Video Article

Chemical Analysis of Water-accommodated Fractions of Crude Oil Spills Using TIMS-FT-ICR MS

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Abstract

Multiple chemical processes control how crude oil is incorporated into seawater and also the chemical reactions that occur overtime. Studying this system requires the careful preparation of the sample in order to accurately replicate the natural formation of the water-accommodated fraction that occurs in nature. Low-energy water-accommodated fractions (LEWAF) are carefully prepared by mixing crude oil and water at a set ratio. Aspirator bottles are then irradiated, and at set time points, the water is sampled and extracted using standard techniques. A second challenge is the representative characterization of the sample, which must take into consideration the chemical changes that occur over time. A targeted analysis of the aromatic fraction of the LEWAF can be performed using an atmospheric-pressure laser ionization source coupled to a custom-built trapped ion mobility spectrometry–Fourier transform–ion cyclotron resonance mass spectrometer (TIMS-FT-ICR MS). The TIMS-FT-ICR MS analysis provides high-resolution ion mobility and ultrahigh-resolution MS analysis, which further allow the identification of isomeric components by their collision cross-sections (CCS) and chemical formula. Results show that as the oil-water mixture is exposed to light, there is significant photo-solubilization of the surface oil into the water. Over time, the chemical transformation of the solubilized molecules takes place, with a decrease in the number of identifications of nitrogen- and sulfur-bearing species in favor of those with a greater oxygen content than were typically observed in the base oil.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55352/>

Introduction

There are numerous sources of environmental exposure to crude oil, both from natural causes and from anthropogenic exposure. Upon release to the environment, particularly in the ocean, the crude oil can undergo partitioning, with the formation of an oil slick on the surface, a loss of volatile components to the atmosphere, and sedimentation. However, low-energy mixing of the poorly soluble oil and the water does occur, and this mixture, which is not classically solubilized, forms what is referred to as the low-energy water-accommodated fraction (LEWAF). The solubilization of the oil components in the water is typically enhanced during exposure of the oil-water interface to solar radiation. This photo-solubilization of the crude oil in the ocean can undergo significant chemical changes due to this exposure to solar radiation and/or due to enzymatic degradation^{1,2}. Understanding these chemical changes and how they occur in the presence of the bulk matrix (*i.e.*, the crude oil) is fundamental to mitigating the effects this exposure has on the environment.

Previous studies have shown that crude oil undergoes oxygenation, particularly the polycyclic aromatic hydrocarbons (PAHs), which represent a highly toxic source of contamination that harms organisms, undergoes bio-accumulation, and is bioactive^{3,5,6}. Understanding the products of the different oxygenation processes is challenging because they occur only in the presence of the bulk matrix. Therefore, a single, standard analysis may not be representative of the changes occurring in nature. The preparation of the LEWAF must replicate the natural processes that take place in an environmental setting. Of particular interest is the oxygenation of PAHs, which occurs due to solar radiation.

The second challenge in the study of the water-accommodated fraction is the molecular identification of the different chemical constituents in the sample. Due to the complexity of the sample, caused by its high mass and degree of oxygen, the oxygenation products are typically unsuitable for the traditional analysis carried out by gas chromatography combined with MS analysis^{7,8}. An alternative approach is to characterize the changes in the chemical formula of the sample by utilizing ultra-high mass resolution MS techniques (*e.g.*, FT-ICR MS). By coupling TIMS to FT-ICR MS, in addition to the isobaric separation in the MS domain, the ion mobility spectrometry (IMS) dimension provides the separation and characteristic information for the different isomers present in the sample^{9,10,11}. Combined with an atmospheric pressure laser ionization (APLI) source, the analysis can be selective to the conjugated molecules found in the sample, allowing the changes that the PAHs undergo to be accurately characterized^{12,13}.

In this work, we describe a protocol for the preparation of LEWAFs exposed to photo-irradiation in order to study the transformation processes of the oil components. We also illustrate the changes that occur upon photo-irradiation, as well as the procedure for sample extraction. We will also present the use of APLI with TIMS coupled with FT-ICR MS to characterize the PAHs in the LEWAF as a function of the exposure to light.

Protocol

1. Preparation of the Low-energy Water-accommodated Fractions (LEWAF)

1. Clean 2-L aspirator bottles by rinsing the bottles with methylene chloride in order to remove any potential contaminants.
2. Fill bottles with 50 mL of methylene chloride, close them, and agitate for 30 s. Drain them into the appropriate waste container. Repeat for a total of three washes.
3. Use one aspirator bottle for the irradiation exposure and the other bottle as a control sample (perform duplicate sets of each if possible).
4. Prepare artificial seawater by mixing 35 g of sea salt mixture per 1 L of water used for a final salinity of 33 parts-per-thousand. Filter the solution under little or no negative pressure to prevent the loss of volatile compounds using a filtration apparatus equipped with a pore-size filter of 0.45 μm or smaller.
5. Fill the aspirator bottles with the artificial seawater, leaving ~20%, or 400 mL, of headspace.
6. Add the crude oil to the premeasured seawater using a gas-tight syringe at a nominal concentration of 1 g oil/L.
NOTE: More viscous oils should be delivered by weight.
7. After the addition of the oil, mix the solutions for 24 h in the dark (or by covering the aspirator bottles with aluminum foil) using a stirring plate operated at low speed without the generation of a vortex (approximately 100 rpm) in order to allow all the water-soluble components to go into solution and to avoid the inclusion of particulate oil.

2. LEWAF Photo-irradiation, Sample Collection, and Handling

1. Prepare two sets of bottles for each photo-irradiation experiment.
2. Cover one set of aspirator bottles with aluminum foil; this will be the dark control.
3. Place the aspirator bottles in a temperature-controlled water bath set to maintain the aspirator bottles at a temperature of 25 °C inside of the solar irradiator equipped with a 1,500-W xenon arc lamp at a light intensity of 765 W/m².
4. Irradiate the samples continuously for the desired time (this experiment lasted 115 h); there is no maximum time.
5. After set times, (15, 24, 48, and 115 h in this experiment) collect 150 mL of water from the bottom of the glass aspirator by uncorking the aspirator bottle and opening the drain valve.
6. Utilizing a separatory funnel perform a liquid-liquid extraction with methylene chloride by adding 50 mL aliquots of methylene chloride, shaking the sample for 2 min regularly venting the funnel to avoid the stopper from blowing off.
7. Allow the phases to separate, and remove the bottom organic phase by removing the stopper from the funnel and slowly open the drain valve. Drain the sample into a flat bottom flask equipped with a funnel filled with 100 g of Na₂SO₄. Rinse funnel with additional methylene chloride. Perform the extraction 3 times in order to increase the extraction efficiency.
NOTE: Multiple extractions are performed in order to increase the extraction efficiency of the procedure.
8. Remove the funnel, add a Snyder column and place the flat bottom flask in a hot water bath set to 58 °C. Evaporate until 15 mL of sample remains.
9. Transfer the sample into a concentrating tube and dry the samples using a gentle stream of nitrogen careful to avoid loss of sample by splashing.

3. Preparation of the Sample for Analysis

NOTE: Sample preparation for analysis is key, and care must be taken to avoid the introduction of foreign contaminants, particularly through the use of any plastics, which will cause leaching into the sample.

1. Prepare a mixture of 1:1 v:v methanol:toluene utilizing LC-MS optima-grade solvents.
2. Using a positive-displacement micropipette, add solvent to the glass vials and then add the samples at a ratio of 1:100, first by adding 990 μL of solvent and then by adding 10 μL of sample.
NOTE: Glass micropipettes are used in order avoid leaching due to the methylene chloride or toluene.
3. Optional: Use tuning mix to calibrate the ion mobility.
NOTE: An API source will often leach if it is regularly used as a calibration standard. If the calibration ions are not present in the spectra, then a mixture can be prepared by adding some of the standard solution to the sample at a 1:1,000 dilution.

4. Fourier Transform–Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) Analysis

1. Set the instrument to the "Operate" mode by clicking the operate button.
2. Load the sample into a sample syringe.
3. Under the API source tab, set the infusion rate at the 200 $\mu\text{L}/\text{h}$ rate.
4. Set the nebulizer pressure to 1 bar and the vaporizer temperature to 300 °C.
5. Set dry gas rate to 1.2 L/min and the temperature to 220 °C.
NOTE: This procedure is applicable to both APPI and APCI.
6. Set the instrument to "Tune" mode by clicking the tune button. A spectrum should appear on the screen.

NOTE: If needed, the instrument may be optimized in order to improve ion transmission and detection in the m/z range of the analytes by changing the instrumental parameters, such as voltages, rf frequencies, and amplitudes. Note that the mass spectrometer will need to be recalibrated.

7. Stop "Tune" mode by clicking the stop button.
 8. Set the number of scans to average, typically between 20-200 scans per mass spectrum, and press the acquisition button to acquire a mass spectrum.
- NOTE: Averaging multiple spectra improves the overall signal-to-noise ratio by decreasing the noise and increasing the signal for low-abundance species¹⁴.

5. Trapped Ion Mobility Spectrometry (TIMS) Analysis

1. Enable "Chromatography" mode on the FT-ICR acquisition program.
2. Enable "TIMS" mode by turning the switch on the external breaker to TIMS.
NOTE: The voltages used in TIMS are controlled externally from the instrument. A second software will control the entrance funnel of the instrument to perform the IMS analysis.
3. Turn on the supplementary roughing pump and, using the control valve of the TIMS, adjust the inlet and outlet pressures to desired ranges (in this experiment, P1 was set to 2.8 mbar and P2 to 1.4 mbar).
NOTE: The external pump should be turned on a few hours prior to analysis to ensure that the pump is warm and that performance is uniform during the IMS analysis, since changes in gas velocity will cause errors in the spectra.
4. First identify the voltage range that is required to perform the IMS analysis; this is done with a fast, cursory search over a wide voltage range (250 V) at a low IMS resolution (1 V/frame).
5. If the voltages used are sufficient to analyze the whole spectrum, then repeat the experiment, adjusting the TIMS for a higher-resolution analysis. To do so, decrease the V/frame to 0.1 V and change the number of frames to cover all of the ΔV_{ramp} .
NOTE: The user may need to optimize the number of TIMS experiments that are accumulated into the collision cell prior to the FT-ICR MS analysis.

6. Data Analysis

1. Open the MS data in a data analysis software.
2. Set the peak-picking criteria to select those above 6 signal-to-noise and use the "Find Peaks" function.
NOTE: The resulting spectrum is internally recalibrated using standards present in the solution. This is used to identify chemical series and is then recalibrated again in order to improve the accuracy for broad-range elemental assignment.
3. Perform elemental assignment using a formula assignment tools or software.
NOTE: Formula limits of $C_{1-100}H_{1-100}N_{0-2}O_{0-7}S_{0-2}$, odd and even electron configurations, are allowed, and a mass tolerance of 0.5 ppm M^+ and $[M+H]^+$ ions forms
4. Import the IMS-MS data to a data analysis software and generate an average mass spectrum for the whole analytical range.
5. Select peaks above 6 signal-to-noise. Using the series that was identified in the ultrahigh-resolution MS analysis, internally recalibrate the spectrum
6. Using the formula identifications from the FT-ICR MS analysis, generate a list of all the target m/z , and save this as "Flist.txt" in the data folder. This list will be used in the first step of data processing
7. Load and run the provided script in order to extract an extracted ion chromatogram (EIC) for every target m/z in the list.
8. Modify the python script to input the file directory, the starting voltage, the step size, and the number of steps in that target analysis¹¹.
9. Run the IMS deconvolution script.
NOTE: This will open each EIC and identify the peaks by iterative Gaussian fitting. Results will be output in Excel file format¹³.
NOTE: After fitting, peaks below a certain criterion are excluded, typically based on the height, width, and area of the peak (peaks below a certain width are typically excluded from the analysis because of random noise spikes).
10. Utilizing the tuning peaks, calibrate the ion mobility from voltage to mobility for the ions¹¹. This can be converted to a collision cross-section using the Mason-Schamp equation.

Representative Results

LEWAF analysis by TIMS-FT-ICR MS results in a two-dimensional spectrum based on m/z and TIMS trapping voltage. Each of the samples, taken at different time points, can therefore be characterized based on the changing chemical composition, as observed by the distribution of chemical formulas and the isomeric contribution identified by the IMS (see **Figure 1**). Typically, the m/z information can be utilized to assign elemental formulas to the analyzed peaks. The use of APLI allows for the analysis of molecules with aromatic and double bonds with greater sensitivity¹⁵, ionizing a broad range of molecular classes (e.g., HC, O_{1-4} , NO_{0-4} , and SO_{0-4})¹³. This information is organized into plots that show the distribution of points as a function of the heteroatom content in the formula, the carbon number, and the DBE of the formula. This allows the changes in the chemical formula to be observed as differences, as a function of the carbon number and the number of rings and double bonds in the molecule (see **Figure 2**).

Two-dimensional characterization of the samples allows the samples to be characterized by the m/z , ion formula, and collisional cross-section for each molecule. This is illustrated as a plot of carbon number versus mobility, where, in the color scale, the DBE of the molecule can be represented. This allows for a correlation of molecular size for specific chemical families (the same heteroatoms and DBE, see **Figure 3**). The CCS information allows for the analysis of the isomeric content, showing changes between analyses, and providing information regarding potential structures for the molecule.

Figure 1. 2D-TIMS-FT-ICR MS of LEWAFs. This represents the typical 2D spectrum acquired from the TIMS-FT-ICR MS for the fraction collected after 24 h. Note how a single trend is observed in the data; the signal in this data comes from highly aromatic chemical structures, which are very condensed. [Please click here to view a larger version of this figure.](#)

Figure 2. DBE versus carbon number. A typical plot showing the distribution of assignments for six different chemical classes (HC, N, O, O₂, O₃, and OS). The x-axis is the number of carbons in the structure, and the y-axis is the double-bond equivalence, described by $DBE = \#C - \frac{\#H}{2} + \frac{\#N}{2} + 1$. The color for each dot is the log of the intensity. [Please click here to view a larger version of this figure.](#)

Figure 3. Mobility versus carbon number. This plot shows the change in inverse mobility, which is linear with size, and the carbon number plots, where the color scale is the DBE. The same six heteroatom classes are shown individually (HC, N, O, O₂, O₃, and OS). [Please click here to view a larger version of this figure.](#)

Discussion

Critical Steps within the Protocol

The chemical complexity of LEWAFs requires accurate preparation in order for the laboratory experiments to accurately reflect what occurs naturally. A valid assessment of the data hinges on three criteria: minimizing the introduction of artifacts throughout sample handling (e.g., preparation of the LEWAF, sampling, extractions, and preparation of the sample for analysis), validating the experimental protocol (i.e., using dark controls for the photo-irradiation experiment), and validating the instrument performance (i.e., validating MS and IMS performance through the use of standards).

Data processing and interpretation can also present a challenge. The first challenge is the assignment of molecular formulas to the observed peaks. This requires high mass accuracy in order to avoid the potential incorrect assignment of the data. IMS interpretation is also complex and requires validation. The current system characterizes the data as a series of Gaussian peaks within 5% deviation of the experimental spectrum, and the data is then filtered to remove peaks that do not meet the peak width, area, and signal-to-noise ratio criteria.

Modifications and Troubleshooting

The prepared LEWAF can be used for a series of different experiments and analytical techniques that explore the potential for photo-oxidation, microbial degradation, or a combination of both. These can then be evaluated on different criteria, such as the resulting toxicity, the rate of intake into an organism, and the molecular characterization, to try to identify novel molecular signatures for the degraded oil.

Another challenge can be the lower sensitivity when performing high IMS-resolution analysis; this can be addressed by increasing the number of accumulations in the collision cell, increasing the overall signal-to-noise ratio of the sample. An alternative solution is to reduce the pressure difference at the entrance and exit of the TIMS analyzer, typically increasing sensitivity.

Limitations of This Technique

TIMS-FT-ICR MS can be applied in the LEWAF molecular weight region ($m/z = 100-900$). However, the ionization source (APLI) can limit the type of molecular ions that can be introduced to the TIMS-FT-ICR MS. Therefore, the ionization step needs to be tailored in order to take into consideration the molecular class target-of-interest. This particular experiment focuses on the aromatic products of oxygenation. Other ionization sources, such as electrospray ionization, atmospheric pressure photo-ionization, atmospheric pressure chemical ionization, or laser desorption ionization, can be used to target complementary molecular classes of the LEWAF. Because of the time-scale of the TIMS-FT-ICR MS analysis, online chromatographic separation is not possible; however, offline fractionation strategies are possible.

Significance of This Technique with Respect to Alternate Methods

The use of APLI-TIMS-FT-ICR MS allows for the characterization of heteroatom-PAHs, evaluating the chemical distribution as well as the isomeric contribution of the molecules. Typically, this has been limited to PAHs and none of their degradation products. However, typical results show that, over time, there are significant changes in the distribution of molecules, with pure hydrocarbons reduced over time while greater numbers of oxygenated molecules are observed. Therefore, the traditional PAH analysis may insufficiently characterize the sample. Unlike other chromatographic methods, there is no limitation in molecular size due to volatility requirements, such as GC. Also, the IMS measurement provides characteristic information on the molecules structure, which is universal, unlike LC methods.

Further Applications of This Technique

This technique is not limited to the study of LEWAFs, and it can also be applied to the untargeted analysis of complex mixtures, particularly those with high isobaric interferences that require ultra-high mass resolution and those with isomeric interferences that need to be resolved. This can be applied to environmental samples, both the targeted analysis of contaminants and the broad-range analysis of dissolved organic matter, petroleum, and even biological material.

Disclosures

The authors declare no competing financial interests.

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References

- King, S. M., Leaf, P. A., Olson, A. C., Ray, P. Z., & Tarr, M. A. Photolytic and photocatalytic degradation of surface oil from the Deepwater Horizon spill. *Chemosphere*. **95** (0), 415-422 (2014).
- Ray, P. Z., Chen, H., Podgorski, D. C., McKenna, A. M., & Tarr, M. A. Sunlight creates oxygenated species in water-soluble fractions of Deepwater Horizon oil. *J Hazard Mater*. **280** (0), 636-643 (2014).
- Duesterloh, S., Short, J. W., & Barron, M. G. Photoenhanced toxicity of weathered Alaska North Slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. *Environ Sci Technol*. **36** (18), 3953-3959 (2002).

4. Duxbury, C. L., Dixon, D. G., & Greenberg, B. M. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*. *Environmental Toxicology and Chemistry*. **16** (8), 1739-1748 (1997).
5. Faksness, L. G., Altin, D., Nordtug, T., Daling, P. S., & Hansen, B. H. Chemical comparison and acute toxicity of water accommodated fraction (WAF) of source and field collected Macondo oils from the Deepwater Horizon spill. *Mar Pollut Bull.* **91** (1), 222-229 (2015).
6. Wang, J. *et al.* Biodegradation of dispersed Macondo crude oil by indigenous Gulf of Mexico microbial communities. *Science of The Total Environment*. **557 - 558** 453-468 (2016).
7. McKenna, A. M. *et al.* Expansion of the analytical window for oil spill characterization by ultrahigh resolution mass spectrometry: beyond gas chromatography. *Environ Sci Technol.* **47** (13), 7530-7539 (2013).
8. Fernandez-Lima, F. A. *et al.* Petroleum crude oil characterization by IMS-MS and FTICR MS. *Anal Chem.* **81** (24), 9941-9947 (2009).
9. Benigni, P., Marin, R., & Fernandez-Lima, F. Towards unsupervised polyaromatic hydrocarbons structural assignment from SA-TIMS-FTMS data. *Int J Ion Mobil Spectrom.* **18** (3), 151-157 (2015).
10. Benigni, P., Thompson, C. J., Ridgeway, M. E., Park, M. A., & Fernandez-Lima, F. Targeted high-resolution ion mobility separation coupled to ultrahigh-resolution mass spectrometry of endocrine disruptors in complex mixtures. *Anal Chem.* **87** (8), 4321-4325 (2015).
11. Benigni, P., & Fernandez-Lima, F. Oversampling Selective Accumulation Trapped Ion Mobility Spectrometry coupled to FT-ICR MS: Fundamentals and Applications. *Analytical Chemistry*. (2016).
12. Castellanos, A. *et al.* Fast Screening of Polycyclic Aromatic Hydrocarbons using Trapped Ion Mobility Spectrometry - Mass Spectrometry. *Anal Methods*. **6** (23), 9328-9332 (2014).
13. Benigni, P., DeBord, J. D., Thompson, C. J., Gardinali, P., & Fernandez-Lima, F. Increasing Polyaromatic Hydrocarbon (PAH) Molecular Coverage during Fossil Oil Analysis by Combining Gas Chromatography and Atmospheric-Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). *Energy & Fuels*. **30** (1), 196-203 (2016).
14. Qi, Y. *et al.* Absorption-Mode Fourier Transform Mass Spectrometry: the Effects of Apodization and Phasing on Modified Protein Spectra. *Journal of the American Society for Mass Spectrometry*. **24** (6), 828-834 (2013).
15. Lababidi, S., & Schrader, W. Online normal-phase high-performance liquid chromatography/Fourier transform ion cyclotron resonance mass spectrometry: Effects of different ionization methods on the characterization of highly complex crude oil mixtures. *Rapid Communications in Mass Spectrometry*. **28** (12), 1345-1352 (2014).