeed, their idea that the most surface active components within the asphaltenes could also be the components that are the first to precipitate seems likely, but without a comparison to the bulk asphaltenes this conclusion is essentially not known.
APPENDIX C

CO-PRECIPITATE PROGRESSION COMPARISON AS A FUNCTION OF SOXHLET EXTRACTION TIME BETWEEN A WYOMING CRUDE OIL AND ITS FIELD GENERATED DEPOSIT

Introduction

Data presented in Appendix C outline as parallel study to that conducted in Chapter 7. We conducted the exact same experimental procedure (Soxhlet extraction) on a Wyoming Crude oil and its field generated deposit. Collaborators on this project wanted to determine if any compositional similarities could be observed between the two samples. In addition, they sought to determine how representative their laboratory generated capillary deposit was to the actual field deposit. The analyzed field deposit is formed in pumping pipelines over time. The deposit precipitates out of the crude oil very slowly, just like cholesterol builds up over time in the human body. Refineries refer to this type of deposition as arterial deposition. The results of the study are included below.

Experimental Methods

Sample Preparation

Asphaltenes were precipitated from a Wyoming Crude Oil and Wyoming Field Deposit with a simplified SARA fractionation (IP-143) procedure. After heptane dilution of the crude oil, raw asphaltenes were isolated by simple filtration. Next, Soxhlet extraction of the raw asphaltenes in clean \( n \)-heptane produced the co-precipitate fraction (heptane-soluble). The extraction solvent and solvent reservoirs were replaced with clean \( n \)-heptane after 1 h and then again after 4, 8, 24, 48, and 72 h. Soxhlet washings were dried to constant weight and prepared for electrospray ionization (ESI) coupled with FT-ICR MS. Samples were dissolved to 1 mg/mL in toluene for electrospray analysis. Dilution to a final concentration of 0.5 mg/mL in 1:1 toluene:methanol allowed for spiking with 1% tetramethylammonium hydroxide for
negative electrospray (ESI). This procedure allows for deprotonation of less acidic chemical functionalities and, therefore, broader chemical composition analysis. All solvents were HPLC-grade from VWR International. Samples were delivered to the ionization source via a syringe pump at a rate of 0.5 µL/min.

Limited amounts of the Wyoming Capillary Deposit were obtained; therefore, an asphaltene/co-precipitate analysis was NOT conducted on the capillary deposit. The capillary deposit was simply characterized as a whole by negative mode ESI FT-ICR MS using TMAH as a modifier (just as above).

**Mass Spectrometry**

Samples were analyzed with a custom-built 9.4 T 22 cm diameter bore (Oxford Corp., Oxford Mead, UK) ESI FT-ICR mass spectrometer with a modular ICR data acquisition system (PREDATOR) that was used to collect and analyze the data. A total of 200 time-domain data sets was co-added and Hanning-apodized, followed by a single zero-fill, fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to charge ratio by the quadrupolar electric potential approximation. All observed species were singly charged, as verified by unit m/z separation between mass spectral peaks corresponding to the $^{12}\text{C}$ and $^{13}\text{C}\!^{12}\text{C}_{c-1}$ isotope for each elemental composition.

**Results and Discussion**

To date, the most comprehensive data set to arise out of this data is from the Wyoming field deposit co-precipitate extraction. Contamination from spray rigs, the instrumentation and the extraction procedure itself have inhibited full data presentation for the Wyoming crude oil co-precipitate extraction. Therefore, the trends observed in the Wyoming field deposit extraction and show you that preliminary results from the Wyoming crude oil co-precipitate extraction correlate to the field deposit findings very well.

The aim of this study was to compare structural similarities and differences observed between a crude oil and its corresponding field deposit. Most specifically, to examine what trends are observed within the co-precipitate fractions collected over a 72 hour period. Co-precipitates are isolated through Soxhlet extraction of the SARA
fractionated asphaltenes (the solubility fraction of crude oil that is \( n \)-heptane insoluble but toluene soluble) Therefore, co-precipitates reverse their solubility behavior as they originally precipitate in \( n \)-heptane solvent during the asphaltene drop but re-dissolve in \( n \)-heptane following Soxhlet washing of the asphaltenes.

Presented in Figure C.1 is the class comparison for all classes detected at \( \geq 1\% \) relative abundance in the Wyoming field deposit. Overall, the co-precipitate fractions are dominated by nitrogen containing compounds. Interestingly though, those classes containing \( O_x \) and \( SO_x \) species (in addition to nitrogen) almost uniformly trend to increasing abundance in the later eluting fractions (exceptions include \( N_1O_1 \), \( N_1O_1S_2 \) and \( N_1O_2 \)). This observation is consistent with (but also an extension to) experiments conducted on co-precipitates here at NHMFL in the past (see Chapter 7). Previous data has shown that co-precipitates are enriched in \( O_x \) and \( SO_x \) species relative to the parent crude oil. Those classes containing ONLY nitrogen and sulfur compounds show a decrease in their relative abundances through the co-precipitate fractions.

![Co-precipitate Evolution in Wyoming Field Deposit](image)

**Figure C.1.** Class analysis (detected at \( \geq 1\% \) relative abundance) across the Soxhlet extraction of Wyoming Field Deposit.
In order to examine aromaticity and alkylation trends more closely, data are presented in iso-abundance color contour plots. **Figure C.2** shows detected N₁ species for the Wyoming field deposit co-precipitates. Plotted on the y-axis is the molecule’s calculated DBE value, from the equation

\[
DBE = C - \frac{H}{2} + \frac{N}{2} + 1 \quad \text{(C.2)}
\]

DBE is measured as the number of rings and/or double bonds in a given molecular structure. The x-axis is plotted as carbon number and provides a measure of alkylation for the assigned molecular formula. Molecular compositions in the 0-1 h co-precipitate fraction span broad carbon number values (15-60) and range in DBE of 9-35.

**Figure C.2.** Iso-abundance color-contour plots of the N₁ class of compounds assigned across the co-precipitate Soxhlet extraction of the Wyoming field deposit. The isolated compounds trend to more aromatic (higher DBE values) structures of increasing alkylation as extraction period increases.

Nearly all species detected lie at or near a unique region known as the PAH planar limit. The PAH (polyaromatic hydrocarbon) planar limit is set by the maximum number of carbon and hydrogen atoms that can be allotted (given the DBE and carbon number limitation imposed) before the molecule has to “bend” into Bucky bowl- or ball-
like (fullerenes) structures. To date, research has consistently shown that fullerene structures do not exist in crude oils. Therefore, a graphically diagonal “planar limit” is often times visually observed when dealing with highly aromatic compounds, such as these $N_1$ species.

As the experiment progresses in time, molecular structure in the fractions trend toward more aromatic compounds of higher carbon number (see 1-4 h image through 48-72 h image). The relative abundances of detected species (in each fraction) increase with elution time. Compounds in the 48-72 h fraction shift in DBE value. Most of the compounds are $15 \leq \text{DBE} \leq 40$ and span $C_{20-70}$. Nearly the exact same observations are presented in Figures C.3 and C.4. Structural evolution for the $N_1O_1$ and $N_1S_1$ classes shows an overall shift to molecules of higher alkylation and aromaticity.

**Figure C.3.** Iso-abundance color-contour plots of the $N_1O_1$ class of compounds assigned across the co-precipitate Soxhlet extraction of the Wyoming field deposit. The isolated compounds trend to more aromatic (higher DBE values) structures of increasing alkylation as extraction period increases.
Figure C.4. Iso-abundance color-contour plots of the N$_1$S$_1$ class of compounds assigned across the co-precipitate Soxhlet extraction of the Wyoming field deposit. The isolated compounds trend to more aromatic (higher DBE values) structures of increasing alkylation as extraction period increases.

Figure C.5 presents the class comparison for compounds detected at greater than 1% relative abundance in the Wyoming crude oil. Only two of the 6 samples are included, but these samples make up extremes of the experimental time frame. Unfortunately, general statements with respect of heteroatom composition are hard to conclude. There are dramatic decreases in %RA for the most abundant N$_1$ and N$_1$S$_1$ classes, and dramatic increases in the N$_1$O$_1$ and N$_1$O$_1$S$_1$ classes. Once data handling on the experimental series is completed, more broad and underlying statements may be formed. Two fractions (8-24 h and 48-72 h) initially yielded grossly different chemical compositions from that of all other fractions analyzed. Repeated ICR analysis demonstrated that these samples were highly sensitive to ESI voltages applied to the spray needle. New results show much more consistent results and will be included in later communications.

Figures C.6 and C.7 depict the iso-abundance color contour plots for the N$_1$ and N$_1$S$_1$ classes. They show compositional comparisons for both the experimental time frames and the compounds collected in the 0-1 h vs. the 24-48 h samples (of the
Wyoming crude oil experiments), and offer a direct comparison between the compounds observed in the Wyoming crude oil and that of the deposit. The compounds detected in the crude oil span a much broader carbon number range than in the deposit during the 0-1 h time interval. Yet interestingly, the most intense colors are depicted in similar DBE and carbon number regions.

![Co-precipitate Evolution in Wyoming Crude Oil](image)

**Figure C.5.** Class analysis (detected at ≥1% relative abundance) across the Soxhlet extraction of Wyoming crude oil.

The second interesting observation noted arises in the Wyoming crude oil (Figure 6, 24-48 h fraction). There appears to be an onset of a secondary region of compounds comprised of lower DBE and carbon number values than that observed in the other plots. We suspect that this material (9 ≤ DBE ≤ 20 and C_{20-40}) results from the release of occluded maltenes, most specifically, resins OR from the removal of a full monomeric unit from the experimentally supported “octomer” asphaltene theory. Resins have long been suspected to act as asphaltene aggregate stabilizers and this data clearly shows (with support from other experiments performed in lab) a new type of compounds detected at much lower DBE and carbon number from the most abundant species. While the N_{1}S_{1} plot doesn’t demonstrate this same trend, it has been observed...
Figure C.6. Selected iso-abundance color-contour plots of the \( N_1 \) class of compounds assigned across the co-precipitate Soxhlet extraction of Wyoming crude oil and corresponding field deposit. Similar compounds are observed in each extraction for a given time frame. The isolated compounds trend to more aromatic (higher DBE values) structures of increasing alkylation as extraction period increases in both co-precipitate extractions.

Figure C.7. Selected iso-abundance color-contour plots of the \( N_1S_1 \) class of compounds assigned across the co-precipitate Soxhlet extraction of Wyoming crude oil and corresponding field deposit. Similar compounds are observed in each extraction for a given time frame. The isolated compounds trend to more aromatic (higher DBE values) structures of increasing alkylation as extraction period increases in both co-precipitate extractions.
repeatedly in other samples of different origins (but predominately from co-precipitate extractions preformed on crude oil samples where maltenic material is abundant).

In all classes there is a shift to compounds of higher DBE/carbon number values. This observation is best represented in Kendrick mass plots. In this representation, Kendrick mass defect is plotted versus nominal Kendrick mass. Kendrick mass scale is based on the mass of a CH$_2$ = 14.0000 Da rather than 14.015 65 Da. Kendrick mass is calculated from

$$\text{Kendrick mass} = \text{IUPAC mass} \times \left( \frac{14.0000}{14.01565} \right) \quad \text{(C.1)}$$

Homologous compounds (those that differ in content only by CH$_2$ groups, but contain the same heteroatom(s), and number of rings plus bonds) have identical mass defects,

$$\text{Kendrick Mass Defect (KMD)} = \left( \text{nominal Kendrick mass} - \text{exact Kendrick mass} \right) \quad \text{(C.2)}$$

These calculations allow for easy sorting and selection of mass from all observed ion masses. Figure C.8 shows the Wyoming crude oil’s Kendrick plots for 0-1 h and 24-48 h washings. During the 0-1 h time interval, KMD for all masses between m/z 200-900 range between 0.1 and 0.5, with the highest concentration from 0.0-0.2 KMD. Yet, in the 24-48 h time interval the most abundant species have KMD values of 0.25-0.4, over the same mass range.

A much more dramatic KMD shift is observed for the compounds in the Wyoming field deposit (Figure C.9). All negative ions span a KMD of 0.0 and 0.5 between m/z 200-700 during the 0-1 h time interval, with the most abundant values observed between 0.0-0.2. Note: the mass range in this spectrum is truncated due to a large contaminant at m/z 283. Compounds in the field deposit that are observed in the 48-72 h time interval are most abundant at KMD values between 0.2-0.5.
Figure C.8. Kendrick mass plots of the negative ions detected in the Wyoming Crude Oil 0-1 h extraction (left) and the 24-48 h extraction (right). There is an overall shift in Kendrick mass defect to higher values, an indication of more aromatic species.

Figure C.9. Kendrick mass plots of the negative ions detected in the Wyoming Crude Oil 0-1 h extraction (left) and the 48-72 h extraction (right). There is an overall shift in Kendrick mass defect to higher values, an indication of more aromatic species.
WYOMING CAPILLARY DEPOSIT

Collaborators also wanted to compare how representative a lab-generated capillary deposit from this Wyoming crude oil was to their actual field deposit and parent crude oil. Unfortunately, the amount of sample supplied by the collaborators made it impossible to do a full co-precipitate comparison of the lab-generated deposit. So outlined below, you will see the characterization of the lab deposit as a whole.

Figure C.10 presents the class graph for all classes detected at ≥1% RA in the Wyoming capillary deposit through negative mode ESI with TMAH modifier. N₁ is observed as the class of highest abundance, and is present at very similar levels to that of the Wyoming crude oil. There is a wide array of nitrogen containing compounds, as well as, non-polar hydrocarbons and S₁. The large compositional space that these classes cover is demonstrated in Figure C.11. The onset of N₁ detection begins only at DBE = 9, which suggest that all acidic nitrogen species are contained in dibenzopyrrolic analogs. All highlighted classes in figure 11 show broad carbon number ranges. The most aromatic compounds are observed in the N₁O₁, N₁O₁S₁, and N₁S₁ classes. The iso-abundance color contour plots of these classes show well defined PAH planar limits.

Figure C.10. All classes detected at ≥1% relative abundance in the Wyoming capillary deposit by negative mode ESI with TMAH modifier.
Figure C.11. Iso-abundance color-contour plots of highlighted classes detected in the Wyoming capillary deposit.

**Implications**

The results from this study have yielded very interesting results and we are continuing our examination of these samples. There is significant compositional similarity between the Wyoming crude oil and the field deposit co-precipitates. In addition, the fact that very little bi-modal distribution of the co-precipitates is observed (unlike the co-precipitates in Chapter 7) may indicate that this Soxhlet extraction technique will prove useful in determining whether a crude oil is prone to arterial deposition or not, prior to pumping and refining! These implications could prove very helpful to the refining industry.
APPENDIX D

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Publications


• Ehrmann, Brandie M.; Kim, Do-Gyun; Rodgers, Ryan P.; Marshall, Alan G. Compositional Analysis of Asphaltene Co-precipitates and their Role in Emulsion Formation In preparation

• Ehrmann, Brandie M.; Juyla, Priyanka; Rodgers, Ryan P.; Marshall, Alan G. Characterization of Specific Accumulation of Acidic Species at the Interface of Water-in-Oil Emulsions by FT-ICR MS In preparation

• Ehrmann, Brandie M.; Robbins, Winston K.; Rodgers, Ryan P.; Marshall, Alan G. Asphaltene Co-precipitate Molecular Progression as a Function of Soxhlet Extraction Period: Implications for Asphaltene Aggregation In preparation