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Petroleomics: Applications in the Fingerprinting of the Acidic and Basic Crude Oil Components Detected by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Petroleomics: Applications in the Fingerprinting of the Acidic and Basic Crude Oil Components Detected by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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ABSTRACT

We have previously demonstrated the ability of electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) to resolve and identify the polar species found in all petroleum distillates. The ultrahigh resolving power and mass accuracy of FT-ICR MS allows for the identification of thousands of compounds in crude oils without prior chromatographic separation. In Chapter 3, we compare positive-ion ESI FT-ICR mass spectra of a South American crude oil with spectra of its SARA-isolated asphaltenes, resins, and aromatics, to determine the effect of the other components on the relative mass spectral abundances of the polar aromatics. Saturates are unobservable by ESI. For the chosen oil, little to no signal was obtained for the asphaltenes and resins due to their mostly acidic nature. The mass distributions, heteroatom class distributions, type (rings plus double bonds) distributions, and carbon number distributions of the aromatic fraction and unfractionated crude oil were highly similar. Thus, the saturates, asphaltenes, and resins do not affect the relative abundances of polar aromatics observed by positive-ion electrospray FT-ICR MS. It is therefore not necessary to isolate the polar aromatic fraction in order to characterize its chemical composition in a petroleum crude oil.

The diminishing clean oil reserve is driving the search for new or improved ways to reduce the level of NSO-containing species found in high abundance in heavy crude oils. Hydrotreatment is the currently preferred technique to remove those polar species. Unfortunately, nitrogen compounds are known to cause coke formation on the surface of the hydrotreatment catalyst, leading to partial or complete deactivation. In Chapter 4, we use positive- and negative-ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) to identify those
nitrogen compounds that resist hydrotreatment. ESI preferentially ionizes polar (e.g., heteroatom-containing) species: basic molecules are detected as positive ions and acidic/neutral molecules as negative ions. FT-ICR MS resolves thousands of species in a single mass spectrum, allowing for unambiguous determination of elemental composition, C_{c}H_{h}N_{n}O_{o}S_{s}, for identification of compound class (numbers of N, O, S heteroatoms, type (rings plus double bonds), and carbon number (revealing the extent of alkylation). We find that hydrotreatment-resistant compounds typically contain a single nitrogen atom, both pyridinic benzalogs and pyrollic benzalogs. Compounds with more than one heteroatom, such as N_{x}O_{x}, N_{x}S_{x} and N_{x}, are partially removed. Compound classes with lower double bond equivalents or fewer CH2 groups are preferentially removed. Species that contain an oxygen atom or O_{x}S_{x} are fully removed by hydrotreatment.

A suite of ten crude oils that contain a varied of total acid numbers (TAN), corrosive character, and are derived from different source rock material are analyzed by ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in Chapter 5. A variety of graphical methods are used to visually display each crude oil and reveal the lack of correlation between the basic and acidic species to TAN and corrosive character. The similarity in class and percent relative abundance of the basic polar species indicates that they play no role in the corrosive character of a crude oil and can not be used as biomarkers for geographical identification. The analysis of the acidic species illustrates the lack of correlation of any one specific class or type of compound to corrosivity or TAN. The vast difference in relative abundance of the carboxylic acids suggests that although these compounds contribute to corrosivity, other species, such as the non-polar sulfur compounds, also play a key role in the corrosive nature of a crude oil. These differences, however, do lend to the use of these carboxylic acids as biomarkers for specific geographical location as well as locaters of specific regions with in a reservoir.
Asphaltenes are typically defined by their solubility in benzene and insolubility in pentane or heptane. They are believed to exist in petroleum crude oil as a colloidal suspension, stabilized by surface-adsorbed resins. Their normal equilibrium under reservoir conditions may be disrupted during production by pressure reduction, crude oil chemical composition changes, introduction of miscible gases and liquids, mixing with diluents and other oils, and by acid stimulation, hot oiling, and other oilfield operations. Electrospray ionization preferentially ionizes polar N-, S-, and O-containing compounds and its combination with ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry makes a powerful tool for the compositional analysis of petroleum-derived materials such as asphaltenes. In Chapter 6, we compare the compositional differences between heptane-precipitated asphaltenes and asphaltenes collected by live oil depressurization. We find that the heptane-precipitated asphaltenes contain higher double bond equivalents (number of rings and double bonds) compared to the asphaltenes induced by pressure drop. On the other hand, the pressure drop product exhibits a higher abundance of species containing sulfur. Thus, the solubility criterion for asphaltenes defines a significantly different chemical composition than the (more field-relevant) pressure-drop criterion.

Asphaltenes are also known to deposit in both the petroleum recovery and topside refining processes. There normal equilibrium in both live and dead crude oil may be disrupted during production by pressure reduction; crude oil chemical composition changes, mixing with diluents and other oilfield operations. In Chapter 7, we compare a dead crude oil deposit asphaltene with its crude oil counterpart and a live crude oil deposit asphaltene. Negative-ion electrospray is the desired mode because of the acidic nature of asphaltenes. We find that the dead crude oil deposits contain higher aromatic character (more saturated), are enriched in oxygenated species as well as multiple heteroatomic classes compared to their crude oil counterpart. The two deposit asphaltenes, live and dead, are very similar in the types of classes detected. For some of the classes, O₄S in particular, the
dead oil typically deposits larger (more aromatic, longer alkyl chains) compounds compared to the live oil. This detailed compositional comparison of the dead deposit asphaltenes to its crude oil counterpart will provide information for the development of more cost-effective methods to control the deposition of asphaltenes and to increase the overall efficiency of the processing fields with asphaltene problems.
CHAPTER 1

PETROLEOMICS

Petroleomics\(^1\), \(^2\) is the term used to describe the study of all components of crude oil (petroleum) and how these components affect the properties and reactivity of that specific crude oil. This idea is not new to the industry. The composition of crude oil is the cornerstone of molecular based management of refinery processes. Simply stated, petroleum companies sell molecules and consequently, an oil’s composition determines its economic value. Therefore, compositional knowledge equals power and enables the company to produce oil reserves more efficiently, to predict production problems and prevent pipe fouling, to reduce refining byproducts and waste, to use the oil reserves more efficiently and finally to make money. This need to determine and understand a crude oil’s composition is the force behind all crude oil analysis.

Petroleum varies dramatically in color, odor, composition and flow properties that reflect the diversity of its origin. The terms light or heavy are often used to describe different types of crude oils. Although not derived from composition, light petroleum (sweet crude oil) is usually rich in low-boiling constituents, whereas heavy petroleum (sour crude oil) is composed of more aromatic, heteroatom-containing constituents with higher boiling points. The decrease in light oil reserves around the world has led to the production of petroleum based products from the heavy crude oils that are more readily available.
Compound determination of crude oils is not the only classification system. Since the beginning of crude oil analysis back in the 1850s, boiling point fractionation has been a common means for classification. Boiling point fractions (distillation cuts) are based on the types of compounds that are removed at a certain temperature range. The higher the temperature, the larger and more complex the crude oil molecules. The size and complexity of crude oil components are directly related to the boiling point range. Some molecules are too complex to be separated based on boiling point and other classification systems are needed to describe these types of compounds.

Another typical classification of crude oils is based on the solubility of different types of compounds in various solvents. **Table 1.1** illustrates the four main classifications of crude oil (Saturates, Aromatics, Resins and Asphaltenes) and the solvent systems in which they are or are not soluble. The non-polar nature of the saturates makes them soluble in most alkanes. A more polar fraction requires a solvent with a higher polarity. The aromatic fraction and resins are partially composed of polar NSO-containing species and they are soluble in a more polar solvent system, such as a mixture of heptane and toluene. Resins are more complex and contain higher molecular weight aromatic heteroatoms, such as N, S, and O. Asphaltenes are thought of as complex mixtures of heteroatom-rich polycyclic hydrocarbons, naturally suspended in crude oil. More specifically, they are comprised of condensed aromatic and naphthenic molecules of molar masses up to ~2000 Da. They are the most polar fraction of crude oil, and comprise much of the heteroatom (N, S, O) and metal (Ni and V) content in heavy oils. Asphaltenes are the major focus in Chapters 6 and 7. It was not until a few years ago that the determination of molecular formulae for individual species became a possibility for complex mixtures, such as crude oil.
**Table 1.1.** Crude oil component solubility/insolubility

<table>
<thead>
<tr>
<th>Soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturates</td>
<td>Heptane/Alkanes</td>
</tr>
<tr>
<td>Aromatics</td>
<td>Heptane/Toluene</td>
</tr>
<tr>
<td>Resins</td>
<td>Heptane/Toluene/Methanol</td>
</tr>
<tr>
<td>Asphaltenes</td>
<td>Toluene</td>
</tr>
</tbody>
</table>

It is interesting the note the similarity between the evolution of protein science to proteomics and the current state of crude oil analysis characterization. Proteins, like crude oil components, were classified based on solubility: Albumins (soluble in water and dilute salt solution); globulins (insoluble or sparingly soluble in distilled water; salted out of aqueous solution by half-saturation with ammonium sulfate); prolamins (insoluble in water but soluble in 50 – 90% aqueous ethanol); glutelins (insoluble in all of the above solvents but soluble in dilute acid or base solution); scleroproteins (insoluble in most ordinary solvents), etc., as shown in **Table 1.2**. Today, these different proteins are classified based on detailed amino acid sequences and 3D structures obtained from highly sophisticated analytical methods, such as mass spectrometry. This detailed information leads to a better understanding of how these proteins function and react within a system. Much like the thousands of proteins that function within a system, there are thousands of compounds that all contribute to the physical and chemical properties of a crude oil.
### Table 1.2. Protein Solubility/Insolubility

<table>
<thead>
<tr>
<th></th>
<th>Soluble In</th>
<th>Insoluble In</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumins</td>
<td>H₂O, Dilute Salt</td>
<td></td>
</tr>
<tr>
<td>Globulins</td>
<td>H₂O (Sparingly)</td>
<td>50% (NH₄)₂SO₄</td>
</tr>
<tr>
<td>Prolamins</td>
<td>50 – 90% Ethanol</td>
<td>H₂O</td>
</tr>
<tr>
<td>Glutelins</td>
<td>Dilute Acid/Base</td>
<td>All of Above</td>
</tr>
<tr>
<td>Scleroproteins</td>
<td></td>
<td>All of Above</td>
</tr>
</tbody>
</table>

Similar advancements in petroleum science are beginning to advance petroleomics. A more detailed molecular based characterization within crude oils will provide basic insight into the function of each compound, and their behavior in upstream and downstream processing environments. Although mass spectrometry is not the only analytical technique employed for the studies of these crude oil components, it is the driving force behind molecular-based characterization.

### Molecular Based Characterization

Petroleum crude oil is arguably the world’s most compositionally complex organic mixture, in terms of the number of chemically distinct constituents. As stated earlier, crude oil is composed of unsaturated and saturated hydrocarbons, heteroatoms (such as N, S, and O), as well as a small percentage of metals, particularly vanadium, nickel, iron and copper, Figure 1.1. The hydrocarbon fraction can be as high as 90% by weight in the lighter oils, compared to about 70% in heavy crude oil. From elemental composition data, it appears that the proportions of the elements in petroleum vary over fairly narrow limits, as shown in Table
The minimal variation in composition is due to the origin of the crude oil, more mobile crude oils at one extreme to the heavier asphaltic crude oils at the other extreme. This similarity in composition is surprising when the number of geographical locations and maturation origins are considered, but is very indicative of the similarity in nature of the precursors from one site to another.

**Figure 1.1.** Composition of the petroleome with corresponding percentages of each type of compound. The petroleome is composed of ~70% hydrocarbons, ~5% polar species and ~30% aromatics.
Table 1.3. Variation in Percent Elemental Composition

<table>
<thead>
<tr>
<th>Element</th>
<th>Composition Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>83.0 - 87.0 %</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>10.0 – 14.0 %</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.1 – 2.0 %</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.05 – 1.5 %</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.05 – 6.0 %</td>
</tr>
<tr>
<td>Metals (Ni and V)</td>
<td>&lt; 1000 ppm</td>
</tr>
</tbody>
</table>

Hydrocarbons

It has been widely established that the hydrocarbon components in petroleum are composed of paraffinic, naphthenic and aromatic groups. The paraffins are saturated hydrocarbons with straight or branched chains and do not contain any ring structures. The naphthenes (alicyclic hydrocarbons) are saturated hydrocarbons containing one or more rings, each of which may have one or more paraffinic side chains (or alkyl chains). The aromatics are hydrocarbons containing one or more aromatic cores, ring systems, such as benzene or phenanthrene, which may be linked with naphthene rings or long paraffinic side chains.

The exact proportions of paraffinic components vary with different crude oils but within any crude oil, the proportion of paraffinic hydrocarbons decreases with increasing molecular weight or boiling point, as shown in Figure 1.2. The paraffin-like compounds are only present in the low boiling point fractions and rarely are found in the high boiling point fractions or the vacuum residue (non-volatile sludge). Although they are not found at the higher boiling points, vestiges of the paraffins in the high molecular weight fractions occur as alkyl side
chains on the aromatic and naphthenic systems, and they can contain tens of carbon atoms.

As previously stated, the naphthenes are saturated cyclic ring structures and do not contain any aromatic cores. It is generally thought that crude oils are composed mainly of naphthenes with five- and six-membered rings. It is widely known that these cycloparaffins are represented in all fractions (boiling point fractions) in which the constituent molecules contain more than five carbon atoms. As seen in figure 1.2, as the molecular weight (boiling point range) increases, there is an associated increase in the amount of naphthenic compounds in the fraction. The vacuum residue may not contain napthenic species, but naphtheno-aromatic (contains both unsaturated and saturated ring structures) groups are known to exist.

Crude oil is a mixture of components, and aromatic compounds are common in all crude oils. A majority of the heteroatom free aromatics contain paraffinic chains, naphthene rings, and aromatic rings side by side. These aromatics are mostly found in lower boiling point fractions and are rarely found in vacuum residues without the presence of a heteroatom. There have been numerous studies done on the determination of the number of saturated ring systems typically found in crude oils. A variety of techniques have been employed to examine the structure of these aromatic species, pyrolysis/gas chromatography/mass spectrometry\(^5\), ultraviolet spectroscopy\(^6-8\), and high-performance liquid chromatography (HPLC).\(^9-11\) In all cases, the evidence favored the presence of the smaller (1-4 DBE) aromatic systems.
Heteroatomic Components

Heteroatomic compounds make up a relatively small portion of crude oils, less than 15\%\textsuperscript{12}, but have significant implications. These compounds are the focus of this dissertation and will be discussed in more detail throughout subsequent chapters. **Table 1.4** illustrates typical heteroatomic species found in crude oils, where $R$ represents an alkyl chain attached to a cyclic or aromatic ring core. They are composed of N-, S-, and O-containing species with aromatic and
naphthenic ring systems. They are typically found in the high boiling point fractions and the asphaltene, aromatic and resin fractions.

Table 1.4. Heteroatomic Compounds Present in Crude Oils.

<table>
<thead>
<tr>
<th>Sulfur</th>
<th>Oxygen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenzothiophene</td>
<td>Phenols</td>
<td>Carbazole</td>
</tr>
<tr>
<td>Cyclic Sulfides</td>
<td>Carboxyclic Acids</td>
<td>Quinoline</td>
</tr>
<tr>
<td>Sulfoxides</td>
<td>Amide (quinoline)</td>
<td></td>
</tr>
</tbody>
</table>

Sulfur species are found as both non-polar (thiophene-like) and polar (sulfoxide) compounds. They are usually the most abundant of the heteroatomic species and are broadly distributed across the range of petroleum fractions. The total sulfur in crude oil can vary from 0.04% up to about 5% in the heaviest forms of crude oil. The number of sulfur atoms per molecule increases sharply in the higher boiling point fractions, mainly due to the increase in molecular weight. Sulfur compounds are thought to be one of the most important heteroatomic constituents of petroleum because of the harmful effects, such as corrosion and deposits, they often produce in finished petroleum.
products. Although this may be the case, oxygen and nitrogen compounds, although present in smaller amounts, are also known to contribute to the harmful effects of finished petroleum products.

Oxygen-containing heteroatoms are known to be present in the form of phenols and carboxylic acids. The total oxygen content is normally less than 2% by weight of crude oil. However, oxygen content increases dramatically with boiling point, resulting in the nonvolatile residue with oxygen content up to 8%. These compounds are known to be the main contributors to the corrosion of pipe equipment during the extraction and refining process of petroleum products. They have been and will continue to be the main types of compounds routinely studied in the petroleum field.

Nitrogen compounds can be classified as both basic and nonbasic. The basic nitrogen compounds are composed mainly of pyridine homologs and occur throughout the boiling point ranges, but have a tendency to exist in higher abundance in the high boiling point ranges and residue. The nonbasic nitrogen compounds, which are usually pyrrole, indole, and carbazole types, also occur in the higher-boiling fractions and residue. The nitrogen content of crude oils tends to fall in the range of 0.1% - 0.9%.

**Other Components**

Crude oils also include of metal and porphyrins in small quantities and will not be the focus of this dissertation. Porphyrins are thought to be biomarkers that could establish a link between compounds found in the geosphere and their corresponding biological precursors.\(^4\, 12,\, 13\) Nickel and vanadium are the two most common metals found in petroleum and must be removed to convert the oil to transportation fuel. The metals are known to affect many upgrading processes and cause particular problems because they poison catalysts used in the removal of
harmful heteroatomic compounds present as well as other processes such as catalytic cracking.\textsuperscript{14}

\textbf{Crude Oil Analysis}

To study the constituents of crude oil, a variety of analytical techniques have been used. Compositions of the saturated hydrocarbons have been well characterized by gas chromatography mass spectrometry\textsuperscript{15}, two dimensional gas chromatography coupled to mass spectrometry\textsuperscript{16}, high resolution mass spectrometry\textsuperscript{17}, and liquid chromatography mass spectrometry.\textsuperscript{18-20} However, comparatively little is known about the less abundant polar species or heavy crude oils, whose compositional complexity far exceeds the peak capacity of typical analytical techniques. However, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is capable of achieving the peak capacity needed to resolve individual components of a complex data matrix. In 2000, Zhan and Fenn\textsuperscript{21} pointed out that the most polar species in petroleum distillates could be ionized by Electrospray Ionization (ESI) and detected by mass spectrometry (see \textbf{Figure 1.3} for typical compounds ionized by ESI). The coupling of these two techniques, ESI and FT-ICR mass spectrometry produces a powerful analytical tool for the analysis of these polar species found in the complex sample matrix of petroleum.

\textit{Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS)}

The development of FT-ICR mass spectrometry\textsuperscript{22, 23} made it possible to achieve ultra-high mass resolving power (\(m/\Delta m_{50\%} > 100,000\), in which \(\Delta m_{50\%}\) is the mass spectral peak full width at half-maximum peak height) mass spectra. High mass resolving power is
necessary for the resolution of many compounds present in the petroleum derived material. **Table 1.5** shows some of these typical mass spacings and the mass resolving power necessary for baseline resolution. This mass spectrometric technique also provides high mass accuracy for the assignment of unambiguous elemental composition with error less than 1 ppm. These two features of FT-ICR mass spectrometry allow for the baseline resolution of closely spaced peaks commonly encountered in petroleum-derived materials and the assignment of elemental composition.

**Figure 1.3.** Typical crude oil components selectively ionized by both positive- and negative-ion electrospray.
Table 1.5. Typical mass spacing differences and resolving power necessary for resolution

<table>
<thead>
<tr>
<th>Doublet</th>
<th>Δm</th>
<th>m/Δm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH₄ vs. C₃</td>
<td>3.4 mDa</td>
<td>135,000</td>
</tr>
<tr>
<td>C₂H₃ vs. ¹³CN</td>
<td>17.0 mDa</td>
<td>27,000</td>
</tr>
<tr>
<td>O vs. CH₄</td>
<td>36.4 mDa</td>
<td>13,000</td>
</tr>
<tr>
<td>H₁₂ vs. C</td>
<td>93.9 mDa</td>
<td>5,000</td>
</tr>
</tbody>
</table>

ESI FT-ICR MS has opened the door to the analysis of complex mixtures, such as heavy crude oils. Typically, more than 15,000 chemically distinct species can be detected and analyzed from a single sample. This compositional knowledge is the basis for the field of petroleomics. With molecular based characterization, chemical properties and reactivity of specific crude oil components will lead to a more efficient extraction as well as better refining processes for the removal of the harmful polar constituents found in all heavy crude oils.

**Fingerprinting the Petroleome**

The ability to redefine crude oil components based on elemental composition has led to the term “petroleomics”. The focus of the subsequent chapters is on the use of ESI FT-ICR MS for the fingerprinting of the polar constituents found in heavy crude oils and their derivatives. Much like fingerprints are unique to an individual, polar species are also unique to a specific crude oil and cause unique refining and environmental problems. Chapter 3 will first examine the matrix effects of species not ionized by electrospray (non-polar species) on those crude oil constituents detected in positive-ion ESI. Chapters 4
and 5 will describe the types of polar species that are resistant to a specific hydrotreatment process and the types of polar species that can cause corrosion during the refining process. Chapter 3 will also investigate potential biomarkers (compounds that survived diagenesis) that can distinguish not only between crude oil geographical locations, but also between crude oils from different fields and intervals. Chapters 6 and 7 will tackle the complex fraction of asphaltenes. Specifically, Chapter 6 will address the need for a compositional definition of asphaltenes as opposed to the current solvent based system, and Chapter 7 will compare deposit asphaltenes with the corresponding crude oil.

**Future Prospects**

The polar species are just a small fraction of compounds that make up the composition of petroleum and petroleum derived materials (i.e. asphaltenes). As stated in the first chapter, petroleum is also composed of non-polar species (i.e. aromatic and unsaturated hydrocarbons), metals, and porphyrins. Current work is being done on molecular based characterization of the non-polar species detected by both Field Desorption (FD) and Atmospheric Pressure Photo Ionization (APPI) coupled to the Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. This technique combined with Gas Chromatography with a Flame Ionization Detector for the analysis of long chain alkyl hydrocarbons will lead to the characterization of all compounds in crude oil.

Molecular based characterization of petroleum and petroleum derived materials (i.e. Asphaltenes) will open the door to a variety of possibilities unattainable with current characterization methods. First and foremost, the ability to predict behavioral characteristics of crude oil
will be possible. Problems such as corrosion and scale deposition can then be eliminated before the processing takes place. Elimination of these problems will lower production costs which will then lower the cost of crude oil products to the consumer.

We also find petroleum products in every area of our lives. They are easily recognized in the gasoline we use to fuel our cars and the heating oil we use to warm our homes. Less obvious are the uses of petroleum-based components of plastics, medicines, food items, and a host of other products. Molecular based characterization of crude oil will potentially lead to the discovery of other uses of petroleum derived materials. If we continue to burn the crude oil for energy, these potential uses will never be recognized.
CHAPTER 2

INSTRUMENTATION, EXPERIMENTAL APPROACH AND GRAPHICAL REPRESENTATION

Instrumentation

Electrospray Ionization (ESI)

Electrospray ionization is a technique used in many forms of mass spectrometry. Electrospray ionizes the polar species present within a sample matrix. Briefly, a positive (or negative) potential is applied to the capillary tip through which a sample solution and electrolyte (e.g. HAc or NH₄OH) are moving. See Figure 2.1 for a typical schematic of an electrospray source. A counter electrode is applied to a plate a few millimeters away, thus creating an electric field between it and the needle. The electric field causes the ions of interest to be propelled towards the tip of the needle, creating a Taylor cone. For positive ion electrospray, the negative ions are attracted to the positive potential applied to the needle, while the positive ions are propelled towards the counter electrode. As charge builds up, the Taylor cone releases charged droplets. Droplets reduce to charged ions, 1 of 2 ways:

1. “Budding” – when desolvation occurs, there is a build up of charge and the ions “bud” on the surface of the droplet and are released.
2. Coulombic explosion – As the desolvation occurs (by heating or gas) the charge builds up in the droplet until the Raleigh limit is reached and the droplet explodes.

The positive ions are then attracted to the counter electrode plate and enter the orifice of the mass analyzer for analysis. The exact mechanism of ion formation is still unknown and under much scrutiny.\textsuperscript{24-27}

The major advantage of ESI is its ability to produce multiply charged ions, which makes it a useful technique for compounds that are high in mass. Since most analyzers (including Fourier transform ion cyclotron resonance mass spectrometry) detect below 2000 m/z, species that have masses higher than 2000 Da can only be detected if they are multiply charged. It is also a “soft” ionization technique producing little to no fragmentation. The crude oils that were run for the studies discussed in Chapters 3-7 are known to fragment under other ionization techniques, such as electron impact ionization (EI). Electrospray is the ionization technique of choice because of its ability to produce ions at atmospheric pressure enabling adaptation to many different mass analyzers.

\textbf{Fourier Transform Ion Cyclotron Resonance Mass Spectrometry FT-ICR MS}

Before the ion can be detected in the ICR cell, they must first undergo excitation. The detection plates within the ICR cell detect the frequency of the ions. The MIDAS data station\textsuperscript{28} then performs an inverse Fourier transform and sends it to the arbitrary waveform
Figure 2.1. Schematic of electrospray ionization source. Adapted from Enke, C. *Anal. Chem.* 1997, 69, 4885-4893.
generator. This produces a wave function that is sent to the opposing excitation plates for the excitation of the ions. Once excited, ions moving in a uniform magnetic field are subject to a Lorenz force given by Eq. (1),

\[ \text{Force} = qv \times B \]  

(1)

in which q, v and B are ionic charge, velocity and magnetic field. The magnetic field causes the ion to bend into a circular path with radius (r). Equation 2 shows the inverse relationship between radius and magnetic field, which causes a higher magnetic field to create a smaller ion radius,

\[ r = \frac{mv}{qB} \]  

(2)

in which r is the radius and m is the mass of the ion. The resolving power of the ion peak detected is proportional to the number of cyclotron orbits (frequency) during the detection period. The farther the ion travels the greater the resolving power. An ion in a larger magnetic field has a smaller cyclotron radius and a higher frequency and therefore a greater resolving power. Once the ions are detected by two opposing detection plates, a waveform is sent to a digitizer and then to the MIDAS data station where a fast Fourier transform (FFT) is performed to produce a frequency spectrum. The frequency spectrum is converted to a magnitude spectrum by a calibration equation. Calibration of spectra using the same trapping potential, to account for frequency shift introduced by the trapping electric field, and same dipolar excitation conditions, to account for frequency shifted by the space charge of ions, will allow for the ultra-high mass accuracy necessary to assign elemental composition in complex mixtures.
Experimental Approach

Typical Instrumental Parameters

Mass analyses for the studies discussed in Chapters 3-7 were performed with a homebuilt FT-ICR mass spectrometer equipped with a 22 cm diameter horizontal bore 9.4 T actively shielded magnet, as shown in Figure 2.2 (Oxford Cop., Oxney Mead, England). Data were collected and processed with a modular ICR data acquisition system (MIDAS). Positive and negative ions were generated from a microelectrospray source equipped with a 50 µm i.d. fused silica micro ESI needle. Samples were infused at a flow rate of 400 nL/min. Typical ESI (+) conditions were: needle voltage, 2 kV; tube lens 350 V. ESI (-) conditions were: needle voltage, -2.1 kV; and heated capillary current, 4 A. Ions were accumulated external to the magnet in a linear octopole ion trap (25.1 cm long) equipped with an axial electric field for 20 s and transferred through rf-only multipoles to a 10 cm diameter, 30 cm long open cylindrical Penning ion trap. Multipoles were operated at 1.5 MHz at a peak-to-peak rf amplitude of 70 V, Figure 2.3. Broadband frequency-sweep ("chirp") dipolar excitation (70 kHz to 1.27 MHz at a sweep rate of 150 Hz/µs and a peak-to-peak amplitude, 190 V) was followed by direct-mode image current detection to yield 4 Mword time-domain data. The time-domain data were processed and Hanning-apodized, followed by a single zero-fill before fast Fourier transformation and magnitude calculation. Frequency was converted to mass-to-charge ratio (m/z) by the quadrupolar electric trapping potential approximation to generate a mass-to-charge ratio (m/z) spectrum.
NHMFL 9.4 T ESI-FT-ICR Mass Spectrometer

Figure 2.2. Schematic of the 9.4 T ESI FT-ICR Mass Spectrometer used in the studies presented.
Figure 2.3. Schematic of the ion optics of the 9.4 T FT-ICR mass spectrometer used for these studies. The electrospray source is located at atmosphere with the ICR cell located at approximately 10-9 torr. There are three stages of differential pumping to achieve the low pressure necessary for detection. Three sets of octopoles, front, middle and transfer, for the transfer and accumulation of ions and a quadrupole for the isolation of a single species.
Data Analysis

Mass spectra were converted from the IUPAC mass scale (based on the $^{12}$C atomic mass as exactly 12 Da) to the Kendrick mass scale. The Kendrick mass scale is based on $CH_2 = 14.0000$ Da rather than 14.01565 Da. Kendrick mass is obtained from the IUPAC mass as shown in Equation (3).$^{34, 35}$

$$Kendrick\ mass = IUPAC\ mass \times \frac{14.0000}{14.01565}$$

Members of a homologous series (namely, compounds that contain the same heteroatom and number of rings plus double bonds, but different number of $CH_2$ groups) have identical Kendrick mass defect and are thus easily sorted and selected from a list of all observed ion masses, as shown in Equation (4).

$$Kendrick\ mass\ defect\ (KMD) = (Kendrick\ nominal\ mass - Kendrick\ exact\ mass)$$

Nominal Kendrick mass is determined by rounding the Kendrick mass up to the nearest whole number. Homologous series are then separated based on even and odd nominal Kendrick mass and KMD as described elsewhere.$^{34, 36}$

The composition of a petroleum molecule is generally expressed by its chemical formula, $C_cH_{2c+2}ZX$, in which $c$ is the carbon number, $Z$ is the hydrogen deficiency (measure of aromatic character), and $X$ denotes the constituent heteroatoms (N, S, O) in the molecule. For convenience, we abbreviate molecular formulas according to their type, double bond equivalents (DBE), and class, heteroatomic components. Double bond equivalents are calculated according to Equation 5.
Double bond equivalents (DBE) = c – h/2 + n/2 +1

For example, pyridine, which as a formula C₅H₅N, would be abbreviated as 4 N. Within the type and class of 4 N, there are many compounds that differ by an addition of CH₂ groups, allowing for three levels of comparisons based on type, class, and carbon number distribution.

**Graphical Representation**

*Kendrick Mass Plots*

Crude oil and its components are some of the most complex samples ever examined. The thousands of components typically found in the samples make it difficult to graphically represent each component in a single graph. The use of the Kendrick mass defect (KMD) and nominal Kendrick mass (NKM) determined in the separation and assignment of the species can be used to produce Kendrick plots. A Kendrick plot provides an excellent vehicle to visualize and sort all of the peaks in a mass spectrum. Compounds of the same class and type but different number of CH₂ units will fall on a single horizontal line (nominal Kendrick mass), with peaks separated by 14 Da but zero difference in Kendrick mass defect. Similarly, compounds of the same class but different type will fall on horizontal lines separated by 0.013 difference in Kendrick mass defect, as shown in Figure 2.4.

Kendrick plots offer a variety of other advantages. First, “outlier” data (i.e. noise spike and in situ contaminants), are more readily recognized when they fall outside of normal patterns. Second, peaks at a higher mass of a homologous series that have been identified are easily assigned an elemental composition whose mass would otherwise be too high to allow for unique assignment based on measured mass alone. In
other words, it is possible to assign the high-mass peaks with confidence by extrapolation from lower-mass members of an existing series.

Figure 2.4. Kendrick mass plot of the O₂ species found in a typical crude oil sample. This plot illustrates the increase in the number of rings plus double bonds (DBE) as the KMD increases as well as the alkylation series along the x-axis.

Kendrick mass defect and nominal Kendrick mass plotted along with percent relative abundance on the z-axis provides a unique look at crude oil, as shown in Figure 2.5. All peaks higher than 3σ of baseline noise, including ions of even and odd mass as well as peaks with one or two ¹³C are easily displayed in this single 3D Kendrick mass plot. Six
A thousand peaks are present in this single one page display, and species of different class, type and carbon number distribution can be determined from various patterns.

**Figure 2.5.** Three dimensional Kendrick mass plot with the Kendrick mass defect plotted on the y-axis, the nominal Kendrick mass on the x-axis and percent relative abundance color coded along the z-axis.

*Van Krevelen Diagrams*

The high mass resolving power ($m/\Delta m_{50\%} > 350,000$ for $200 < m/z < 1000$) and high mass accuracy ($\leq 1$ ppm) of FT-ICR MS allows for the unambiguous assignment of elemental composition to thousands of species found in complex environmental samples. The elemental
compositions reveal the compound class (i.e. numbers of N, O, and S atoms), type (number of rings plus double bonds), and degree of alkylation (namely, the number of –CH₂ groups for compounds of a given class and type). The remaining issue is how to sort and display the compositions to best illustrate the differences between various samples. As stated previously, Kendrick plots are an excellent tool for the display of all compounds found in a particular sample matrix. Another important tool is the van Krevelen diagram.

Van Krevelen diagrams were introduced in 1950 as a means to analyze bulk elemental data of coal samples. The diagrams consisted of the molar ratio of hydrogen to carbon as the ordinate and oxygen to carbon as the abscissa. Even in 1950 when analytical instruments were not as powerful, it was evident that samples of different origin could be distinguished by the location of the data in these plots. Kim et al. was the first to recognize that ultrahigh-resolution mass spectrometry enables the extension of van Krevelen’s idea from mole ratio in a bulk sample to atomic ratio for each of the thousands of elemental compositions present in a single sample, so that each distinct species contributes a single data point to the van Krevelen plot. They applied van Krevelen diagrams to dissolved organic matter from a black-water stream in the pinelands of New Jersey.

Here we apply a modified version of the van Krevelen diagrams to crude oil components as a tool to distinguish one sample from the next. We produce three-dimensional plots of H/C vs. O/C vs. percent relative abundance, like the one seen in Figure 2.6. The abscissa can be modified to display the compounds of interest by changing the atomic ratio of O/C to S/C or N/C depending on the heteroatom of interest. What is attractive about the van Krevelen diagram is its ability to separate graphically all the classes of interest for a given sample, and to provide a pattern of unsaturation and alkylation for each class.
In a van Krevelen diagram, heteroatom *classes* may be separated on one axis (e.g., S/C ratio to distinguish classes differing in number of S atoms), and DBE differences spread out along the second axis (H/C ratio). A homologous alkylation series composed of compounds that contain the same number of rings plus double bonds and the same heteroatoms, but different number of CH$_2$ groups shows up as diagonals. *Class* is separated along the x axis and the *type* along the y axis. As the H/C ratio increases the number of rings plus double bonds decreases. The van Krevelen diagram allows for convenient visual separation of heteroatom *class*, DBE *type*, and alkylation pattern.\textsuperscript{94}
CHAPTER 3

USE OF SARA FRACTIONATION FOR THE DETERMINATION OF MATRIX EFFECTS IN THE ANALYSIS OF CRUDE OIL BY ELECTROSPRAY IONIZATION FT-ICR MS

Introduction

Petroleum crude oils are among the most complex organic mixtures in the world, in terms of the number of chemically distinct constituents within a dynamic abundance range of 10,000-100,000. These crude oils are composed of saturated and unsaturated hydrocarbon molecules, polar components containing aromatic compounds with N, S, and O heteroatoms, and metals. Compositions of the saturated hydrocarbons have been well characterized by gas chromatography mass spectrometry (GC-MS)\textsuperscript{15}, two dimensional gas chromatography coupled to mass spectrometry (GCxGC MS)\textsuperscript{16}, high-resolution mass spectrometry\textsuperscript{17, 39}, and liquid chromatography mass spectrometry (LC-MS)\textsuperscript{18-20}. The unsaturated hydrocarbon fraction containing heteroatoms has recently been characterized by electrospray ionization (ESI) ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)\textsuperscript{40, 41}.

The two key features of ultrahigh-resolution FT-ICR mass analysis are its ability to resolve species differing in elemental composition by C\textsubscript{3} vs. SH\textsubscript{4} (0.0034 Da mass difference) and high mass accuracy (<0.001}
Da). That mass accuracy allows for the assignment of a unique elemental composition, \( \text{C}_c\text{H}_h\text{N}_n\text{O}_o\text{S}_s \), to singly-charged ions up to \( \sim 300-400 \) Da. Our 9.4 T ESI FT-ICR mass spectrometer\(^{29} \) provides mass resolving power, \( m/\Delta m_{50\%} > 300,000 \) (in which \( \Delta m_{50\%} \) is the magnitude-mode mass spectral peak full width at half-maximum peak height) from \( 225 < m < 1000 \) Da\(^{42-44} \), enabling the identification of thousands of polar species in a single mass spectrum. Electrospray was first introduced as an ionization source for the study of petroleum by Zhan and Fenn\(^{21} \), who showed that ESI could ionize the polar molecules in various petroleum distillates. Although those polar species constitute less than 15% by weight of crude oil\(^{12} \), they have significant implications in such areas as oil refining\(^{45} \) and fuel stability\(^{46} \), and their combustion produces sulfur and nitrogen oxides (\( \text{SO}_x \) and \( \text{NO}_x \)) known to produce acid rain and air pollution.

Mass spectrometric characterization of crude oils has heretofore relied heavily on pre-chromatographic separations to simplify the analyte composition, due to the lack of resolving power and mass accuracy of conventional MS techniques needed to resolve and identify the thousands of components commonly found in a single crude oil. The most common method of separation is the SARA fractionation, separating the crude oil into saturates, aromatics, resins, and asphaltenes. Jewell \textit{et al.}\(^{47, 48} \) separated the heavy end of crude oils into acid, base, neutral-nitrogen, saturate, and aromatic fraction by use of a combination anion- and cation-exchange chromatography, coordination chromatography, and adsorption chromatography. Subsequently introduced separative techniques include high-pressure liquid chromatography (HPLC) containing silica and alumina columns\(^{49} \) and the development of bonded stationary phases in HPLC columns.\(^{10, 50, 51} \)

The SARA fractionation method usually starts with removal of asphaltenes by precipitation with a saturated hydrocarbon. The following SAR separation is then accomplished by elution with a series of
increasingly polar solvents as the mobile phase. The saturates are eluted first with a non-polar solvent such as hexane, followed by the elution of the aromatics with toluene, and finally the resins are separated with a more polar solvent.

High-field positive-ion ESI FT-ICR MS resolves and enables assignment of elemental composition of thousands of chemically distinct species. However, ESI generates positive ions by protonation of neutrals in the original sample; ergo, the most basic constituents (e.g., aromatics with at least one nitrogen atom) are preferentially observed in positive-ion ESI FT-ICR MS. An obvious question is the extent to which the relative abundances of protonated aromatics in the positive-ion ESI FT-ICR mass spectrum of crude oil are affected by the presence of other species (namely, saturates, resins and asphaltenes). Therefore, in this chapter, we perform a SARA procedure to isolate each fraction, and then compare its positive-ion ESI FT-ICR mass spectrum to that of its parent crude oil. The present effort complements a prior analysis of the effect of other components on the relative abundances of fractionated acidic asphaltenes observed by negative-ion ESI FT-ICR MS.

**Experimental Methods**

*Fractionation of aromatic species*

**Figure 3.1** shows the SARA (saturates/aromatics/resins/asphaltenes) method used for the fractionation of the aromatic compounds found in crude oil. Approximately 1 g of South American
Figure 3.1. "SARA" (saturates/aromatics/resins/asphaltenes) fractionation and isolation scheme for the aromatic compounds found in South American crude oil. We consider here the aromatic fraction, asphaltenes and resins due to their high content of polar species accessible by electrospray ionization.
crude oil was dissolved in 40.0 mL of toluene for introduction to the rotary evaporator (Model R-200, Buchi, New Castle, Delaware). After 1 hour, nitrogen gas was bubbled through the remaining toluene/crude oil to remove toluene and volatile compounds. The volatile compounds comprised 11.2% (by weight) of the original crude oil. To remove asphaltenes, the crude oil sample was then dissolved in 40 mL of hexane (HPLC grade) and vacuum filtered (Whatman #1 paper, Whatman international, Maidstone, England). The asphaltenes were collected and stored in a glass vial. The asphaltenes constituted 7.1% of the original crude. 8 mL of the hexane-soluble fraction was adsorbed onto 3g of alumina (80-200 mesh, Fisher Scientific, Fairlawn, New Jersey). The alumina was then packed on top of 10 g of neutral alumina in a 22 x 400 mm column. The aromatic components (42.2%) were fractionated by elution with 80 mL of toluene while the Resins (14.7%) were collected with 50 mL of 80:20 (v:v) ratio of toluene to methanol through the column.

**Sample preparation for ESI FT-ICR MS**

The crude oil sample, the aromatic, asphaltene, and the resin fractions were each prepared by dissolving 20 mg in 3 mL of toluene, and then diluting to 20 mL with methanol. There was no precipitation at this volumetric ratio of 8.5:1.5 (methanol:toluene). 3 µL of acetic acid was added to 1 mL of the final solution to facilitate protonation of the basic nitrogen compounds to yield [M-H]⁺ ions.

**Results and Discussion**

The aromatic fraction, asphaltenes, and resins were isolated from the heavy crude oil to allow us to determine their effect on electrospray ionization efficiency for polar aromatic species. Figure 3.2 shows the
Figure 3.2  Broadband positive-ion electrospray ionization FT-ICR mass spectra of a South American crude oil and its aromatic fraction derived from SARA fractionation (see fig. 3.1). Note the high similarity of the two spectra, evidenced by their similar number- and weight-average molecular weights, \( M_n \) and \( M_w \). The average mass resolving power ranges from \( 350,000 < m/\Delta m_{50}\% < 450,000 \) for each spectrum.
broadband (250 < m/z < 900) mass spectra of a South American crude oil and its aromatic fraction isolated by the SARA method. Little to no signal was obtained for the asphaltenes and resin fractions. Asphaltenes are acidic in nature and contain few basic components, and are thus more appropriately observed as negative ions, for which acidic species are preferentially ionized, see previous paper. All ions are singly charged, as evident from the 1.0034 Da spacing between \( ^{12}\text{C}_n \) and \( ^{13}\text{C}^{12}\text{C}_{n-1} \) ions of otherwise identical elemental composition. The mass spectra of the crude oil and its aromatic fraction are visually similar, and exhibit similar number-average and weight-average molecular weights, \( M_n \) and \( M_w \): \( (M_n/M_w = 560/580 \) and 570/590, respectively.) The somewhat lower signal-to-noise ratio for the aromatic fraction accounts for the decrease in the number of peaks with magnitude larger than 3\( \sigma \) of the baseline noise.

**Figure 3.3** shows mass scale-expanded segments of the FT-ICR mass spectra of figure 3.2, at a representative even mass, 470 Da. Mass resolving power, \( m/\Delta m_{50\%} \), ranges from 350,000-450,000 throughout the mass range. All 21 different elemental compositions for the South American crude oil have been identified. 16 of 17 peaks were identified in the corresponding nominal mass of the aromatic fraction. The similarity between the two spectra is evident by the 16 common peaks (indicated by the dashed lines). At least some of the 5 peaks identified in the crude oil that are not seen in the aromatic fraction may well be present, but are not identified due to the lower signal-to-noise ratio for that fraction.

According to the "nitrogen rule"\(^5\), even-mass ions contain an odd number of nitrogen atoms, for the even-electron \((M+H)^+\) ions typically observed in positive-ion electrospray. For example, \( N_1 \) species observed at (even) nominal mass (470 Da in Figure 3) include \( N, NO, NS, NOS, \) and \( NS_2 \). Conversely, elemental compositions with an even number of nitrogen atoms at an even mass must contain one \( ^{13}\text{C} \) rather than \( ^{12}\text{C} \).
(indicated by * in figure 3.3). Finally, even-mass ions can contain one nitrogen and two $^{13}\text{C}$, as seen for a few species in figure 3.3. Similarly, at odd masses (not shown), we observed only $\text{N}_2\text{S}$ and $\text{N}_2$, along with some even-mass $^{12}\text{C}_n$ compositions but with $^{13}\text{C}^{12}\text{C}_{n-1}$. The deviation between assigned and calculated mass is very small (0-1 mDa), confirming the elemental assignment of each ion.

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**Figure 3.3.** Narrow segment of the ultrahigh-resolution mass spectra in Figure 2 at a representative even mass, 470 Da. Monoisotopic even-electron ((M+H)$^+$) species correspond to compounds that contain an odd number of nitrogen atoms (nitrogen rule), such as N, NO, NS, NOS, NS$_2$, N$_3$. Asterisks denote species containing one $^{13}\text{C}$ atom in place of $^{12}\text{C}$. Vertical dashed lines denote species found in the aromatic fraction.
Elemental composition immediately yields the class (namely, the numbers of heteroatoms, \(N_nO_oS_s\)) and type (number of rings plus double bonds, reported as double bond equivalents, DBE) of each compositionally distinct component. Figure 3.4 lists the seven most abundant classes found in both the South American crude oil and its aromatic fraction. The % relative abundance is the summed mass spectral peak magnitudes for all members of each class divided by the sum of all peak magnitudes in the spectrum. Only those compounds with a % relative abundance >1% are included; they include N, NO, NS, NS\(_2\), NOS, \(N_2\), and \(N_2S\). The near-identical % relative abundances for crude oil and aromatic fraction classes (e.g., 49.7% vs. 50.3% for the N class), for each of the 7 classes clearly demonstrate that the non-aromatic fractions (saturates, resins, and asphaltenes) do not affect the relative ionization efficiencies for the aromatics.

For an elemental composition, \(C_cH_hN_nO_oS_s\), it can be shown that\(^{52}\):

\[
\text{Rings + Double bonds} = \text{DBE} = c - h/2 + n/2 + 1.
\]  

Figure 3.5 shows the DBE distribution for the class of compounds that contain 1 nitrogen atom (\(C_cH_{2c+z}N\)), in which \(Z\) is called the hydrogen deficiency. \(Z\) is a measure of aromaticity, the more negative the value, the more aromatic the species. DBE is also a measure of aromaticity. The higher the DBE value, the more rings plus double bonds the compound will contain. Both the range, \(3 < \text{DBE} < 26\), and the % relative abundance for each DBE value are strikingly similar. For the \(N_1\) class, both crude oil and its aromatic fraction the highest abundance is for DBE = 8 (most likely diaromatic), consistent with a prior suggestion that most neutrals and basic nitrogen compounds contain at least one aromatic ring or one sulfur.
Figure 3.6 shows the carbon number distributions for $N_1$ compounds that contain 9 rings plus double bonds. The carbon number, which indicates the degree of alkyl substitution, ranges from 20 to ~64 for both the crude oil and its aromatic fraction. Again, the % relative abundances for each carbon number match well between the crude oil and its aromatic fraction, further demonstrating the lack of matrix affects other species found in crude oil have on the basic, polar components.
Type Distribution for the N Class

Another way to graphically display species within a particular class and type is by plotting double bond equivalents vs. carbon number, with signal color-coded according to percent relative contain the heteroatoms, N and S, Figure 3.7. Comparison of the range in both DBE and carbon number distribution clearly illustrates the similarity between these two samples. The South American crude oil and its aromatic fraction both

Figure 3.5. Type distribution for the basic N-compounds identified in the positive ion ESI FT-ICR mass spectra of South American crude oil and its aromatic fraction. DBE represents the double bond equivalents and indicates the number of rings plus double bonds for species containing one nitrogen. The high similarity between the two distributions ranging from $4 < \text{DBE} < 28$ shows that it is not necessary to isolate the aromatic fraction in order to identify its polar components.
have a DBE range of 6 - 27, with the most abundant being centered around 9 – 15 rings plus double bonds. The carbon number distributions are approximately the same, ranging from 20 to ~60 carbon atoms. Figure 3.8 is also a plot of DBE vs. carbon number, with percent relative abundance color-coded for those species containing two nitrogen heteroatoms. The similarity in both the DBE range and carbon number
Double bond equivalents vs. carbon number for those species containing the heteroatoms, N and S. Signal is color-coded according to percent relative abundance. Both the South American crude oil and its aromatic fraction are plotted to illustrate the similarity in both the DBE distribution and carbon number distribution.

**Figure 3.7.**
Figure 3.8. Double bond equivalents vs. carbon number for those species containing two nitrogen atoms of the whole South American oil and its aromatic fraction.
distribution once again supports the lack of matrix affects the saturates, resins and asphaltene have on the basic, polar species typically seen in positive-ion electrospray ionization. Detailed information is available for all classes found in both samples and all support this same trend.

This type of 3D graph is an excellent analytical tool to visually display all types of compounds found within a particular class. They not only fully illustrate the double bond equivalents distribution, but also show the carbon number range as well as the percent relative abundance of each individual species. Only FT-ICR mass spectrometry affords the high mass accuracy and high mass resolving power to uniquely assign each peak in a mass spectrum, providing unprecedented detail of crude oil components. These attributes allow for a compound to compound comparison between two samples. Even at this level of scrutiny, these two samples, South American Crude oil and its aromatic fraction, are almost identical. This similarity at the class, type, and carbon number distribution clearly demonstrates that the ionization of the basic aromatic species generated in positive-ion electrospray is unaffected by the saturates, resins, and asphaltene also present in crude oil samples.

**Conclusion**

FT-ICR mass spectrometry is known for its qualitative abilities because of its ability to do a compound to compound comparison between two or more samples. This paper, along with a previous study by Qian et. al. examines the lack of matrix effects in both positive and negative ion electrospray. In both studies, it was found that compounds such as the saturates, resins, and non-polar aromatics play no role in the ionization efficiency of the polar species detected in positive and negative -ion electrospray ionization. This knowledge has laid the groundwork for potential use of FT-ICR mass spectrometry for
quantitative analysis of petroleum samples. The information from these studies combined with future work in the examination of ionization efficiencies of individual classes within each polar group will open the door for unprecedented detail in not only the types of components found in petroleum samples, but also the concentrations of individual species.
CHAPTER 4

IDENTIFICATION OF HYDROTREATMENT-RESISTANT HETEROATOMIC SPECIES IN A CRUDE OIL DISTILLATION CUT BY ELECTROSPRAY IONIZATION FT-ICR MASS SPECTROMETRY

Introduction

Removal of NSO-containing species from petroleum crude oil is expensive. Compounds that contain oxygen and sulfur are known to cause corrosion, ultimately requiring replacement of production equipment.\textsuperscript{53, 54} Upon combustion, compounds that contain nitrogen and sulfur form NO\textsubscript{x} and SO\textsubscript{2}, which in turn react with water in the air, leading to acid rain.\textsuperscript{55} Nitrogen-containing species in finished fuels also form gums and solid precipitates during storage.\textsuperscript{46} One of the few practical techniques for the removal of these harmful species is catalytic hydrotreatment. Currently, hydrotreatment is the preferred process of removing the NSO containing polar species from crude oils. Unfortunately, some nitrogen-containing species promote catalyst deactivation through coke formation on the catalyst surface.\textsuperscript{56-58}

Hydrotreatment is performed on a very large scale commercially: e.g., 26 million barrels of feedstocks per day worldwide and 8.2 million barrels per day in the U.S.A. in 1995.\textsuperscript{59} A number of studies\textsuperscript{60} indicate that the N-containing ring of unsaturated heterocycles must be saturated before C-N bond cleavage can occur. The overall
hydrodenitrogenation rate for the N-heteroatomic compounds is limited by the extent of hydrogenation of the unsaturated N-containing rings. The basic nitrogen (e.g., pyridinic) species are more readily saturated and have a higher hydrogenation rate than the more acid/neutral indole and pyrrole compounds. A catalyst is required to lower the activation energy and reduce the amount of (expensive) H\(_2\) consumed during the reduction. Two widely used catalysts are Ni-Mo/Al\(_2\)O\(_3\) and Co-Mo/Al\(_2\)O\(_3\). Saben et al.\(^61\) found that the Ni-Mo/Al\(_2\)O\(_3\) catalyst was best for the removal of nitrogen.

Increasing legal restrictions on the permitted maximum level of sulfur-, nitrogen- and oxygen-containing species in petroleum have led to widespread adoption of catalytic hydrodenitrogenation. The development of better catalysts and technology requires the identification of nitrogen compounds that survive hydrotreatment. Model compound studies\(^62\) can shed light on the fundamental aspects of the catalysis, but cannot reliably predict the reactivity of all of the heteroatom-containing components of actual heavy gas oil feedstocks.

Crude oils are one of the most complex mixtures, containing molecules with thousands of different elemental compositions within an abundance dynamic range of \(\sim\)10,000:1. Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) is now recognized as a powerful technique for resolution and identification of complex mixtures, including crude oil\(^40, 41, 43, 44, 63-67\), humic and fulvic acids\(^68-70\), and other natural organic matter.\(^71, 72\) Electrospray ionization allows for preferential ionization of polar species (e.g., NSO-compounds), without prior chromatographic separation. FT-ICR mass spectrometry affords high mass resolving power (\(m/\Delta m_{50\%} > 300,000\), in which \(\Delta m_{50\%}\) denotes mass spectral peak full width at half height) and high mass accuracy (<1 ppm), enabling the baseline resolution and unambiguous assignment of elemental composition of thousands of species in a single
mass spectrum. Here, we apply positive- and negative-ion ESI FT-ICR MS to characterize the relative abundance distributions for different heteroatom-containing classes, rings plus double bonds (double bond equivalents, or DBE), and carbon distribution (to reveal the extent of alkylation of aromatic cores) in narrow distillation cut (483-504 °C). It thus becomes possible to determine, in unprecedented detail, the efficiency of hydrotreatment-based removal of molecules in terms of their individual chemical nature.

**Experimental Methods**

The crude oil feed was distilled into seven narrow distillation cuts (the one analyzed here is 483-504 °C), and each cut was hydrotreated in a bench-scale hydrotreater at 385 °C. The high nitrogen content of this distillation cut before and after hydrotreatment made it the focus of this chapter. Both feed and product narrow distillation cuts were then separated by retention chromatography to produce nitrogen-concentrated samples. Approximately 20 mg of each sample was completely dissolved in 3 mL of toluene and then diluted with 17 mL of methanol. One milliliter of the solution mixture was removed and spiked with either 5 μL of pure (99.9%) acetic acid (for positive–ion ESI) or 10 μL of 30% NH₄OH (for negative-ion ESI).

**Results and Discussion**

**ESI FT-ICR mass spectra**

Positive- and negative-ion electrospray FT-ICR mass spectra (Figure 4.1) reveal the number of basic and neutral nitrogen species resistant to hydrotreatment. Comparison of the number of mass spectral peaks for the untreated and hydrotreated samples (figure 4.1) establishes
the removal of 1200 acidic/neutral components (negative-ion ESI) and 1600 basic components (positive-ion ESI) by hydrotreatment. Furthermore, the high mass resolving power and high mass accuracy of FT-ICR MS allow for a more detailed inspection of the data.

At higher magnification (100 or 10 in m/z range), the negative-ion ESI FT-ICR mass spectrum of the untreated sample (Figure 4.2) reveals that all species are singly charged, based on the m/z = 1 spacing between each monoisotopic peak (all carbons are $^{12}$C) and its corresponding isotopic variant in which one $^{12}$C is replaced by $^{13}$C. The mass scale expansions also show families of peaks separated by spacings of 14.0156 Da (species differing by one -CH$_2$ group) and 2.0156 Da (species differing by one ring or double bond).

The "nitrogen rule" of mass spectrometry$^{52}$ states that an even-electron ion of odd (even) mass contains an even (odd) number of nitrogen atoms. Nitrogen-containing negative ions generated by electrospray ionization are typically deprotonated even-electron species [M – H$^-$. Thus, in the present examples, a compound containing an odd number of nitrogen atoms appears at even mass, whereas a compound containing an even number of nitrogen atoms or one nitrogen and one $^{13}$C appears at odd mass. Qian et. al. previously reported that in negative-ion electrospray ionization, those species containing two oxygen atoms (carboxylic acids) are ionized with an order of magnitude higher efficiency relative to neutral nitrogen species.$^{40}$ Since we observe the highest-magnitude peaks at even masses in negative-ion mass spectra, the present samples must contain neutral nitrogen species in high abundance; the O$_2$-containing species must have been fully or partially removed by retention chromatography performed before mass analysis.
Figure 4.1. Broadband positive- and negative-ion electrospray ionization FT-ICR mass spectra of the nitrogen-concentrated species found in a crude oil distillation cut (483 – 504 °C) and a corresponding hydrotreated sample, at mass resolving power, 350,000 < $m/\Delta m_{50\%}$ < 450,000. Hydrotreatment significantly reduces the number of peaks in both spectra.
**Hydrotreatment-resistant basic nitrogen species (positive ion ESI FTICR MS)**

A Kendrick plot (Kendrick mass defect vs. nominal Kendrick mass) provides an excellent vehicle to visualize and sort all of the peaks in a mass spectrum. **Figure 4.3** shows Kendrick plots for positive-ion ESI FT-ICR mass spectra of untreated (top) and hydrotreated (bottom) samples, with each signal color-coded according to percent relative abundance. Relative abundance was determined by dividing the magnitude of each peak by the summed magnitudes of all above-threshold peaks in the mass spectrum. Upon visual inspection of these two samples, it is clear that the untreated distillation cut has a broader KMD range, 0.02 < KMD < 0.35 than the hydrotreated sample, 0.03 < KMD < 0.25. KMD is directly related to the double bond equivalents (DBE) of a particular compound, the higher the KMD value, the higher the DBE value (higher number of rings plus double bonds). The hydrotreatment process preferentially removes or reduces the double bond equivalents of those compounds that contain a greater number of rings plus double bonds and can lead to the generation of species at lower double bond equivalents (DBE).

As noted above, positive-ion ESI preferentially ionizes basic species (e.g., molecules containing a pyridine ring). **Figure 4.4** shows positive-ion ESI FT-ICR mass spectral windows extending from 450-450.5 Da for
Figure 4.2. Two mass scale-expansions of the full range negative-ion ESI FT-ICR mass spectrum of untreated crude oil (fig. 4.1, upper-left). In the lower mass segment, peaks that are members of a homologous series (species containing the same heteroatom, but differ by a CH₂ group, 14.0156 Da apart) are indicated by the red arrows. Top: A mass difference of 2.0156 Da (i.e., two hydrogen atoms) corresponds to members of the same class (same numbers of heteroatoms) but different type (i.e., different number of rings plus double bonds, or DBE). The highly abundant species at even nominal masses contain a single nitrogen atom, as expected after the removal of all other heteroatoms by retention chromatography (see text).
Figure 4.3. Kendrick mass defect (KMD) vs. nominal Kendrick mass (NKM), with each signal color-coded according to percent relative abundance for all species detected in positive ion electrospray for the untreated (top) and hydrotreated (bottom) samples. The hydrotreated sample clearly has a narrower Kendrick mass defect distribution, indicating a smaller double bond equivalents (DBE) distribution.
untreated (top) and hydrotreated (inverted, bottom) samples. Possible structures corresponding to two elemental compositions are identified. These are possible representative structures and are only presented to illustrate typical compounds ionized in positive ion electrospray. The figure shows that those species that contain a higher DBE value, 17 NS, are preferentially removed compared to the species of lower DBE value of the same class, 10 NS. Those compounds that contain multiple heteroatoms, such as 18 NOS, 12 NS\textsubscript{2} and 17 NO\textsubscript{2}, are also preferentially removed or reduced to other compounds of lower DBE values. Compounds with oxygen and sulfur heteroatoms are preferentially removed compared to those species that contain a nitrogen heteroatom. When compounds contain multiple heteroatoms, such as NOS, NS and NO, the hydrotreatment process may only remove the oxygen or sulfur heteroatom producing an N-containing species (pyridinic benzenes).\textsuperscript{73} The presence of the single nitrogen-containing species in the hydrotreated sample clearly suggests that these compounds (present in the original sample, or generated from multiple heteroatom containing species) are resistant to the treatment.

**Figure 4.5** shows the relative abundance distribution among heteroatom-containing classes for positive-ion ESI FT-ICR mass spectra of untreated and hydrotreated distillation cuts. The hydrotreated sample contains ~94% N-containing species, ~62% for the untreated sample. The *relative* abundance of N-containing species increases because other classes (e.g., NS, NO) are preferentially removed by hydrotreatment or these pyridinic benzenes are being produced from the reduction of the
Figure 4.4. One Dalton mass scale-expanded segment of the positive-ion ESI FT-ICR mass spectra of untreated (plotted normally) and hydrotreated (inverted) N-concentrated crude oils of figure 4.1 (right). Of the 16 above-threshold peaks identified in the untreated oil, 6 (shown by boxes), predominantly pyridine-like compounds containing a single nitrogen atom, persist after hydrotreatment. Two possible representative structures illustrate the ability of positive-ion ESI FT-ICR MS to preferentially ionize pyridine-like compounds.

multiple heteroatomic species. The inset in figure 4.5 shows the relative abundance distribution as a function of double bond equivalents (DBE) for the N-class. The DBE distributions for both the treated and untreated samples are similar, because all members of the N-class are highly resistant to removal by hydrotreatment. A possible explanation
for a shift of the most abundant DBE type from 9 (in the untreated sample) to 8 (in the treated sample) is the reduction of NS and NO compounds to single N-containing compounds. The removal of the sulfur and oxygen heteroatom, when they are present as furan-like structure and thiophene-like structure causes the DBE value to decrease by 1. Those single N-containing species at low DBE, 2 and 3, in the
The hydrotreated sample illustrates the production of low DBE species. A likely explanation is the first step in the hydrotreatment process. The first step is the hydrogenation of the aromatic rings or the reduction of the number of rings plus double bonds (DBE values). Figure 4.6 shows the relative abundance distribution for the NS-containing class in positive-ion ESI FT-ICR mass spectra of untreated (top left) and hydrotreated samples (top right), as a function of DBE and carbon number. For a given DBE, the compounds most resistant to hydrotreatment contain a higher number of carbon atoms, corresponding to more and/or longer alkyl chains, presumably due to increased steric hindrance of contact between the heteroatom and the catalyst. The carbon number distribution for NS-containing compounds with 11 rings plus double bonds, shown at the bottom of figure 4.6, clearly shows preferential hydrotreatment-induced depletion of lower carbon-number species (again due to less steric hindrance for fewer and shorter alkyl chains). Of course, depletion depends on the placement of the alkyl chain on each aromatic core; steric hindrance is clearly highest when alkyl chains are placed on the carbons on either side of the heteroatom of interest. Previous results by Rodgers et. al. revealed similar patterns of alkyl chain steric hindrance when the alkyl groups are located at the 4,6 position on benzothiophene like compounds. A similar pattern is seen for all classes (except for the N-class, which resist hydrotreatment, irrespective of the extent of alkylation). Those NS-species that are produced, 1-5 rings plus double bonds, are compounds that have undergone the first step of hydrogenation but have not been subjected to the treatment long enough to break the S-C bond for complete removal of the sulfur heteroatom as H₂S.
Figure 4.6. Top: DBE vs. carbon number for the CcH2c+ZNS species in untreated and hydrotreated fractionated crude oil samples. The average carbon number distribution increases from ~31-37 at 1-17 rings plus double bonds to ~21-39 at 6-21 rings plus double bonds upon hydrotreatment. Hydrotreatment clearly removes those NS-compounds with high double bond equivalents and low carbon number. Bottom: Relative abundances as a function of carbon number for species containing 11 DBE. Note the lack of low carbon number species in the hydrotreated sample.

Hydrotreatment-resistant neutral nitrogen species (negative ion ESI FTICR MS)

Figure 4.7 is a plot of Kendrick mass defect (KMD) vs. nominal Kendrick mass (NKM), with percent relative abundance color-coded in the z-axis for both the untreated (top) and hydrotreated (bottom)
Figure 4.7. Kendrick mass defect (KMD) vs. nominal Kendrick mass (color-coded in the remaining axis according to percent relative abundance for all resolved above-threshold peaks in the broadband ESI FT-ICR negative-ion mass spectrum) for the untreated (top) and hydrotreated (bottom) N-concentrated crude oil. Note the shift to lower KMD (due to removal of more highly aromatic and/or O\textsubscript{x}S\textsubscript{x} species upon hydrotreatment.)
samples. The Kendrick mass defect (KMD) increases directly with increasing aromaticity of a particular compound class (i.e., a particular combination of N, O, and S heteroatoms). For untreated crude oil (figure 4.7-top), the highest-abundance species (green/yellow/and red) range between nominal Kendrick mass, 300 - 500, and Kendrick mass defect, 0.100 – 0.200. After hydrotreatment (figure 4.7-bottom), the KMD values decrease (due to preferential elimination of oxygen- and sulfur-containing species of high KMD and removal or reduction of highly aromatic N-containing species), and the most abundant species are now found in the nominal Kendrick mass (NKM) range, 400-500 (due to preferential elimination of some lower-mass species). This centralization of high abundance species shifts to higher NKM and lower KMD. Although the 3D Kendrick plot provides for sensitive visual assessment of changes in type (aromaticity), the signals for different classes (heteroatoms) are overlaid, and are better displayed by other graphical means (see below).

As stated earlier, the high mass resolving power and high mass accuracy (< 1 ppm) of FT-ICR MS allow for the unequivocal assignment of elemental composition based on accurate mass measurement. Figure 4.8 shows a 0.4 Da mass window for both the untreated (top) and hydrotreated (inverted, bottom) samples. Hydrotreatment eliminates 12 of the 17 identified species from the mass spectrum. The resolving power, elemental composition and error for each of the peaks in the both samples (figure 4.8) are listed in Table 4.1. Compounds removed by hydrotreatment contain multiple heteroatoms, such as NS, NOS, OS and NO. Hydrotreatment-resistant compounds contain a single nitrogen atom or have multiple heteroatoms and lower aromaticity (i.e., smaller number of rings plus double bonds). Possible representative structures shown in figure 4.8 illustrate pyrollic-like compounds ionized by negative-ion electrospray. Those structures are based on compounds normally seen in crude oils as well as model compounds.66
Figure 4.8. One Dalton mass scale-expanded segment of the negative-ion ESI FT-ICR mass spectra of untreated (plotted normally) and hydrotreated (inverted) N-concentrated crude oils of figure 4.1 (left). Of the 17 (out of 18 with magnitude larger than 3 standard deviations of baseline noise) peaks identified in the untreated oil (see table 4.1), only 5 (shown by boxes), from N and NO classes, remain after hydrotreatment. Three possible representative structures illustrate the carbazole-like compounds typically detected as negative ions.
Table 4.1. Elemental composition assignments for untreated and hydrotreated fractionated crude oils.

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Peak Location m/z</th>
<th>Resolving Power (m/∆m50%)</th>
<th>Elemental Composition</th>
<th>Error (+/- ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400.14609</td>
<td>473000</td>
<td>C_{25}H_{24}O_{2}S (^{13}C)</td>
<td>+0.8</td>
</tr>
<tr>
<td>2</td>
<td>400.17110</td>
<td>394000</td>
<td>C_{29}H_{23}NO</td>
<td>+1.0</td>
</tr>
<tr>
<td>3</td>
<td>400.17435</td>
<td>394000</td>
<td>C_{26}H_{27}NOS</td>
<td>+0.7</td>
</tr>
<tr>
<td>4</td>
<td>400.18239</td>
<td>337000</td>
<td>C_{26}H_{28}OS (^{13}C)</td>
<td>+0.6</td>
</tr>
<tr>
<td>5</td>
<td>400.19092</td>
<td>394000</td>
<td>C_{27}H_{29}N (^{34}S)</td>
<td>+0.8</td>
</tr>
<tr>
<td>6</td>
<td>400.19223</td>
<td>473000</td>
<td>C_{26}H_{27}NO_{3}</td>
<td>+1.0</td>
</tr>
<tr>
<td>7</td>
<td>400.20032</td>
<td>473000</td>
<td>C_{28}H_{28}O_{3} (^{13}C)</td>
<td>+1.0</td>
</tr>
<tr>
<td>8</td>
<td>400.20176</td>
<td>394000</td>
<td>C_{25}H_{29}NS (^{13}C_{2})</td>
<td>+0.6</td>
</tr>
<tr>
<td>9</td>
<td>400.20720</td>
<td>394000</td>
<td>C_{30}H_{27}N</td>
<td>+0.3</td>
</tr>
<tr>
<td>10</td>
<td>400.21056</td>
<td>394000</td>
<td>C_{27}H_{31}NS</td>
<td>+0.3</td>
</tr>
<tr>
<td>11</td>
<td>400.22828</td>
<td>337000</td>
<td>C_{27}H_{31}NO_{2}</td>
<td>+0.2</td>
</tr>
<tr>
<td>12</td>
<td>400.23628</td>
<td>472000</td>
<td>C_{27}H_{32}O_{2} (^{13}C)</td>
<td>-0.1</td>
</tr>
<tr>
<td>13</td>
<td>400.26457</td>
<td>337000</td>
<td>C_{28}H_{36}NO</td>
<td>-0.0</td>
</tr>
<tr>
<td>14</td>
<td>400.27252</td>
<td>295000</td>
<td>C_{28}H_{36}O (^{13}C)</td>
<td>-0.4</td>
</tr>
<tr>
<td>15</td>
<td>400.28401</td>
<td>394000</td>
<td>C_{27}H_{36}N_{2} (^{13}C)</td>
<td>+0.2</td>
</tr>
<tr>
<td>16</td>
<td>400.29193</td>
<td>337000</td>
<td>C_{27}H_{37}N (^{13}C_{2})</td>
<td>-0.3</td>
</tr>
<tr>
<td>17</td>
<td>400.29857</td>
<td>590000</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>400.30088</td>
<td>590000</td>
<td>C_{29}H_{39}N</td>
<td>-0.2</td>
</tr>
</tbody>
</table>

Figure 4.9 shows the relative abundance distribution according to compound class, for untreated (12 assigned classes) and hydrotreated (7 assigned classes) crude oil. Compounds containing only a single nitrogen heteroatom (carbazole-like species) are the only class completely resistant to hydrotreatment. O-containing species present in the untreated sample after retention chromatography are preferentially removed by hydrotreatment. Interestingly, addition of one or more oxygen or sulfur atoms to compounds containing one nitrogen results in partial (NO_{x}, NS) or complete NOS and O_{x}S_{x} (x = 0-3) removal upon hydrotreatment. Finally, note that relative abundance is scaled
separately for each mass spectrum; thus, the relative abundance of the N class increases by virtue of reduction in the amounts of other classes or the production of carbazole-like species with lower DBE values from these multiple heteroatom O- and S-containing species as evident in Figure 4.10.

**Figure 4.9.** Relative abundances for various compound classes in the negative-ion ESI FT-ICR mass spectra of untreated and hydrotreated fractionated crude oils. Only those classes with relative abundance > 1% are shown. The % relative abundance is the individual compound mass spectral peak magnitude divided by the summed magnitudes of all peaks in the spectrum.
The members of a given heteroatomic class differ in double bond equivalents (DBE = number of rings plus double bonds) and number of carbons. Figure 4.10 shows negative-ion ESI FT-ICR mass spectral relative abundance (color-coded) as a function of DBE and carbon number for two heteroatomic classes, N (top) and NO (bottom), for untreated (left) and treated (right) crude oils. For the C\textsubscript{c}H\textsubscript{2c+2}N class, hydrotreatment increases the relative abundance of species containing 9-12 rings plus double bonds and 30 – 38 carbons, by preferential removal of other classes as well as the production of N-species at low DBE and low carbon numbers. This production of lower DBE species illustrates either the partial removal of compounds containing multiple heteroatoms, such as NS\textsubscript{x} and NO\textsubscript{x}, or the hydrogenation of the aromatic rings present in the carbazole-like compounds. In contrast, hydrotreatment lowers the relative abundance of virtually all members of the C\textsubscript{c}H\textsubscript{2c+2}NO class, irrespective of DBE or carbon number. Of special note, the production of the 4 NO species in the hydrotreated sample is most likely due to the scission of the O-C bond in the 5 NO species in the untreated sample. This presents another possible explanation for the presence or production of compounds with a smaller number of rings plus double bonds. Also, heteroatom classes with oxygen or sulfur atoms with higher carbon number appear to be more resistant to hydrotreatment, perhaps because the longer alkyl chains hinder the access of the molecules to achieve close contact with the catalyst.
Figure 4.10. Double-bond equivalents vs. carbon number for the N- and NO- classes of untreated and hydrotreated fractionated crude oils.
CHAPTER 5

ANALYSIS OF NSO POLAR SPECIES FOR CORRELATION OF ELEMENTAL COMPOSITION TO TOTAL ACID NUMBER (TAN), CORROSIVITY, AND GEOGRAPHICAL LOCATION DETECTED BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

Introduction

One of the greatest concerns to the oil refining industry is the presence of polar species, such as N-, S-, and O-containing compounds in crude oils. Although these species make up a relatively small percentage of crude oils (less than 15%), they can cause major concerns during many stages of the production process and to the environment. For example, upon combustion, compounds that contain nitrogen and sulfur form NO\textsubscript{x} and SO\textsubscript{2}, which in turn react with water in the air, forming acid rain.\textsuperscript{55} Asphaltenes (highly saturated NSO-containing compounds) are known to precipitate and deposit during petroleum production and processing.\textsuperscript{75-77} Some of the nitrogen-containing species are known to promote catalyst deactivation through coke formation on the catalyst surface during hydrotreatment processes.\textsuperscript{56-58} These nitrogen species are also known to form gums and solid precipitates in finished fuels during storage.\textsuperscript{46} Finally, one of the greatest concerns of the oil refining industry is the presence of petroleum acids because of corrosion they cause of the pipe equipment.\textsuperscript{53, 78, 79}
The main culprits linked to the corrosion problems associated with production are those species that contain 2 oxygen atoms, or carboxylic acids. In this chapter, all species containing O$_2$ will be considered carboxylic acids. Naphthenic acids will refer to those compounds that include a carboxylic group and one or more saturated ring structures. Crude oils can contain up to 4 wt % of these carboxylic acids and the characterization of these acids is a major topic within the oil industry because of the corrosion problems associated with these compounds. The acidity of crude oils is measured by the total acid number (TAN), which is defined as the mass of potassium hydroxide (in milligrams) required to neutralize one gram of crude oil. Typical industry standards state that a high corrosive crude will have a TAN > 0.5 mg KOH/g of crude oil. Oils can vary in TAN from < 0.1, to as high as 8 mg KOH/g of crude oil. Recent results have shown that there are deficiencies in relying upon this method for direct correlation. There is little detailed work published on identifying the compound groups responsible for high TAN values and/or high corrosive character. Here we will attempt to identify specific compounds that cause high TAN values or high corrosive character.

The polar species found in crude oils also have positive implications. These heteroatomic species can be useful in the identification of specific crude oils to geographical location based on geochemical clues traced back to biological precursors. Certain polar species can be found at much higher concentration than others of similar structure. These have survived diagenesis of petroleum molecules and are called biomarkers. Many hydrocarbons, oxygen compounds, sulfur and nitrogen compounds have been identified as biomarkers. The prime importance of biomarkers is in the enabling of geochemists to relate crude oils to their parent kerogen and thus draw conclusions about the origin of a crude oil. These biomarkers also are an indicator of oil maturity and biodegradation. Hughey et. al
recently published the first study of the identification of crude oils of different geochemical origin and found that these polar species can be used to identify geographical location.\textsuperscript{66} There appear to be little or no studies on the comparison of NSO polar species of crude oils from the same geographical location, but from different location within the reservoir.

We have recently demonstrated electrospray ionization\textsuperscript{84} Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)\textsuperscript{85} for analysis of polar species in crude oil\textsuperscript{40, 41, 43, 44, 63, 65-67} and coal.\textsuperscript{95, 107, 108} ESI coupled to high-field (9.4 T) FT-ICR MS has resolved and identified elemental compositions (C\textsubscript{c}H\textsubscript{h}N\textsubscript{n}O\textsubscript{o}S\textsubscript{s}) of thousands of polar species in crude oils and coal. From those elemental compositions, the samples may be further characterized according to the relative abundance distributions for different heteroatom-containing classes, rings plus double bonds (DBE) (to reveal the degree of aromaticity for each compound), and carbon distribution (to reveal the extent of alkylation of aromatic cores) Here we apply electrospray ionization Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry to a suite of ten crude oils of different corrosive character, TAN and production site (geographical location). This effort is the first of its kind to attempt correlation of corrosivity, TAN and elemental composition. We will also illustrate the use of these polar species in the identification of crude oil origin and location within the production reservoir.

**Experimental Methods**

**Crude oil samples – determination of TAN and corrosivity**

Ten crude oils exhibiting different total acid number (TAN) and corrosivity are shown in Table 5.1. TAN was determined by the amount of potassium hydroxide (KOH) needed to neutralize one gram of crude oil.
A probe that contained a wire (made of the sample material as the production pipes) with electrical resistance applied was inserted into the production stream to test for corrosion. The measure of the electrical resistance is a function of the diameter of the wire. Changes in the wire diameter (because of corrosion) cause the electrical resistance, in the form of current, to change. Unfortunately, within a single day the variations within the current can be rather significant. However, this method allows for immediate responses after a different oil is inserted into the blend to be processed. Highly experienced equipment and process engineers used this change in current, along with field data to assign the corrosivity to each crude oil tested in this study. The measure of TAN and corrosive character to each of our crude oils in this suite, illustrate the deficiencies in relying on TAN alone for a measure of corrosive character. Although A5 has a low TAN value (0.3 mg KOH/g crude oil) it still exhibited high corrosivity. C1 has a relatively high TAN value (0.8 mg KOH/g crude oil) but exhibits negligible corrosive character.

Two of the crude oils (A5 and B3) are from production site one, while the remaining eight come from production site two. Both production sites are located in South America but at different geographical locations.

**Sample preparation**

The crude oil was used as received from the oil company. 20 mg of crude was dissolved in 3 mL of toluene, and then diluted to 20 mL with methanol. 1 mL of the final solution was spiked with either 3 µL of acetic acid or 10 µL of ammonium hydroxide to facilitate protonation or deprotonation.
Table 5.1. Crude oils analyzed

<table>
<thead>
<tr>
<th>Crude Oil</th>
<th>TAN</th>
<th>Corrosivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>4.0</td>
<td>High</td>
</tr>
<tr>
<td>A2</td>
<td>2.3</td>
<td>High</td>
</tr>
<tr>
<td>A3</td>
<td>1.8</td>
<td>High</td>
</tr>
<tr>
<td>A4</td>
<td>1.5</td>
<td>High</td>
</tr>
<tr>
<td>A5</td>
<td>0.3</td>
<td>High</td>
</tr>
<tr>
<td>B1</td>
<td>1.0</td>
<td>Medium</td>
</tr>
<tr>
<td>B2</td>
<td>0.5</td>
<td>Medium</td>
</tr>
<tr>
<td>B3</td>
<td>0.5</td>
<td>Medium</td>
</tr>
<tr>
<td>C1</td>
<td>0.8</td>
<td>Negligible</td>
</tr>
<tr>
<td>C2</td>
<td>0.3</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

Results and Discussion

Both positive and negative ion mass spectra were collected for all ten crude oils to illustrate the similarities and differences in the acidic (negative) and basic (positive) –NSO polar compounds. **Figure 5.1** illustrates mass spectra, both positive (right) and negative (left) electrospray, for three representative crude oils, A1 (top), B1 (middle), and C1 (bottom). All mass spectra exhibit an average mass resolving power range from 350,000 < m/Δm_{50%} < 400,000. The distribution of m/z is similar for all three crude oils, 280 < m/z < 825 for negative ions.
Figure 5.1. Representative full-range Positive (right) and negative (left) electrospray ionization Fourier transform ion cyclotron resonance mass spectra of three representative crude oils, A1 (high corrosivity, top), B1 (medium corrosivity, middle), and C1 (negligible corrosivity, bottom). The combined spectra for a single crude oil have approximately 9000 chemically distinct species. The average mass resolving power ranges from $350,000 < m/?m50\%$, $< 400,000$ over the full mass range in both positive- and negative- ion mode.
and $300 < m/z < 750$ for positive ions, despite the difference in TAN and corrosivity. This indicates that the molecular weight distribution plays no role in the corrosive nature of a particular crude oil. In the sample, A1, over 8,500 chemically distinct species were detected above the noise threshold in both positive and negative electrospray ionization. In all 10 crude oils, approximately 100,000 chemically distinct species were detected. This number is astronomical when considering that these polar species only account for less than 15% of the whole crude oil. Only FT-ICR MS affords the high mass resolving power and high mass accuracy ($<1$ ppm error) for the unambiguous assignment of elemental composition to almost 100% of the ions detected.

This complexity makes crude oils very difficult to not only analyze but also to visualize each of the components of a single spectrum. As stated in the previous chapter, conversion of the IUPAC mass to Kendrick mass allows for the sorting and assignment of species above the m/z of 400. Plotting Kendrick nominal mass vs. Kendrick mass defect allows for the visual display of all detected species in a single graph. **Figure 5.2** shows Kendrick plots for both negative (left) and positive (right) –ion FT-ICR mass spectra of A1 (top), B1 (middle) and C1 (bottom), with each signal color-coded according to percent relative abundance. Relative abundance was determined by dividing the magnitude of each peak by the summed magnitudes of all above-threshold peaks in the mass spectrum. Upon visual inspection of the acidic components (negative electrospray ionization), it is clear that all three samples contain roughly the same nominal Kendrick mass (NKM) range, 250 – 850, and the same Kendrick mass defect (KMD) range, 0.05 – ~0.4. Kendrick mass defect is directly related to the number of rings plus double bonds associated with a particular species. A higher KMD indicates a higher number of rings plus double bonds (DBE value). There are differences in the most
Figure 5.2. Kendrick mass plots of the negative and positive ions in A1 (top), B1 (middle), and C1 (bottom), in which the z-axis (color) denotes contours of percent relative abundance of each individual component. The similarity in the nominal Kendrick mass and Kendrick mass defects of all three samples in positive electrospray indicate these basic species play no role in the corrosive nature of a crude oil. The differences in the negative ions indicate that an oil's corrosive nature comes from the acidic species.
abundant species between the three crude oils with different corrosivity, which originate from the same production site. This suggests that ESI FT-ICR has the ability to distinguish species from different fields or intervals within the same geographical location. The differences between Kendrick plots of crude oils with the same corrosivity (not shown) indicate that the acidic polar species does not alone correlate to the corrosive character.

The observation of basic components is illustrated in the Kendrick plots of the positive-ion FT-ICR mass spectra (right). The NKM range for all three samples, A1, B1, and C1 is 300 < NKM < 750, whereas the KMD range is 0.05 < KMD < 0.35. The similarity between these ranges as well as the similarity in the abundance of the components indicates that the basic components play no role in the corrosivity of the crude oil. These basic species cannot be used as markers to distinguish crude oils from different fields or intervals within the same geographical location.

The next two sections will examine the basic and acidic polar species detected by positive and negative electrospray ionization, separately. The determination of the classes (heteroatoms), type (number of rings plus double bonds, DBE) and alkylation patter will possibly lead to a better understand of physical and chemical properties of a crude oil, such as corrosivity. This detailed assignment will also lead to the determination of biomarkers within crude oils which can then be used to not only locate specific geographical locations, but also specific fields and intervals within a production site. Although the complexity of crude oils is a hindrance for analysis by conventional analytical techniques, we use the ability of FT-ICR MS to take advantage of this complexity as a tool to distinguish between oils.
**Positive-ion Electrospray FT-ICR MS (Basic Components)**

Kendrick plots provide excellent information on the types of components found in crude oils but are unable to shed any light on the specific classes detected. **Figure 5.3** is a class comparison of the basic polar species detected. Only those classes with a relative abundance > 1% are shown. Percent relative abundance of each class is determined by dividing the sum of the relative abundances of all species within a class by the relative abundance of species detected in the crude oil. Four chemically distinct classes were found in the four crude oils.

**Figure 5.3.** Comparison of compound classes and double bond equivalents (inset) for species containing a single nitrogen atom in positive electrospray for 4 crude oils, A1, B1, C1 (site 2) and A5 (site 1). Only 4 distinct heteroatom classes were detected and all 4 crude oils have similar % relative abundances of each class. The distribution of double bond equivalents (inset) illustrates the similar range of rings plus double bonds, 4 – 27.
analyzed in positive-ion electrospray; N, NS, NO, and N\(_2\). Even though this graph illustrates crude oils of different corrosive character from production site one (A1, B1, and C1) and production site two (A5), they all contain the same classes with similar percent relative abundances. Those species containing a single nitrogen atom have an abundance of ~82% for all four crude oils. The inset graph illustrates the double bond equivalents (DBE, number of rings plus double bonds) for those species containing a single nitrogen atom. Much like the percent relative abundance of the class, the DBE distribution for all four crude oils is similar, 4 – 27 rings plus double bonds. Similarity in class and type indicates the lack of corrosive character in the basic species of crude oils and the inability to use these components as distinguishing markers in crude oils.

**Negative-ion Electrospray FT-ICR MS (Acidic Components)**

Negative electrospray ionization selectively ionizes the acidic components (carboxylic acids and neutral nitrogen compounds) in a sample matrix. Previously, our group has published a deprotonation efficiency study on acidic species and found that carboxylic acids are preferentially ionized an order of magnitude over the neutral nitrogen (pyrrole benzalogs) species.\(^6\) The preferential ionization of the acidic species by negative electrospray ionization makes it the desired technique because it is thought that these acidic components, specifically the carboxylic acids, are the key contributors to corrosivity. These acids are also known to be biomarkers, which can provide clues to oil’s origin, maturity, and degree of biodegradation.

**Correlation of Corrosivity, TAN and Elemental Composition.** It has been proposed that the main compounds that contribute to the corrosivity of a crude oil are those that contain a carboxylic acid group
While ESI FT-ICR MS is unable to quantitatively compare species between two or more oils, it has the ability to qualitatively compare species within crude oil samples. **Figure 5.4** plots the relative abundance of the O$_2$-containing acids relative to all species vs. TAN (a). It is clear that there is no direct correlation between TAN and corrosivity (represented by color and shape) with the overall percent relative abundance of the carboxylic acids. Next, the abundance of the naphthenic acids (1-3 rings plus double bonds) were examined by

![Diagram of percent relative abundance of O$_2$-containing species vs. TAN (a) and the ratio of percent relative abundance of naphthenic acids to percent relative abundance of other carboxylic acids vs. TAN (b).](image)

**Figure 5.4.** Plot of percent relative abundance of O$_2$–containing species vs. TAN (a) and the ratio of percent relative abundance of naphthenic acids to percent relative abundance of other carboxylic acids vs. TAN (b). All ten crude oil are represented, high corrosive crudes (blue, diamond), medium corrosive crudes (red, square) and low corrosive crudes (green, triangle). Clearly, no correlation is present between all carboxylic acids and TAN and the corrosive character of the crude oils.
plotting the ratio of naphthenic acid percent relative abundance to the percent relative abundance of all other carboxylic acids vs. TAN (b). Previous results by other groups indicate that the unsaturated carboxylic acids (naphthenic acids) play a key role in the overall corrosivity of a crude oil.\textsuperscript{79, 80} Here, we show no correlation between these unsaturated acids and the TAN or corrosivity of the oil. These results clearly show that although these acids may be contributing to the overall corrosive character of the crude oils, they are not alone. Other species, such as the non-polar sulfur components (not detected by ESI FT-ICR MS) may also be a contributing factor.\textsuperscript{86}

**Correlation of acidic components to particular geographical location.** In Figure 5.5, the relative abundance for each compound class is summed over all nominal masses, divided by the total relative abundance for all peaks, and depicted in bar graph form to provide a comparison of crude oils by compound class (i.e., NSO compounds). As noted above, acids are ionized more efficiently than neutral nitrogen compounds so that ion signal alone is not a quantitative estimate of the relative amount of a given neutral in the original crude oil. However, these percentages can be used to qualitatively compare the suite of 10 crude oils. There are 14 chemically distinct classes found in the suite of 10 crude oils with a percent relative abundance > 1%. As evident by the different abundances of all of these classes, the acidic polar species can possibly be used as markers for particular production sites, intervals and fields within a basin. In all 10 crude oils, those species containing a single nitrogen atom and those containing two oxygen atoms are the dominate components and account for approximately 75\% - 90\% the acidic polar species detected.

As stated earlier, A5 and B3 are oils from the same geographical location and should have very similar biomarkers. Both of these crude
Figure 5.5. Compound class comparison of the major –NSO species found in negative-ion FT-ICR MS for all 10 crude oils. 14 chemically distinct classes were detected with a relative abundance > 1%. Crude oils, A5 and B3 are from production site 1; whereas the eight remaining crude oils are from production site 2. The differences within specific sites allows for differentiation of crude oils not only from different production sites, but also different intervals and fields within the same basin.

oils have a high percent relative abundance of O$_2$ species compared to the other eight oils from production site 2. Only A2 (from site 2) has a higher relative abundance of these carboxylic acids. Acids, specifically O$_2$ class, are common components in young or immature crude oils. The data suggests that production site 1 is geographically located at a younger reservoir than site 2. The acids present in the crude oil are the
original components of the oil and can be used as biomarkers or they are formed by chemical or biochemical oxidation of the original crude molecules after migration into the reservoir. As evident by the vast differences in the percent relative abundance of classes between oils from the same production site, it is clear that this detailed class identification can also be used to identify oils at different intervals or fields within the same production site.

Next we are going to take a closer look at the types of compounds found in the O₂ class, **Figure 5.6.** Percent relative abundance is plotted on the y-axis with double bond equivalents (DBE) on the x-axis. Those species containing 1 – 3 rings plus double bonds are known as naphthenic acids, see representative structure in figure. Those species that contain more than 3 rings plus double bonds can have structures that contain saturated rings, or are considered aromatic. Possible structures for these compounds can also be found in figure 5.6. Typical biomarkers are composed of hopane derivatives which are composed of unsaturated rings. A large part of petroleum napthenes, from mono-ring compounds to those with six rings, can be assumed to be directly derived from biological matter. Those species that contain 1 – 3 rings plus double bonds in our data are composed of this hopane skeleton. It is evident from the high relative abundance of these naphthenic acids in A5 and B3 that they come from a different geographical location than the other eight crude oils present.

**Figure 5.7** shows iso-abundance contours as a function of double bond equivalents and carbon number for O₂ species in six crude oils, A2, A4, B1, B2, A5, and B3. All six crude oils have approximately the same DBE range, 1 - ~19 rings plus double bonds, and carbon number range from 17 < carbon number < 60. The crude oils from site 2, A2, A4, B1, and B2, have a high abundance of O2 species containing 4 rings plus double bonds and 31-34 carbon atoms. This is in contrast to those species from production site 1, A5 and B3, which have a high relative
Figure 5.6. Comparison of double bond equivalence (DBE) and % relative abundance in the $O_2$ class, grouped according to possible ring structure (cyclic vs. aromatic structures). A5 and B3, from the same production site, contain the highest percent relative abundance of the naphthenic acids (those species containing 1-3 rings plus double bonds), indicating the use of these compounds as biomarkers for this particular production site. Structures illustrate possible representative compounds with corresponding number of rings plus double bonds for components typically found in crude oil.

abundance of those species that contain 2 rings plus double bonds and 31-34 carbon atoms. This clearly illustrates that this $O_2$ class can be used as biomarkers to link specific oils to their appropriate geographical location. Unfortunately, distinguishing between neighboring fields and
Figure 5.7. Double bond equivalents (DBE) vs. carbon number (scaled in the third direction according to percent relative abundance contours) for negative ions that are members of the O₂ class (with a generic formula of CₓHᵧO₂). Those species with 2 rings plus double bonds and 31 – 35 carbon atoms found as the most abundant O₂ compounds in A5 and B3 (of production site 1, c) illustrates the use of these naphthenic acids as biomarkers of this production site. This is in contrast to those most abundant O₂ species with 4 rings plus double bonds found in the other 4 crude oils illustrated here (a, b).
different intervals within a geographical location can not be accomplished by looking at the O₂ class alone.

The negative-ion ESI FT-ICR mass spectral relative abundance (color-coded) as a function of DBE and carbon number for those species containing a single nitrogen atom are illustrated in Figure 5.8. The number of rings plus double bonds (DBE) range from 2 < DBE < ~30 for all except A2. A2 has a smaller DBE range because the overall percent relative abundance of these species is relatively low compared to the other 5 crude oils. This is due to the high abundance of O₂ compounds found in this oil (see figure 5.5). The carbon number range for all six oils is relatively the same, 20 – 60 carbon atoms. These nitrogen containing compounds are also known to be considered biomarkers and can be used to identify specific geographical location, but in the case of these 10 crude oils, this is not the case. The similarity in the types of compounds found within this class prohibits their use as identifying species to a particular production site.

Conclusion

ESI FT-ICR MS affords the selective ionization of the polar species (acidic and basic) from a suite of 10 crude oils for their correlation with corrosivity, TAN, and geographical location. The similarity evident in the basic components indicates that these species play no role in the corrosive nature of crude oils and can not be used as identifying markers of specific geographical location. Our data suggests that although carboxylic acids (O₂ class) are known to be the main contributor to the corrosive character of crude oils this is not the case for the suite of 10 oils analyzed here. Other species must also be contributing to the overall corrosivity of the crude oils. Although we were unable to correlate corrosivity and TAN to elemental composition of the polar species, the
Figure 5.8. Double bond equivalents (DBE) vs. carbon number (scaled in the third direction according to percent relative abundance contours) for negative ions that are members of the N class (with a generic formulae of C$_c$H$_h$N). The differences in the abundance and the types of compounds present in the 6 representative crude oils indicate the inability to use this specific class of compounds as biomarkers.
vast differences found in the abundance of these acidic polar species can be used to not only link crude oils to specific production sites, they can also be used to differentiate oils extracted from different intervals and fields within a reservoir. This is accomplished by the identification of specific biomarkers inherent to a particular geographical location.
CHAPTER 6

REDEFINING ASPHALTENES: COMPOSITIONAL DIFFERENCES IN PRESSURE DROP AND SOLVENT DROP ASPHALTENES DETERMINED BY ELECTROSPRAY IONIZATION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

Introduction

Crude oil is one of the most complex mixtures in the world, and is composed of thousands of species that define its chemical and physical properties. The constituents of crude oils are typically classified by solubility: saturates, aromatics, resins, and asphaltenes (“SARA”). SARA fractionation starts with removal of asphaltenes by precipitation with a saturated hydrocarbon (e.g., heptane). Subsequent elution with a series of increasingly polar solvents as the mobile phase yields saturates (eluted with a non-polar solvent such as hexane), followed by the elution of aromatics with toluene. The resins are separated with a more polar solvent. Here, we focus on the asphaltenes because they can generate major problems in production, and because it is important to establish the degree of correspondence between laboratory and field definitions of asphaltenes.

Problems associated with asphaltene precipitation and deposition have been widely reported in the petroleum production and processing industry. Asphaltenene precipitation and subsequent deposition in production tubing and topside facilities impose significant cost
penalties to crude oil production. Some of the parameters reported to affect asphaltene precipitation/deposition include pressure, composition, and temperature. A primary criterion to study asphaltene precipitation/deposition for upstream operation is live oil depressurization\(^{89, 90}\), to determine the onset pressure for asphaltene precipitation, and the quantity of asphaltenes precipitating out of solution and depositing on the cell wall.

In the laboratory, asphaltenes are conveniently defined by solubility as those compounds in "dead" oil (i.e., oil at atmospheric pressure) that are insoluble in hexane (IP 143 and ASTM 6560). In the field, asphaltenes (precipitation /deposition) were induced by pressure reduction as crude oil is produced from the reservoir pressure to the surface. Structurally, asphaltenes are typically thought of as complex mixtures of heteroatom-rich polycyclic hydrocarbons. More specifically, asphaltenes are comprised of condensed aromatic and naphthenic molecules of molar masses up to ~2000 Da.\(^{91, 92}\) They are also the most polar fraction in crude oil, and comprise much of the heteroatom (N, S, O) and metal (Ni and V) content of heavy oils. Due to its inherent compositional complexity, the actual chemical structure of asphaltenes is difficult to define with existing analytical tools. Here we apply microelectrospray ionization\(^84\) FT-ICR mass spectrometry (ESI FT-ICR MS)\(^85\) to two asphaltenes samples collected from \(C_7\) precipitation and live oil depressurization experiments, from the same well. FT-ICR mass spectrometry affords high mass resolving power \(|m/\Delta m_{50\%} > 300,000|\), in which \(\Delta m_{50\%}\) denotes mass spectral peak full width at half height) and high mass accuracy, < 1ppm, for the unambiguous assignment of elemental composition to thousands of components per mass spectrum. ESI FT-ICR MS has been successfully applied to complex mixtures such as crude oil \(^{40, 41, 43, 44, 63, 65-67}\) and coal.\(^{95, 107, 108}\) Such results have laid the groundwork for the new field of "petroleomics"\(^1, 2\), namely, the correlation (and ultimately prediction) of the properties and behavior of
petroleum and its distillates from their detailed chemical compositions. Positive and negative ion electrospray serve to reveal the basic and acidic components of the two samples. Accurate mass measurement generates elemental compositions \((C_{c}H_{h}N_{n}OoS_{s})\) of thousands of components of each of the two differently defined asphaltene samples. From those elemental compositions, the samples may be further characterized according to the relative abundance distributions for different heteroatom-containing classes (i.e., \(N_{n}OoS_{s}\)), rings plus double bonds (double bond equivalents, or DBE) to reveal the degree of aromaticity for each compound, and carbon distribution (to reveal the extent of alkylation of aromatic cores). It thus becomes possible to determine, in unprecedented detail, the differences in chemical composition of asphaltenes defined by solubility vs. pressure-drop method. The differences turn out to be substantial.

**Experimental Methods**

**P-drop asphaltenes**

The pressure-drop asphaltenes were collected by live oil depressurization. The experiment was performed with a high-pressure pressure-volume-temperature (PVT) cell equipped with a near-infrared laser-based solid detection system described previously.\(^{89}\)

The oil was equilibrated on a rocker to a single-phase fluid at the bottomhole temperature and an initial pressure of 12,000 psia for one week. Forty-five mL of the previously conditioned oil was charged into the PVT cell, by use of a positive displacement pump. The temperature of the cell and its contents were kept at 138 °C and 12,000 psia for 8 hours to further condition the oil inside the cell. A baseline transmittance of the laser was initially established. Depressurization consisted of stepping down the pressure in 100 psia decrements. A relaxation time of 7 min
between pressure steps allowed the transmittance of the laser to equilibrate. As the pressure on the live oil decreased, it became less dense, thus allowing more light to be transmitted through it. The intensity of transmitted light increases until asphaltenes in the oil started to precipitate, and then decreased. The point at which the transmittance starts to decrease is the onset pressure of asphaltene precipitation and corresponds to the pressure at which asphaltene precipitation begins in the wellbore. After the oil was depressurized, the asphaltene deposits on the cell walls, on the mixer, and endcap were washed off with toluene. The toluene was evaporated and the solids analyzed gravimetrically. The fraction of asphaltenes that precipitated but that did not stick to a surface was filtered out of the oil by use of an attached bulk filtration apparatus. The results from the live oil experiments are summarized in Table 1.

**Heptane-precipitated (C$_7$) asphaltenes**

The C$_7$–insoluble asphaltenes were prepared by a modified IP-143 method.$^{93}$ The asphaltene content of the dead crude oil was 9% by weight.

**Sample preparation for ESI FT-ICR MS**

10 mg of each sample was dissolved in 5 mL of toluene and then diluted with 5 mL of methanol to produce a final concentration of 1 mg of asphaltene per mL of solvent. 2 µL of base was added to facilitate deprotonation for negative-ion ESI and the sample was sprayed as for positive-ion ESI.
### Table 6.1. Deposition and depressurization results for P-drop sample.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottomhole Pressure</td>
<td>6,539 psia</td>
</tr>
<tr>
<td>Initial Depressurization</td>
<td>12,000 psia</td>
</tr>
<tr>
<td>Asphaltene Onset Pressure</td>
<td>4,500 psia</td>
</tr>
<tr>
<td>Saturation Pressure</td>
<td>4,000 psia</td>
</tr>
<tr>
<td>Total Asphaltene in Oil</td>
<td>3.44 g</td>
</tr>
<tr>
<td>Precipitated Asphaltenes</td>
<td>0.138g</td>
</tr>
<tr>
<td>Asphaltene Deposited</td>
<td>0.148g</td>
</tr>
</tbody>
</table>

### Results and Discussion

**Positive and negative ion ESI FT-ICR MS**

Both positive and negative ion mass spectra were collected for the two asphaltene samples to illustrate the difference in the acidic (negative ions) and basic (positive ions) polar species. Asphaltenes are routinely detected in negative ion electrospray and are rarely observed in positive mode. Here, we present a unique look at asphaltenes detected in both negative and positive electrospray. Samples, such as asphaltenes, are only observable in positive electrospray when they contain a significant concentration of basic species. The mass spectra for C₇-insoluble and pressure-drop asphaltenes are shown in Figure 6.1. All four spectra exhibit an average mass resolving power, m/Δm₅₀% of ~400,000 at m/z 500. Elemental compositions could be assigned to approximately 12,000 peaks per sample between 300 < m/z < 750 to within ≤ 1 ppm error. The
Figure 6.1. Broadband negative- and positive- ion electrospray ionization FT-ICR mass spectra of the C7 insoluble and pressure drop (P-drop) asphaltenes. All four mass spectra range from approximately 320 < m/z < 750, with little difference in the molecular weight range for the two samples. The average mass resolving power, m/Δm50% is ~350,000 with a maximum signal to noise ratio of ~1000:1.
similarity in m/z range of the two samples for both positive and negative ions indicates similar molecular weight distributions for the two samples.

FT-ICR MS can baseline-resolve tens of compounds per nominal mass. Figure 6.2 shows mass scale-expanded segments (ranging over m/z ~ 0.5) from all four mass spectra of figure 6.1. The difference not only in the number of peaks, but also position, clearly illustrates the differences between these two asphaltenes samples. The two samples have 4 peaks in common for both negative and positive ions. All peaks above a threshold of 3 times the standard deviation of baseline noise (3σ), are assigned an elemental composition. Each spectrum has several peaks labeled according to the double bond equivalents (DBE), and heteroatoms of its assigned elemental composition (e.g., 4 O₂). For both positive and negative ions of this nominal mass, the P-drop asphaltenes are enriched in compounds that contain both oxygen and sulfur atoms whereas the C₇-insoluble asphaltenes are composed more of compounds that contain nitrogen.

The next two sections will examine the similarities and differences between these two asphaltenes. First, the examination of the classes, those compounds that contain the same heteroatom, will be compared. Both positive and negative ions will be looked at. Next, a variety of graphs will be used to compare and contrast the different types of compounds (those species that contain the same heteroatom, but differ by the number of rings plus double bonds). Positive ions will be compared first, in order to examine the basic species that are preferentially ionized with this mode of electrospray. Negative ions, acidic species, will be examined next using similar graphical methods, such as Kendrick plots and van Krevelen diagrams.37, 38
**Class comparison**

The different heteroatom classes are best visualized in relative abundance distributions. The class comparisons of the basic species detected as positive ions are illustrated in Figure 6.3. The P-drop sample displays higher abundance of species containing sulfur, such as NS and NS\(_2\). Although the P-drop asphaltenes show higher abundance of species containing a single nitrogen atom, the C\(_7\)-insoluble asphaltenes are relatively enriched in species containing multiple nitrogen heteroatoms, such as N\(_2\) and N\(_3\).
Negative-ion electrospray selectively ionizes acidic components: typically, carboxylic acids and pyrollic type compounds. **Figure 6.4** shows a relative abundance distribution for various heteroatom classes for negative ions from both asphaltenes samples. The P-drop and C7-insoluble asphaltenes contain 19 and 14 different heteroatom classes.
The P-drop asphaltenes are dominated by species containing both oxygen and sulfur, of which the most abundant class is $O_4S$. In contrast, the $C_7$-insoluble asphaltenes are dominated by compounds that contain nitrogen, such as NS, NOS, and NO. Sulfur and oxygen heteroatoms are present only if nitrogen is present, except for relatively low-abundance (~2.5%) for $O_4S$.

![Negative Ions](image-url)

**Figure 6.4.** Compound classes in negative-ion electrospray FT-ICR MS for P-drop and $C_7$-insoluble asphaltenes. Only those classes with relative abundance >1% are shown. Inspection reveals an increase/presence of those compounds containing oxygen and sulfur in the P-drop asphaltenes, such as $O_4S$, $O_2$, and OS suggesting that these species preferentially flocculate as the pressure increases. The increase/presence of those compounds containing nitrogen (such as NS, N$_2$S, and N$_2$) in the $C_7$-insoluble asphaltenes evidently results from their insolubility in heptane.
**Type comparisons**

Compound type refers to the number of rings plus double bonds (DBE) associated with increasing aromaticity of a particular species. It can be graphically displayed in a Kendrick plot (Hughey et al. 2001) of Kendrick mass defect (KMD) vs. nominal Kendrick mass (NKM), with % relative abundance (%RA) shown as iso-contours on the third axis. Kendrick mass defect varies directly with aromaticity, and thus provides a quick visual measure for comparison of aromatic character for different samples. Another graphical representation is a plot of double bond equivalents (DBE) vs. carbon number, with signal color-coded according to per cent relative abundance. *Type* is easily displayed along the y-axis along with the carbon number (x-axis) for a single *class* of compounds identified in the sample.

**Positive ion electrospray.** In positive electrospray ionization, basic species such as pyridinic benzagols are preferentially ionized. **Figure 6.5** shows Kendrick plots for all positive ions in ESI FT-ICR mass spectra of both heptane-insoluble (C\textsubscript{7}) and pressure drop (P-drop) asphaltenes. The C\textsubscript{7}-insoluble asphaltene sample has a KMD range from 0.05 – 0.50 with the most abundant species from 0.10 – 0.20, whereas the P-drop asphaltene sample KMD's range from 0.05 – 0.45 with the most abundant species from 0.05 – 0.10, the C\textsubscript{7}-insoluble asphaltenes are thus more highly aromatic than the P-drop asphaltenes.

Close inspection of N\textsubscript{1} species, detected in positive electrospray, reveals that the heptane-insoluble (C\textsubscript{7}-insoluble) asphaltenes have a higher abundance of species containing more rings plus double bonds. **Figure 6.6** shows iso-abundance contours as a function of double bond equivalents and carbon number for N species (as positive ions) for both asphaltenes samples. The heptane-insoluble asphaltenes range from 4 < DBE < 32 with highest abundance from 7 < DBE < 26. In contrast, the most abundant P-drop asphaltene N\textsubscript{1} species range over 10 < DBE < 12. The carbon number range for both samples is about the same, 23 – 52;
Figure 6.5. 3D Kendrick plot of C₇-insoluble (top) and pressure drop (bottom) asphaltenes, in which the z-axis (color) denotes contours of percent relative abundance of each component.
Figure 6.6. Double bond equivalents (DBE) vs. carbon number (scaled in the third direction according to per cent relative abundance contours) for positive ions that contain a single nitrogen atom. The C7-insoluble asphaltenes have lower %RA of single pyridine-like compounds, and higher aromatic character. The most abundant species range from ~7 to 26 rings plus double bonds compared to ~10-12 rings plus double bonds in P-drop asphaltenes.
i.e., similar number of CH$_2$ groups attached to the aromatic core of each compound. Although the P-drop asphaltenes exhibit higher overall abundance of N$_1$ compounds, the C$_7$-insoluble asphaltenes have higher DBE values and are thus more aromatic.

Figure 6.7 contains similar graphs for two more classes of compounds detected in positive ion electrospray. One class, NS, illustrates a similar trend seen for the N$_1$ species, while the N$_2$ class follows a slightly different trend. Although the P-drop asphaltenes exhibit a higher overall abundance of NS compounds, the C$_7$-insoluble asphaltenes have higher DBE values and thus contain compounds with a higher degree of aromatic character. The NS-class found in the P-drop asphaltenes range from 6 < DBE < 25 with the highest abundant species from 6 < DBE < 17. This is in direct contrast to the same class found in the C$_7$-insoluble asphaltenes which has a range from 8 < DBE < 32, with the most abundant from 19 < DBE < 27. For both classes, N and NS, previously shown, the classes in the P-drop sample had the highest percent relative abundance (%RA). The class that contains two nitrogen atoms, the C$_7$-insoluble asphaltenes contains the highest %RA. The P-drop asphaltenes have a range 8 < DBE < 24 with the most abundant between 9 and 20 rings plus double bonds. The C$_7$-insoluble asphaltenes has a range of 8 < DBE < 32, with the most abundant from 10 < DBE < 27. This difference between the two samples is most likely due to the difference in percent relative abundance of the detected N$_2$-containing species between the two samples. What is important to note is the fact that although both samples contain many similar classes, this is not indicative of similar types as illustrated by the previous two figures.
Figure 6.7. DBE vs. carbon number (scaled in the third direction according to per cent relative abundance contours) for positive ions that contain a N and S heteroatoms as well as those that contain 2 nitrogen atoms. Those species that contain N and S follow a similar trend to that seen in figure 6.6, whereas those species that contain two nitrogen heteroatoms follow a slightly different trend.
**Negative ion electrospray.** Figure 6.8 contains Kendrick mass plots as in figure 6.5, but this time for negative rather than positive ions. Negative ions derive from acidic species such as pyrrolic benzalogs and carboxylic acids. The KMD range for the C_7-insoluble asphaltenes (figure 6.8, top) in negative ion mode is ~0.05 – 0.50. The most abundant species have a KMD range from 0.20 – 0.45 indicating that the acidic species are more highly aromatic than the basic species seen in figure 6.5. Although the overall KMD range for all species in the P-drop asphaltenes (figure 6.8, bottom) is roughly the same as for the heptane-insoluble sample, the most abundant species are concentrated over a KMD range of 0.15 – 0.20. The highest-abundance components (KMD ~0.16) are those that contain one ring or double bond and four oxygen and one sulfur atom, 1 O_4S. The shift to lower KMD indicates lower aromatic character for the P-drop asphaltenes sample.

Figure 6.9 shows a comparison of the double bond equivalents and carbon number distributions for the O_4S class for both the P-drop and C_7-insoluble asphaltenes. The DBE distributions for the two asphaltene samples are clearly very similar except for the uniformly lower relative abundance for O_4S species in the C_7-insoluble sample. The inset illustrates the carbon number distribution for O_4S class members that contain 1 or 2 rings plus double bonds. Again, the carbon number distributions are about the same, ~16 – 48, except for the difference in %RA (27% vs. 2.5%--see figure 6.4) between the two samples.

Much like positive ion electrospray, just because the two asphaltene samples have some of the same classes, does not mean they contain the same *types* of compounds. Figure 6.10 includes graphs of two *classes*, N and NS, which are present in both the P-drop and C_7-insoluble asphaltenes. Those species that contain one nitrogen heteroatom in the P-drop asphaltene have a range 8 < DBE < 30, with the most abundant from 15 – 23 rings plus double bonds. The same class in the C_7-insoluble sample contains roughly the same DBE range
Figure 6.8. Kendrick mass plots of the negative ions in C₇-insoluble (top) and pressure-drop (bottom) asphaltenes. The negative ions (as for the positive ions) from the C₇ insoluble asphaltenes show a more highly aromatic character than the P-drop asphaltenes. The most abundant class and type for the pressure-drop asphaltenes is 1 SO₄, with a KMD of 0.16, indicated by the green, yellow and red colors.
but with the most abundant from $19 < \text{DBE} < 27$, indicating a shift to higher aromatic character of this $N_1$-containing class for the $C_7$-insoluble asphaltenes. This same trend is seen for those species that have both a single nitrogen and a single sulfur heteroatom. Those species in the P-drop asphaltenes have a range of $11 < \text{DBE} < 30$, while the same species in the $C_7$-insoluble asphaltenes have a range of $15 < \text{DBE} < 35$. This
Figure 6.10. DBE vs. carbon number (scaled in the third direction according to per cent relative abundance contours) for negative ions that contain a single nitrogen atom and those that contain N and S heteroatoms.
slight shift in the number of rings plus double bonds indicates the presence of more aromatic species in the C$_7$-insoluble asphaltenes, similar to the trend seen for those species that contain a single nitrogen atom. The shift to higher number of rings plus double bonds for the most abundant species within this class for the C$_7$-insoluble asphaltenes also indicates this shift to more aromatic species.

Another graphical representation of crude oil type and class is a van Krevelen diagram, consisting of isoabundance contours as a function of (e.g.) H/C and S/C ratio for each compound containing those atoms. First conceived for bulk elemental composition (van Krevelen 1950), the idea was first applied to all of the individual elemental compositions in humic substances by Kim et. al. to distinguish condensed hydrocarbons from lipids and other components. In a van Krevelen diagram, heteroatom classes may be separated on one axis (e.g., S/C ratio to distinguish classes differing in number of S atoms), and DBE differences spread out along the second axis (H/C ratio). A homologous alkylation series composed of compounds that contain the same number of rings plus double bonds and the same heteroatoms, but different number of CH$_2$ groups shows up as diagonals. The van Krevelen allows for convenient visual separation of heteroatom class, DBE type, and alkylation pattern.

Van Krevelen diagrams for negative-ions containing a single nitrogen atom and 1 or 2 sulfur heteroatoms are illustrated in Figure 6.11 for both asphaltene samples. Class is separated along the x axis and the type is separated along the y axis. As the H/C ratio increases the number of rings plus double bonds decreases. There is a slight shift to lower H/C ratio (i.e., higher number of rings plus double bonds) for the C$_7$-insoluble asphaltenes compared the P-drop sample. There is also an increase in the %RA of those negative ions containing 2 sulfur
Figure 6.11. Van Krevelen diagrams for negative ion species that contain NS\textsubscript{x} heteroatoms in the C\textsubscript{7} insoluble (top) and P-drop (bottom) asphaltenes. The abscissa is the ratio of sulfur atoms to carbon atoms; the ordinate is the ratio of hydrogen atoms to carbon atoms; and the third axis shows relative abundance contours. The shift to higher H/C ratio for the P-drop asphaltenes indicates lower aromatic character relative to the heptane-insolubles. The greater number of species and higher %RA of NS\textsubscript{2} compounds illustrates an enrichment of sulfur compounds in the P-drop asphaltenes relative to the C\textsubscript{7}-insoluble asphaltenes.
heteroatoms in the P-drop sample, consistent with the enrichment of sulfur-containing positive ions seen in figure 6.3.

**Conclusion**

Compositional variations between asphaltenes obtained from pressure drop and C₇ precipitation are readily apparent in classes and types of species identified by ESI FT-ICR MS. Interestingly, for those shared compound classes and types identified by both positive and negative ESI FT-ICR MS, the carbon number only varied slightly. Positive ion ESI FT-ICR MS exposed compositional differences in the basic species and showed that the C₇ asphaltene sample contained a smaller relative abundance of the N₁-containing species but this class is composed of higher rings plus double bonds compared to the P-drop asphaltenes. The pressure drop asphaltenes are enriched in compounds that contain sulfur such at NS and NS₂ as well as fewer rings plus double bonds compared to the C₇ asphaltenes. Negative ion ESI FT-ICR MS exposed compositional differences in the acidic species and exposed the enrichment of oxygen and sulfur containing species in the pressure drop sample. Similar to the aromaticity differences seen in the basic species, the pressure drop sample is composed of mainly less aromatic heteroatomic compounds compared to the C₇ asphaltene sample. The analysis of dead oil asphaltenes may be very misleading since, if the oil lost material upon pressure reduction, only a subset of the asphaltene fraction is being observed. Based on this study, that subset is very different than what was lost upon depressurization.

Depressurization favors the flocculation of low DBE, highly polar species that comprise OₓS (x is any integer 2-5), O₂, NS, NS₂ and O₄S₂. The analysis of crude oil components by ESI FT-ICR MS has opened the door to compound to compound comparison between crude oils which is the backbone to
petroleomics. This ability to baseline resolve and assign elemental composition on a molecular level allows us to not only study crude oils but also predict production issues involving asphaltene-like compounds. It is clear that a better definition for asphaltenes is needed and the need to find a logistical method for the analysis of elemental composition of live oil samples.
CHAPTER 7

COMPOSITION COMPARISON OF DEAD CRUDE OIL DEPOSIT ASPHALTENES TO ITS CRUDE OIL COUNTERPART AND LIVE CRUDE OIL DEPOSIT ASPHALTENES DETECTED BY ELECTROSPRAY IONIZATION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

Introduction

Deposition of crude oil components into piping for topside facilities during the refining process and petroleum recovery operations is a major economical concern for the crude oil industry. The replacement of clogged pipes can cost millions of dollars, which eventually finds its way to the consumer in increased cost of petroleum derived materials. In the petroleum recovery process, it is known that these deposits tend to precipitate out of solution mainly due to depressurization from the reservoir pressure, at which the system must be assumed to be in equilibrium. The basic reason for this precipitation must be the interactions between different molecules in the fluid. The deposition in the topside processing facilities is not completely understood and under continuous study. The deposits in both recovery operations and processing are known to be comprised mainly of asphaltenes.

Asphaltenes are present in crude oils in the range of 0 % - 10 % or more and have a large effect on chemical properties of the crude oil. They are the heaviest and the most polar fraction and are enriched in heteroataoms (N, S, O), typically a few mass %. The “lab” asphaltene is...
defined by its insolubility in \( n \)-heptane (or some other light \( n \)-alkane) and solubility in toluene and are obtained from dead oil (oil at atmospheric pressure). The “field” asphaltene is known as the components that deposit on the production equipment walls during the depressurization of the live crude oil from reservoir pressures to atmospheric pressure. A crude oil that possesses all chemical components including light ends in proper amounts as found in the subsurface formation (at reservoir pressures) is termed a live oil.

Asphaltenes have been routinely studied using vapor pressure osmometry\(^{100-102}\), size exclusion chromatography\(^{100, 101, 103}\), low resolution mass spectrometry\(^ {102, 104-106}\) and UV-fluorescence\(^ {91, 92}\) for a better understanding of molecular weight distribution. The actual chemical structure of asphaltenes is difficult to define with these existing analytical tools. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)\(^ {85}\) is the only analytical technique that provides adequate resolving power and mass accuracy for the assignment of elemental composition. FT-ICR mass spectrometry affords high mass resolving power (\( m/\Delta m_{50\%} > 300,000 \), in which \( \Delta m_{50\%} \) denotes mass spectral peak full width at half height) and high mass accuracy, < 1 ppm, for the unambiguous assignment of elemental composition to thousands of components per mass spectrum. Microelectrospray\(^ {84}\) (ESI) is the desired mode of ionization because of its ability to selectively ionize the polar species within a sample matrix without matrix effects, without pre-chromatographic separation. ESI FT-ICR MS has been successfully applied to complex mixtures such as crude oil\(^ {40, 41, 43, 44, 63, 65-67}\), coal\(^ {95, 107, 108}\), humic and fulvic acids\(^ {68-70}\), and other natural organic matter\(^ {71, 72}\).

Here we present a direct compositional comparison of a dead crude oil and its deposit asphaltenes to better understand the compounds that are being preferentially deposited. The data obtained from this study will provide information on the development of a cost-effective method to control the deposition of asphaltenes and to increase the overall
efficiency of the processing fields with asphaltene problems. We will also present a direct comparison of these dead oil deposit asphaltenes to live oil deposit asphaltenes. Although these deposit asphaltenes are derived from different source oils, the information can be used to gather detailed information about not only the components deposited but also will provide hints to the composition of live oil. A previous study was performed on asphaltenes from dead and live-oil samples but was unable to get detailed class (heteroatom content), type (aromatic character, and alkylation pattern data. To the best of our knowledge, this is the first detailed (compound to compound) comparison of dead deposit asphaltenes to its crude oil counterpart and a live deposit asphaltene.

**Experimental Methods**

**Dead crude oil and dead deposit asphaltene sample prep**

The dead crude oil was prepared for analysis by dissolving 20 mg of sample in 10 mL of toluene and then diluted with methanol to a final volume of 20 mL. The dead deposit asphaltenes were collected from the pipe walls used in the refining process. A modified electrospray solvent system with methanol and toluene was used to dissolve 20 mg of the dead deposit asphaltenes to make a final concentration of 1 mg/mL. For negative-ion electrospray analysis, 10 µL of 28 wt. % ammonium hydroxide was added to 1 mL of crude oil sample to facilitate deprotonation of the acidic species present in the sample.

**Live deposit asphaltene sample prep**

The live deposit asphaltenes were collected by live oil depressurization experiments described in Chapter 6. 10 mg of each sample was dissolved in 5 mL of toluene and then diluted with 5 mL of
methanol to produce a final concentration of 1 mg of asphaltene per mL of solvent. 2 µL of 28 wt. % ammonium hydroxide was added to facilitate deprotonation of acidic species for negative-ion electrospray ionization.

**Results and Discussions**

*Dead Crude Oil vs. Dead Deposit Asphaltenes*

Both the dead crude oil and its dead deposit asphaltenes were analyzed by negative ion electrospray FT-ICR MS. Asphaltenes are known to be acidic in nature, therefore negative ion mode the desired mode of ionization for this comparison study of the deposit asphaltene to its parent whole crude oil and a live deposit asphaltene. Negative ion electrospray selectively ionizes the acidic species within a sample matrix without interference from other non-polar or basic components. Figure 7.1 shows the mass spectra for the dead crude oil (top) and the dead deposit asphaltenes (bottom). Each spectrum has an m/z range of approximately 320 to 750 indicating little difference in the molecule weight range of the two samples. Mass resolving power, m/Δm<sub>50%</sub> (in which Δm<sub>50%</sub> is the magnitude-mode mass spectral peak full width at half-maximum peak height), for both samples range from ~350,000-~500,000. This ultra high resolving power is achieved throughout the whole mass range in both mass spectra. There are over 6,500 and 7,000 peaks in the dead crude oil and its corresponding dead deposit asphaltenes. The increase in the number of peaks for the dead
Figure 7.1. Broadband negative-ion electrospray ionization FT-ICR mass spectra of the dead crude oil (top) and its dead deposit asphaltenes (bottom), at a mass resolving power, $350,000 < \frac{m}{\Delta m_{50\%}} < 450,000$. 

Dead Crude Oil
6691 peaks

Dead Deposit Asphaltene
7201 peaks
deposit asphaltene is accounted for by the increased concentration of polar species in the deposit. The two peaks found in the dead deposit asphaltenes, at m/z of 475 and 710 were not a part of a homologous series (assumed to be chemical noise) and were unassigned due to the high m/z, but do not affect the ability to assign the other peaks found within the spectrum.

Viewing the samples at the broadband spectrum level does not give detailed information on the types of components detected within the sample matrix. Zooming in on the ultra high mass resolution mass spectrum provides a more detailed look at the number and type of components found at a single nominal mass. Figure 7.2 shows mass scaled-expanded segments (ranging over m/z ~ 0.5) for both the dead crude oil (top) and the dead deposit asphaltenes (bottom). There are 15 and 13 peaks detected at m/z 470 for the dead crude oil and dead deposit asphaltene. The dashed lines indicate the 8 peaks that are common to both mass spectra. All 20 chemically distinct species lying above a threshold of 3 standard deviations of baseline noise (3σ) have been assigned an elemental composition with an error < 1ppm as shown in Table 7.1. All the species contain a single nitrogen atom, two nitrogen atoms with one $^{12}\text{C}$ replaced by $^{13}\text{C}$, or a single nitrogen atom with 2 $^{12}\text{C}$ replaced with 2 $^{13}\text{C}$, as stated by the nitrogen rule.$^{52}$ The shift to lower mass defect for the dead deposit asphaltenes indicates that these species are more aromatic (more unsaturated) than the species found in the dead crude oil. This shift is due to the negative mass defect associated with a reduction in hydrogen atoms (1.007825 Da), characteristic of components containing greater rings plus double bonds. The same trend is seen for species that appear at odd nominal
Figure 7.2. One Dalton mass scale-expanded segment of the negative-ion ESI FT-ICR mass spectra of dead crude oil (top) and dead deposit asphaltenes (bottom) of figure 7.1. 15 and 13 above-threshold peaks are identified in the dead crude oil and dead deposit asphaltenes. Vertical dashed lines highlights peaks present in both samples. The shift to lower mass defect in the dead deposit asphaltenes indicates the presence of higher aromatic species (higher number of rings plus double bonds), see text for explanation. Heteroatoms are represented in red for some of the peaks, see table 7.1 for assignment of all peaks detected.

Dead Crude Oil
15 peaks

Dead Deposit Asphaltene
13 peaks

Shift to lower mass defect (more aromatic structure) & higher heteroatom content

masses. Those species detected at an m/z 471 (not shown) are enriched with oxygen atoms. There are 12 and 20 peaks above the threshold of 3σ for the dead crude oil and dead deposit asphaltene. This is in contrast to the number of peaks found at even masses. This difference is due to the
increase in oxygen-containing species in the dead deposit asphaltenes, which are found at odd nominal masses.

Table 7.1. Elemental compositional assignments for both the dead crude oil and dead deposit asphaltene.

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Peak Location (m/z)</th>
<th>Resolving Power (m/Δm50%)</th>
<th>Elemental Composition</th>
<th>Error (+/- ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>470.15517</td>
<td>402000</td>
<td>C_{30}H_{21}NO</td>
<td>+0.3</td>
</tr>
<tr>
<td>2</td>
<td>470.17610</td>
<td>402000</td>
<td>C_{32}H_{25}NO_{3}</td>
<td>-0.1</td>
</tr>
<tr>
<td>3</td>
<td>470.19157</td>
<td>335000</td>
<td>C_{36}H_{25}N</td>
<td>+0.3</td>
</tr>
<tr>
<td>4</td>
<td>470.19495</td>
<td>503000</td>
<td>C_{33}H_{29}NS</td>
<td>+0.3</td>
</tr>
<tr>
<td>5</td>
<td>470.21269</td>
<td>402000</td>
<td>C_{33}H_{29}NO_{2}</td>
<td>+0.3</td>
</tr>
<tr>
<td>6</td>
<td>470.23189</td>
<td>503000</td>
<td>C_{32}H_{30}N_{2}O{^{13}C}</td>
<td>+0.0</td>
</tr>
<tr>
<td>7</td>
<td>470.23358</td>
<td>402000</td>
<td>C_{30}H_{33}NO_{4}</td>
<td>-0.2</td>
</tr>
<tr>
<td>8</td>
<td>470.24002</td>
<td>402000</td>
<td>C_{32}H_{31}NO{^{13}C_{2}}</td>
<td>+0.0</td>
</tr>
<tr>
<td>9</td>
<td>470.24911</td>
<td>503000</td>
<td>C_{34}H_{33}NO</td>
<td>+0.4</td>
</tr>
<tr>
<td>10</td>
<td>470.26829</td>
<td>402000</td>
<td>C_{33}H_{34}N_{2}{^{13}C}</td>
<td>+0.0</td>
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<tr>
<td>11</td>
<td>470.27002</td>
<td>503000</td>
<td>C_{31}H_{37}NO_{3}</td>
<td>-0.1</td>
</tr>
<tr>
<td>12</td>
<td>470.27661</td>
<td>402000</td>
<td>C_{33}H_{35}N{^{13}C_{2}}</td>
<td>+0.5</td>
</tr>
<tr>
<td>13</td>
<td>470.28541</td>
<td>503000</td>
<td>C_{35}H_{37}N</td>
<td>+0.2</td>
</tr>
<tr>
<td>14</td>
<td>470.30654</td>
<td>503000</td>
<td>C_{32}H_{41}NO_{2}</td>
<td>+0.2</td>
</tr>
<tr>
<td>15</td>
<td>470.34294</td>
<td>310000</td>
<td>C_{33}H_{45}NO</td>
<td>+0.2</td>
</tr>
<tr>
<td>16</td>
<td>470.35101</td>
<td>402000</td>
<td>C_{33}H_{46}O{^{13}C}</td>
<td>+0.1</td>
</tr>
<tr>
<td>17</td>
<td>470.37039</td>
<td>402000</td>
<td>C_{32}H_{47}N{^{13}C_{2}}</td>
<td>+0.2</td>
</tr>
<tr>
<td>18</td>
<td>470.37920</td>
<td>503000</td>
<td>C_{34}H_{49}N</td>
<td>-0.1</td>
</tr>
<tr>
<td>19</td>
<td>470.40841</td>
<td>402000</td>
<td>C_{31}H_{54}O_{2}{^{13}C}</td>
<td>-0.1</td>
</tr>
<tr>
<td>20</td>
<td>470.44489</td>
<td>402000</td>
<td>C_{32}H_{58}O{^{13}C}</td>
<td>+0.1</td>
</tr>
</tbody>
</table>
Figure 7.3. Kendrick mass defect (KMD) vs. nominal Kendrick mass (NKM), with each signal color-coded according to percent relative abundance for all species detected in negative ion electrospray for the dead crude oil (top) and dead deposit asphaltene (bottom) samples. The dead deposit asphaltene sample clearly has a greater abundance at higher Kendrick mass defect, indicating a higher double bond equivalents (DBE) distribution.
Zoomed mass insets are not the ideal method for determining the aromaticity of species found within a sample matrix (every nominal mass would have to be interrogated separately). Kendrick plots (Figure 7.3) are an excellent tool for the visualization of all species found in a sample in a single graph. Kendrick plots consist of Kendrick mass defect plotted on the ordinate, nominal Kendrick mass on the abscissa and each signal color-coded according to percent relative abundance for all species detected in negative ion electrospray for the dead crude oil (top) and dead deposit asphaltene (bottom) samples. Kendrick mass defect is directly related to the aromaticity of a particular species. A higher Kendrick mass defect (KMD) indicates higher double bond equivalents (number of rings plus double bonds) for a given species. The nominal Kendrick mass (NKM) range for both samples, dead crude oil and dead deposit asphaltenes, are approximately the same. The dead crude oil has a KMD range from 0.05 – 0.45 with the most abundant species from 0.12 – 0.22, whereas the dead deposit asphaltenes have KMD range from 0.05 – 0.5 with the most abundant species from 0.25-0.40. This shift clearly illustrates the increase in the abundance of species with a greater number of rings plus double bonds in the dead deposit asphaltenes and corroborates the shift to lower IUPAC mass defect in the zoomed mass inset of figure 7.2. Unfortunately, Kendrick mass plots can only give information on the double bond equivalents of species found in a sample and provides little to no information on the classes detected.

Figure 7.4 is a two dimensional bar graph of the classes that are detected in both the dead crude oil (blue) and dead deposit asphaltene (red). Only those classes with relative abundance > 1% are shown. The percent relative abundance is the summed abundance for each class divided by the abundance of all species detected in the sample. Eight and 13 chemically distinct classes were detected in the dead crude oil and dead deposit asphaltenes, respectively. The dead deposit asphaltenes are enriched in species that contain oxygen. This directly
supports the increase in the number of peaks per even nominal mass as stated earlier. The relative abundance is scaled separately for each mass spectrum; thus, the relative abundance of the N class may decrease by virtue of the increase in the amounts of other classes, specifically any compound containing O$_2$, which are known to be preferentially ionized by a factor of 10 over the neutral nitrogen species.$^{66}$
As stated earlier, Kendrick plots are an excellent tool for the visualization of all species found in a crude oil sample matrix for the determination of the *types* of compounds (the number of rings plus double bonds) found. Another important graphical tool is the van Krevelen diagram. They were first employed for use towards FTICR mass spectrometry data by Kim *et al.* to distinguish condensed hydrocarbons from lipids and other components. Van Krevelen diagrams consist of iso-abundance contours as a function of (e.g.) H/C ratio and O/C ratio for each compound containing those atoms. In a van Krevelen diagram, heteroatom *classes* are separated on the x-axis (e.g., O/C ratio to distinguish classes differing in number of O atoms), and DBE difference spread out along the y-axis (H/C ratio). Homologous alkylation series composed of compounds that contain the same number of rings plus double bonds and the same heteroatoms, but different number of CH\(_2\) groups show up as diagonals. The van Krevelen allows for convenient visual separation of heteroatom *classes*, DBE *type*, and alkylation pattern.

**Figure 7.5** is a van Krevelen diagram of the NO\(_x\) classes found in both the dead crude oil (top) and dead deposit asphaltenes (bottom). The abscissa consists of the ratio of oxygen atoms to carbon atoms to visually separate species containing a different number of oxygen atoms. The ordinate is composed of the heteroatomic ratio of hydrogen to carbon. Although there is little difference in the H/C ratio range, 0.6 – 1.5 for both samples, there is a large increase in the relative abundance of species with multiple oxygen atoms. The boxes indicate, roughly, the O/C ratio that corresponds to those species that contain NO, NO\(_2\), and NO\(_3\). The increase in the amount and number of compounds with multiple oxygen atoms clearly illustrates that these compounds are preferentially deposited. The similarities between the H/C ratios for both samples indicates that similar ranges of DBE (number of rings plus
Figure 7.5. Van Krevelen diagrams for negative ion species that contain NO\textsubscript{x} heteroatoms in the dead crude oil (top) and dead deposit asphaltene (bottom). The abscissa is the ratio of oxygen atoms to carbon atoms; the ordinate is the ratio of hydrogen atoms to carbon atoms; and the third axis shows relative abundance contours. Although there is no shift in the H/C ratio there is an abundance shift to a lower H/C ratio in the dead deposit asphaltenes indicating a shift to higher aromatic species. There is also a sharp increase in the number of species containing multiple oxygen atoms for the dead deposit asphaltene sample.
Figure 7.6. Van Krevelen diagrams for negative ion species that contain $N_x$. Not only is there a shift lower H/C ratios for the dead deposit asphaltenes, there is also a shift in abundance from a single nitrogen atom to compounds that contain 2 nitrogen atoms.
double bonds) in those species that contain a single nitrogen and oxygen atom.

A similar graph (Figure 7.6) can be constructed for those species that contain multiple nitrogen atoms, such as N, N₂, and N₃, by plotting the atomic ratio of N/C on the x-axis. Unlike the previous figure, there is great contrast between both the H/C ratio range and the N/C ratio range. The dead crude oil (top) has an H/C ratio range of 0.8 – 1.6, with the most abundance ranging from 1.2 – 1.4. This is in direct contrast to the dead deposit asphaltenes (bottom) which contain an H/C ratio from 0.6 – 1.4 with the most abundant species (for species containing a single nitrogen atom) ranging from 0.8 – 0.9. This shift to lower H/C ratio clearly indicates that those compounds that contain a single nitrogen atom are only deposited when they contain a higher number of rings plus double bonds. There is a great increase in the range along the x-axis (atomic ratio of N/C) for the dead deposit asphaltenes. A higher N/C ratio indicates an increase in the number of nitrogen atoms present in a particular compound. The boxes roughly indicate those species that contain N, N₂, and N₃ heteroatoms. This increase illustrates that those species that contain multiple heteroatoms, such as N₂ and N₃ are preferentially deposited on the pipe walls during refining.

As stated earlier, those species that are preferentially deposited are enriched in oxygen as evident by the increase in the number of species that contain multiple oxygen atoms. Figure 7.7 illustrates van Krevelen diagrams for those species that contain O, O₂, O₃ and O₄. The dead crude oil is located on top and is dominated by species containing a single oxygen atom, as evident by the low O/C ratio range, 0.02 - ~0.04. The dead deposit asphaltenes (bottom) have very little single oxygen species, but contain a high abundance of multiple oxygenated species. There is little difference between the H/C ratio of these compounds but the data does suggest that those species containing a single oxygen atom
Figure 7.7. Van Krevelen diagrams for negative ion species that contain $O_x$. While the Dead crude oil contains a high abundance of those compounds that contain a single oxygen atom, the dead deposit asphaltenes have a higher abundance of species with multiple oxygen atoms.
that do deposit tend to have a higher number of rings plus double bonds as evident by the lower H/C ratio associated with these species. This van Krevelen diagram is an excellent tool to illustrate the enrichment of oxygenated species in the deposited asphaltenes from the dead crude oil during the refining process.

**Dead Deposit Asphaltenes vs. Live Deposit Asphaltenes**

Although these two deposit asphaltenes are isolated from different parent oils, the comparison gives detailed information on the types of compounds that are preferentially deposited on production equipment during the production of live crude oil and the refining of dead crude oil. This information will lead to a better understanding of the similarities and differences in these two different types of deposit asphaltenes, and how the respective deposits relate to the types of compounds found in both dead and live crude oils. **Figure 7.8** shows the mass spectrum for the live deposit asphaltene. It has an m/z range of approximately 300 to 800 with a mass resolving power, m/Δm_{50%} of ~400,000 at m/z 500. Elemental composition could be assigned to approximately 12000 peaks to within < 1 ppm error. The inset graph displays the 19 chemically distinct classes found in the live deposit asphaltene. Only those classes with a percent relative abundance > 1% are shown. The percent relative abundance was determined in the same method as stated earlier in this paper. The live oil deposit, like the dead oil deposit asphaltene, is enriched in species that contain an oxygen atom. Although the most abundant class in each sample matrix is not the same, O₄S for the live deposit asphaltene and NO for the dead deposit asphaltene, the same trend of oxygen enriched species is evident. 10 of the 13 classes found in the dead deposit asphaltenes (figure 7.4) are also found in the live deposit asphaltenes, only N₂O, N₂O₂ and O₃ are not present. While there are many similarities in the classes found in the two samples, the type
Figure 7.8. Broadband negative-ion electrospray ionization FT-ICR mass spectra of the live deposit asphaltenes. The inset graph reveals the class distribution for the acidic species present in negative-ion electrospray. The sample is dominated by species that contain multiple heteroatoms and are enriched in oxygen. There are only 4 classes present that do not contain an oxygen atom.
(double bond equivalents or the number of rings plus double bonds) has to be examined further.

3D Carbon number vs. Double bond equivalents (DBE) plots (with percent relative abundance color-coded in the third dimension) are an ideal graphical representation to expose type differences of compounds found in a particular class. **Figure 7.9** contains two plots for the O₄S class found in both the live deposit asphaltenes (left) and the dead deposit asphaltenes (right). Upon visual inspection, it is obvious that the two different samples contain different types of compounds in this class. The carbon number range for the live deposit asphaltenes is 16-45, while

![Figure 7.9](image-url)

**Figure 7.9.** DBE vs. carbon number (scaled in the third direction according to percent relative abundance contours) for negative ions that contain 4 oxygen atoms and a single sulfur heteroatom in both the live deposit asphaltenes (right) and the dead deposit asphaltenes (left).
the dead deposit asphaltene have a range of 25-45. This suggests that the dead asphaltenes only deposit species that contain a longer alkyl chain while the live asphaltenes deposit species with a larger range of CH₂ groups attached. Another indication of the deposit of larger compounds from the dead oil is the higher DBE (number of rings plus double bonds) values. The live deposit asphaltenes have a DBE range from 0 – 12 rings plus double bonds with the most abundant at 1 ring or double bond in contrast to the dead deposit asphaltenes with a DBE range from 0 – 15 rings plus double bonds with the most abundant between 3 – 7. Clearly, the two crude oils, live and dead, preferentially deposit different types of compounds even though both preferentially deposit the O₄S class.

Figure 7.10 illustrates the type distribution for the classes that contain a single nitrogen atom and 1, 2 or 3 oxygen atoms. Carbon number is plotted along the x-axis, the double bond equivalents along the y-axis, with each signal color-coded according to percent relative abundance. Not only are the percent relative abundance for these three classes in both samples relatively the same, but also the type of compounds found within the classes are similar. Although for each class in the dead deposit asphaltenes (right) tend to have a more narrow high abundance species (red, yellow, green colors) than the live deposit asphaltenes (left) they both have roughly the same carbon number distribution of ~25 - ~50 carbons. For each class, the double bond equivalent distribution is relatively the same as well. Unlike the O₄S class, not only is the percent relative abundance similar for both the asphaltenes samples, but also the types of compounds that are found within each class. This clearly illustrates the similarity in the NOₓ compounds deposited from both dead and live crude oils.
Figure 7.10. Double-bond equivalents vs. carbon number for the NOx classes of live deposit asphaltenes (right) and dead deposit asphaltenes (left).
Conclusion

There are major differences between the dead crude oil and dead deposit asphaltenes. The dead deposit asphaltenes are enriched in compounds that contain a higher number of rings plus double bonds (DBE), as evident by the high abundance of high Kendrick mass defect species (correspondingly low IUPAC mass defect) found at each nominal mass and the shift to lower H/C ratios (van Krevelen plots) in the classes identified. The dead deposit asphaltenes are also dominated by species containing oxygen, indicating that these oxygenated species are preferentially deposited. Other species that are preferentially deposited are those that contain multiple numbers of heteroatoms, such as N₂, N₃, O₂, O₃ and O₄. The dead deposit asphaltenes and live deposit asphaltenes are very similar (they have 10 classes in common), and are uniformly enriched in oxygenated species. However, although the two samples contain the same classes, they don’t always preferentially deposit the same types of compounds (number of rings plus double bonds), as in the case of O₄S. These results suggest that both before and after depressurization, many classes (O₃S, O₄S, NO, NO₂, NO₃, and N₂) preferentially form production deposits. The similarity in their class, type, and carbon number suggest that they are very similar materials. However, SOₓ classes with an associated low DBE preferentially flocculate/deposit during pressure reduction. This selectively depletes the low DBE members of these classes, leaving the higher DBE class members in the dead oil and subsequently available to form dead oil production deposits. Thus, the presence of SOₓ classes is associated with both live (low DBE members) and dead oil (high DBE members) production deposits.
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BIOGRAPHICAL SKETCH

EDUCATION

Florida State University, Tallahassee, FL
PhD candidate in Analytical Chemistry with environmental forensic concentration

College of William and Mary, Williamsburg, VA
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HONORS AND AWARDS

Hoffman Teaching Merit Fellowship, Florida State University 2000-2001

PROFESSIONAL AFFILIATIONS

American Society for Mass Spectrometry since 2001
American Chemical Society since 2002

LEADERSHIP AND DEPARTMENTAL INVOLVEMENT

Participated in graduate student recruitment, Florida State University 2001-present
Regional Science Fair Judge 2003-present

TEACHING EXPERIENCE

Instructor, Mad Science of Tallahassee 2004-2005
Prepared and taught educational activities for classroom and assembly type presentations. Motivate young children, preschool to high school age, to get excited about science in their world.

Teaching Assistant, Honors General Chemistry II, Florida State University 2002
Prepared, taught and administered grades
Revised/modified Honors lab manual
Teaching Assistant, Honors General Chemistry I, Florida State University 2001
Prepared “pre-lab” lectures, stimulated interests of students towards Chemistry and administered grades

Chemistry Tutor, Tutored students in General Chemistry 2000-2004
Teaching Assistant, General Chemistry for non-majors, Florida State University 2000-2001
Taught basic chemistry lab techniques to students not interested in the sciences as a career, prepared “pre-lab” lectures and administered grades.

RESEARCH EXPERIENCE

Graduate Research Associate with Dr. Alan G. Marshall, Department of Chemistry, Florida State University, 2001-present

Areas of study include:
- Analysis of Crude Oils, Particulate Matter, and Natural Organic Matter
- Kendrick Mass Analysis
- SARA Method of Fractionation for Crude Oils
- Application of Van Krevelen diagrams to Crude oils
- Electrospray Ionization, Field Desorption, Atmospheric Pressure Photo Ionization and MALDI

Undergraduate Research Associate with Dr. Robert Orwoll, Department of Chemistry, College of William and Mary, 1999-2000

Areas of Study include:
- Separation of Enantiomers using Molecular Imprinting Polymers
- Use of HPLC for quantification of polymer uptake

INSTRUMENTAL SKILLS

- Homebuilt high-field micro-electrospray ionization FT-ICR mass spectrometers
- Hewlett Packard 6890 gas chromatograph coupled to a flame ionization detector and a mass selective detector
- Bruker III biflex MALDI-TOF with Reflectron capabilities (manager)
SELECTED PRESENTATION


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