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Isolation and Determination of the Composition and Structural Components that Comprise Dissolved Organic Matter Using Normal Phase Liquid Chromatography

Jonathan A. Long Jr

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1 Isolation and Determination of the Composition and Structural Components that Comprise
2 Dissolved Organic Matter Using Normal Phase Liquid Chromatography
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9 A Thesis
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16 Submitted to the Graduate Faculty of the
17 University of New Orleans
18 in partial fulfillment of the
19 requirements for the degree of
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27 in
28 Chemistry
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35 By
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37 Jonathan Allen Long Jr.
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39 B.S. University of New Orleans, 2017
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41 May 2021

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ACKNOWLEDGEMENTS

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I would like to begin by thanking my family for their continuous love and support throughout my graduate school experience. Specifically, I would like to thank my mother, Debra Long, and my aunt, Tracey Long.

This milestone in my life would not have been possible without the support I've received from my friends along the way. There are five people that I'd like to specifically acknowledge in this section, the first of which is Nichole Pianovich. We met in the spring of 2015, and as time went on, she went from being a stranger to someone that I consider to be family. The next person I'd like to acknowledge is Jason Hood. He's basically the brother I never had. Whether it be helping me with physics or showing me how to change a taillight, he always finds a way to help me when I need it. Sarah Gauthier is the next person I would like to recognize, and she's probably the most important of the five people. This is because her journey in grad school has already come to an end. This allows her to empathize with what I have going on. It also allows her to offer perspective, on what's going on so I can focus on the long term gain. Janelle Do is the last person I would like to acknowledge. Janelle helped me stay true to my processes and avoid taking shortcuts, always reminding me that shortcuts may get you to the end of the journey faster, but without all the memories and learning that comes with the traditional path.

I would like to thank both of my advisors, Dr. Zito and Dr. Podgorski, for their continuous support and guidance throughout my time in grad school. Lastly, I'd like to acknowledge the financial support from the Board of Regents (Reference #: 20130010705).

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182	Silver, H. F., Liquid-Chromatography Field-Ionization Mass-Spectrometry in the Analysis of High-
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226 **Abstract**

227
228
229 To study the reactivity of complex mixtures, understanding the different processes
230 affiliated with them are key. A separation procedure was developed to obtain a complete
231 representation of the aromatic subfraction of the light and heavy crude oil sample. The crude oils
232 were separated into four fractions based on the number of condensed aromatic rings present. The
233 dissolved organic matter (DOM) was generated from each fraction via thin films (30 μm) and a 12
234 h photodegradation time. DOM generated from each fraction was subjected to a photodegradation
235 time series from 0 – 96 h in 24 h increments. Each DOM degradation series was measured using
236 excitation emission matrix spectroscopy (EEMs) and statistical analysis were applied using
237 parallel factor analysis (PARAFAC), to identify the underlying fluorophores present in the sample
238 set. The 2% loading of sample provided the desired fractions. Dissolved organic carbon and
239 absorbance measurements collectively decreased with increasing exposure time.

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Keywords: Dissolved Organic Matter, Normal-Phase Liquid Chromatography, Parallel Factor Analysis, photodegradation

267 Introduction

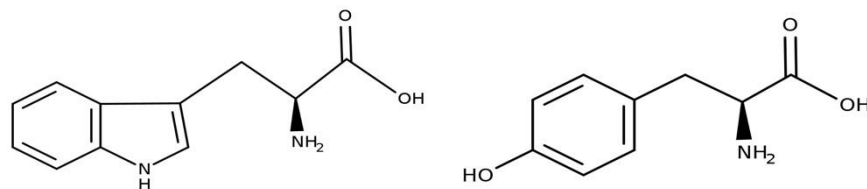
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270 *Structural Complexity of DOM and the Carbon Cycle*

271 The genesis of dissolved organic matter (DOM) can be linked to two overall sources, the
272 first being terrestrial derived DOM and the second being marine derived DOM. Each giving rise
273 to slightly different variations of generated DOM. Marine derived DOM is understood to come
274 from the decay of phytoplankton and is believed to consist of 25-50% proteins, 5-25% lipids, and
275 up to 40% carbohydrates.⁹ While terrestrial derived DOM is the byproduct of biological
276 degradation (i.e. biomass, plant litter, and soil organic matter) that reaches the ocean via rivers,
277 lakes, and glaciers. Degradation of vascular plants yields DOM containing 10% proteins, 30-50%
278 carbohydrates, some lipids found in the roots and leaf cuticles, 15-25% lignin, and other
279 biomacromolecules.⁹⁻¹²

280 Of the byproducts formed, carbohydrates and proteins are considered to be biolabile due
281 to the nature of their chemical structure. This is due to their susceptibility to hydrolysis at the hands
282 of different enzymes present in the environment. The hydrolysis reaction takes place at the areas
283 where peptide and glycosidic bonds are located.⁹ Lignin, on the other hand, exists as a branched
284 macromolecular system, comprised of repeating phenylpropanoid units, which are connected by
285 ether and carbon-carbon bonds at differing locations throughout the structure (Figure 2). This
286 structural design supports the chemical stability of lignin, which results in a chemical resistance to
287 microbial degradation.⁹

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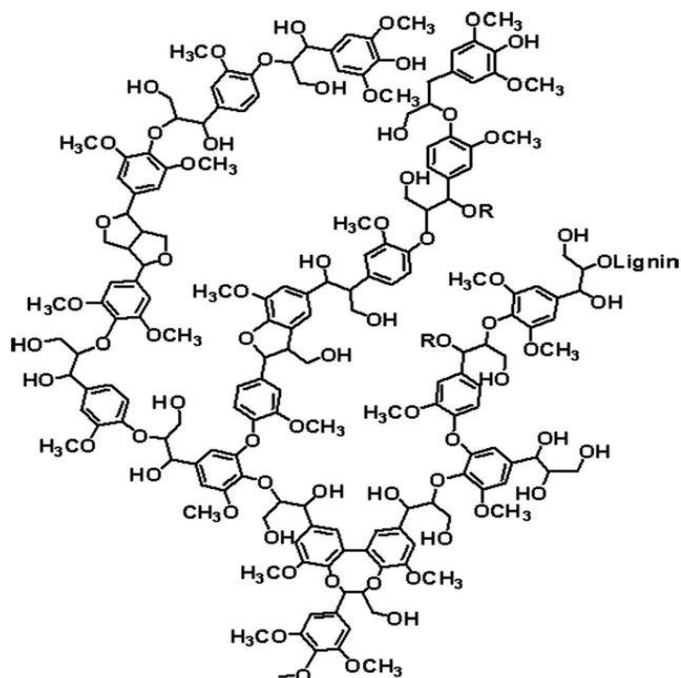
291 **Figure 1.** Chemical structure of tryptophan (left) and tyrosine (right) amino acids.

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294 As DOM is photodegraded, a cascade effect begins to take place that inevitably ends when
295 the starting compounds reach a point where they cannot be reduced anymore. When compounds

296 cannot be reduced, one of a few classifications can remain: tyrosine-like, tryptophan-like, humic-
297 like, or fulvic-like (Figure 1).

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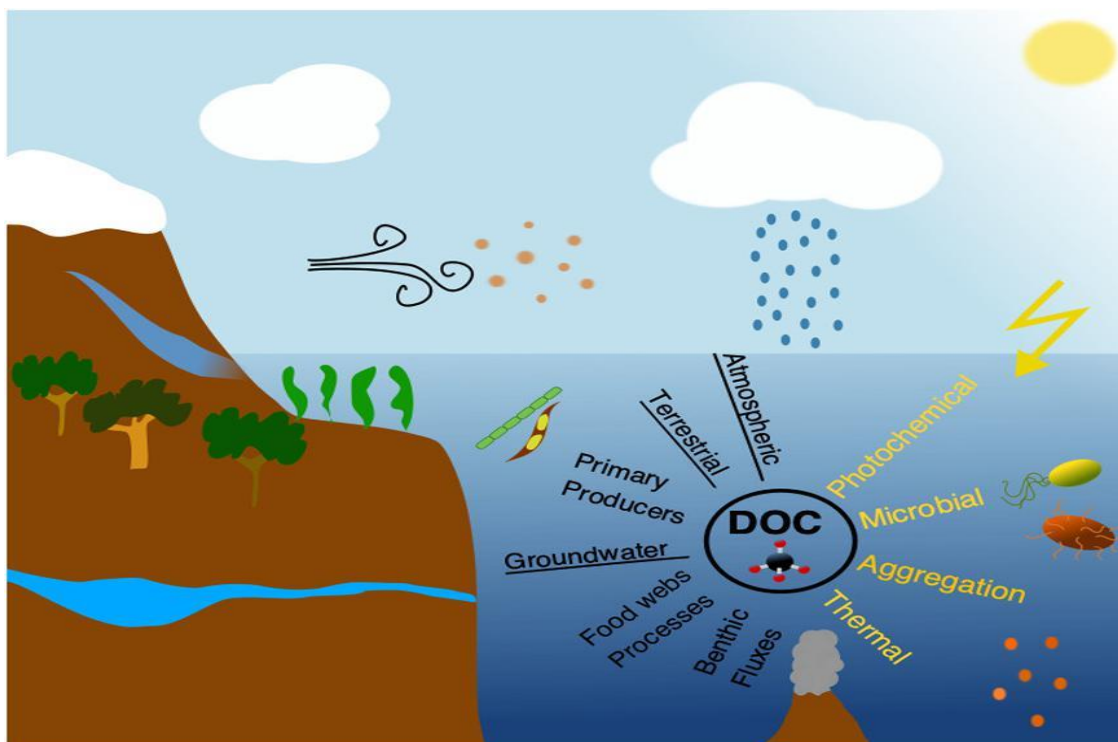


300
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302 **Figure 2.** A structural depiction of one of the chemical byproducts of vascular plant decay known as
303 lignin¹. Reprinted with permission from Prieur, B., et al., *Phosphorylation of lignin: characterization and*
304 *investigation of the thermal decomposition*. RSC Adv., 2017. 7: p. 16866-16877. Copyright 2017 by The
305 Royal Society of Chemistry.

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308 Understanding the complex organic components that comprise DOM has become the
309 primary focus of many studies over the recent years. Figure 2, illustrates a traceable structure
310 affiliated with DOM, known as lignin, due to its resistance to degradation processes in the global
311 carbon cycle. A detailed understanding of the molecular structures and associated reactivities of
312 specific classes of molecules that are present in the complex mixture of DOM can allow for
313 inferences to be made regarding how they will be broken down and subsequently cycled. Figure 3
314 shows the different pathways by which dissolves organic carbon (DOC) is cycled.



315 **Figure 3.** To the left the main input sources can be seen (black) and to the right the degradation are
 316 degradation (DOC removal) pathways (yellow).¹³
 317
 318

319 Insights into the structures that comprise this complex mixture can provide vital
 320 information that will inevitably help to develop an understanding of their reactivities by various
 321 degradation pathways.^{6, 13-18} Photochemical degradation of DOM molecules found in surface
 322 waters have already been found to considerably affect the composition and quantity of dissolved
 323 organic carbon (DOC) carried through marine systems¹⁹.

324 ***Composition of Crude Oil***

325 Crude oil is comprised of hundreds of different hydrocarbons and other organic and
 326 inorganic substances including sulfur, nitrogen, and oxygen, as well as metals like vanadium and
 327 nickel.²⁰ The percent abundance for the different elements found in petroleum are shown in table

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Elemental Analysis	Light Crude (wt %)	Heavy Crude (wt %)
Carbon	86.0	87.0
Hydrogen	13.5	10
Nitrogen	0.2	0.3-0.5
Oxygen	<0.5	<0.1
Sulfur	<2.0	3.0
Nickel (ppm)	<10.0	16
Vanadium (ppm)	<10.0	50

Table 1. Weight percentage for carbon, hydrogen, nitrogen, oxygen, sulfur, nickel, and vanadium.^{4, 21}

Variations in the amount of each element present gives rise to crude oils that differ in physical properties. The two types of oil used in this study are Macondo surrogate oil (MC252) and Maya sour crude oil. The properties of the two oils are detailed in Table 3. Macondo surrogate oil has an API gravity of 37.2, a low viscosity, and is classified as light oil. Whereas the Maya crude oil has an API gravity of 22.2, a highly viscous oil, and is classified as a heavy oil. Light oil contains a low concentration of heteroatoms. This can be seen by the low weight percent for nitrogen, oxygen, sulfur, nickel, and vanadium displayed in table 1. In contrast, heavy oils possess higher concentrations of heteroatoms, which is supported by the higher weight percentages found for nitrogen, oxygen, and sulfur in table 1.

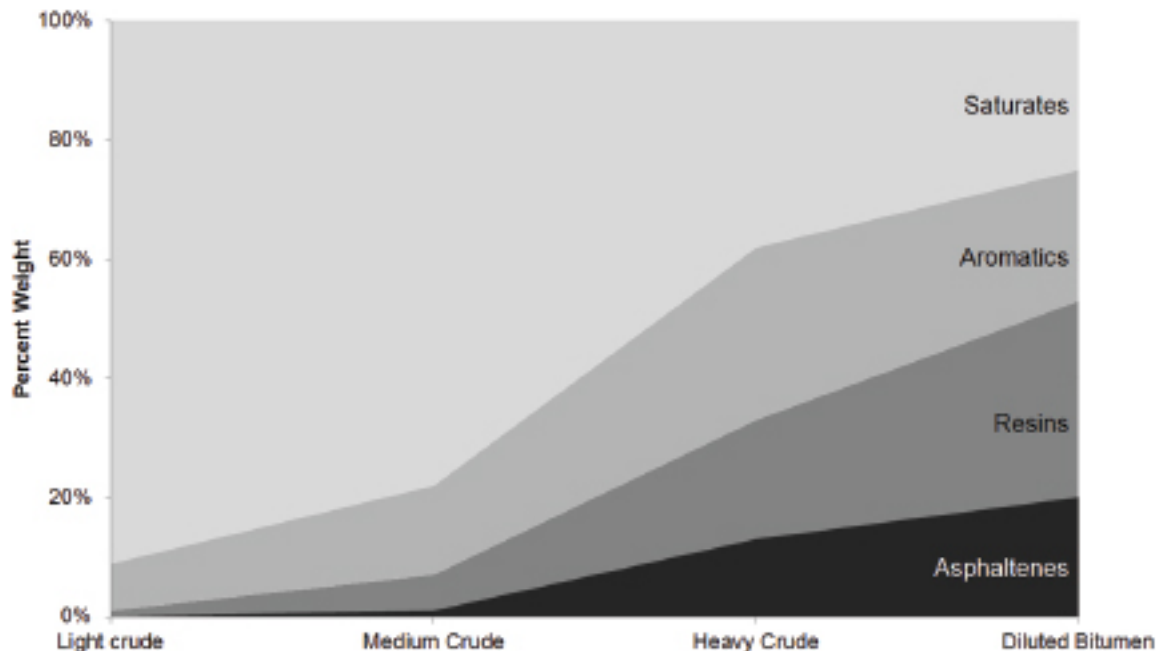
Aspect	Macondo	Maya
API ^o	37.2	22.2
Specific Gravity	0.839	0.922
Density (g/cm ³)	0.839	0.921
Viscosity	Low	High
Classification	Light	Heavy

Table 2. Comparison of the characteristics exhibited by Macondo surrogate oil and Maya sour crude oil.⁴

A mixture of a heavy and light oil was used to obtain a full distribution of compounds that adequately represent the structural continuum. This is necessary because, at the molecular level, the variations found between the light and heavy oil, complement one another. The light oil

354 provides saturated, as well as, mono and dicyclic compounds. Heavy oil possesses the larger
355 aromatic systems needed to represent the structural continuum (Figure 4).

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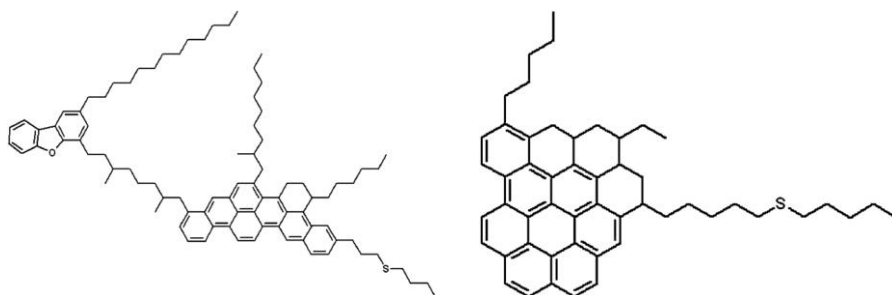


358
359 **Figure 4.** The distribution of weight percentages of typical components found in light, medium, and heavy
360 crude oil's². Reprinted in part with permission from Environment, C.; Technology, B.; Studies, D.;
361 Medicine, N., *Spills of diluted bitumen from pipelines: A comparative study of environmental fate, effects,*
362 *and response.* 2016; p 1-166. Copyright 2016 by the National Academy of Sciences.

363
364
365 Asphaltenes are the portion of crude oil that are insoluble in alkanes, such as n-heptane and
366 n-pentane, and are defined by their solubility characteristics in place of a specific chemical
367 characterization.^{5, 22-24} Given their unique composition, heteroatoms are able to influence
368 surrounding asphaltene compounds into sticking together by playing off the polarizability of the
369 aromatic systems that are locked into configuration in combination with subtle variations in charge
370 separation.²² Light oils has lower concentrations of asphaltenes than heavy oils. Oils that contain
371 higher concentrations of asphaltenes are darker in color and exhibit a higher viscosity than that of
372 oils with lower concentrations of asphaltenes.²² The chemical composition of the Maya crude oil
373 used in this study is classified as a heavy oil, and as such, is prone to having higher concentrations
374 of asphaltenes than the Macondo surrogate oil.

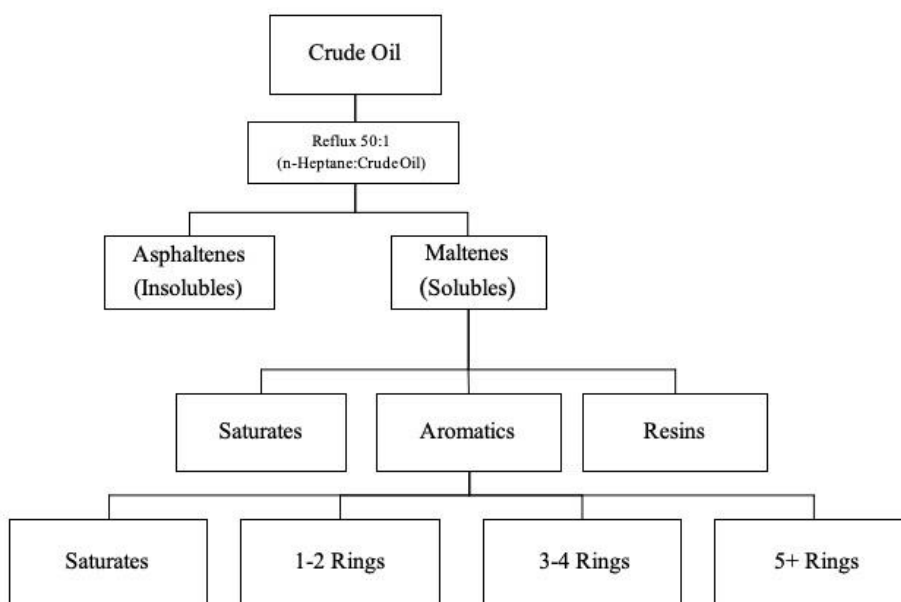
375 The structure of asphaltenes can vary in type which can influence their properties. An
376 example of an archipelago style asphaltene can be seen on the left in Figure 5. It is comprised of
377 two condensed aromatic cores that are linked together by an aliphatic chain. Another style of
378 asphaltene is the island configuration which is comprised of a single condensed aromatic core with
379 aliphatic chains branching from outside rings.³ Asphaltenes, regardless of the structural
380 orientation, are described as being insoluble in excess solvents made of nonpolar compounds (i.e.
381 pentane and heptane).²⁵

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384
385 **Figure 5.** Two examples of asphaltene chemical structures. On the left, an example of an archipelago type
386 asphaltene. On the right, an example of an island type asphaltene³. Reprinted in part with permission from
387 Boek, E., T. Headen, and J. Padding, *Multi-scale simulation of asphaltene aggregation and deposition in*
388 *capillary flow*. Faraday discussions, 2010. **144**: p. 271-84; discussion 323. Copyright 2010 by Faraday
389 Discussions.

390
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392 Once the asphaltenes are precipitated out of solution, the remaining product is comprised
393 of what is described as ‘maltenes’. The maltenes fraction of crude oil is made up of saturated,
394 aromatic, and resin like compounds.^{5, 26} Humic acids vary in structure depending on the source
395 material from which it is derived. In comparison with fulvic acids, humic acids are larger have a
396 lower oxygen content and a low solubility in water at neutral pH. The lack of solubility can be
397 attributed to the lack of oxygen in the structure. In contrast, fulvic acids have a much higher oxygen
398 content and dissolve more rapidly in water.



399 **Figure 6.** SARA fractionation of crude oil with subsequent fractionation of the ‘aromatic’ division into
 400 discrete bins based on the number of rings^{4, 5}. Adapted with permission from Gaspar, A., et al.,
 401 *Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by*
 402 *Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.*
 403 *Energy & Fuels*, 2012. **26**(6): p. 3481-3487. Copyright 2012 by the American Chemical Society.

404
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 406 Sub fractionation of the aromatic portion of maltenes and Macondo surrogate oil mixture
 407 provides insight into the underlying components that gave rise to the features that define the overall
 408 sample. In order to understand the whole picture, each individual fraction possesses a unique
 409 characteristic that plays a role in the final answer. From the separated aromatic rings, photo
 410 exposure experiments can be performed to introduce oxygen atoms into the separated ring fractions
 411 to resemble compounds found in aquatic environments. This will lead to insight into identifying
 412 which core structural bins these different compounds originate from. This can be achieved because
 413 specific fluorophores are defined by specific excitation and emission wavelengths.²⁷

414 ***Boduszynski Continuum Model***

415 Mieczyślaw Boduszynski published a series of articles that focused on the composition of
 416 heavy petroleum.^{6, 28, 29} The first publication in the series explores variations in molecular weight,
 417 hydrogen deficiency, and heteroatom concentrations in response to the atmospheric equivalent
 418 boiling point (AEBP).³⁰ The data obtained shows that heavy petroleum, and its residues, are not
 419 comprised of predominately high molecular weight components. But rather, contain a distribution
 420 of molecular weights, with the highest molecular weight observed not exceeding 2000³⁰. It was

421 also concluded that with an increase of AEBP the concentration of heteroatoms and hydrogen
422 deficiency were seen to increase.³⁰

423 The next publication in the series looks at molecular characterization utilizing two different
424 methods³¹. The data obtained from the first method demonstrated changes in the concentrations of
425 the molecular building blocks as AEBP increases and solubility decreases.^{30,31} The second method
426 centers on utilizing high-performance liquid chromatography (HPLC) to separate compound class
427 fractions for more in-depth molecular characterization.³¹ Results obtained from the second method
428 showed a diversity of molecular types, with multiple homologous series of compounds, each series
429 having a broad carbon distribution.³¹

430 Boduszynski and his team also completed a separation of 50 polycyclic aromatic
431 hydrocarbons (PAH) using an amino functionalized silica stationary phase⁶. This allowed the PAH
432 compounds to elute based on the number of aromatic rings. By separating the PAHs based on their
433 core ring structure, the method could potentially be applied to a more complex sample, such as the
434 maltenes fraction of crude oil. Separating the maltenes based on their core ring structure would
435 provide a way to reduce the complexity of the sample by yielding different subclasses that could
436 be studied individually.

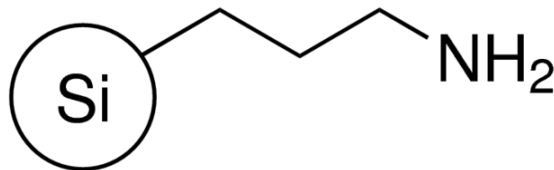
437 *Normal-Phase Liquid Chromatography (NPLC)*

438 The properties and behavior exhibited by crude oil can be better understood by developing
439 a fundamental understanding of the different chemical components that comprise it.^{26,32} To obtain
440 a better understanding the SARA (Saturates, Asphaltenes, Resins, and Aromatics) fractionation
441 procedure is frequently used to separate crude oil samples into their underlying constituents.^{5,33} In
442 Figure 8 illustrates the procedure for how the SARA method fractionates crude oil into its
443 underlying components on the basis of each fractions solubility. For this study, the aromatic
444 fraction was chromatographically separated in a second dimension by number of aromatic rings.
445 of the aromatics based on the number of rings, DOM was generated by photochemical oxidation
446 of the residues that corresponded to each fraction.

447 The complex mixture can be separated into different classes, which will be comprised of
448 compounds that share similar structural characteristics, by employing normal phase liquid
449 chromatography (NPLC).^{6,34,35} This process can be achieved because of the differences in
450 structure, and subsequently the polarity, exhibited by each of the fractions. The differences will
451 allow the fractions to interact with the stationary phase at different strengths to increase the quality

452 of the separation. The stationary phase used in this study was 3-aminopropyl functionalized silica
453 gel and is shown in Figure 7 below.

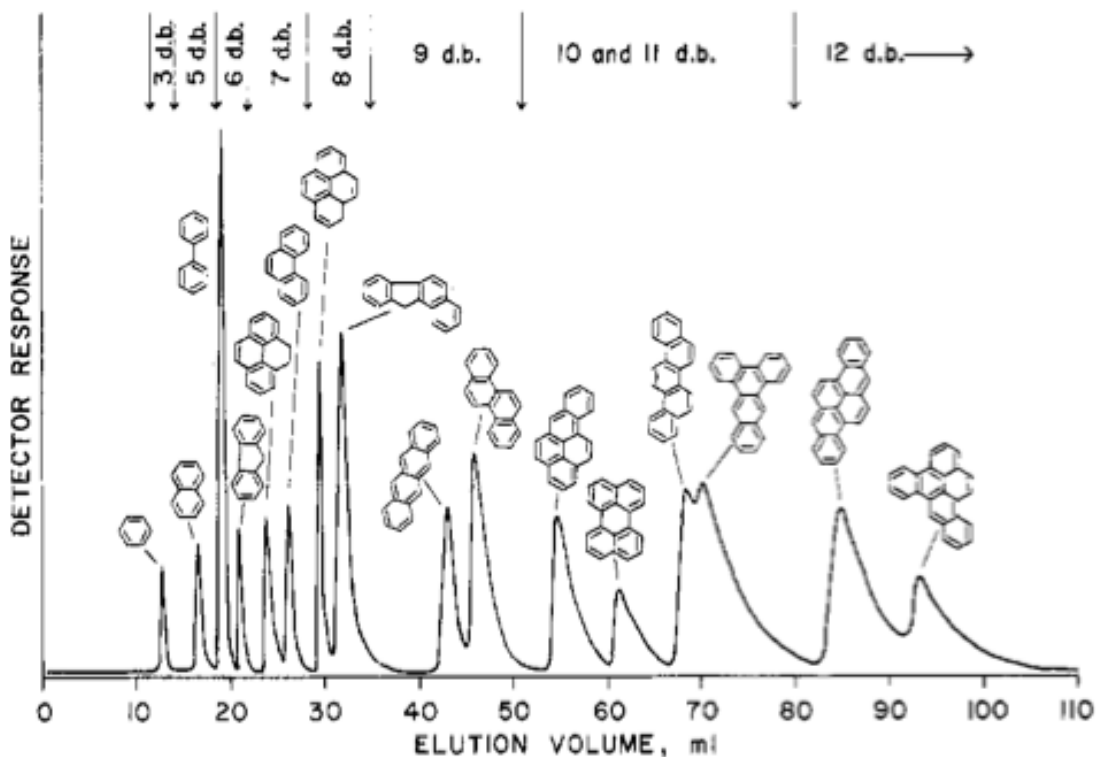
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457 **Figure 7.** Structural depiction for 3-aminopropyl silica functionality used in the chromatographic
458 separation.

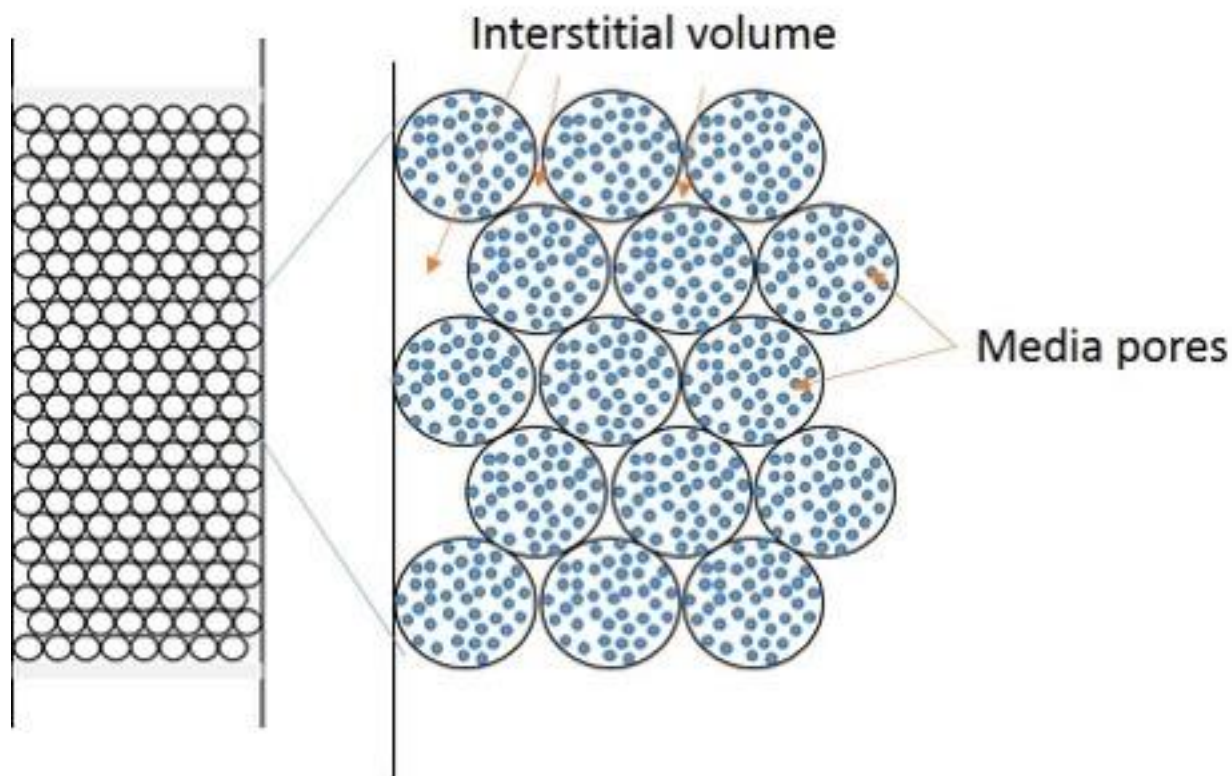
459
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461 The elution will yield the saturates fraction first, since they are the least polar and will
462 interact the least with the more polar stationary phase. To get the more polar fractions to elute,
463 alterations to the mobile phase will have to be made by adding a more polar solvent to the mobile
464 phase to mitigate the interactions occurring between them and the stationary phase.

465 Boduszynski and his team found that by using functionalized silica the mixture could be
466 separated into different classes based on the number of aromatic rings found in the sample.⁶ In
467 Figure 8, the results obtained by Boduszynski et al. (1983) for the separation of heavy oil at the
468 analytical level can be seen.



469 **Figure 8.** Elution profiles of Polyaromatic Hydrocarbons (PAH) on a semipreparative μ Bondpak NH_2
 470 Column⁶. Adapted with permission from Boduszynski, M. M.; Hurtubise, R. J.; Allen, T. W.; Silver, H.
 471 F., Liquid-Chromatography Field-Ionization Mass-Spectrometry in the Analysis of High-Boiling and
 472 Nondistillable Coal Liquids for Hydrocarbons. *Analytical Chemistry* **1983**, 55 (2), 225-231. Copyright
 473 1983 American Chemical Society.

474
 475
 476 The use of 3-Aminopropyl functionalized silica provided the necessary means to separate
 477 the mixture based on the number of rings present, with adequate selectivity and resolution.^{17, 36, 37}
 478 Preparative chromatography was used to scale up the separation procedure so that enough of each
 479 ring fraction could be obtained to make a 30 μm oil film for the DOM generation section of the
 480 study. Upon scaling up the reaction, issues began to arise with maintaining reproducibility with
 481 respect to the column volume which affected the integrity of the separation. As a result, it can be
 482 seen how to appropriately account for interstitial volume between particles as well as pore size
 483 (Figure 9).



484 **Figure 9.** Visualization of intersitial and pore volume in relation to column volume.^{7,8}

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487 For the purpose of this study, a combination of Macondo surrogate oil and Maya Sour
488 Crude oil were combined to makeup the sample (Table 2). This was to provide a sufficient balance
489 of low molecular weight compounds with high molecular weight compounds.

490 ***Removal of Carbon via Photochemical Pathways***

491 The degradation of DOM involves a complex overlay of different disciplines including
492 biological, chemical, and photochemical pathways.³⁸ Photochemical removal is important when
493 considering the fate of DOM because it yields products that are then exposed to microbial
494 degradation.³⁸ The production of oxygenated compounds as a function of time in the presence of
495 sunlight has been proven to occur when petroleum is exposed to water.³⁹⁻⁴¹ Upon photooxidation,
496 compounds that started off being oil soluble are now closer to being water soluble.³⁹ To elucidate
497 the pathways by which these newly water soluble compounds are transported, the ring specific
498 fractions collected from the separation described in the previous section were used to produce
499 aromatic DOM.³⁹

500
501

502 ***Parallel Factor Analysis (PARAFAC)***

503 Advancements in spectroscopic instrumentation (i.e. absorbance and fluorescence) and
504 data processing programs have yielded a rapid and highly sensitive way to extract information
505 from samples to help in the characterization of DOM.^{39, 42} Parallel factor analysis (PARAFAC) is
506 a multivariate modeling statistical analyses which highlights the underlying fluorescent
507 components of the sum of 40 spectra reducing them to four specific components.^{25, 43}

508 The first step in obtaining any PARAFAC model begins with preprocessing the data set
509 being used. This preprocessing step is to adjust for any biases, remove any scattering that may be
510 present, and to normalize the data. Normalizing the signals of all samples is crucial because it
511 allows all samples in the dataset, regardless of high or low concentration, similar weight. This
512 allows the generated PARAFAC model to focus, not on the intensity of all the signals in the set,
513 but rather the chemical variations exhibited between the samples.⁴⁴

514 Next, multiple exploratory phases will be completed to develop potential models in order
515 to locate and remove any outliers present in the sample set. In this exploratory phase it will be key
516 to identify the correct number of components, such that there is significant distribution of variation
517 across the data, resulting in a model that only encompasses random errors.⁴⁴ The model that yields
518 the highest core consistency at the end of the exploratory phase will indicate the appropriate
519 number of components to be used to represent the model as a whole.^{44, 45}

520 The final step in the PARAFAC process is the model validation step and its success is
521 heavily reliant on using the appropriate number of components when fitting the model.⁴⁴ Under or
522 overcompensating the number of components in the dataset, when attempting to validate the
523 method, will prevent the model from validating. When a model is fitted with an insufficient number
524 of components, the validation failure occurs as a result of the model erroneously grouping signals
525 that are representative of components that are distinctly different. Attempting to validate a model
526 using too many components will fail due to an overlap of multiple components in the dataset,
527 resulting in the model mistakenly coupling multiple PARAFAC moieties as one component.⁴⁴

528 Once the PARAFAC model is completed, the model can be uploaded to OpenFluor, an
529 online repository containing already published fluorescence spectra, to identify similar spectra so
530 that more information can be extracted from the model.

531

532 **Experimental Methods**

533
534

535 ***Asphaltene Precipitation***

536 Maya sour crude oil and n-heptane were added to a 1000 mL round bottom flask in a ratio
537 of 1:50. The mixture was then heated and refluxed at a temperature of 100 °C for 1 hour. After 1
538 hour, the solution was removed from the heat and allowed to continue refluxing for 30 minutes
539 and then was capped and stored overnight. The solution was then filtered through a Whatman #1
540 filter (11 µm) to isolate the maltenes fraction of the crude oil.

541 ***Column Packing and Separation Parameters***

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543

Contents	Fraction	Column Volumes	Solvent
Saturates	1	1	n-Heptane
1-2 Rings	2	1	n-Heptane
3-4 Rings	3	3	n-Heptane
5 Rings	4	2	2% Dichloromethane in n-Heptane
5+ Rings	5	2	10% Dichloromethane in n-Heptane

544 **Table 3.** Separation Parameters for Crude Oil Mixture.

545
546

547 ***Maltenes and MC252 Sample Preparation***

548 Maltenes and MC252 oil were added to a vial in a 2:1 ratio respectively. The total mass of
549 the sample was kept at a 2 % sample load with respect to the amount of stationary phase.

550 ***Photodegradation Time Series***

551 Using the solar simulator (Atlas Suntest CPS+, Atlas Material testing Technology LLC)
552 set at 765 W/m² one 24 hour interval is equivalent to four days of natural sun-light in the Gulf of
553 Mexico¹⁸. Films were produced using each ring fraction obtained in Section 2.2. For a 30 µm film
554 thickness, 87 mg of each respective fraction is required. To provide a uniform film 87 mg of sample
555 was dissolved into 10 mL of n-heptane and 2 mL was poured over 50 mL of nanopure water inside
556 a 250 mL jacketed beaker thermostatically controlled at 27°C. Generation of DOM from each ring
557 fraction was subjected to simulated sunlight for 24 hours. The DOM from each subsequent ring
558 fraction (30 mL) was photodegraded from 0 to 96 hours in 24 hour increments. Quartz lids securely
559 placed over the jacketed beakers to reduce evaporation yet still allow sufficient light through
560 during all simulations.

561

562 ***Total Organic Carbon Analysis***

563 DOC concentrations were obtained via the Shimadzu total organic carbon (TOC) analyzer
564 using a high-temperature combustion method and a platinized alumina catalyst. A ratio of 1:3 (v/v)
565 was used to dilute the sample in nanopure water that was then acidified to a pH of 2 utilizing 12
566 M HCl. Samples were then sparged for 5 min at 75 mL/min with ultra high purity air to remove
567 the inorganic carbon from the samples prior to analysis. A five-point calibration curve was used
568 to determine the samples concentration utilizing a range from 0 to 50 ppm using potassium
569 hydrogen phthalate (KHP). To ensure the efficiency of the instrument, calibration curves ranging
570 from 0 to 50 ppm were conducted every 10 samples as well as at the end of the run. A 5 ppm check
571 was also integrated into the sample analysis to confirm maximum instrument efficiency. Blank
572 samples were run before and after all checks and calibration curves. To determine the DOC
573 concentration present in the sample, the best 3 injections of 5 were used to determine the DOC
574 present in a sample at an injection volume of 150 μ L. The best 3 injections obtained should result
575 in a coefficient of variance of <2% for duplicate injections³⁹.

576 ***Absorbance Analysis***

577 All samples in the study were measured on an Agilent UV-Vis absorbance instrument, from
578 200 to 600 nm. Absorbance values corresponding to 254 nm were used for the specific UV-
579 absorbance (SUVA₂₅₄) calculation in conjunction with DOC values²⁷.

580 ***Fluorescence Analysis***

581 Excitation emission matrix spectroscopy (EEMs) measurements were collected at an
582 excitation range from 240 to 800 nm and emission from 240 to 800 nm using a 10 mm quartz
583 cuvette. Excitation spectra were collected every 5 nm and emission every 3 nm at a 1.5 second
584 integration time. Sample intensities were normalized to Raman scattering units (RSU) and
585 statistical analysis were applied using PARAFAC analysis. Inner-filter effects were mitigated by
586 correcting to 0.1, based on the absorbance value found at 254 nm, using nanopure water.^{18, 46, 47}
587 The drEEM toolbox was utilized to produce a validated model, to yield a four component
588 PARAFAC model from 40 samples.^{25, 44, 48}

589

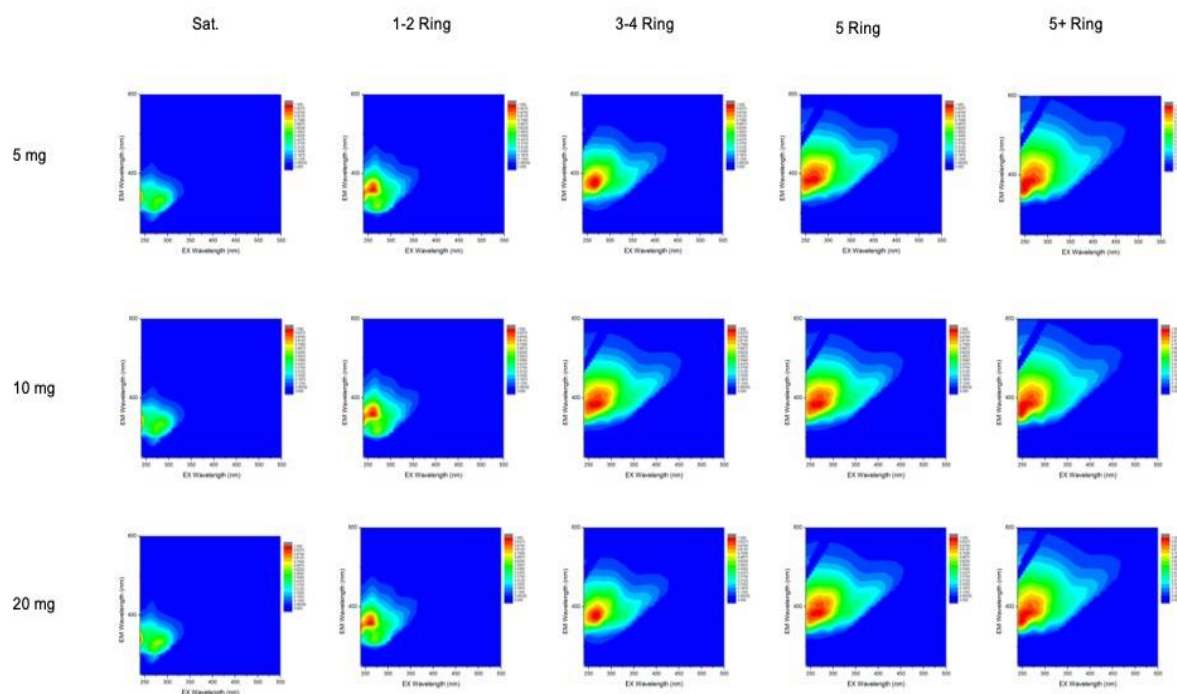
590 **Results and Discussion**

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593 ***Determination of Load Capacity***

594 Loadings of 5, 10, and 20 mg were applied to HF-Mega BE-NH₂ solid phase extraction
595 (SPE) cartridges to identify the optimal loading capacity. Based on the EEMs shown in Figure 10
596 below, it was determined that a 2 % loading of sample to stationary phase was optimal.

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598



599 **Figure 10.** Fluorescence spectra confirming the 2 % loading had minimal carryover for each fraction.
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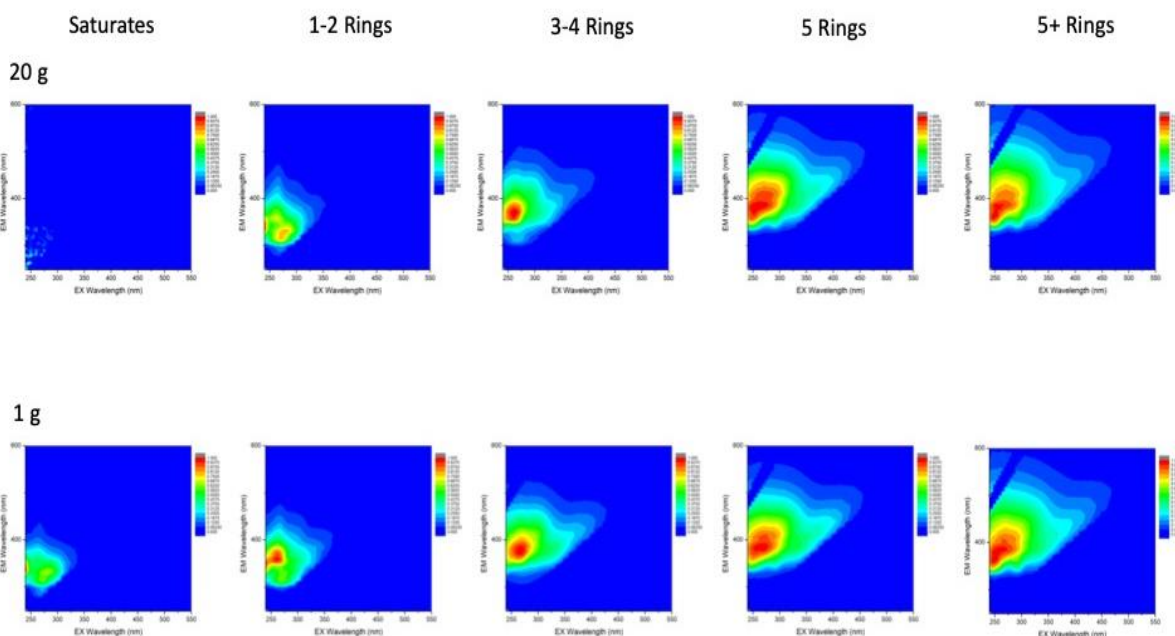
601
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603 The saturate fraction of oil is known to possess compounds that are aliphatic and alicyclic
604 in structure.⁵ Therefore, the EEMs taken for the saturate fraction should have a very small, if any,
605 fluorescent signature. The signatures seen in Figure 10 for the saturate fraction were determined
606 to be the 1 - 2 ring fraction that eluted earlier than anticipated. This occurred as a result of a
607 miscalculation for the total column volume. This error was adjusted before the preliminary scale
608 up was conducted.

608 ***Preliminary Scale Up***

609 Before the final scale up, a preliminary scale up was completed to ensure that the separation
610 could be sufficiently reproduced without losing quality. In Figure 11 below, a comparison of the

611 scaled down model at 1 gram of functionalized silica alongside the preliminary scale up at 20
612 grams of functionalized silica can be seen. The EEMs shown demonstrate that there is minimal
613 loss of quality upon scaling up. After adjusting the column volume, the EEMs of the saturates
614 fraction in the preliminary scale up reflects no coelution between the saturates and 1 - 2 ring
615 fractions.
616
617



618 **Figure 11.** Comparison of scaled down EEMs with preliminary scale up EEMs for 20 g (top) and 1 g
619 (below).
620
621

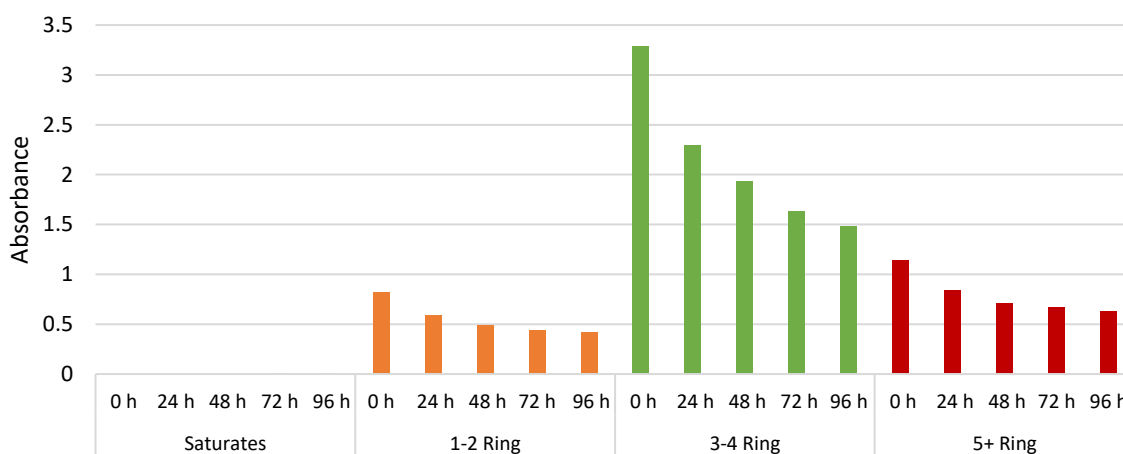
622 The alterations made to the column volume between the scaled down separation and the
623 preliminary scale up returned results that accurately depict what is expected with respect to
624 chemical theory, as well as supports the findings of previously completed studies that examined
625 the chemical composition of the subfractions that makeup oil. As a result of their structural nature,
626 compounds eluted within the ‘saturates’ fraction lack the conjugated π bond system necessary to
627 yield a fluorescent signature. The lack of fluorescent signature found for the saturate fraction of
628 the preliminary scale up depicted in Figure 11, displays some small sporadic signatures, but isn’t
629 representative of any specific moiety. For the preliminary scale up the EEM representing the
630 saturate fraction of the separation possess some nondistinctive signatures that are shifted to the
631 shortest wavelengths. Figure 11 shows that as the number of rings increase, the EEM’s become

632 shifted to longer wavelength. EEMs collected for the 5 - ring and 5+ ring fractions displayed
633 contour plots showing little difference between the two, therefore they were combined to represent
634 the 5 + ring fraction.

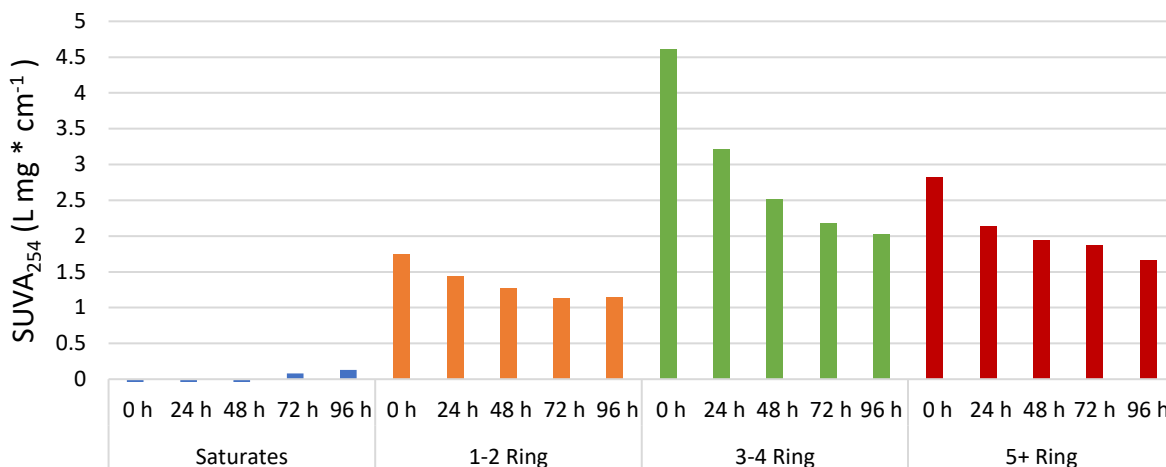
635 *Photodegradation of Fractionated Aromatic Ring Fractions*

636 The adjustment to the column volume previously described previously, allowed for the
637 subsequent final scale up, using the verified ratios between then preliminary scale up and the
638 scaled down studies. The next portion of the study explores how these different fractions
639 individual breakdown in the presence of sunlight. It can be seen in Plot 1 below, the general
640 trend described by previous studies, that as exposure time increases the ratio of hydrogen to
641 carbon (H/C) decreases and the ratio of oxygen to carbon (O/C) increases as oxygenation
642 increases as a function of time.

643
644



645
646 **Plot 1.** Absorbance values illustrating 1 - 2 ring DOM (orange), 3 - 4 ring (green) and 5 + ring (red)
647 photodegradation time series.



648
 649 **Plot 2.** SUVA₂₅₄ (L mg⁻¹ C⁻¹ m⁻¹) for saturates (blue), 1 - 2 ring (orange), 3 - 4 ring (green) and 5+ ring
 650 (red). A decrease in SUVA₂₅₄ is observed as the photodegradation time is increased.
 651

652
 653 The application of SUVA₂₅₄ can be used as a proxy for the aromatic content in DOM.^{49, 50}
 654 In Plot 2, it can be seen for all ring fractions, a decrease in aromaticity occurs with increasing
 655 exposure times.

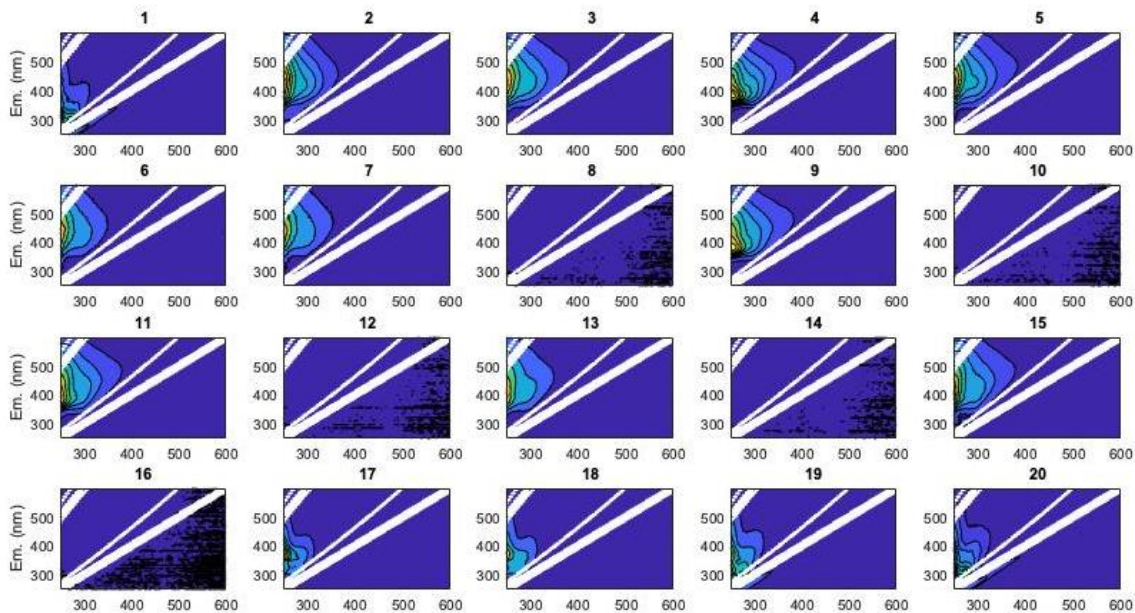
656 **PARAFAC Analysis**

657 The EEMs for the photodegraded ring fractions were processed in MATLAB to remove
 658 outliers, excise and smooth the scatter regions, and to normalize to unit variance (Figure 12). The
 659 saturates timeseries displays no fluorescent signature due to the lack of conjugated double bonds.
 660 As a result, they are omitted from the PARAFAC process as outliers, because it will introduce
 661 additional variance into the model. After removal, a model can be constructed that better explains
 662 the differences between the degradation products that makeup the remaining samples.

663 For each fraction, the contour plot should decrease in intensity at its core and as stretch
 664 around the edges as the condensed aromatic rings begin to break and yield smaller compounds.
 665 This results as a byproduct of photooxidation. The smaller compounds will begin to introduce
 666 oxygen containing functionalities such as carboxylic acids, ketones, and aldehydes. A good
 667 representation of this trend can be seen for the 3 - 4 ring degradation series. In the 0 hour timepoint
 668 (Figure 12 - 4), a high intensity yellow core can be seen with surrounding regions that vary in
 669 shape and distance from the epicenter. The 24 hour timepoint (Figure 12 - 9), the concentric rings
 670 begin to gradually shift out as photooxidation occurs. This trend occurs throughout the remaining
 671 timepoints, and when the ninety-six hour time point is reached (Figure 12 - 15), the original

672 epicenter is now reduced to a small, less intense region with the outside bands stretching towards
673 longer excitation wavelengths.

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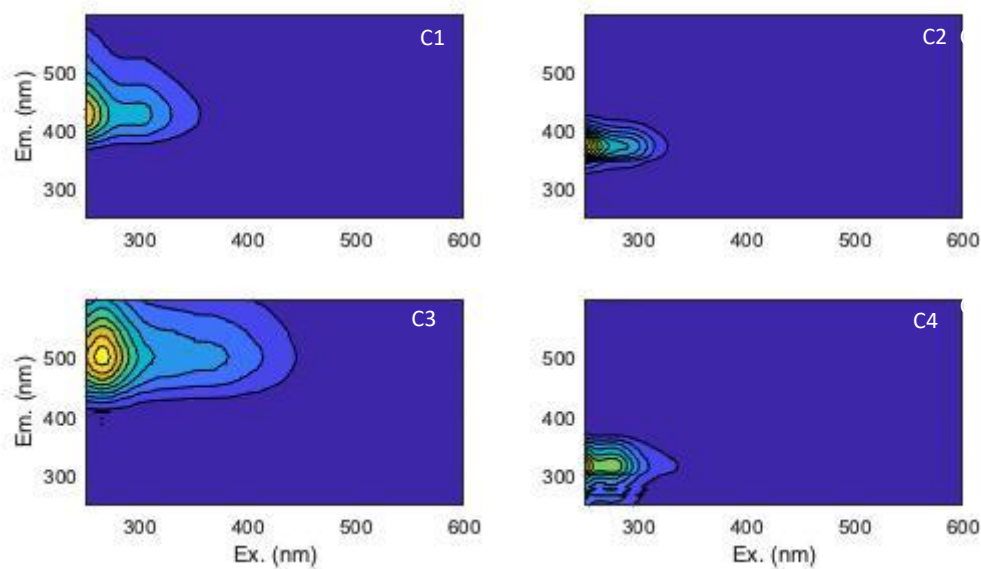
676

677 **Figure 12.** Corrected excitation emission scans of the photodegraded ring fractions.[†]

678
679

680 The validated PARAFAC model (Figure 13) portrays the fluorescent signatures that were
681 present in all 40 samples. The model output was uploaded into OpenFluor, to identify similar
682 components from other studies, to aid in building a more complete picture of the data that is
683 present. Based on the fractions expected, an increase in emission is to be expected as the number
684 of core aromatic rings per fraction increases. Any overlap in fractions can initially be attributed to
685 overlap of the desired fractions.

[†] The corresponding file list for EEMs can be found in appendix.



686

687 **Figure 13.** Validated 4 component model (C1 – C4) from the PARAFAC analysis.

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A four component PARAFAC model was validated (Figure 13) showing a shift in excitation/emission wavelengths from shorter to longer wavelengths from C4, C2, C1 and C4, respectively. The first component, C1, (Figure 13) shows a initial excitation at 250 nm and then a secondary excitation at 305 nm, resulting in an emission maximum at 427 nm. This peak is found in a region characterized as marine humic-like in nature and is most likely derived from the 5 + ring fraction.^{51, 52} This region can also be described as Peak A and is representative of blue shifted aromatic compounds that have a high molecular weight.⁵²

Component 2 reflects characteristics described as protein-like and/or amino acid-like in nature.⁵³ Component 2 (C2) has a primary excitation at 250 nm and a secondary excitation at 290 nm, that result in an emission of 373 nm. With respect to C1, C2 is slightly more blue shifted and as a result is characteristic of the 3 - 4 ring fraction degradation products. The compounds present can be described as possessing low molecular weight and are high in aromaticity.⁵²

Component 3 (C3) displayed a primary and secondary excitation at 265 nm and 370 nm, respectively. This resulted in an emission of 501 nm that can be related to the 5 + ring fraction. Despite the 5 ring and 5 + ring fractions being combined upon scale up, the PARAFAC model depicted these two fractions as two distinctly different components within the model. Since the C3 exhibited the longest excitation/emission wavelengths of all the components in this model, it can be inferred that it is representative of the 5 + ring fraction in the separation. Component 3

708 demonstrates a chlorophyll like terrestrial dominance in regard to chemical structure, which
709 corroborates its affiliation with the 5+ ring fraction.⁵⁴

710 Component 4 (C4) is the most blue-shifted of all the components, and as a result is
711 considered to be best representative of the 1 - 2 ring fraction degradation products. The peak for
712 this component can be described as mixture of tryptophan and tyrosine-type compounds, that
713 resemble a protein like confirmation.⁵⁵ C4 also displays characteristics of photobleaching, which
714 corroborates the nature of the component in terms of the model as a whole.⁵⁶ At this point in the
715 photo degradation process, when the compounds yield a ‘protein-like’ structure, they tend to be
716 less photolabile than that of humic substances.⁵⁶

717 **Conclusion**

718
719
720 Utilizing a 2 % sample loading, with respect to the mass of the stationary phase, a uniform
721 scale up was achieved with minimal overlap of the fractions desired. The error encountered during
722 the scale up, using an open column separation approach, was identified to be the column volume
723 used to define each respective fraction. When the components obtained from the validated model
724 are rearranged, a linear relationship appears that represents the four separated fractions. The final
725 component is indicative of compounds that are less likely, if not completely, exempt to
726 photolability.

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- 728
729
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890 **Appendix**

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893 File list for exported corrected EEMS in Figure 12.

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895

1	1-2R 96hrSEM.dat'
2	5+ 0hrSEM.dat'
3	5+ 24hrSEM.dat'
4	3-4R 0hrSEM.dat'
5	5+ 48hrSEM.dat'
6	5+ 72 hrSEM.dat'
7	5+ 96hrSEM.dat'
8	Sat 0hrSEM.dat'
9	3-4R 24hrSEM.dat'
10	Sat 24hrSEM.dat'
11	3-4R 48hrSEM.dat'
12	Sat 48hrSEM.dat'
13	3-4R 72hrSEM.dat'
14	Sat 72hrSEM.dat'
15	3-4R 96hrSEM.dat'
16	Sat 96hrSEM.dat'
17	1-2R 0hrSEM.dat'
18	1-2R 24hrSEM.dat'
19	1-2R 48hrSEM.dat'
20	1-2R 72hrSEM.dat'

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899 **VITA**

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902 The author was born in Greer, South Carolina. He obtained his bachelor's degree in
903 chemistry from the University of New Orleans in 2017. He joined the University of New Orleans
904 chemistry graduate program to pursue a master's based thesis in analytical chemistry, and became
905 a member of Dr. Phoebe Zito and Dr. David Podgorski's research groups in 2018.