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An Experimental Study into Tholin's Solubility with Liquid Hydrocarbons on Titan

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An Experimental Study into Tholin's Solubility with Liquid Hydrocarbons on Titan

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Space and Planetary Sciences

by

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Abstract

Titan's production of organics in its atmosphere and the liquid hydrocarbon lakes present on its surface make it a prime target for astrobiologists. Many previous studies on laboratory analogs of these organics, called "tholins", have found amino acids, nitriles, and other building blocks necessary for life. The potential solubility of tholins in the lakes of Titan presents an opportunity to create physical and chemical changes within the organics. While the lakes of Titan are primarily comprised of nonpolar compounds (methane and ethane), many predict that very small amounts of polar hydrocarbons are also present. To better understand these processes, researchers create "tholins" to utilize in these studies. Since the production of tholins is also a randomized process, variations in the structure are to be expected in tholin production. Studies have been developed to better understand variability in the compounds and how best to simulate conditions being investigated.

Chapter 1 investigates the effects of varying production times on tholin molecular structure. While the process of tholin production is randomized, general structural trends resulting from the effects of certain variables can be observed. Through these experiments, we seek to characterize the effects of different production time scales on the structure and composition of tholins. Matrix-Assisted Laser Desorption Ionization (MALDI) was utilized to observe large variations and spectral shifts on 1-hour, 4-hour, 6-hour, and 12-hour production times of tholins. Resulting spectra do not show large changes in the molecular weights of tholins produced at different timescales. The resulting organics produced however might be different, with more aromatics and ring structures being present as production time increases. Therefore, the tholins produced at longer production times might not be dissimilar to tholin produced at shorter timescales.

Chapters 2, 3, and 4 investigate tholins in the lakes on Titan. To better understand the impact these liquid polar compounds within the lakes might have on tholins, we utilize a mixture of both polar and nonpolar solvents. Acetonitrile, hexane, and a mixture solvent of hexane saturated with acetonitrile act as analogs to the Titan lake environment. Dynamic Light Scattering (DLS) was used in Chapter 2 to observe the physical characteristics (such as hydrodynamic diameter and dispersity indices) of the tholin particles within each solution. Measurements showed that the presence of acetonitrile did increase particle diameter and polydispersity values. Additionally, Fourier Transform infrared spectroscopy (FTIR) and MALDI were utilized in Chapter 3 and 4 for spectroscopic analysis of organics with and without oxygen-containing species, respectively. Results from both of these studies showed no chemical changes in the samples containing mixtures of solvents. These projects can provide insight into the environment of Titan and further our knowledge of its potential habitability.

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Dedication

To Daniel and our family, and to my parents for all their sacrifices and support during my education.

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Introduction

Science Background

Since the Miller-Urey experiment, it was found that the compounds necessary for the biological processes occurring on Earth could be created from non-biological processes (Miller 1953). The Miller-Urey experimental study conducted a simulation of the conditions of early earth, including a representative atmospheric gas mixture and an electrical discharge in the presence of water. This experimental set-up produced simple amino acids such as glycine, α -alanine, and β -alanine; all compounds required for life to occur (Miller 1953) Miller's concept was expanded upon by Carl Sagan and his studies into interstellar grains that found organic production from cosmically abundant gas mixtures and various energy sources (Sagan & Khare, 1979). It was from this study that the term "tholins" was coined, closely beating out the alternative "star tar" (Sagan and Khare 1979). From these investigations, we can see that organic production in the universe occurs in a variety of environments with carbon-containing compounds and an energy source, such as UV and cosmic rays.

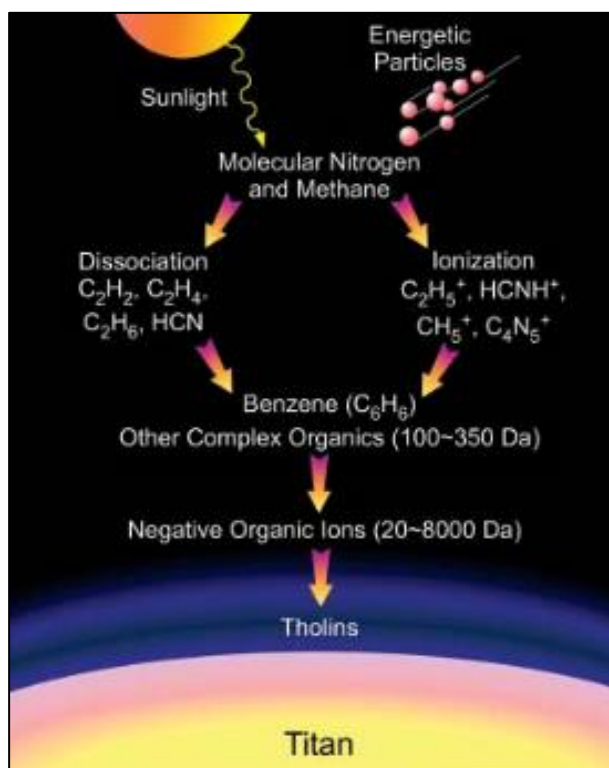


Figure 1: Cartoon showing the chemical process leading up to the formation of tholins in Titan's upper atmosphere. The process begins with free energy from solar UV radiation and energetic particles impinging on Titan's atmosphere. The most abundant constituents (CH_4 and N_2) combine through a number of reaction pathways to form larger organic and nitrile compounds (100 to 350 daltons) that eventually lead to the formation of negatively charged tholin aerosols (20 to 8000 daltons) observed at ~1000 km. (Figure and caption taken from Waite et al. 2007).

Compounds	Mixing ratio	Location with ^{reference#}
<i>Main constituents</i>		
Dinitrogen N ₂	0.98	} Mid stratosphere, mid latitude ³⁶
Methane CH ₄	0.0148	
Dihydrogen	~0.001	
<i>Other hydrocarbons</i>		
Ethane C ₂ H ₆	1.1 × 10 ⁻⁵	} Mid stratosphere, 70°N ⁴⁷
Ethene C ₂ H ₄	5.5 × 10 ⁻⁷	
Ethyne C ₂ H ₂	5.1 × 10 ⁻⁶	
C ₂ HD	2.0 × 10 ⁻⁹	
Propane C ₃ H ₈	6.9 × 10 ⁻⁷	
Propene C ₃ H ₆	~3 × 10 ⁻⁶	} Ionosphere ⁴⁸
Propyne C ₃ H ₄	2.4 × 10 ⁻⁸	
Butadiyne C ₄ H ₂	2.3 × 10 ⁻⁸	} Mid stratosphere, 70°N ⁴⁷
Benzene C ₆ H ₆	4.2 × 10 ⁻⁹	
Toluene C ₇ H ₈	Inferred	} Ionosphere ⁴⁸
Anthracene C ₁₄ H ₁₀	Inferred	
<i>N-organics</i>		
Methanenitrile HCN	9.7 × 10 ⁻⁷	} Mid stratosphere, 70°N ⁴⁷
Propynenitrile HC ₃ N	4.6 × 10 ⁻⁸	
Ethanedinitrile C ₂ N ₂	1.4 × 10 ⁻⁸	
Ethanenitrile CH ₃ CN	2.0 × 10 ⁻⁸	} 300 km disk-average ⁴⁹ Stratosphere ⁵⁰
Butynedinitrile C ₄ N ₂	Solid phase	
Propanenitrile C ₃ H ₃ CN	~2 × 10 ⁻⁷	} Ionosphere ⁴⁸
Propenenitrile C ₂ H ₃ CN	~4 × 10 ⁻⁷	
<i>O-compounds</i>		
Carbon monoxide CO	4.7 × 10 ⁻⁵	Stratosphere ⁵¹
Carbon dioxide CO ₂	1.4 × 10 ⁻⁸	Stratosphere ⁴⁷
Water H ₂ O	4.0 × 10 ⁻¹⁰	Stratosphere ⁵²
<i>Rare gases</i>		
Argon ⁴⁰ Ar	3.4 × 10 ⁻⁵	} Mid stratosphere, mid latitude ³⁶
Argon ³⁶ Ar	2.1 × 10 ⁻⁷	
Neon ²² Ne	2.8 × 10 ⁻⁷	

Figure 2: Compounds found in Titan's atmosphere from various courses at each layer of the atmosphere. (Figure taken from Raulin et al., 2012).

primary constituents being nitrogen (N₂) and methane (CH₄; Niemann et al. 2005; Waite et al. 2007). Data from Voyager 1 and Pioneer 11 found Titan to have organics in the atmosphere (Martin G. Tomasko and Smith 1982) supporting laboratory and observational studies. Within Titan's atmosphere, energetic sources will interact with the molecules present in the atmosphere (mostly [N₂] and methane [CH₄]) leading to the creation of organics that will increase in complexity and weight as they continue to react (Figure 2).

Organics that are being produced will begin as molecular nitrogen (N₂) and methane (CH₄) and form into larger complex molecules as described in Figure 1. Since this is a

Many studies by Sagan and others specifically investigated the atmosphere of Saturn's largest moon, Titan, including experimental investigations with analog gas mixtures of Titan's atmosphere and a high-frequency electrical discharge that showed that organics were able to be produced, many of which have biological relevance (Sagan 1971; 1973; Sagan and Khare 1981; Sagan et al. 1993; Sagan and Thompson, n.d.). These experiments were able to produce these reddish-brown tholins that were found to contain around 75 different compounds we find commonly in life on Earth (Hörst et al. 2012; Khare et al., n.d.).

Titan stands as one of the few planetary bodies that holds a thick atmosphere, with its

randomized process, there is not a consistent molecular structure being created through these interactions (Waite et al. 2007; M.G. Tomasko et al. 2008). However, the Huygens probe's GCMS and the aerosol collector & pyrolyser (ACP) have provided insight into some of the compounds being produced in Titan's atmosphere, as seen in Figure 2, (Raulin et al. 2012).

The search into the biological potential of Titan has provided great insight into the moon's potential habitability. Multiple missions, laboratory studies, and models have been produced to help better understand the chemistry of the Titan atmosphere (He et al. 2012; Hörst 2017; Ralph D Lorenz et al. 2018; McKay 1996; Cordier et al. 2009). With the many similarities between early Earth and Titan, understanding organic production and the rise of biologically relevant compounds might not only inform our understanding of Titan's potential habitability, but also the origin of life on Earth (Trainer et al. 2006).

In addition to the production of organics, there are other features present on Titan that pique the interest of astrobiologists. One such feature would be the presence of liquid lakes on the surface of polar regions (Stofan et al. 2007). Titan is the only planetary body, other than Earth, to have liquid on its surface (Stofan et al. 2007). However, unlike Earth, these lakes are primarily comprised of liquid methane and ethane (Tan et al. 2013; Stofan et al. 2007; Raulin et al. 2010; McKay 1996). While these will be the main hydrocarbons present, the lakes are thought to include small amounts of polar compounds, such as ammonia (NH_3), cyanoacetylene (HC_3N), and hydrogen cyanide (HCN) in the 1 - 50 ppm range (Raulin et al. 2012; Cordier et al. 2009; Raulin et al. 2010).

Interactions between these lakes and organics are highly likely as the compounds fall towards the surface and potentially act as nucleation cores for rain droplets (Rannou et al. 2006), especially if there is indeed precipitation occurring in the polar regions (Rannou et al. 2006; Turtle et al. 2011). Tholin solubility in non-polar and polar solvents has been investigated and been found to be greater in polar solvents compared to non-polar solvents (McKay 1996; Carrasco et al. 2009; Coll et al. 1999). Most of the tholins would not be expected to be soluble

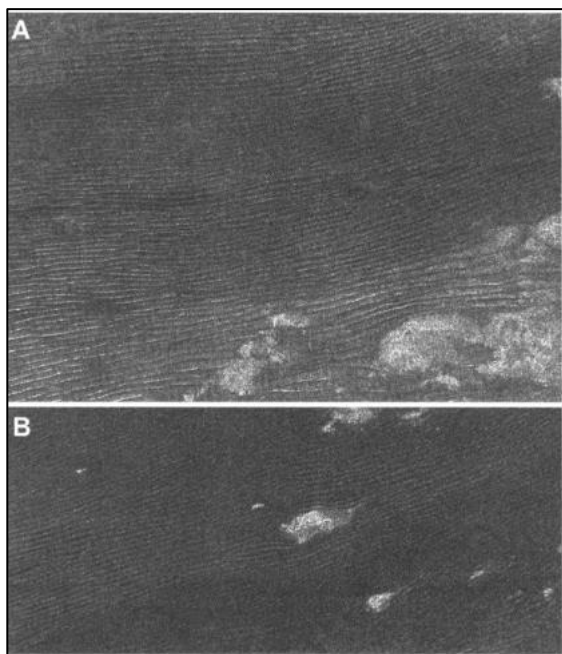


Figure 3: (A) A 235 km by 175 km segment (north up) of the Belet sand sea at $\sim 12^{\circ}\text{S}$, 100°W . Topographic glints are evidence of slopes facing the observer (radar illumination is from above). The subparallel orientation of the dunes and the 1:2 dune:interdune gap ratio are typical of longitudinal dunes on Earth. (B) Segment (235 km by 80 km) to the east of (A). Bright areas are small hills; the flow diverges around them and reconverges. A tail toward the right is evident in the lower hill, suggesting an east-northeast transport direction (Figure and caption from Lorenz et al., 2006).

with non-polar methane (CH_4)/ ethane (C_2H_6) lakes. It is important to note that laboratory studies have found that even in polar solvents, not all of the tholins are soluble. Carrasco et al. found that only 35% of organic material dissolved in polar solutions leaving 65% left behind (Carrasco et al. 2009). While some of the tholins will not be soluble in the lakes, a fraction of the organics might be soluble with the polar liquid hydrocarbons.

In addition to the lakes, dunes in the equatorial regions of Titan also hold potential clues as to how organics move on Titan. Previous studies have observed dune-like structures on the surface of Titan (see Figure 3). Dunes have also been observed in large desert regions on Earth where they are largely comprised of silicate particles

(Lorenz et al. 2006). Unlike Earth, many think the dunes on Titan will be comprised of the organics produced in Titan's atmosphere, with spectra from these regions broadly matching those taken from various organics (Clark et al. 2010; Barnes et al. 2015). One issue with this line of thinking is the sizes necessary for particle transport. The diameters required will be in the 300-micron range; a far distance from the 1-10 micron diameter modeled to be in the atmosphere (Barnes et al. 2015; M.G. Tomasko et al. 2008).

Previous studies have speculated on processes that could contribute to organics growth once on the surface. Barnes et al. suggested that interactions within the lakes could contribute to the size of particles increasing through flocculation (Barnes et al. 2015). Larger, insoluble organics could clump and be held together by Van der Waals forces. This process could very well create the size ranges necessary to fill in the blanks regarding the composition and creation of Titan's dunes.

These questions and more regarding Titan have led to further investigations, including a recently selected mission, Dragonfly. During this mission, investigation into the dune's particles will be conducted; providing vital answers to the composition and biological potential in these regions (Lorenz et al. 2018). Our research seeks to investigate the organics' solubility and physical characteristics within the lakes of Titan through experimental laboratory work with tholins and observe changes within these laboratory analogs with varying production times, providing a reference for Dragonfly and future investigations of the astrobiological potential of Titan.

Methodology

Chapters will discuss in depth the experimental protocols used in each project. However, some general techniques were used in the studies and will be discussed in this section.

Tholin production typically incorporates the use of an energetic source, such as an electrical spark, UV, or plasma, and a gas mixture containing methane (CH_4). For this investigation, tholin production will simulate the processes occurring in Titan's upper atmosphere, thus creating tholins for laboratory use. Our methods implement concepts from previous studies. Szopa et al. designed the Aerosols Production in Microgravity by REactive Plasma (PAMPRE in the original French acronym) method, utilizing capacitively coupled radio-frequency (RF) cold plasma system discharges in conjunction with relevant gas mixtures (Szopa et al. 2006). The PAMPRE method allowed for the interaction of molecules in the gas phase with plasma discharges while in suspension. Our project used a variation of this method with the Technics Hummer II Sputtering machine utilizing a DC electrical discharge cold plasma (Figure 4) in place of the radio frequency found in the PAMPRE protocol.

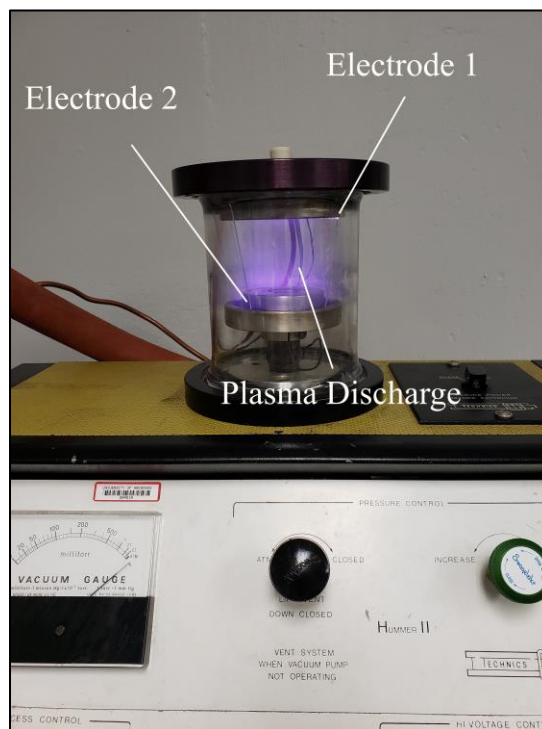


Figure 4: Technics Hummer II sputtering system at the University of Arkansas W.M. Keck Lab with DC cold plasma discharge at 30mA before the production of tholins.

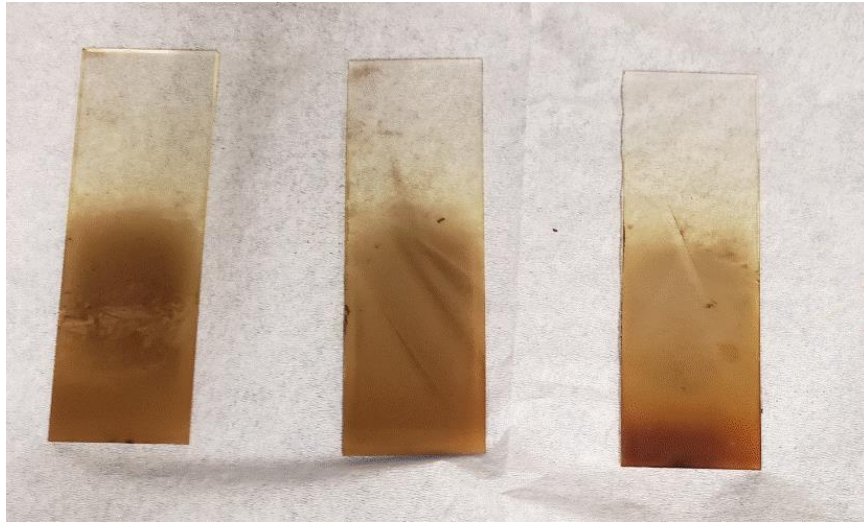


Figure 5: Tholins produced at the University of Arkansas Keck lab. A cold plasma discharge (27 mA) was produced for ~ 72 hours within a gas mixture of a 95% nitrogen (N_2), 5% methane (CH_4) at 0.5 Torr - 3.00 Torr. These tholins were collected from these slides and utilized for the experimental investigations in chapter 1,2, and 3.

This study used a cold plasma discharge (~ 27 mA) within a gas mixture, which simulated the charged particle radiation interacting with Titan's atmosphere, to synthesize Titan-like tholins. Chamber conditions of 0.5 Torr - 3.00 Torr were maintained

at ambient laboratory temperature for approximately three days with a continuous flow at <0.5 SCFM of a 95% nitrogen (N_2), 5% methane (CH_4) gas mixture for tholins investigated in Chapters 1, 2, and 3. The flow rate is kept low in order to prevent the organics from being removed from the chamber. A gas mixture of 96% nitrogen (N_2), 2% methane (CH_4), and 2% carbon monoxide (CO) was incorporated into the experimental protocol in Chapter 4. It is important to note that CO will be present in much lower volumes on Titan compared to our experimental conditions. While the carbon monoxide (CO) fraction within the gas mixture was not exact in comparison with Titan's atmospheric composition, this ratio will allow observation of compounds being produced in such conditions and was in line with previous investigations incorporating carbon monoxide (CO) (Hörst et al. 2012) .

Radiolysis of the methane (CH_4) and (N_2) induce a reaction where nitrogen/carbon compounds will recombine with other nitrogen/carbon compounds increasing their molecular

weights and complexity as the reaction continues. At this point, the organics will deposit as fine dust atop all areas inside the sputtering machine chamber. Glass slides positioned inside the chamber collect tholin deposits for collection and allow for tholin sampling while minimizing outside contamination (Figure 5).

Production of tholins in such a matter is a randomized process, as previously mentioned, and can be affected by differing production times and gas mixtures. For this reason, we compare the tholins produced at the University of Arkansas to others in the literature. As seen in Figure 6, large bands of nitrogen and carbon containing groups may be observed as well as nitrogen containing aromatic hydrocarbons and carbon double bonds. While some variation was expected, the results are qualitatively similar to organics in previous studies (Figure 7) (Gautier et al. 2012; Quirico et al. 2008). Spectra in Gautier et al. were measured in absorption coefficient ϵ , as their experimental protocol considered the effect of the film thickness. However, peaks in their resulting absorbance spectra remained consistent with Keck lab tholins.

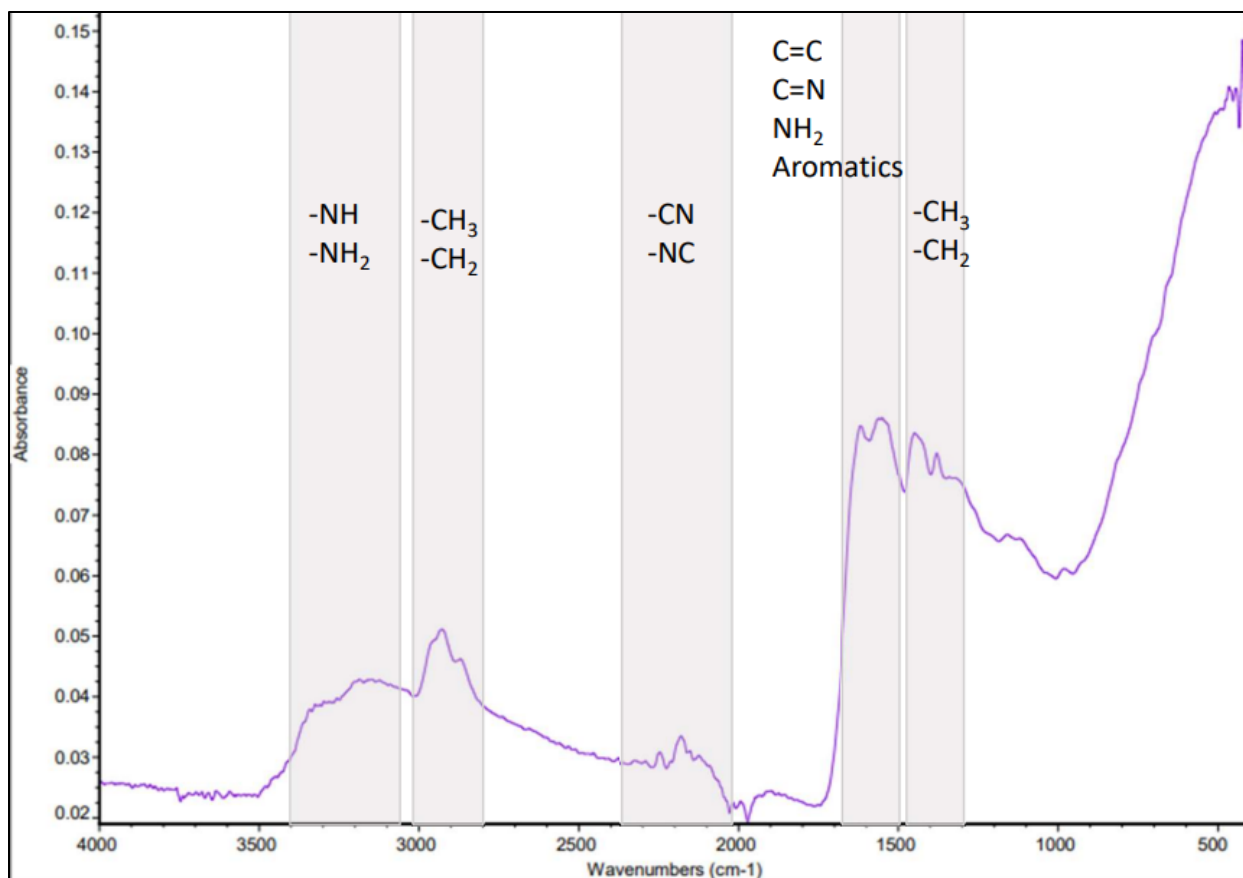


Figure 6: FTIR spectrum of tholins produced at the University of Arkansas utilizing a methane (CH₄) and nitrogen (N₂) gas mixture. While tholin production is a randomized process created by the dissociation and ionization of the carbon and nitrogen, regular peaks are observed in varying absorbances corresponding to specific bonds and structures. Keck Lab tholins display similar features as to organics produced in published literature, including producing a large number of C=C, C=N, NH₂, and aromatic groups.

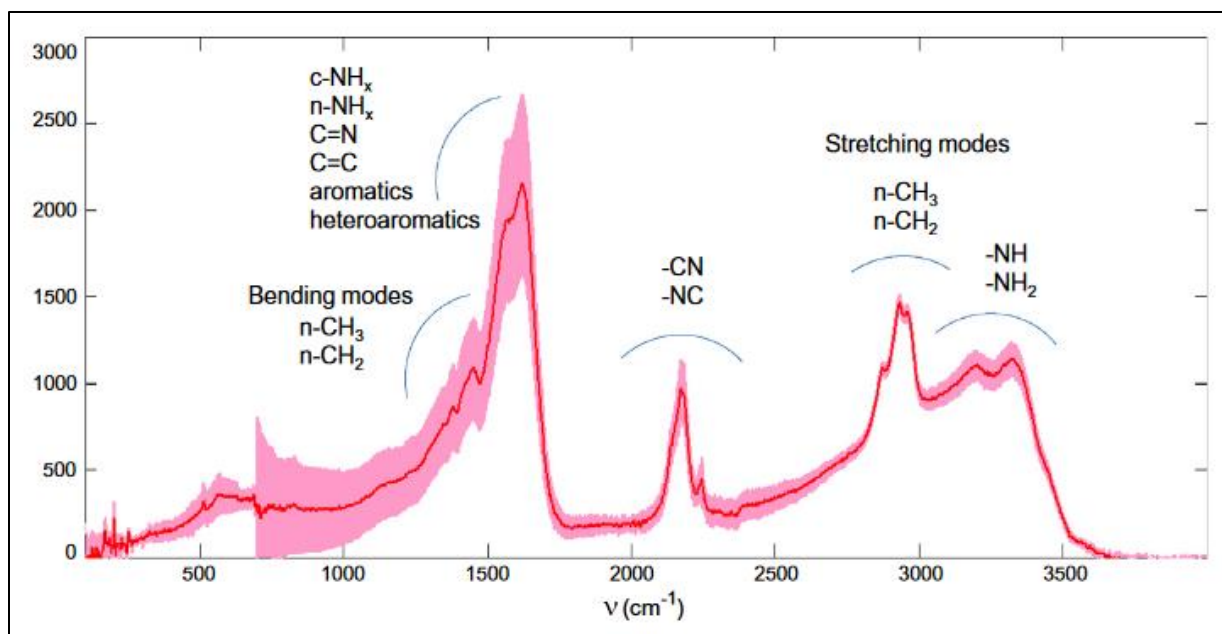


Figure 7: Evolution of ϵ from the far- to the mid-infrared for tholin produced in PAMPRE with 5% methane. Average spectrum is given by the red curve. Pink envelopes indicate this standard deviation (1σ) of the spectra, representing the variation of the spectrum from one measure to another on the same sample. Possible attributions are given for major bands of the spectrum. *n*-X and *c*-X mean that the functional group X is attached to, respectively, an aliphatic or an aromatic skeleton (Figure and caption from Gautier et al., 2012).

After production, tholins were kept in a sealed desiccator to reduce oxygen contamination. Samples were kept under argon along with Drierite absorbent to capture and reduce residual ambient moisture. Samples remained in the desiccator until analysis. Sample storage can be seen in Figure 8.

To better simulate the conditions on Titan, mixtures of non-polar and polar solvents were combined with ~5 mg of tholins at room temperature. Acetonitrile, hexane, as well as mixtures of acetonitrile and hexane, served as solvents. While methane and ethane are the abundant liquids on Titan, both are in the gas phase under ambient lab temperature and pressure. Therefore, hexane was used as a non-polar alkane solvent instead. While hexane and acetonitrile are typically assumed to be immiscible, previous studies have shown low amounts of mutual

solubility. Within the primarily hexane solution, a 0.035 mole fraction of acetonitrile in hexane has been found during studies around room temperature (Nagata 1987; Rusling et al. 1969). Low fractions of acetonitrile that would be present in this hydrocarbon phase would be most analogous to Titan conditions, with levels of polar compounds existing in the ppm range within the lakes (Corrales et al. 2017).



Figure 8: Tholin storage apparatus utilized. A sealed desiccator is kept at room temperature under argon (Ar) limiting the organics' potential reaction with ambient oxygen (O₂) and water (H₂O).

As both physical and chemical components of the sample needed to be investigated, a range of instrumentation was required.

Tholins within solvents of hexane, acetonitrile, and hexane saturated with acetonitrile were analyzed with Dynamic light scattering (DLS) to measure physical properties of the tholin particles, specifically their size and size ranges.

DLS utilizes Brownian motion to measure the particle hydrodynamic diameter distribution and a mean value of the tholin particles within the samples.

Additionally, DLS analysis provides the dispersity index, which will quantify the particle size

range on a scale of 0 to 1, with 0 describing a sample with all particle sizes the same (monodisperse) and 1 describing a sample with every particle size different (polydisperse). Samples were analyzed with the DLS instrument Brookhaven ZetaPALS with 10 mm pathway optical cuvettes.

Fourier-transform infrared spectroscopy (FTIR) was incorporated to observe band shifts and changes within the solid precipitate in the tholin solvent mixtures. A Shimadzu IRAffinity-1S FTIR with an IR source and KBr beamsplitter which was fitted with an attenuated total reflection (ATR) attachment was utilized to analyze the solid tholin precipitate of the samples.

To investigate how various production timescales impact tholin structure within a laboratory setting, a method was needed to look at the molecular compounds and structures within the samples. Laser desorption/ionization (LDI)- time-of-flight mass spectrometer (TOF) and matrix-assisted laser desorption/ionization (MALDI) were utilized in the investigations described in chapters 1, 3 and 4. The LDI and MALDI utilized a 337 nm nitrogen laser and 355 nm YAG laser 10 to 50 Hz.

All methods are incorporated and expanded upon in each chapter. Projects will include their own experimental protocols in line with their science questions and objectives. Protocols will be discussed in each corresponding chapter's experimental methods section chapter.

Dissertation Outline

This dissertation is comprised of an introduction with a description of the science background and a general methodology followed by four chapters, each with a corresponding project. Chapters will explain each project's science background, methodology, and results. Chapter 1 will describe an investigation into the effects of varying production times on the structures of tholins. Chapter 2 will explain a study into the particle sizes of tholins in polar, non-polar, and a mixture of polar and nonpolar solvents relevant to the lakes and the dune regions of Titan. Chapter 3 will explain the solubility of tholins produced with a 95% nitrogen (N_2) and 5% methane (CH_4) gas mixture within polar, non-polar, and a mixture of polar and nonpolar solvents relevant to Titan. Both the solid and liquid fractions within each sample will be discussed. Chapter 4 will follow up on previous solubility experiments and utilize a gas mixture incorporating carbon monoxide (CO). The dissertation will end with the conclusions and synthesis chapter relating the conclusions and results from all studies and their specific relevance to Titan.

References

- Barnes, W., Lorenz R., Radebaugh J., Hayes A., Arnold K., and Chandler C. 2015. "Production and Global Transport of Titan's Sand Particles." *Planetary Science* 4 (1): 1. <https://doi.org/10.1186/s13535-015-0004-y>.
- Carrasco, N., I. Schmitz-Afonso, J-Y. Bonnet, E. Quirico, R. Thissen, Odile Dutuit, A. Bagag, et al. 2009. "Chemical Characterization of Titan's Tholins: Solubility, Morphology and Molecular Structure Revisited." *The Journal of Physical Chemistry A* 113 (42): 11195–203. <https://doi.org/10.1021/jp904735q>.
- Clark, Roger N., John M. Curchin, Jason W. Barnes, Ralf Jaumann, Larry Soderblom, Dale P. Cruikshank, Robert H. Brown, et al. 2010. "Detection and Mapping of Hydrocarbon Deposits on Titan." *Journal of Geophysical Research* 115 (E10): E10005. <https://doi.org/10.1029/2009JE003369>.
- Coll, P., D. Coscia, N. Smith, M.-C. Gazeau, S.I. Ramírez, G. Cernogora, G. Israël, and F. Raulin. 1999. "Experimental Laboratory Simulation of Titan's Atmosphere: Aerosols and Gas Phase." *Planetary and Space Science* 47 (10–11): 1331–40. [https://doi.org/10.1016/S0032-0633\(99\)00054-9](https://doi.org/10.1016/S0032-0633(99)00054-9).
- Cordier, D., Olivier M., Jonathan I. L., Panayotis L., and Véronique V.. 2009. "An Estimate of the Chemical Composition of Titan." *The Astrophysical Journal* 707 (2): L128–31. <https://doi.org/10.1088/0004-637X/707/2/L128>.
- Corrales, L. R., Thomas D. Yi, Trumbo S., Shalloway D., Lunine J., and Usher D. 2017. "Acetonitrile Cluster Solvation in a Cryogenic Ethane-Methane-Propane Liquid: Implications for Titan Lake Chemistry." *The Journal of Chemical Physics* 146 (10): 104308. <https://doi.org/10.1063/1.4978395>.
- Gautier, T., Carrasco N., Mahjoub A., Vinatier S., Giuliani A., Szopa C., Anderson C., Jean-Jacques C., Dumas P., and Cernogora G.. 2012. "Mid- and Far-Infrared Absorption Spectroscopy of Titan's Aerosols Analogues." *Icarus* 221 (1): 320–27. <https://doi.org/10.1016/j.icarus.2012.07.025>.
- He, Chao, Guangxin L., Upton K., Imanaka H., and Smith M. 2012. "Structural Investigation of Titan Tholins by Solution-State ^1H , ^{13}C , and ^{15}N NMR: One-Dimensional and Decoupling Experiments." *The Journal of Physical Chemistry A* 116 (19): 4760–67. <https://doi.org/10.1021/jp3016062>.

- Hörst, S. M. 2017. "Titan's Atmosphere and Climate." *Journal of Geophysical Research: Planets* 122 (3): 432–82. <https://doi.org/10.1002/2016JE005240>.
- Hörst, S.M., R.V. Yelle, A. Buch, N. Carrasco, G. Cernogora, O. Dutuit, E. Quirico, et al. 2012. "Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere Simulation Experiment." *Astrobiology* 12 (9): 809–17. <https://doi.org/10.1089/ast.2011.0623>.
- Khare, B.N., Sagan C., E.T. Arakawa, H. Ogino, T.O. Willingham, and B. Nagy. n.d. "Amino Acid Analysis of Titan Tholins." In , 843.
- Lorenz, R. D., S. Wall, J. Radebaugh, G. Boubin, E. Reffet, M. Janssen, E. Stofan, et al. 2006. "The Sand Seas of Titan: Cassini RADAR Observations of Longitudinal Dunes." *Science* 312 (5774): 724–27. <https://doi.org/10.1126/science.1123257>.
- Lorenz, R. D, Turtle E., Barnes J., and Trainer M. 2018. "Dragonfly: A Rotorcraft Lander Concept for Scientific Exploration at Titan." *Johns Hopkins APL Technical Digest* 34 (3): 14.
- McKay, C. P. 1996. "Elemental Composition, Solubility, and Optical Properties of Titan's Organic Haze." *Planetary and Space Science* 44 (8): 741–47. [https://doi.org/10.1016/0032-0633\(96\)00009-8](https://doi.org/10.1016/0032-0633(96)00009-8).
- Miller, S. 1953. "A Production of Amino Acids under Possible Primitive Earth Conditions." *Science* 117 (3046): 528–29.
- Niemann, H. B., S. K. Atreya, S. J. Bauer, G. R. Carignan, J. E. Demick, R. L. Frost, D. Gautier, et al. 2005. "The Abundances of Constituents of Titan's Atmosphere from the GCMS Instrument on the Huygens Probe." *Nature* 438 (7069): 779–84. <https://doi.org/10.1038/nature04122>.
- Quirico, E., Montagnac G., Lees V., McMillan P., Szopa C., Cernogora G., Rouzaud J.N., et al. 2008. "New Experimental Constraints on the Composition and Structure of Tholins." *Icarus* 198 (1): 218–31. <https://doi.org/10.1016/j.icarus.2008.07.012>.
- Rannou, P., F. Montmessin, F. Hourdin, and S. Lebonnois. 2006. "The Latitudinal Distribution of Clouds on Titan." *Science* 311 (5758): 201–5. <https://doi.org/10.1126/science.1118424>.

- Raulin, F., Brassé C., Poch O., and Coll P.. 2012. “Prebiotic-like Chemistry on Titan.” *Chemical Society Reviews* 41 (16): 5380. <https://doi.org/10.1039/c2cs35014a>.
- Raulin, F., Hand K. P., McKay C.P., and Viso M. 2010. “Exobiology and Planetary Protection of Icy Moons.” *Space Science Reviews* 153 (1–4): 511–35. <https://doi.org/10.1007/s11214-009-9610-x>.
- Rusling, J. F., R. J. Bertsch, R. A. Barford, and H. L. Rothbart. 1969. “Liquid Equilibria in the System Methyl Oleate-Methyl Palmitate-Acetonitrile-Hexane. Significance in Craig Countercurrent Extraction.” *Journal of Chemical & Engineering Data* 14 (2): 169–73. <https://doi.org/10.1021/jc60041a018>.
- Sagan, C. 1971. “The Solar System beyond Mars: An Exobiological Survey.” *Space Science Reviews* 11: 827–66.
- . 1973. “The Greenhouse of Titan.” *Icarus* 18: 649–56.
- Sagan, C., and Khare B. N.. 1979. “Tholins: Organic Chemistry of Interstellar Grains and Gas.” *Nature* 277: 102–7.
- Sagan, C., Khare B. N., W. Reid Thompson, G.D. McDonald, Michael Wing, Jeffrey Bada, Tuan Vo-Dinh, and E.T. Arakawa. 1993. “Polycyclic Aromatic Hydrocarbons in the Atmospheres of Titan and Jupiter.” *The Astrophysical Journal*, no. 414: 399–405.
- Sagan, C., and Khare B.N. 1981. “The Organics Clouds of Titan.” In , 13:701.
- Sagan, C., and Thompson W.R., n.d. “Production and Condensation of Organics Gases In the Atmosphere of Titan.” *Cornell University for Radiophysics and Space Research*, 56.
- Stofan, E. R., C. Elachi, J. I. Lunine, R. D. Lorenz, B. Stiles, K. L. Mitchell, S. Ostro, et al. 2007. “The Lakes of Titan.” *Nature* 445 (7123): 61–64. <https://doi.org/10.1038/nature05438>.
- Szopa, C., Cernogora G., Boufendi L., Correia J. J., and Coll P. 2006. “PAMPRE: A Dusty Plasma Experiment for Titan’s Tholins Production and Study.” *Planetary and Space Science* 54 (4): 394–404. <https://doi.org/10.1016/j.pss.2005.12.012>.

- Tan, S. P., Kargel J. S., and Marion G.M. 2013. "Titan's Atmosphere and Surface Liquid: New Calculation Using Statistical Associating Fluid Theory." *Icarus* 222 (1): 53–72. <https://doi.org/10.1016/j.icarus.2012.10.032>.
- Tomasko, M. G., and Smith P. H. 1982. "Photometry and Polarimetry of Titan: Pioneer 11 Observations and Their Implications for Aerosol Properties." *Icarus* 51 (1): 65–95. [https://doi.org/10.1016/0019-1035\(82\)90030-6](https://doi.org/10.1016/0019-1035(82)90030-6).
- Tomasko, M.G., L. Doose, S. Engel, L.E. Dafoe, R. West, M. Lemmon, E. Karkoschka, and C. See. 2008. "A Model of Titan's Aerosols Based on Measurements Made inside the Atmosphere." *Planetary and Space Science* 56 (5): 669–707. <https://doi.org/10.1016/j.pss.2007.11.019>.
- Trainer, M. G., Pavlov A. A., DeWitt L. H., Jimenez J. L., McKay C. P., Toon O. T., and Tolbert M. A. 2006. "Organic Haze on Titan and the Early Earth." *Proceedings of the National Academy of Sciences* 103 (48): 18035–42. <https://doi.org/10.1073/pnas.0608561103>.
- Turtle, E. P., J. E. Perry, A. G. Hayes, R. D. Lorenz, J. W. Barnes, A. S. McEwen, R. A. West, et al. 2011. "Rapid and Extensive Surface Changes Near Titan's Equator: Evidence of April Showers." *Science* 331 (6023): 1414–17. <https://doi.org/10.1126/science.1201063>.
- Waite, J. H., D. T. Young, T. E. Cravens, A. J. Coates, F. J. Crary, B. Magee, and J. Westlake. 2007. "The Process of Tholin Formation in Titan's Upper Atmosphere." *Science* 316 (5826): 870–75. <https://doi.org/10.1126/science.1139727>.

Chapter 1: Effect of production timescales on the properties and compositions of CH₄-N₂-derived tholins

Introduction

The haze layer present in the atmosphere of Titan is comprised of organics produced through photolysis of primarily methane (CH₄) and nitrogen (N₂). The Voyager and Cassini-Huygens missions sampled compounds from Titan's atmosphere and found a large prevalence of ammonia (NH₃) and hydrogen cyanide (HCN) (Yung et al. 1984; Hanel et al. 1981; de Kok et al. 2007; Israël et al. 2005). Organics produced in the atmosphere of Titan have been found to have varying levels of complexity. Structures ranging from primary methane (CH₄) to larger, more complex molecules such as simple polycyclic aromatic hydrocarbons (PAHs) and complex nitrogen containing polycyclic aromatic hydrocarbons (N-PAHs) have been found through experimental studies (Niemann et al. 2005; Waite et al. 2007; McGuigan et al. 2006; Coll et al. 1999; Gautier et al. 2012; Imanaka et al. 2004).

While some missions are planned to investigate these organics in situ, current experimental studies rely on laboratory analogs to these organics, also called 'tholins' (Lorenz et al. 2018). Previous investigations have compared the tholins produced in laboratories to the compounds found in the Cassini missions. Gautier et al. compared tholins in various studies to Cassini-CIRS data and found that spectra are in good agreement. During Gautier's analysis many absorption peaks, specifically those at 1450 cm⁻¹, 1380 cm⁻¹, 515 cm⁻¹ and 325 cm⁻¹ were present across tholins and Cassini-CIRS spectra. While some variations are to be expected from the randomized process of tholin production, the agreement of these results support tholins as an analog to investigate Titan organics on Earth.

During laboratory studies, production of tholins is carried out through various techniques incorporating a range of gas mixtures, pressures, production times, and energy sources. Some variations in the tholins are to be expected as each compound is produced through a randomized process. However large variations in tholins' structures and compositions have been observed based on changing any one of the previously mentioned variables (Gautier et al. 2012; Imanaka et al. 2004; Sagan and Khare 1979).

Even though, the structure of tholins most often referred to as $(\text{CH}_2)_m(\text{HCN})_n$ co-polymers based on the existing limited amount of high resolution mass spectrometry data, it is without doubt that much more complex polymers are also present (Rüger et al. 2019). Studies have shown that variability in certain experimental protocols can affect the structure of resulting tholins. Various protocols have been implemented and tested such as pressure (Imanaka et al. 2004), energy source (Sagan and Khare 1979), gas composition (Gautier et al. 2012), and time dependencies (Imanaka et al. 2004) and have been observed to have structural implications on the resulting tholins.

Imanaka et al investigation of varying pressure effect on tholin structures shows that the incorporation of nitrogen was affected by the pressures at which the production of tholins occurred. Results indicated that nitrogen was incorporated in higher quantities at lower pressures, specifically into carbon chains. Carbon diimides ($-\text{N}=\text{C}=\text{N}-$) and isocyanides ($-\text{C}-\text{N}\equiv\text{C}$) were both mentioned as possible structural incorporation. Higher deposition experimental protocols were found to produce tholin structures that incorporate nitrogen into terminal groups, such as nitriles ($-\text{C}\equiv\text{N}$; Imanaka et al. 2004). Additionally, Imanaka et al observed the differences in tholins structures produced for 1, 3, 5, and 9 hr. IR absorbance spectra showed a proportional increase in peak height as production times increased.

Sagan and Khare investigated the effects of UV and spark discharge on tholins produced with various gas mixtures (Sagan and Khare 1979). From their gas chromatography-mass spectroscopy spectra, compounds produced from spark electrical discharges were observed to have more complex structures. Results showed a higher number of cyclical compounds (such as pyrroles, pyridines, and aromatics) being observed within tholin samples produced with electrical spark discharge.

Gautier observed the influence of various methane (CH_4) concentrations in $\text{N}_2:\text{CH}_4$ gas mixtures utilized in tholin production. Methane concentrations of 1%, 2%, 5% and 10% balanced with nitrogen (N_2) were observed. IR absorbance spectra found that the lower concentrations of methane contained higher amounts of nitrogen within the structures, primarily amines and a movement of unsaturated and saturated nitriles. Additionally, lower concentrations of methane were found to contain lower amounts of saturated aliphatic carbons.

Tholins Reactivity

Recent studies on the methanol-soluble and on the water-soluble portion of tholins using ion mobility coupled to Fourier transform mass spectrometry reveals the existence of more than 1500 compounds, with a majority of the structures related to CHN_x ($x=3-12$) class compounds (Rüger et al. 2019; Gautier et al. 2014). Rüger et al. found that tholins contain a stepwise addition of $\text{C}_2\text{H}_3\text{N}$ into different compounds present in the samples. Gautier et al. (2014) found that in a water/methanol solvent, amines, triple bonded nitrogen and cyclic compounds were able to be found in tholin samples.

While previous studies have observed structural changes of tholins produced over various timescales (Imanaka et al. 2004), none have investigated the earliest observations of tholin production. Since consistency is important in laboratory investigations utilizing tholins, our

study investigates the larger molecular themes within tholins produced over time periods of 1, 4, 6, and 12 hours. We have studied any changes between tholins produced at different timescales and narrowed down at which time point consistency can be seen.

Methods

Tholin Synthesis

Tholins were produced using a modified Hummer Technics II sputtering machine utilizing a cold plasma discharge at 45 DC mA, 10.5 V. A gas mixture of 95% CH₄/5% N₂ was exposed to these conditions at a flow rate of < 0.5 standard cubic feet per minute (SCFH) and an ambient pressure of ~ 4.0 - 5.0



Figure 9: MALDI sample disks placed into holding tray after tholin deposition. Tholins were produced for 1 hour (middle left disk), 4 hours (lower left disk), 6 hours (lower middle disk), and 12 hours (lower right disk). The control disk is located on upper right corner.

Torr. Tholins were deposited onto MALDI disks for easy analysis (Figure 1). Prior to tholins deposition, each MALDI disk underwent cleaning using DI water, HPLC grade methanol, 140 proof ethanol, and HPLC grade acetone successively before a final heating for at least 30 minutes at 80 °C, ensuring all solvents had been fully evaporated. A range of tholin deposition times were utilized to observe changes over different production times on stainless steel MALDI disks. Tholin production was run for 1, 4, 6, and 12 hours. After the organic deposits were made, the stainless steel MALDI disks were held under Ar in a desiccator with DrieRite desiccant for

no more than 72 hours to mitigate oxygen contamination. Tholins were only removed for analysis to limit atmospheric exposure.

Analytical Methods

The synthesized tholins were analyzed with direct desorption ionization mass spectroscopy using a 355 nm YAG laser coupled to a Bruker Ultraflex II matrix-assisted laser desorption/ionization – time of flight- mass spectrometer (MALDI-TOF-MS). After the direct desorption studies, MALDI disks with the tholin deposits were returned to the lab and submerged in 5 mL of DI solvent water to remove the tholins from the surface of the disks. The resulting DI water and tholin solution was then subjected to electrospray ionization-mass spectrometry (ESI-MS) by a Shimadzu-IT-TOF-MS.

Results

MALDI spectra of tholins as a function of synthesis duration are shown in Figure 2. Overall, peaks observed were similar in 1, 4, 6, and 12 hours with a clear evolution of the spectral pattern changing as function of time. Peaks found in a control MALDI plate without tholins deposited were considered as background peaks (see Figure 2, top spectrum) and were subsequently ignored. Before analysis, a brown color of the organic deposit was observed that become more intense in each increasing time of production sample.

Peaks were observed at every mass, specifically in the lower m/z ranges under 200 m/z . Peak distributions were observed in the 1 hour sample around 80.4, 96.4, 112.5, 122.5, 140.5, 156.6., 196.6 m/z all with heights under 1×10^4 intensity (au). Peak distributions were observed in the 4 hour sample at 69.4, 86.5, 96.4, 112.5, 124.7, and 152.7 m/z with heights around 0.5×10^5 intensity (au). In the 6-hour samples, peak distributions were found at 68.4, 85.5, 97.6, 123.7, 151.8, and 190.9 m/z with heights around 0.8×10^4 intensity (au). Finally, peak distributions were

seen in the 12 hour samples at 68.4, 85.5, 97.6, 123.7, 151.8, and 190.9 m/z with heights around 3.0×10^4 intensity (au).

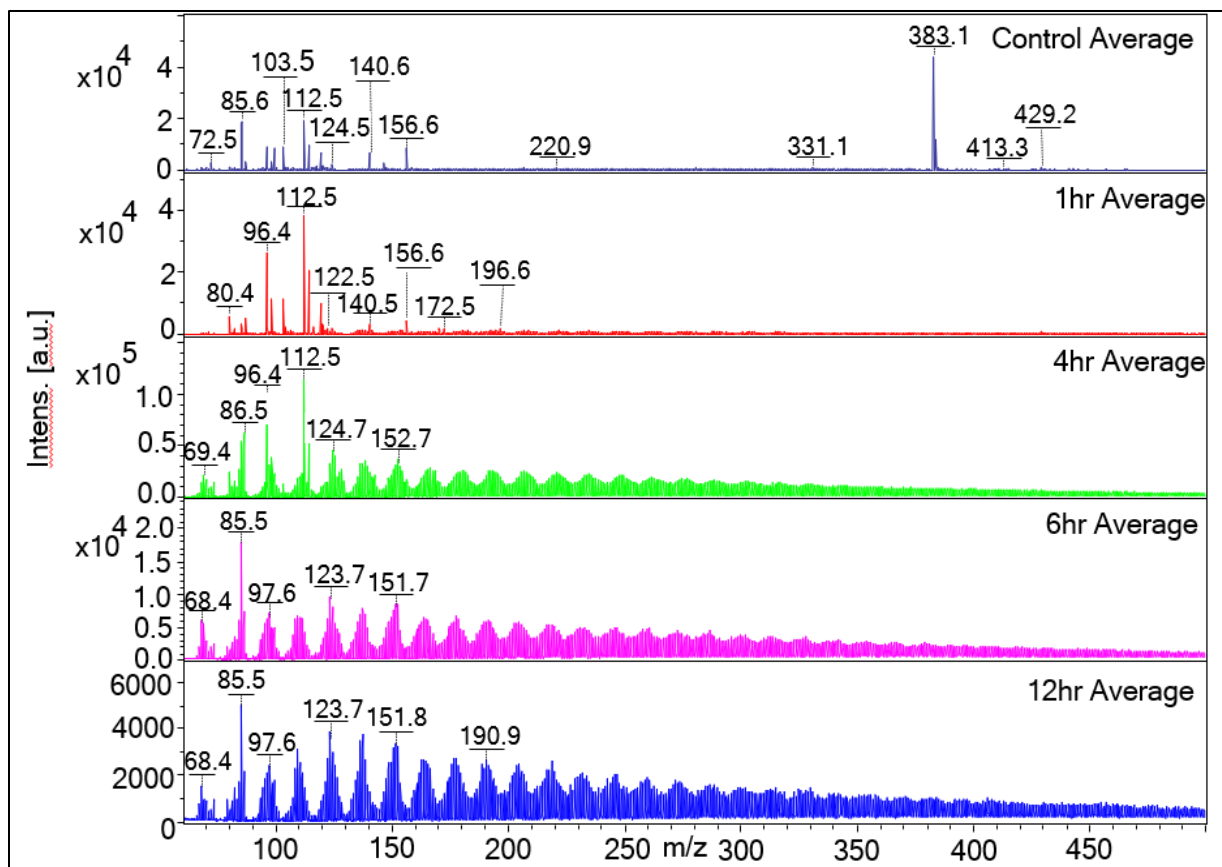


Figure 10: Direct desorption ionization mass spectra of tholins produced at times of 1 hour (red), 4 hours (green), 6 hours (pink), and 12 hours (blue, bottom). Control was taken of a sample disk without any tholins produced on it (navy, top). Note that relative peak heights above m/z 200 increase with the tholin production time.

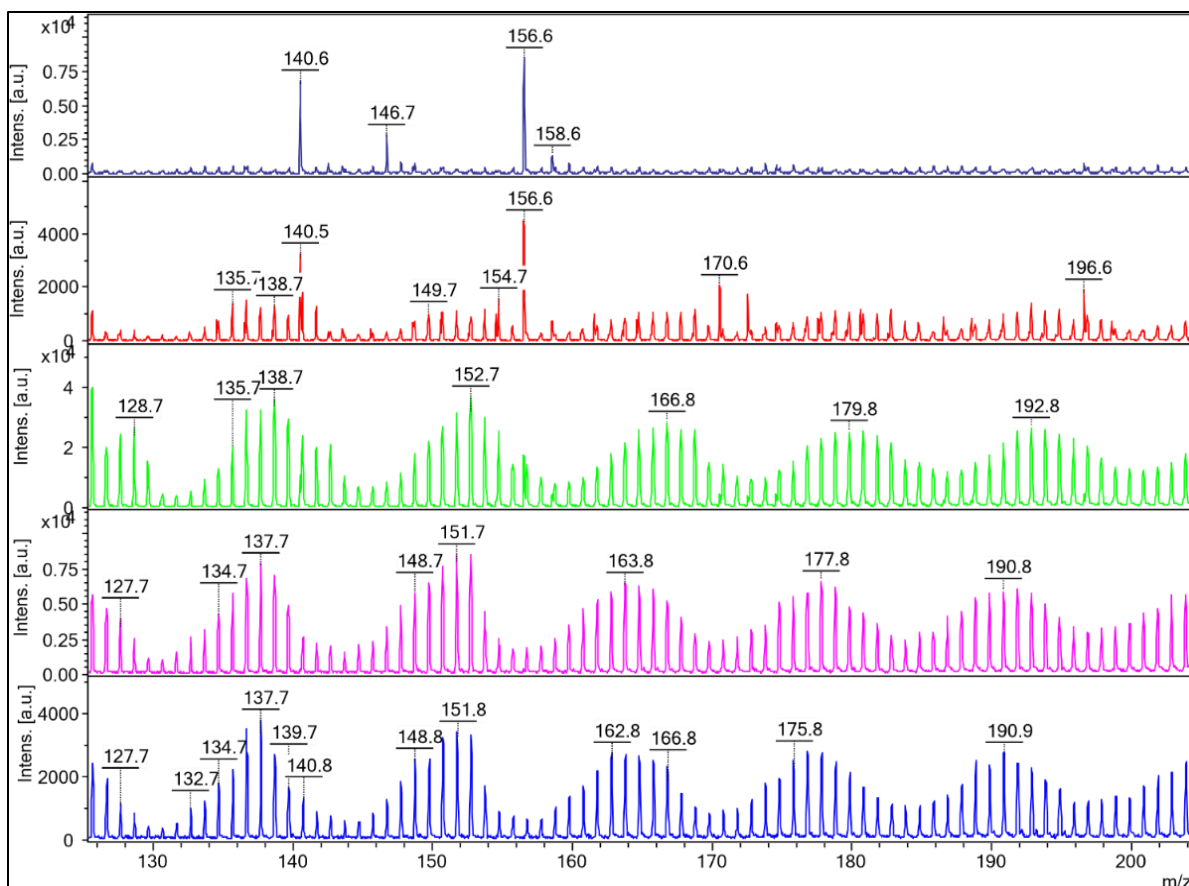


Figure 3: Mass spectra taken from 1 hour (red), 4 hour (green), 6 hour (pink), and 12 hours (blue, bottom) focused on the 120-200 m/z range. Control spectra of sample disk without tholin depositions is also displayed (navy, top).

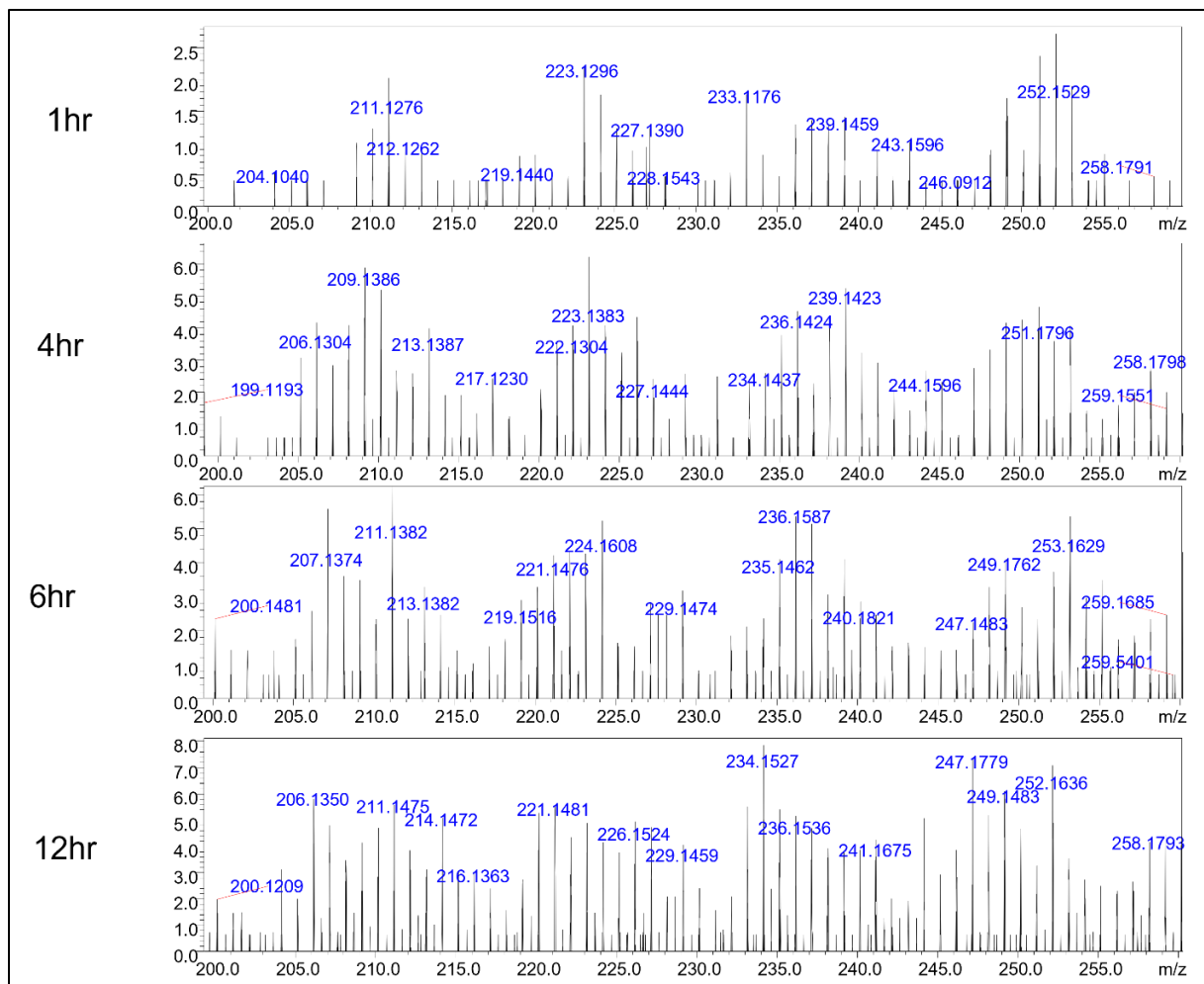


Figure 4: ESI mass spectra after MALDI disks with tholin deposits were returned to the lab and submerged in DI solvent water to remove the tholins from the surface of the disks. Resulting spectra showed similarities to the shift of peaks to lower m/z observed within the direct desorption ionization.

ESI-MS analysis was done on the DI liquid that was used to wash off the organics after MALDI analysis. Spectra collected from this analysis is shown in Figure 4. While the resolution and limitations of ESI-MS constrain any conclusion about molecular composition, a potential trend is possible between the samples, similar to that observed in the solid samples. For example, peaks can be seen at every molecular weight with Gaussian distributions observed in the 230-245 m/z range, with the middle peaks in the gaussian distribution moving from 239 m/z in the 1 hour sample, to 236 m/z in the 4 hour sample, to 234 m/z in the 12 hour sample. For additional data on repeated experiments, see the appendix.

Tholin Experimental Structures

Previous experiments have investigated compounds created in the atmosphere of Titan with tholins on Earth. Spectra from our study show a slight spectral shift in the Gaussian distributions with each increasing production time. While focusing on lower mass compounds (120-200 m/z range) in the direct desorption ionization spectra, with the larger gaussian distribution of the peaks shifts to lower m/z can be clearly seen as the deposition time increase (Figure 3). For example, this can be seen in the spectral distributions within the 230-245 m/z range, with the middle peaks in the gaussian distribution moving from 239 m/z in the 1 hour sample, to 236 m/z in the 4 hour sample, to 234 m/z in the 12 hour sample. We interpret this result to be an indication that as production times increase, a preference for carbon double bonds (C=C) and ring structures (as these would be the most thermodynamically stable at higher molecular weights) instead of carbon hydrogen bonds (C-H). The absence of hydrogens within the structures at such as slight rate might cause in the spectral shifts within our data.

Our results are in line with previously conducted studies regarding a shift from saturated to unsaturated structures within tholins (Rüger et al. 2019; Carrasco et al. 2009). Rüger et al.

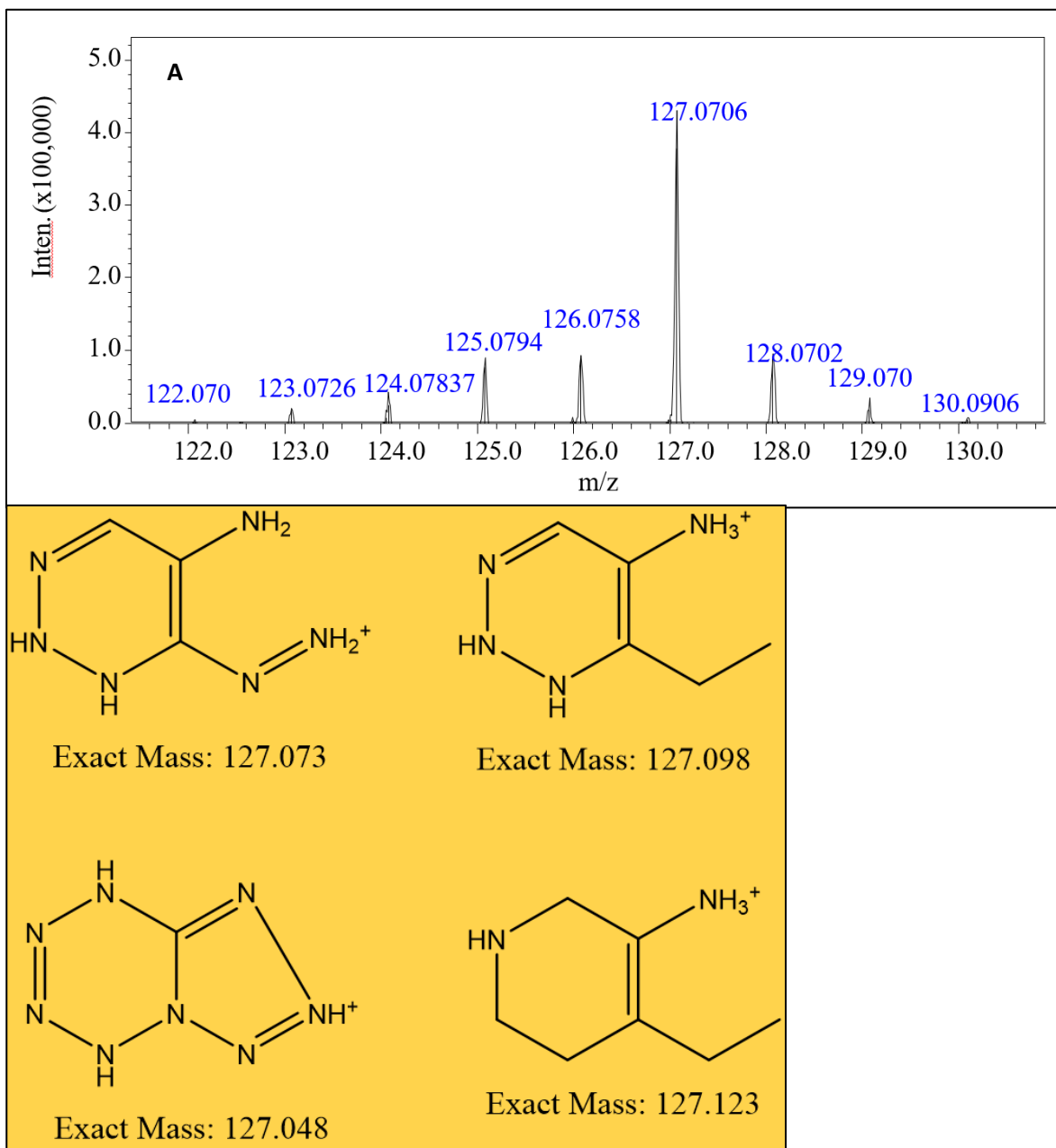
2019 reported similar results, concluding that C_2H_3N building blocks were being primarily incorporated in higher molecular weights. Carrasco et al. 2009 found that lower molecular weight structures of aliphatic chains ending in a methyl, amine, or nitrile group (Carrasco et al. 2009). Previous experiments have also shown that the aromatics within the tholins samples produced at higher timescales will have higher concentrations of nitrogen and carbon as unsaturation increases (Gautier et al. 2014). Therefore, N-PAH's production might increase with increasing deposition time.

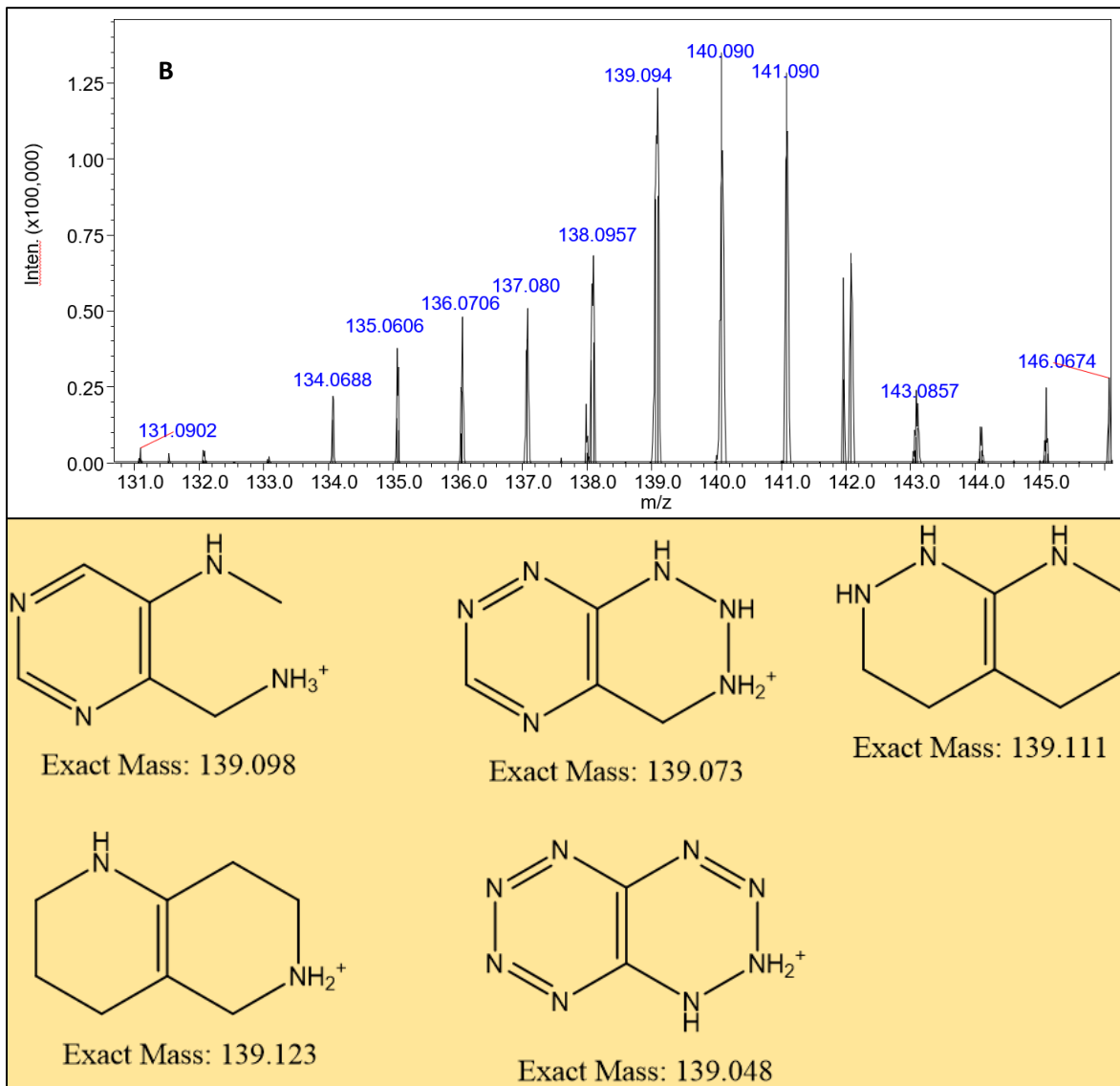
Our observations of rings and double bonds increasing as the as production times increases, echo the results found in these previous studies. With our and previous studies observing increasing number of C-C bonds seen within our results, we can conclude that the carbon within the higher timescales are bonding at increasing rate with other carbon atoms instead of other atoms present (such as hydrogen or nitrogen in this study) leading to this increase in unsaturated bonds. As such this will lead to an increase in larger carbon-based molecules in higher deposition times, specifically aromatics ring structures as they are the more thermodynamically stable.

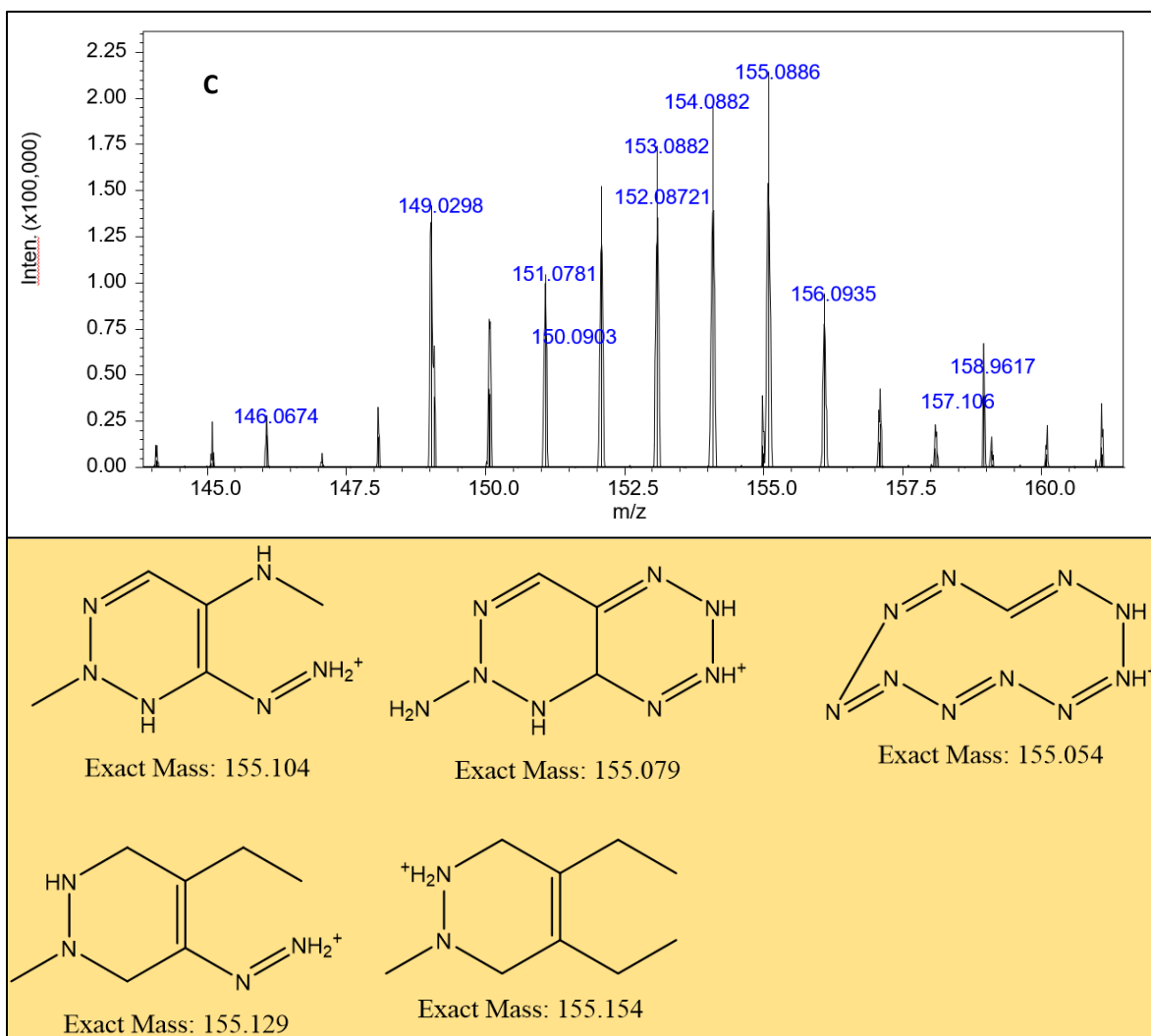
It is possible that the production of tholins is more analogous to a closed cyclization process, with the smaller aliphatic chains initially produced reacting over again, each time gaining weight and complexity, eventually moving into the more thermodynamically stable N-PAH structures. From the perspective of tholin production as a cyclical process, each observed peak in low resolution mass spectrometers might be representative of multiple individual compounds' molecular weights and isotopic distributions. Peaks might incorporate a variety of compounds within a particular peaks range. Figure 5 displays possible structures that fit various peaks observed within our study protocols.

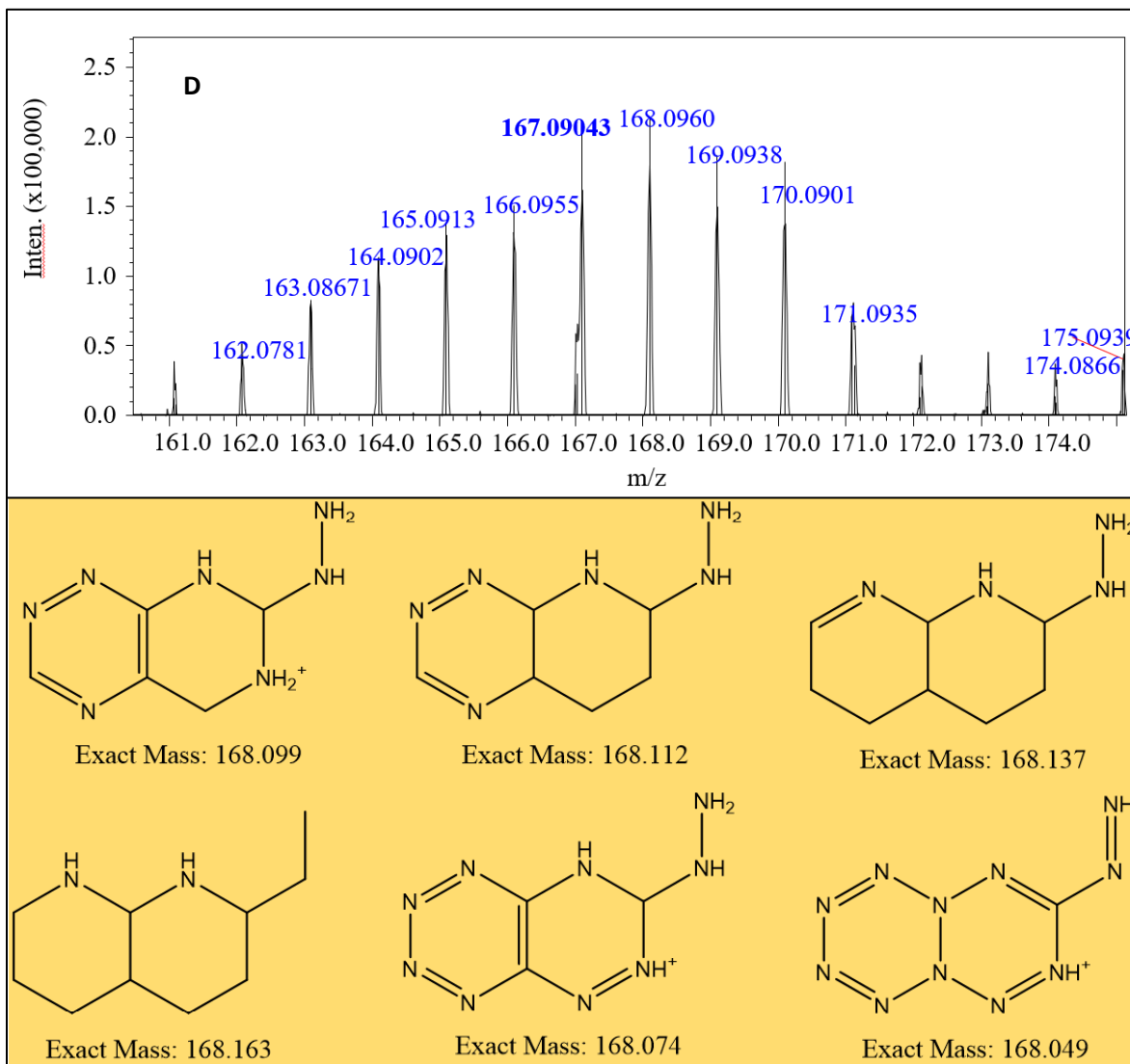
Structures proposed in Figure 5, incorporate attributes from ours and previous studies results. As our methodology utilized a gas mixture of 90% nitrogen (N₂)/10% methane (CH₄), molecules proposed are comprised of primarily carbon, along with nitrogen and hydrogen. Stable rings structures with high numbers of unsaturated bonds can be created within peaks in each range. Additionally, many of these compounds proposed are aromatic, as they will be the most stable. If present within the Titan atmosphere, these structures could be included in reactions producing biologically important compounds, as aromatic structures as necessary for some biological molecules (e.g. nucleotides, some proteins).

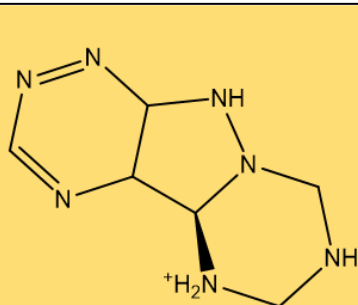
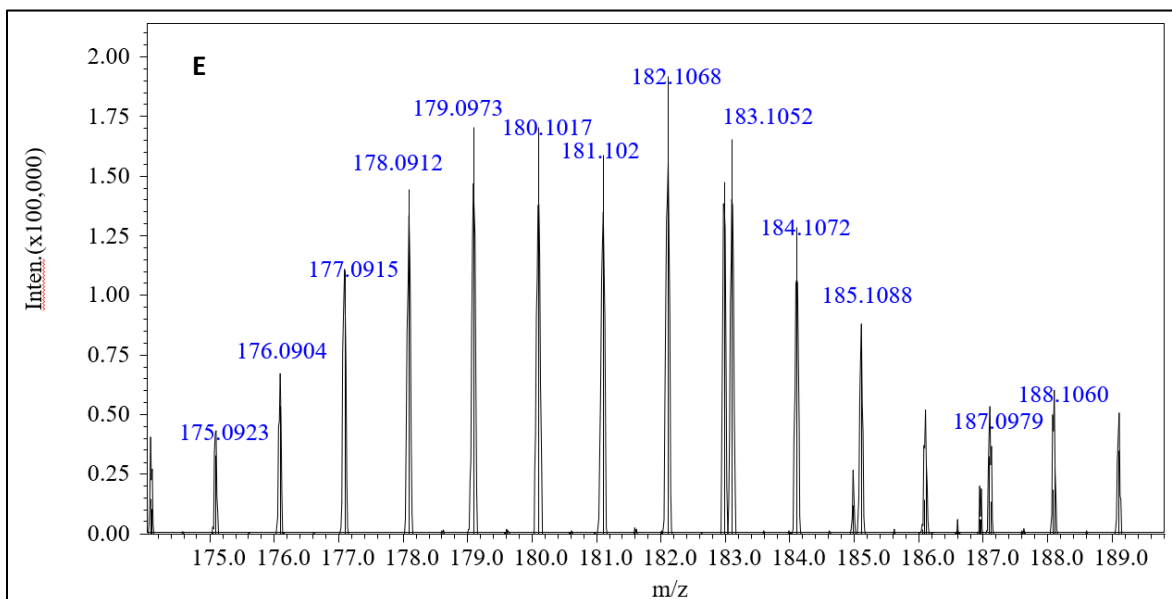
Figure 5: Potential structures that could be present within our sample and being displayed in a singular direct desorption peak. Peaks in the 120-130 m/z (A), 130-150 m/z (B), 145-160 m/z (C), 160-174 m/z (D), and 175-189 m/z (E) are shown below along with their potential structures and corresponding molecular weight.



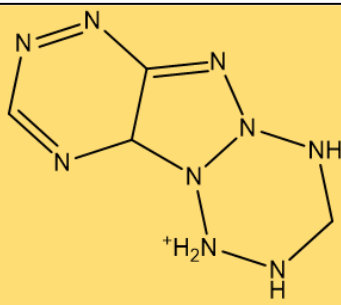




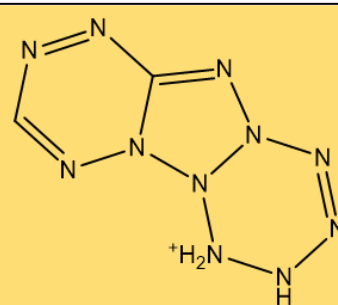




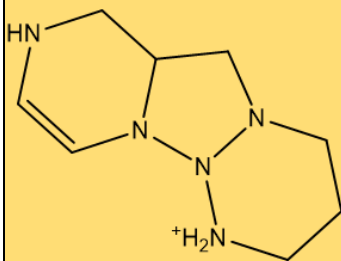
Exact Mass: 182.115



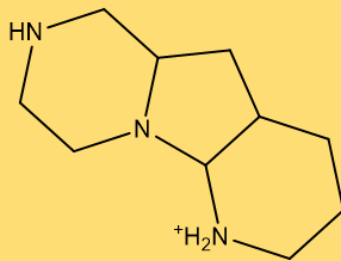
Exact Mass: 182.090



Exact Mass: 182.065



Exact Mass: 182.140



Exact Mass: 182.165

Discussion

Our study is currently limited by low resolution compared to previous tholin studies. However, our results could show minor differences between organic compounds produced within different production time scales. Interestingly, minor peaks are still observed during the 1 hour time scales with similar peaks to those observed in the 4, 6, and 12 hour time periods. Furthermore, trends of observing organics present at every molecular weight can also be seen in the liquid ESI spectra, however the 1 hour sample was not able to be replicated. This was likely do to a mechanical error in the gas line for the sputtering machine that occurred during the 1 hour production repeat.

While previous studies have shown no differences between time scales of 1, 3, 5, and 9 hours, to the best of our knowledge, no experiments have been conducted on these longer time scales, specifically of 12 hour timescales (Imanaka et al. 2004). Imanaka et al. (2004) utilized infrared spectroscopy and found no or very little differences between samples that were deposited at 1, 3, 5, and 9 hour time scales, only that the volume of tholins were directly proportional to production time (Imanaka et al. 2004).

Our study shows evidence of changes within the molecular weight of compounds produced within different production time scales. One explanation for this shift is an increase in unsaturation or rings and double bonds as the as production times increase. Carbon structures constituting of primarily single bonds will be able to bond with more atoms, increasing their overall weight. As double bond and ring structures are produced, less atoms can be added, and therefore the molecular weight will decrease. From the larger quantities of peaks observed in the gaussian distributions of direct infusion ESI mass spectra taken from the water-soluble portion of tholin as shown in Figure 4 the shifting of the gaussian distributions the lower molecular weight

in not as clear (as compared to the MALDI spectra). However, one can still observe the same behavior in some of the peaks, such as the shift in the 230-245 m/z gaussian distribution peak from 236 m/z to 234 m/z.

From these results, it is possible that tholin production is of an increasing cyclic nature of nitrogen containing organic compounds, with molecules produced having an affinity to react with other compounds to reach a thermodynamically stable complex structure. This is indicated by overall shift in MALDI and electrospray ionization mass spectra. These are most likely N-PAHs with the capability of trapping UV light. Obviously, this could be related to generally the total produced mass, but also to the increased number of nitrogen containing PAHs. Finally, our shows that chemistry might be occurring at early timescales that are less than 1 hour. Spectra from the MALDI show that organics are being produced at 1 hour producing similar spectral features that are observed in the 4, 6, and 12 hour production times.

Conclusion

In this work we attempted to synthesize Titan aerosol simulants (or tholins) using various production timescales from 1 to 12 hours using a mixture of N₂ and CH₄ in a 95 - 5 vol% ratio. From direct desorption ionization and MALDI-ESI analysis, we conclude that tholin production and chemistry is occurring at very early stages with timescales less than 1 hour. It is possible that processes producing these tholins are occurring <1 hour of production time.

The production of tholins in a laboratory setting is crucial for experimental investigations. It is important to characterize the structural changes that can occur while producing tholins in order to provide context to experimental results and their relation to the Titan environment. If these types of compounds and trends are observed on Titan (by future

missions such as Dragonfly (Lorenz et al. 2018)), then the reactions occurring in the moon's atmosphere might be able to provide insight into the organics that are located on the surface.

Future studies should include elemental and compositional analyses to further understand the resulting compounds from these different time scales. While our study can provide insight into general trends for compounds produced through radiolysis, investigations utilizing analysis into molecular composition, such as GC-MS, could confirm the presence of molecules proposed in this study.

References

- Carrasco, N., I. Schmitz-Afonso, J-Y. Bonnet, E. Quirico, R. Thissen, Odile Dutuit, A. Bagag, et al. 2009. "Chemical Characterization of Titan's Tholins: Solubility, Morphology and Molecular Structure Revisited." *The Journal of Physical Chemistry A* 113 (42): 11195–203. <https://doi.org/10.1021/jp904735q>.
- Coll, P., D. Coscia, N. Smith, M.-C. Gazeau, S.I. Ramírez, G. Cernogora, G. Israël, and F. Raulin. 1999. "Experimental Laboratory Simulation of Titan's Atmosphere: Aerosols and Gas Phase." *Planetary and Space Science* 47 (10–11): 1331–40. [https://doi.org/10.1016/S0032-0633\(99\)00054-9](https://doi.org/10.1016/S0032-0633(99)00054-9).
- Gautier, T., Carrasco N., Mahjoub A., Vinatier S., Giuliani A., Szopa C., Carrie M. Anderson C. M., Correia J. J., Dumas P., and Cernogora G. 2012. "Mid- and Far-Infrared Absorption Spectroscopy of Titan's Aerosols Analogues." *Icarus* 221 (1): 320–27. <https://doi.org/10.1016/j.icarus.2012.07.025>.
- Gautier, T., Carrasco N., Schmitz-Afonso I., Touboul D., Szopa C., Buch A., and Pernot P.. 2014. "Nitrogen Incorporation in Titan's Tholins Inferred by High Resolution Orbitrap Mass Spectrometry and Gas Chromatography–Mass Spectrometry." *Earth and Planetary Science Letters* 404 (October): 33–42. <https://doi.org/10.1016/j.epsl.2014.07.011>.
- Hanel, R, B Conrath, F. M. Flasar, V Kunde, W. Maguire, J Pearl, J. Pirraglia, et al. 1981. "Infrared Observations of the Saturnian System from Voyager 1," 10.
- Imanaka, H., Khare B. N., Elsila J. E., Bakes E. L.O., McKay C. P., Cruikshank D. P., Sugita S., Matsui T., and Zare R. N. 2004. "Laboratory Experiments of Titan Tholin Formed in Cold Plasma at Various Pressures: Implications for Nitrogen-Containing Polycyclic Aromatic Compounds in Titan Haze." *Icarus* 168 (2): 344–66. <https://doi.org/10.1016/j.icarus.2003.12.014>.
- Israël, G., C. Szopa, F. Raulin, M. Cabane, H. B. Niemann, S. K. Atreya, S. J. Bauer, et al. 2005. "Complex Organic Matter in Titan's Atmospheric Aerosols from in Situ Pyrolysis and Analysis." *Nature* 438 (7069): 796–99. <https://doi.org/10.1038/nature04349>.

- Kok, R. de, P.G.J. Irwin, N.A. Teanby, E. Lellouch, B. Bézard, S. Vinatier, C.A. Nixon, et al. 2007. “Oxygen Compounds in Titan’s Stratosphere as Observed by Cassini CIRS.” *Icarus* 186 (2): 354–63. <https://doi.org/10.1016/j.icarus.2006.09.016>.
- Lorenz, R. D, Turtle E. P., Barnes J. W., and Trainer M. G.. 2018. “Dragonfly: A Rotorcraft Lander Concept for Scientific Exploration at Titan.” *Johns Hopkins APL Technical Digest* 34 (3): 14.
- McGuigan, M., Waite H. J., Imanaka H., and Sacks R. D. 2006. “Analysis of Titan Tholin Pyrolysis Products by Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry.” *Journal of Chromatography A* 1132 (1–2): 280–88. <https://doi.org/10.1016/j.chroma.2006.07.069>.
- Niemann, H. B., S. K. Atreya, S. J. Bauer, G. R. Carignan, J. E. Demick, R. L. Frost, D. Gautier, et al. 2005. “The Abundances of Constituents of Titan’s Atmosphere from the GCMS Instrument on the Huygens Probe.” *Nature* 438 (7069): 779–84. <https://doi.org/10.1038/nature04122>.
- Rüger, C. P., Maillard J., Maître J. L., Ridgeway M., Thompson C. J., Schmitz-Afonso I., Gautier T., et al. 2019. “Structural Study of Analogues of Titan’s Haze by Trapped Ion Mobility Coupled with a Fourier Transform Ion Cyclotron Mass Spectrometer.” *Journal of the American Society for Mass Spectrometry* 30 (7): 1169–73. <https://doi.org/10.1007/s13361-019-02205-7>.
- Sagan, C., and Khare B. N. 1979. “Tholins: Organic Chemistry of Interstellar Grains and Gas.” *Nature* 277: 102–7.
- Waite, J. H., D. T. Young, T. E. Cravens, A. J. Coates, F. J. Crary, B. Magee, and J. Westlake. 2007. “The Process of Tholin Formation in Titan’s Upper Atmosphere.” *Science* 316 (5826): 870–75. <https://doi.org/10.1126/science.1139727>.
- Yung, Y. L., M. Allen, and J. P. Pinto. 1984. “Photochemistry of the Atmosphere of Titan - Comparison between Model and Observations.” *The Astrophysical Journal Supplement Series* 55 (July): 465. <https://doi.org/10.1086/190963>.

Appendix

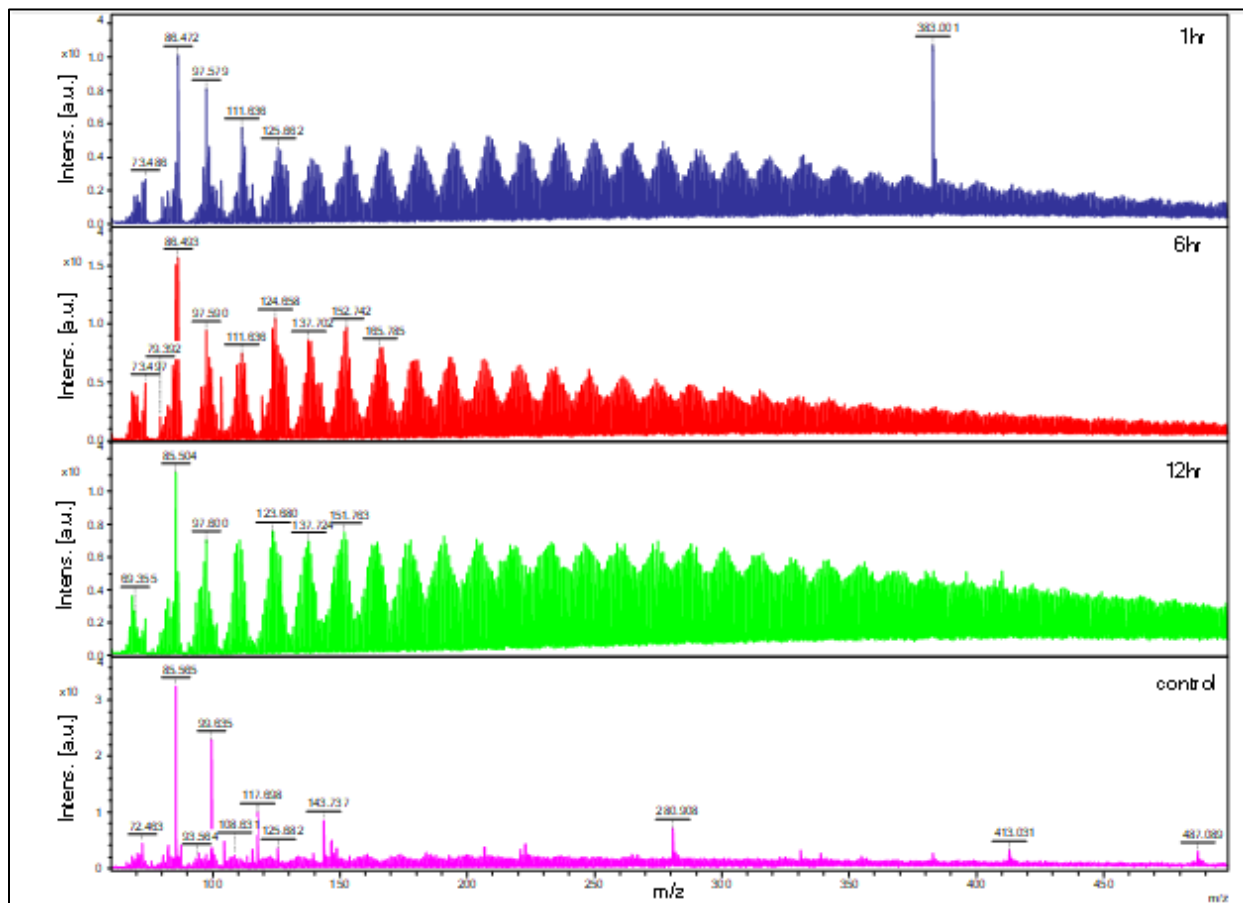


Figure 6: Replicate direct desorption ionization mass spectra of tholins produced at times of 1 hour (blue), 6 hours (red), and 12 hours (green). Control was taken of a sample disk without any tholins produced on it (pink). The 4-hour sample was not viable and subsequently not included.

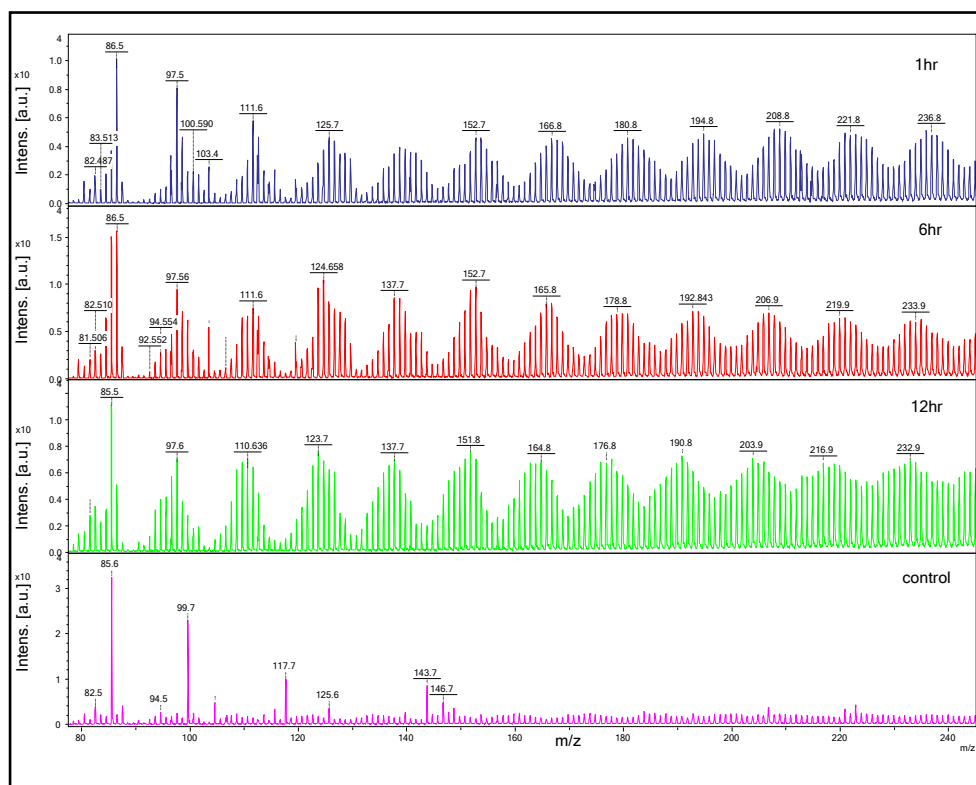


Figure 7: Replicate mass spectra taken from 1 hour (blue), 6 hours (red), and 12 hours (green) focused on the 120-200 dm/z range. Control spectra of sample disk without tholin depositions is also displayed (pink). The 4-hour sample was not viable and subsequently not included.

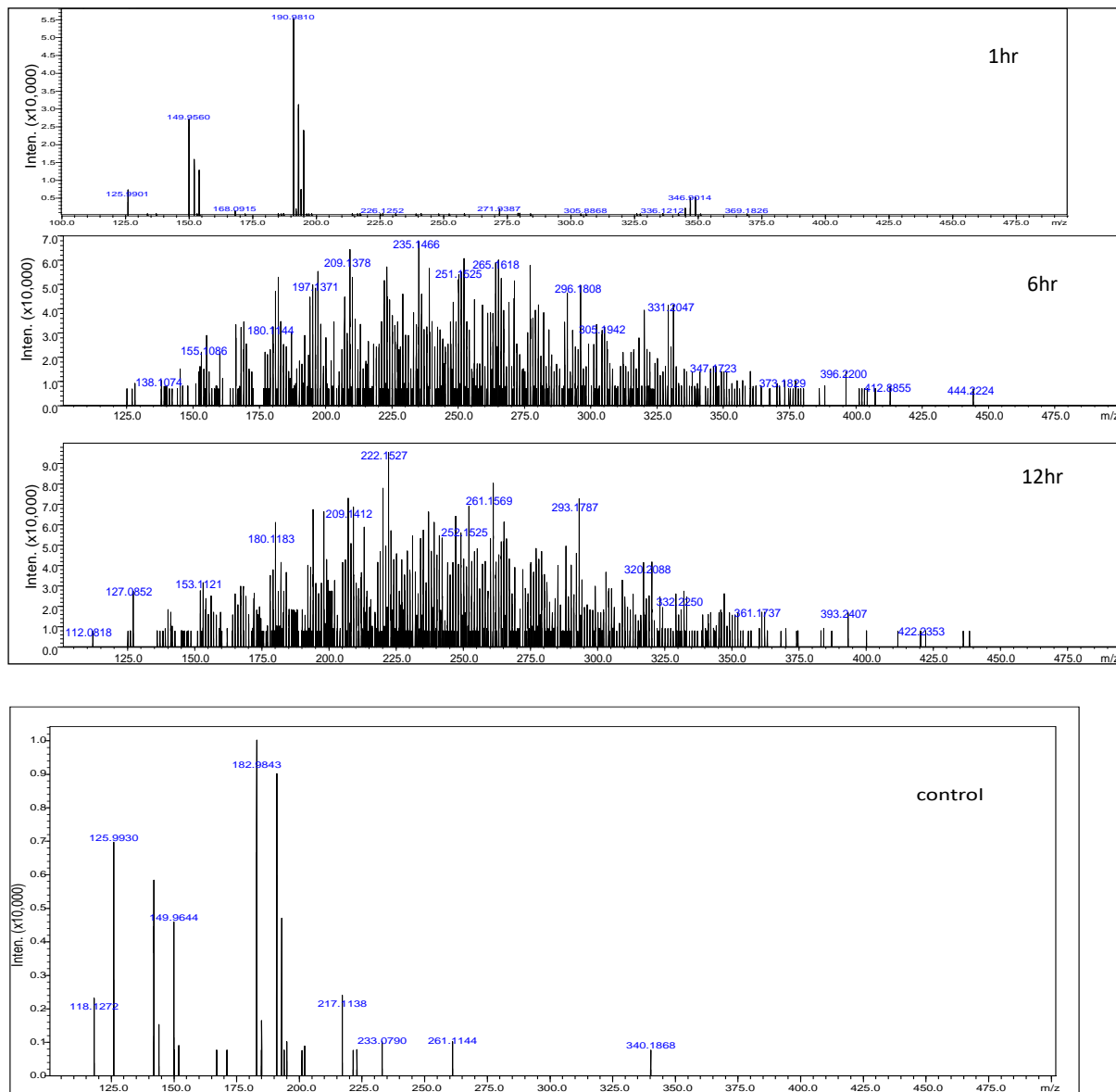


Figure 8: Replicate ESI mass spectra after MALDI disks with tholin deposits were returned to the lab and submerged in DI solvent water to remove the tholins from the surface of the disks. The 4-hour sample was not viable and subsequently not included.

Chapter 2: DLS Analysis of Tholins in Mixtures of Hydrocarbons Simulating Titan Lakes

Introduction

Interactions between complex organics produced in Titan's atmosphere and the surface features of this moon are highly likely and a unique area of research. Understanding these interactions can not only provide insight into the chemical reactions occurring on Titan but also how these interactions affect features observed on the moon's surface. Some of the biggest questions currently surrounding Titan are regarding the interactions between the lakes present in the polar regions and complex organics produced in Titan's atmosphere. While these questions have looked at potential chemical reactions, others have focused on the physical attributes of tholins in the lake environments.

Lakes on Titan are known to consist of primarily methane, ethane and nitrogen (Lunine, Stevenson, and Yung 1983; Mitri et al. 2007; Stofan et al. 2007; Tan, Kargel, and Marion 2013) with minor amounts of polar hydrocarbons (Daniel Cordier et al. 2009; D. Cordier et al. 2012; Corrales et al. 2017). Previous studies have investigated the interactions between organics and the Titan lakes speculating on whether tholins will float on the surface, sink to the bottom, or be dispersed throughout the lakes (Yu et al. 2020; Daniel Cordier and Carrasco 2019). Based on knowledge of densities and surface tension between tholins and liquid hydrocarbons, it has been argued that tholins could either sink into the depths of the lake, or float on the surface. It is important to note that any organics floating atop the lakes would lead to wave dampening affecting shorelines and lake structure (Daniel Cordier and Carrasco 2019). Cordier & Carrasco, 2019 observed that due to the predicted densities of the liquid hydrocarbons present on the surface compared to the aerosol densities, it would be expected that a majority of organics would sink towards the bottom (Yu et al. 2020; Daniel Cordier and Carrasco 2019). If these organics do

indeed sink towards the bottom of the lake, it is reasonable to assume that these would contribute to the lakebed sediments and potential evaporites.

Previous investigations have found a range of tholin particle sizes produced in laboratory experiments (see Table 1), however, most of these studies have focused on particles after production and analysis. While this method can help investigate the organic production in the atmosphere, these interactions in the lakebed might change the structures of organics.

Table 1: Diameters found in Previous Tholin Experiments

Production Variables	Diameter	Reference
5% CH ₄ gas mixture N ₂ balance spark and UV energy sources	20 nm	Hörst et al. 2012
Various N ₂ /CH ₄ mixtures and energy sources	0.01 to 0.5 μm range 10-500 nm	Coll et al. 1998
5% CH ₄ N ₂ gas mixture to a glow plasma discharge (pressure: 3 Torr, temperature: 100 K)	20-30 nm	Yu et al. 2020

Barnes, 2015 speculated possible mechanisms increasing the organic particles size by orders of magnitude (Barnes et al. 2015). One of these proposed mechanisms was described as the “Bottom Up: flocculation” method. In this method, flocculation of the organic particles occurs within the lakes, increasing their particle size. These flakes will eventually settle to the bottom of the lakes and be incorporated into the sediment and any evaporites left over. These larger particles will be removed and separated from the lakes through aeolian process (Barnes et al. 2015).

Cassini images of Titan’s surface revealed large longitudinal dunes in the equatorial regions (R. D. Lorenz et al. 2006). Spectra taken from these dune-like regions show a small abundance of water ice, with some spectral areas showing similarities to various organics (Barnes et al. 2015 and references within). However, questions regarding the size of the particles needed to create such structure have been raised, as the organic particles sizes postulated are much smaller than those required for the dune structures. Specifically, the particle size range in relation to the transport of the organics from the polar regions and the large volume needed to create dunes have been investigated. These studies found that if organics were making up the dunes, additional processes, such as the organics undergoing flocculation, would be required in order to achieve the required particle size (Barnes et al. 2015).

One of the larger questions regarding these processes is the size discrepancy between sand particles. Dune particle diameter are calculated to be around 200-300 microns on Titan. However, models predict tholins particle diameter to be around 1 micron in the atmospheric haze, with the diameter increasing to around 10 microns around the surface. In order for the organics to create and move within the dunes, an increase in organic size would be required for saltation and particle transport (Barnes et al. 2015).

However, the behavior of tholins in Titan solvent has not been investigated, and therefore this hypothesis can't be validated or refuted. This study will investigate the possibility of organic flocculation in Titan lakes and particles sizes in different solvents. We utilize a mixture of solvents at room temperature to simulate the expected presence of both polar and nonpolar hydrocarbons in the lakes and observe if there are any changes in particle size or physical reactions such as coagulation.

Methods

Tholin Production

Tholins were produced in the University of Arkansas Keck lab with a Hummer Technics II sputtering machine. A gas mixture of 95% nitrogen and 5% methane at approximately 4.0 Torr was used with a DC cold plasma at 45 DC mA, 10.5 V. Production was allowed to run for approximately 3 days with a flow rate < .5 SCFH and deposited on VWR Microslides. After production, tholins samples were kept in a desiccator under Ar to mitigate atmospheric oxygen contamination. Drierite desiccant was also placed in the bottom of the desiccator to absorb any ambient moisture. Samples were removed only for analysis.

Experimental Protocol

Three Titan relevant solvents were selected to study interactions with tholins: hexane, acetonitrile, and a solution of hexane saturated with dissolved acetonitrile. While methane and ethane are the primary liquids on Titan, both are in the gas phase under room temperature and pressure. Therefore, hexane (liquid at ambient conditions) was used as a non-polar analog solvent instead. Our experimental set up includes the hexane saturated with acetonitrile to create a solution analogous to Titan lake conditions, as previous studies have found an hexane saturated with acetonitrile solution to have a mole fraction of ~ 0.035 (Nagata, 1987; Rusling et al., 1969).

These experiments were performed in triplicate, with experiments 2 and 3 utilizing solvents that were strained with a 2 μm filter to ensure no large particle contamination before experimental use and the addition of tholins.

Samples were prepared with 5 mg of tholins dispersed in 1 mL of hexanes, 5 mg of tholins dispersed in 1 ml of acetonitrile, and 5 mg of tholins dispersed in 1 mL of hexane saturated with acetonitrile. Prior to analysis, samples were diluted to 2 mL using the respective solvents in which they were dispersed. The dilutions of the samples were then subjected to sonication, vortexing and measurement of UV-Vis spectrum with the respective solvents acting as baseline for the UV-Vis measurements. The samples were sonicated and vortexed and immediately measured using the DLS instrument.

Changes were measured utilizing dynamic light scattering (DLS) analysis, not only to measure average particle diameter, but also the dispersity of the particle sizes to measure any variation between each solution. For DLS analysis, sample used was 0.1 mL of tholins diluted to 2 mL with solvent. The hydrodynamic diameter and polydispersity index of the tholin samples were measured using dynamic light scattering (DLS) instrument (Brookhaven ZetaPALS).

Results

DLS analysis was performed on each sample to determine the hydrodynamic diameter (D_h), and polydispersity. This can provide information not only on the particles size of the solid tholins in the solvent, but also how ubiquitous the sizes are across the sample. While the hydrodynamic diameter is only an estimation based on ideal circumstances and a spherical particle, the measurement provides a good estimation of macromolecule sizes and can provide insight into discrepancies between the three solvents. Dispersity can inform about the

distribution of particle sizes on a scale of 0-1, with values closer to 0 describing a monodisperse sample and 1 a polydisperse sample.

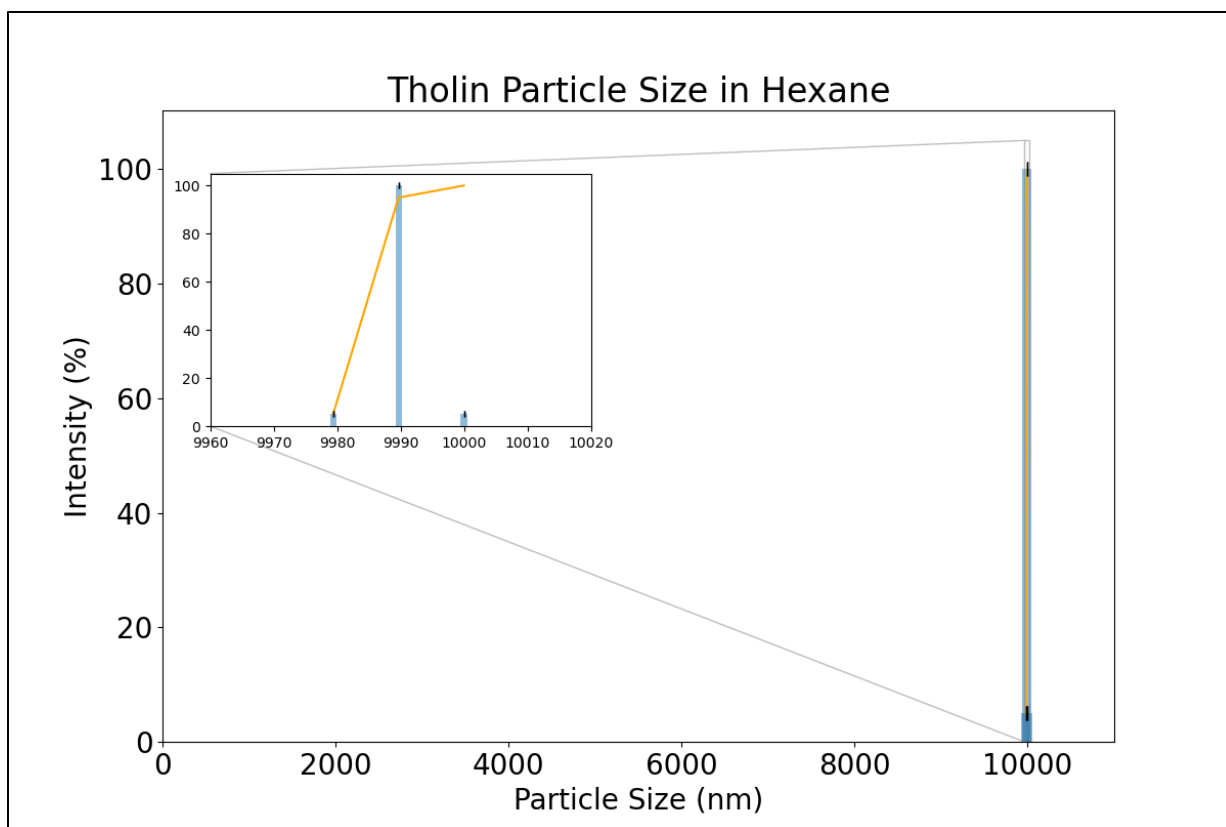


Figure 1: Averaged multimodal size distribution results from hexane and tholins. Three peaks were observed in close succession at 9979.4 nm, 9989.7 nm, 10000 nm. These were observed in each one of the triplicates and therefore had no error bars in the averaged spectra.

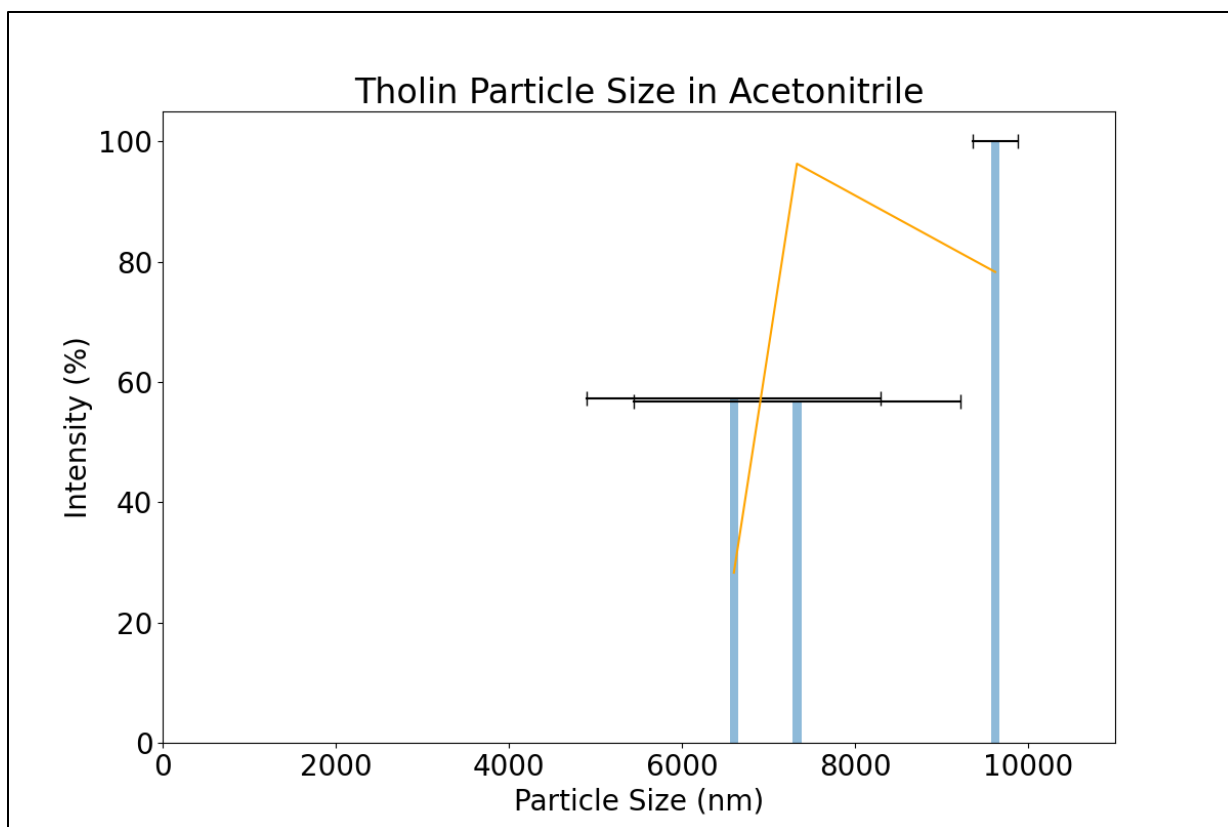


Figure 2: Averaged multimodal size distribution results from tholins in acetonitrile. Averaged particle peak are observed in 6597.70 nm, 7325.93 nm, 9615.43 nm. Peaks were not consistently observed in any of the experiments, and therefore large fluctuations in average particle sizes were seen in each experiment. Large error bars resulted from the inconsistent data. Data from each experiment is located in the appendix.

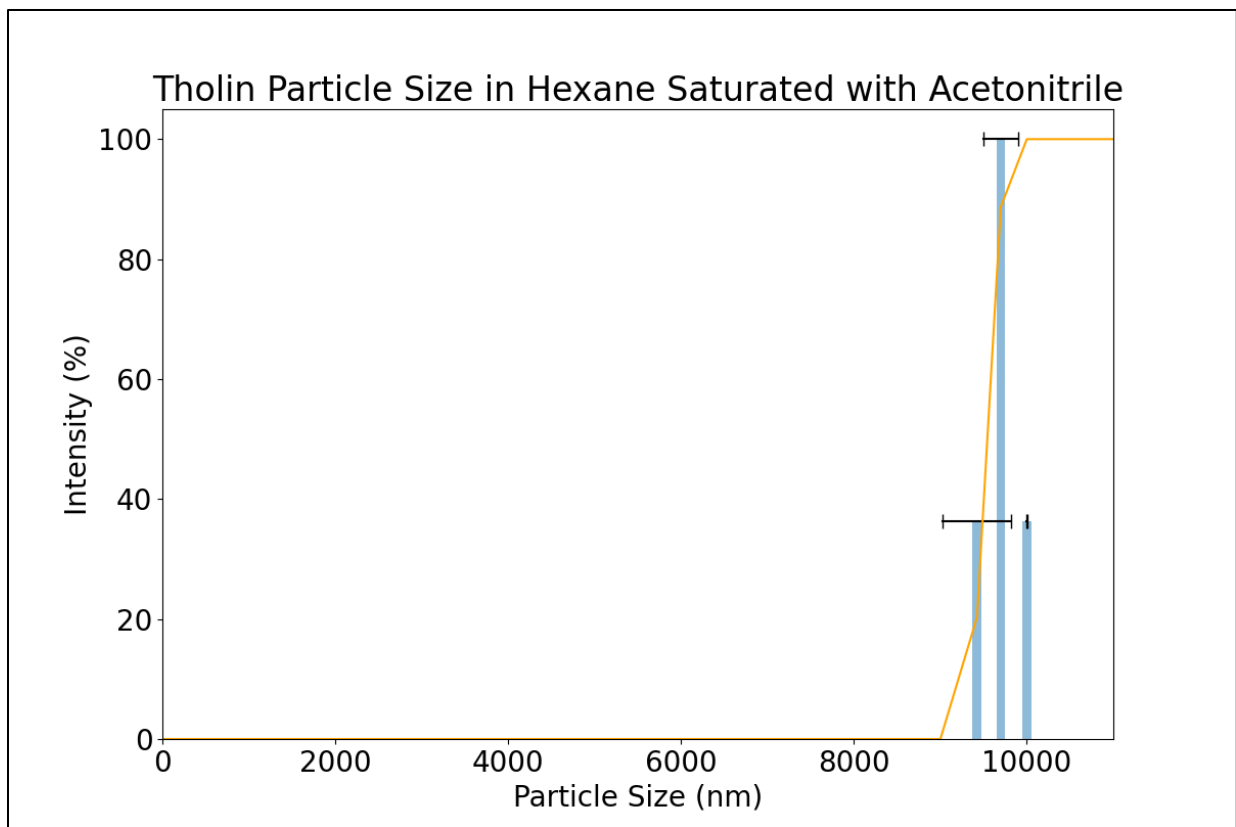


Figure 3: Averaged multimodal size distribution results from tholins in hexane saturated acetonitrile. Peaks were observed at 9423.3 nm, 9698.7 nm, and 10000.0 nm.

Spectra taken from each of the solvent samples (as shown in Figure 1, 2, and 3) found peaks with distributions ranging from $\sim 6 \mu\text{m}$ - $\sim 10 \mu\text{m}$. Particle sizes were observed in the hexane sample that measured $9.980 \mu\text{m}$, $9.99 \mu\text{m}$, and $10.000 \mu\text{m}$. In the acetonitrile sample, particle sizes were observed to be $6.598 \mu\text{m}$, $9.615 \mu\text{m}$, and $7.326 \mu\text{m}$. Finally, particle sizes of $9.423 \mu\text{m}$, $9.699 \mu\text{m}$, $10.00 \mu\text{m}$. No large differences were observed between the hexane and the hexane saturated with acetonitrile samples with single Gaussian distributed peaks around 10.00 were observed in both samples. Particle sizes were smaller in the polar acetonitrile sample ranging from $\sim 6 \mu\text{m}$ - $\sim 10 \mu\text{m}$.



Figure 4: Tholins and hexane sample after the transfer of the liquid into the cuvette. The soild brown residue left behind show the adhesion of the tholin to the glassware.

Values obtained from these studies (as shown in Table 2) show the average raw data obtained in the three experiments for each solvent. Unprocessed data from each experiment can be seen in the appendix. It is important to note that measurements were most likely affected by the tholin adhering to the cuvette during analysis, specifically in the hexane sample (see Figure 4). While this was remedied with utilizing one cuvette per sample, the adhesion of the tholins to the side of the cuvette might still be occurring. Additionally, it is important to note that the tholins in the sample would eventually settle on the bottom of the cuvettes in all solvents. If the tholins settled too quickly the measurements might not be accurate.

Therefore, the singular peaks observed in the DLS measurements might be

Table 2: Averaged DLS Results

Sample Name	Hydrodynamic diameter (μm)	Polydispersity
Hexane + Tholins	86.33 (±59.21)	0.317 (±0.246)
Acetonitrile + Tholins	69.65 (±59.15)	0.444 (±0.254)
Hexane Saturated with Acetonitrile + Tholins	117.96 (±99.97)	0.491 (± 0.270)

Table 2: Averaged results from three different sets of experimental tests. The diameters of tholins in the non-polar hexane and the mixture of solvents are much larger than those seen in the acetonitrile samples, with the tholins in the hexane saturated with acetonitrile being almost double. The polydispersity observed in the hexane samples are less than the polydispersity observed in the samples containing any amount polar compounds. Plus/minus range is derived from the standard deviation.

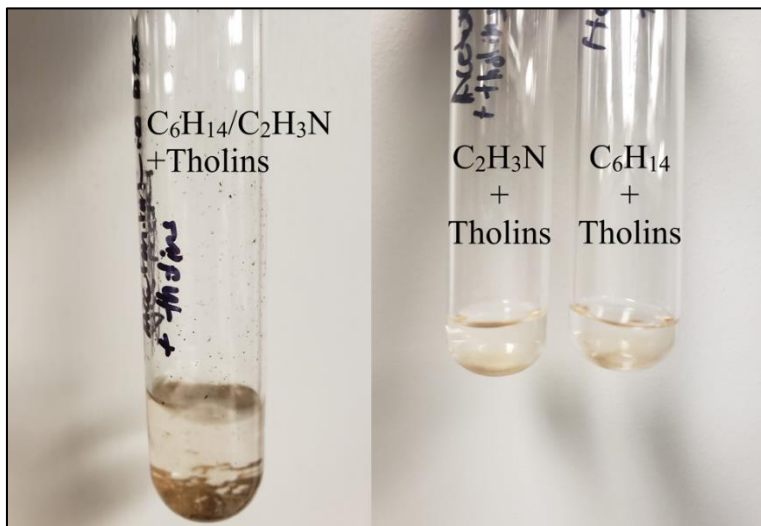


Figure 5: Tholins after their addition to acetonitrile, hexane (right), and hexane saturated with acetonitrile (left). Note the particles sticking to the sides of the glass within the hexane saturated with acetonitrile solution. No such particles were observed within the singular solvent samples.

In the hexane and tholin sample, the hydrodynamic diameter is larger, around $86.332 \mu\text{m}$ with a polydispersity index of .317. Tholins added to acetonitrile had a smaller hydrodynamic diameter around $69.651 \mu\text{m}$ with a polydispersity of .444. Hexane saturated with acetonitrile showed properties of both polar and non-polar solvents, with larger particles sizes of $117.966 \mu\text{m}$ observed, but a polydispersity of .491 that is more similar to the acetonitrile sample. DLS measurements are typically averaged, therefore the output value will be a combination of all the measurement taken during analysis. While this will not affect the interpretation of a single data point, it is important to note that large variations were observed in many of the samples. Data from each experiment can be viewed in the appendix.

Discussion

DLS measurements showed larger, more monodispersed particles being observed in hexane while smaller more varied sizes were seen in acetonitrile. The sample of hexane saturated with acetonitrile showed attributes of both the polar and non-polar solvent sample, with particle having the larger diameter observed in the hexane, but the dispersity index of acetonitrile. Our hexane saturated with acetonitrile solvent would be most analogous to the environment present in the lakes on Titan, and therefore provides insight into the processes present on the moon's surface. Throughout this study, we have found that changes in organics reactions can be observed in different solutions with mixtures of non-polar and polar solvents.

While it might be assumed that the low amount of acetonitrile present in the solution would be negligible in the largely non-polar solution, these results show that there is an effect on the organics. Even within the largely non-polar hexane solvents, the addition of a polar hydrocarbon (in the case acetonitrile) had a great effect on the resulting particles increasing the variation and distribution of the organic particles as seen by the large dispersity index. The presence of a polar compound, even in extremely low volumes, might induce changes in the tholins that have impact on their possible reaction in Titan lakes.

Previous studies have postulated as to how tholins might aggregate into larger particles, making it possible to allow for the 200 micron sized "grains" necessary to form the dunes present on the surface of Titan (Ralph D. Lorenz 2014). One theory of particular interest was postulated in Barnes et. al. 2015 in which subaqueous processes could lead to flocculation or coagulation of organics into the size ranges needed for aeolian transport to the equatorial dunes (Barnes et al. 2015). Our findings of particle diameter changes based on the polarity of the solvents provides experimental evidence possibly supporting this theory, as larger particles were found in the

mixtures of polar and nonpolar liquids. However, our measurements do not find the particle above the 200-micron grain size necessary for the organics in the polar regions to be comprising the dunes. Therefore, processes might be occurring in the lakes of Titan to increase the particle size, the resulting diameters might not be large enough to transport over large distances.

It is also important to mention these measurements might not be indicative of the entire tholins volume within the samples. In some experiments particle sizes were observed to be over 200 microns (see hexane saturated with acetonitrile data for experiment 1 and 3 in the appendix). However, these measurements were inconsistent and could not be replicated. One explanation could be that they are sinking too quickly for analysis. Even with our implementation of vortexing the samples, the tholins would sink to the bottom in seconds. Since DLS measurements are dependent on the particle remaining in suspension, it may be possible that the larger sizes might be sinking towards the bottom faster than the instrument can take a measurement, as the equipment requires ~1 min for sample measurements and the particles settle within seconds.

Conclusions

Our investigation detected hydrodynamic diameter differences in Titan tholins in polar, non-polar, and mixtures of solvents. Most importantly, we found tholins in the hexane saturated with acetonitrile have an increase in the particle diameter that, while not in the range necessary for saltation, could be indicative of process supporting the aggregation of tholins within the lakes. While many other variables would affect the potential for this mechanism, the particle size increase could lead to the 200 microns over longer time periods, different temperature, etc.

It is important to note that DLS measurements are highly dependent on the viscosity of the liquid utilized. While this investigation is conducted at room temperature and pressure,

variations in the reactions are to be expected under environments like that on Titan. Results obtained under room temperature conditions show that some previously unreported processes are occurring with the tholins in the mixture of solvents that lead to particle growth. Viscosity variation that would result from repeating these experiments under Titan conditions might change the processes possible. Further experimental investigation into particle size under Titan temperature and pressure is needed.

References

- Barnes, Jason W, Ralph D Lorenz, Jani Radebaugh, Alexander G Hayes, Karl Arnold, and Clayton Chandler. 2015. "Production and Global Transport of Titan's Sand Particles." *Planetary Science* 4 (1): 1. <https://doi.org/10.1186/s13535-015-0004-y>.
- Coll, Patrice, David Coscia, Marie-Claire Gazeau, Lionel Guez, and Franc Ois Raulin. 1998. "Review and Latest Results of Laboratory Investigations of Titan's Aerosols." *Origins of Life and Evolution of the Biosphere* 28 (2): 195–213.
- Cordier, D., O. Mousis, J.I. Lunine, S. Lebonnois, P. Rannou, P. Lavvas, L.Q. Lobo, and A.G.M. Ferreira. 2012. "Titan's Lakes Chemical Composition: Sources of Uncertainties and Variability." *Planetary and Space Science* 61 (1): 99–107. <https://doi.org/10.1016/j.pss.2011.05.009>.
- Cordier, Daniel, and Nathalie Carrasco. 2019. "The Floatability of Aerosols and Wave Damping on Titan's Seas." *Nature Geoscience* 12 (5): 315–20. <https://doi.org/10.1038/s41561-019-0344-4>.
- Cordier, Daniel, Olivier Mousis, Jonathan I. Lunine, Panayotis Lavvas, and Véronique Vuitton. 2009. "An Estimate of the Chemical Composition of Titan." *The Astrophysical Journal* 707 (2): L128–31. <https://doi.org/10.1088/0004-637X/707/2/L128>.
- Corrales, L. René, Thomas D. Yi, Samantha K. Trumbo, David Shalloway, Jonathan I. Lunine, and David A. Usher. 2017. "Acetonitrile Cluster Solvation in a Cryogenic Ethane-Methane-Propane Liquid: Implications for Titan Lake Chemistry." *The Journal of Chemical Physics* 146 (10): 104308. <https://doi.org/10.1063/1.4978395>.
- He, Chao, Guangxin Lin, Kathleen T. Upton, Hiroshi Imanaka, and Mark A. Smith. 2012. "Structural Investigation of Titan Tholins by Solution-State ^1H , ^{13}C , and ^{15}N NMR: One-Dimensional and Decoupling Experiments." *The Journal of Physical Chemistry A* 116 (19): 4760–67. <https://doi.org/10.1021/jp3016062>.

- Hörst, S.M., R.V. Yelle, A. Buch, N. Carrasco, G. Cernogora, O. Dutuit, E. Quirico, et al. 2012. "Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere Simulation Experiment." *Astrobiology* 12 (9): 809–17. <https://doi.org/10.1089/ast.2011.0623>.
- Kawai, Jun, Seema Jagota, Takeo Kaneko, Yumiko Obayashi, Bishun N. Khare, Christopher P. McKay, and Kensei Kobayashi. 2013. "Titan Tholins as Amino Acid Precursors and Their Solubility in Possible Titan Liquidospheres." *Chemistry Letters* 42 (6): 633–35. <https://doi.org/10.1246/cl.130101>.
- Lorenz, R. D., S. Wall, J. Radebaugh, G. Boubin, E. Reffet, M. Janssen, E. Stofan, et al. 2006. "The Sand Seas of Titan: Cassini RADAR Observations of Longitudinal Dunes." *Science* 312 (5774): 724–27. <https://doi.org/10.1126/science.1123257>.
- Lorenz, Ralph D. 2014. "Physics of Saltation and Sand Transport on Titan: A Brief Review." *Icarus* 230 (February): 162–67. <https://doi.org/10.1016/j.icarus.2013.06.023>.
- Lunine, Jonathan I., David J. Stevenson, and Yuk L. Yung. 1983. "Ethane Ocean on Titan." *Science* 222 (4629): 1229–30. <https://doi.org/10.1126/science.222.4629.1229>.
- Mitri, Giuseppe, Adam P. Showman, Jonathan I. Lunine, and Ralph D. Lorenz. 2007. "Hydrocarbon Lakes on Titan." *Icarus* 186 (2): 385–94. <https://doi.org/10.1016/j.icarus.2006.09.004>.
- Stofan, E. R., C. Elachi, J. I. Lunine, R. D. Lorenz, B. Stiles, K. L. Mitchell, S. Ostro, et al. 2007. "The Lakes of Titan." *Nature* 445 (7123): 61–64. <https://doi.org/10.1038/nature05438>.
- Tan, Sugata P., Jeffrey S. Kargel, and Giles M. Marion. 2013. "Titan's Atmosphere and Surface Liquid: New Calculation Using Statistical Associating Fluid Theory." *Icarus* 222 (1): 53–72. <https://doi.org/10.1016/j.icarus.2012.10.032>.
- Yu, Xinting, Sarah M. Hörst, Chao He, Patricia McGuiggan, Kai Kristiansen, and Xi Zhang. 2020. "Surface Energy of the Titan Aerosol Analog 'Tholin.'" *The Astrophysical Journal* 905 (2): 88. <https://doi.org/10.3847/1538-4357/abc55d>.

Appendix

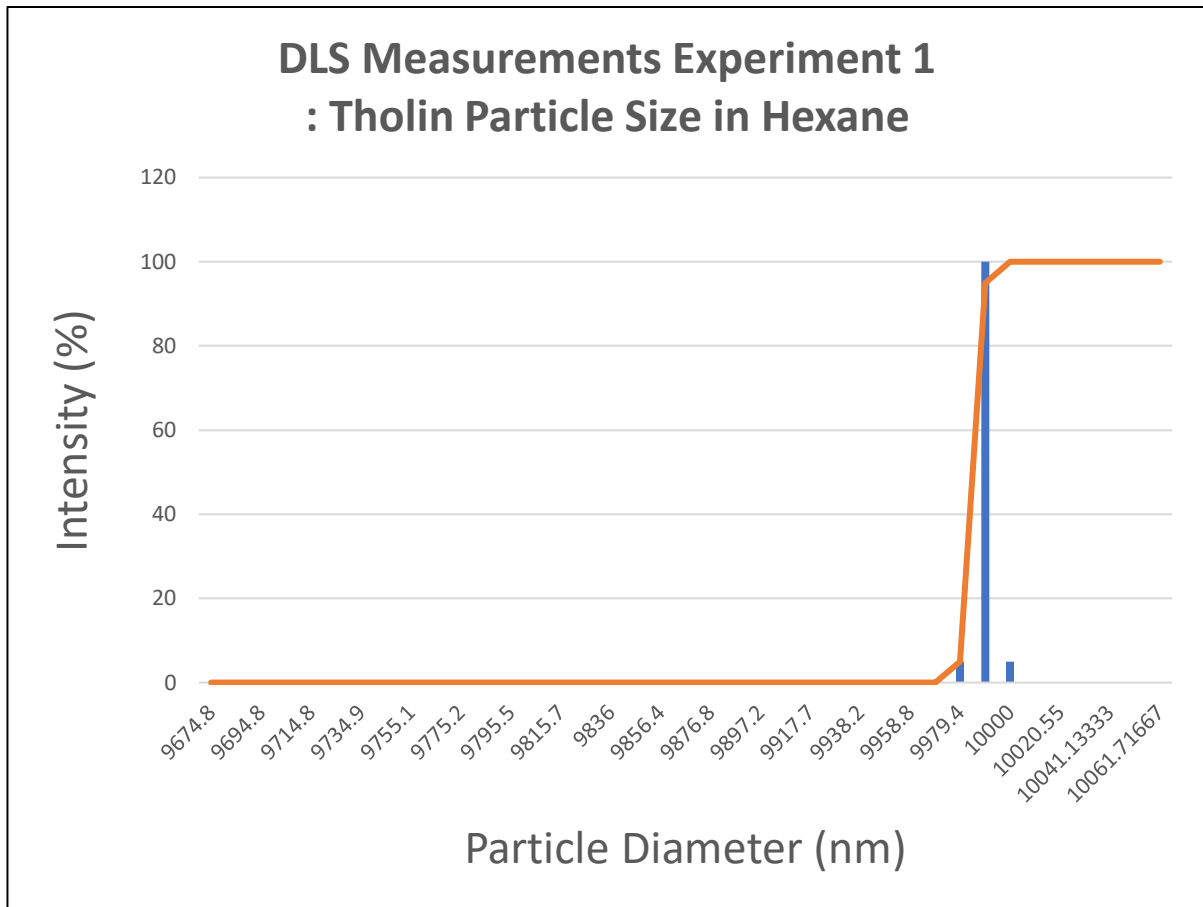


Figure 6: Multimodal size distribution results from DLS Measurements from experiment 1 of tholins in hexane.

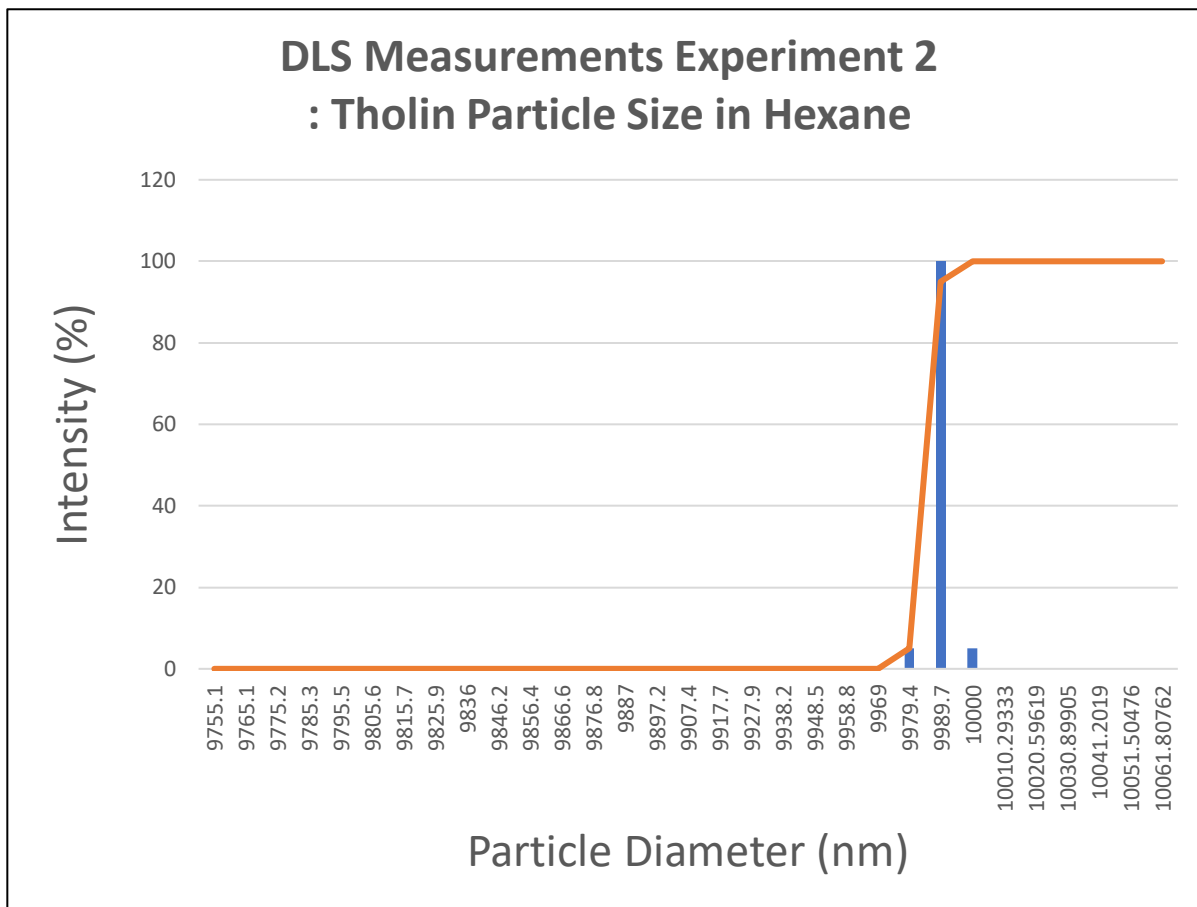


Figure 7: Multimodal size distribution results from DLS Measurements from experiment 2 of tholins in hexane.

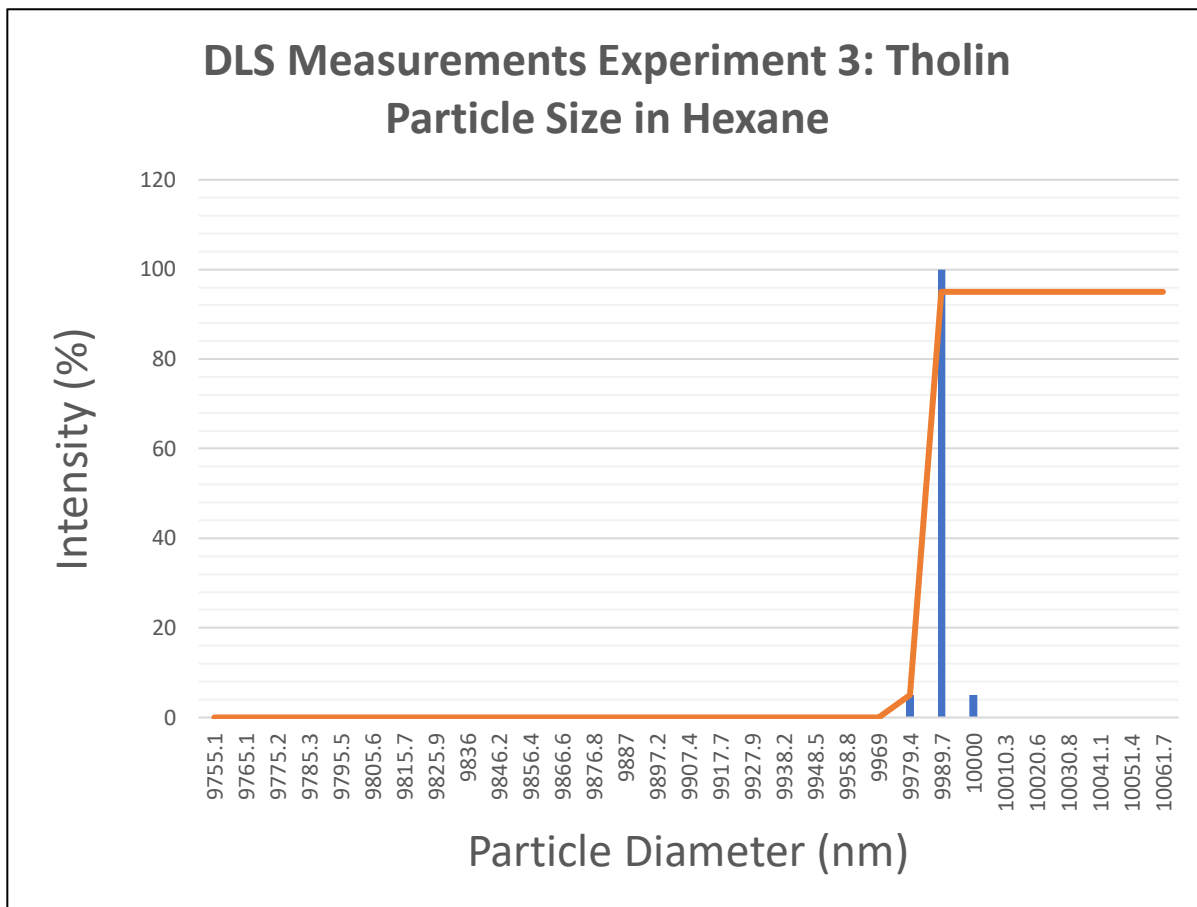


Figure 8: Multimodal size distribution results from DLS Measurements from experiment 3 of tholins in hexane.

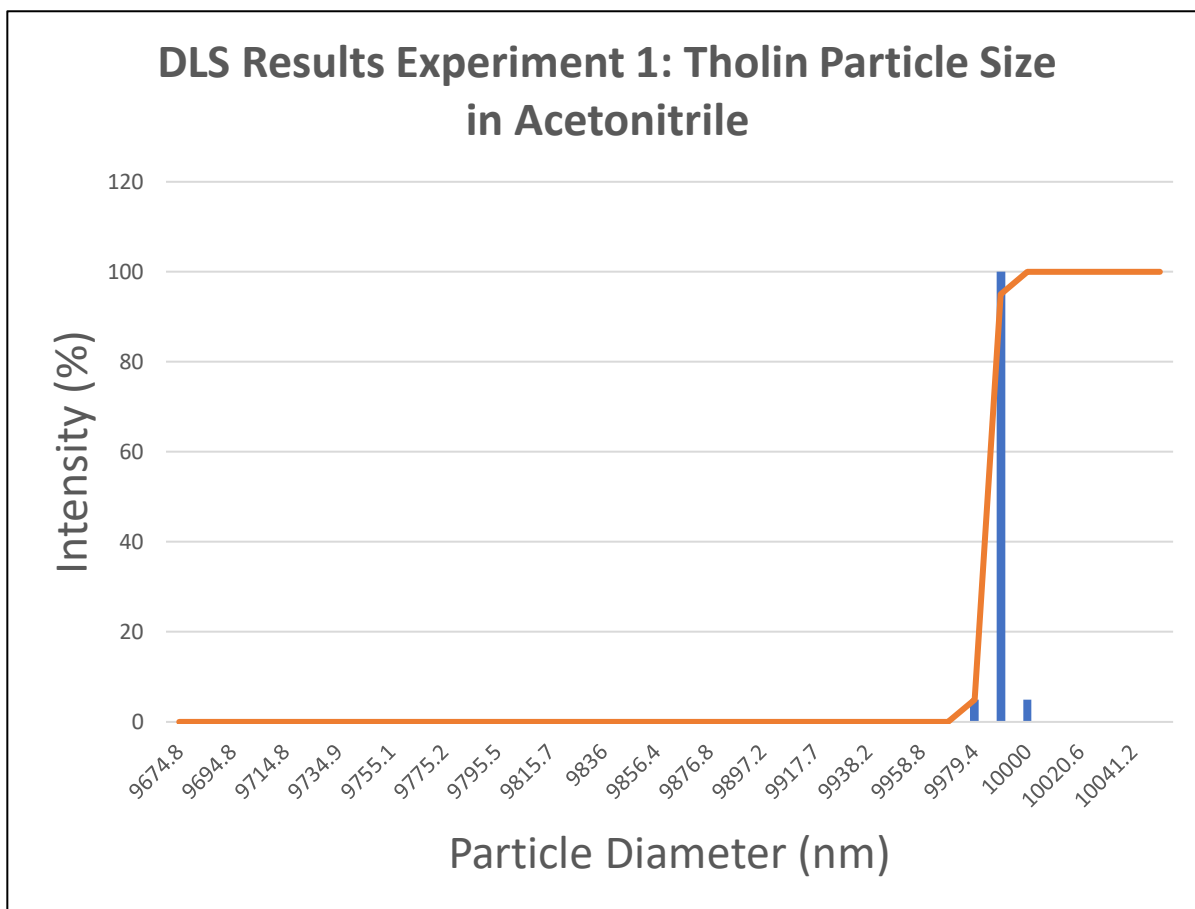


Figure 9: Multimodal size distribution results from DLS Measurements from experiment 1 of tholins in acetonitrile.

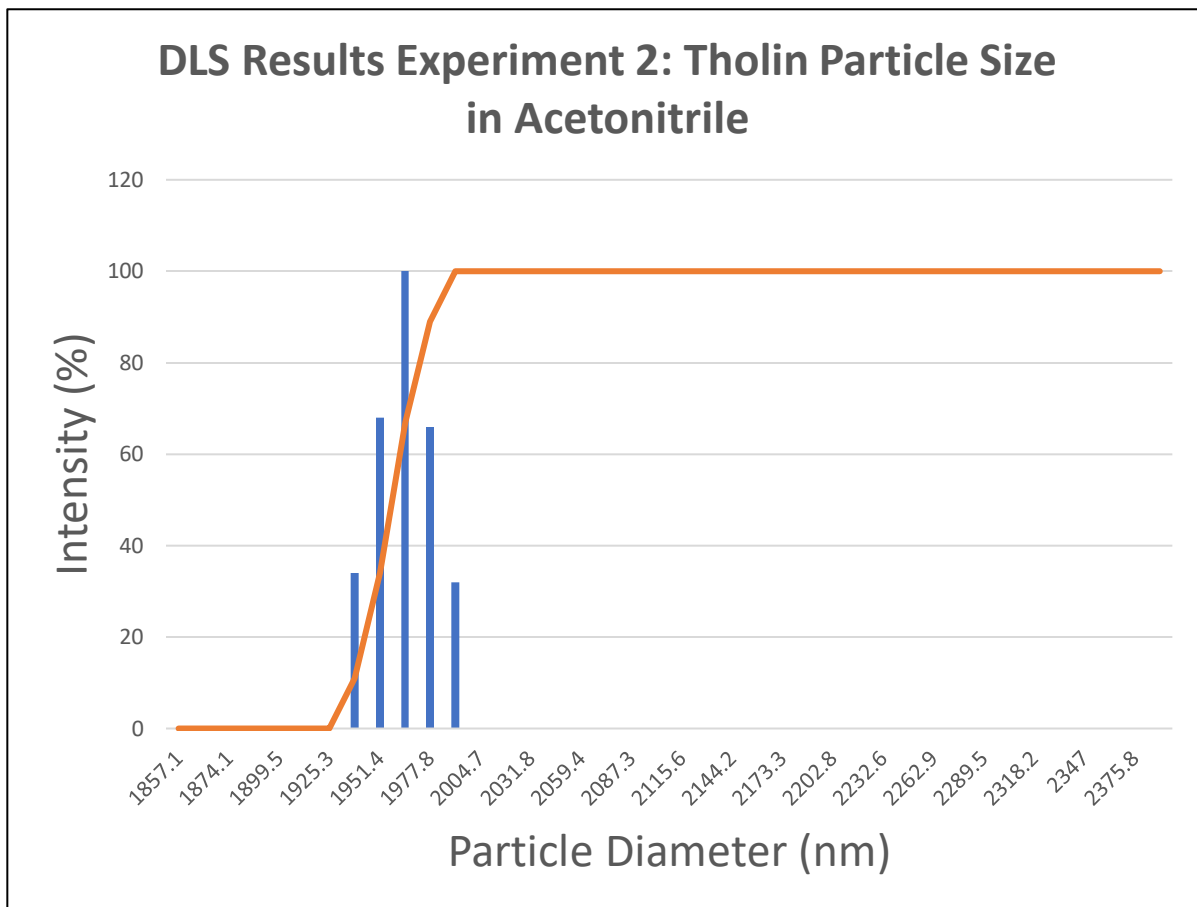


Figure 10: Multimodal size distribution results from DLS Measurements from experiment 2 of tholins in acetonitrile.

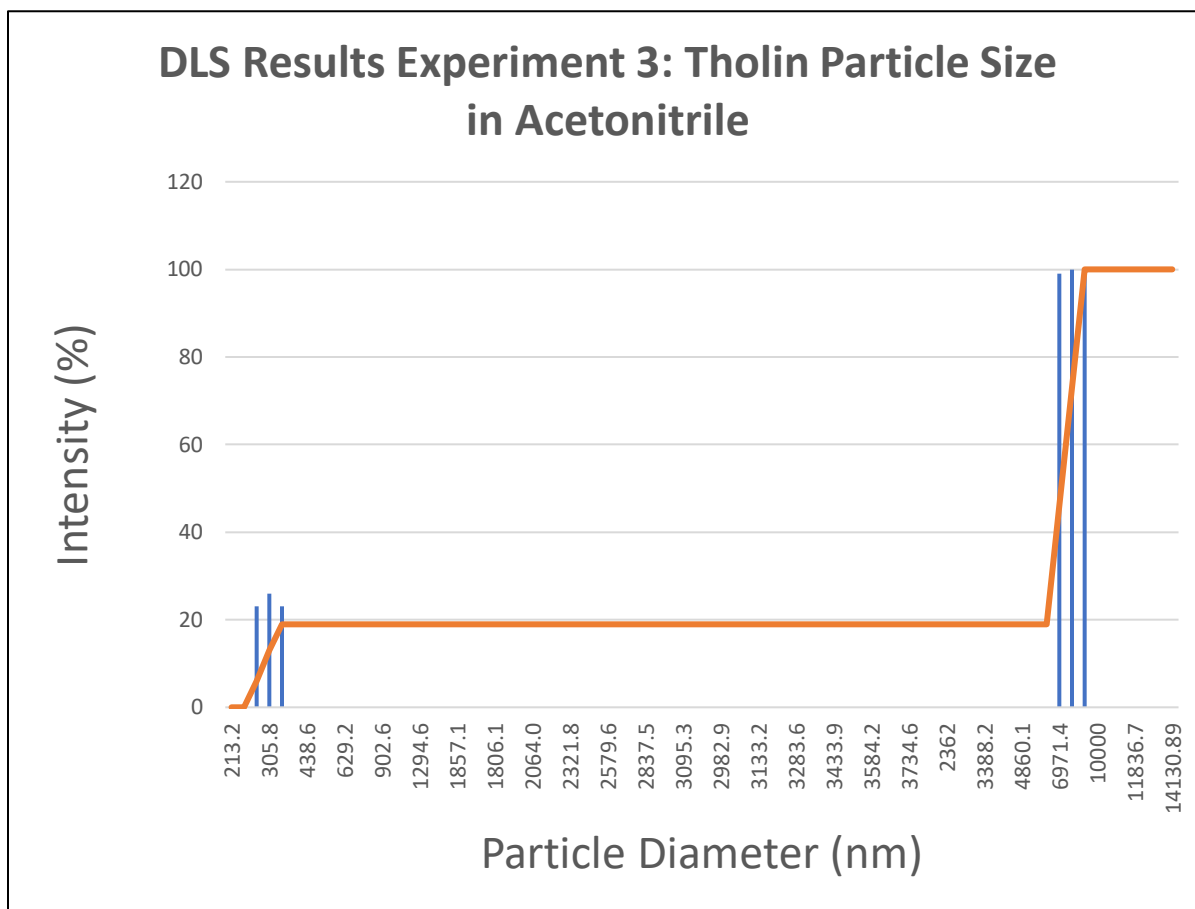


Figure 11: Multimodal size distribution results from DLS Measurements from experiment 3 of tholins in acetonitrile. A peak was observed in this sample around 305nm that was not able to be repeated in any other experiment and was not included in the averaged peak data. We attribute this as a residual peak from the solvent.

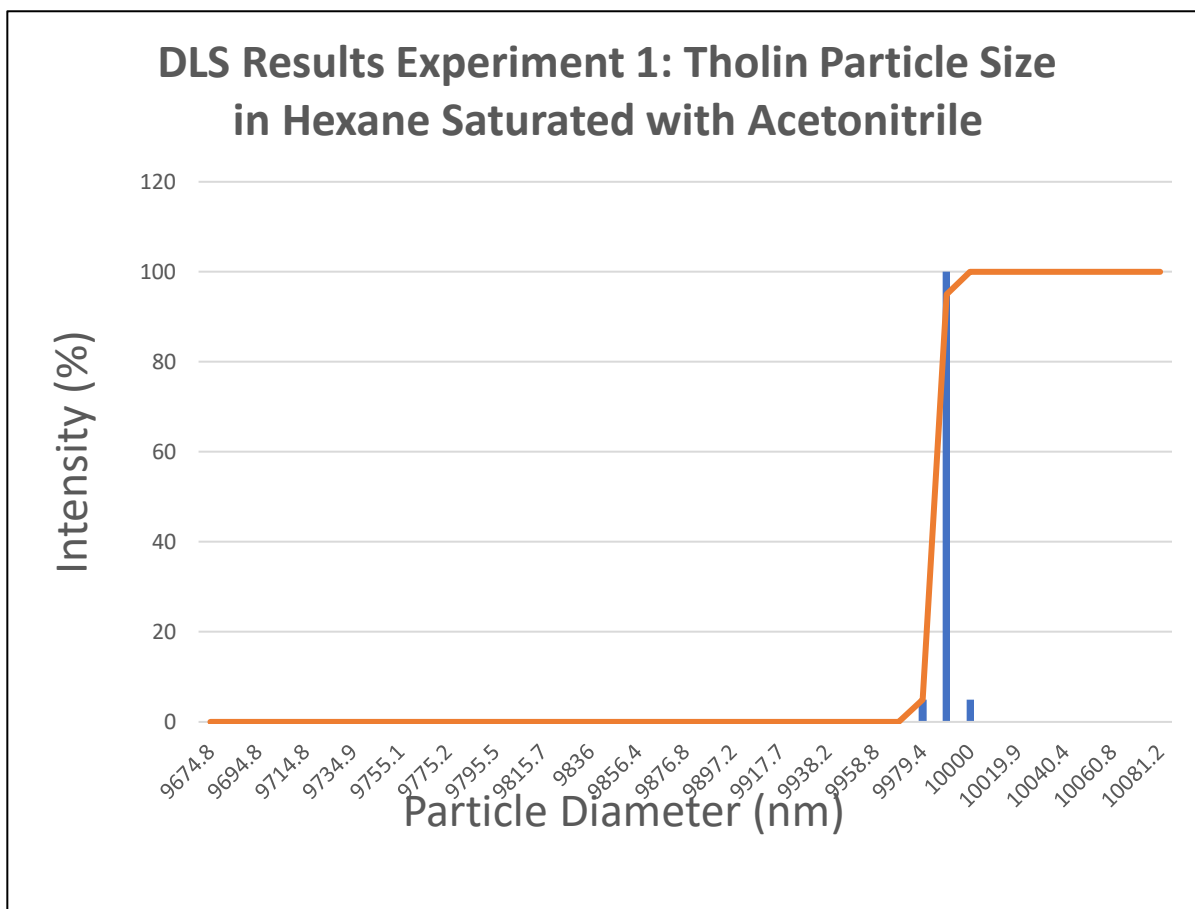


Figure 12: Multimodal size distribution results from DLS Measurements from experiment 1 of tholins in hexane saturated with acetonitrile.

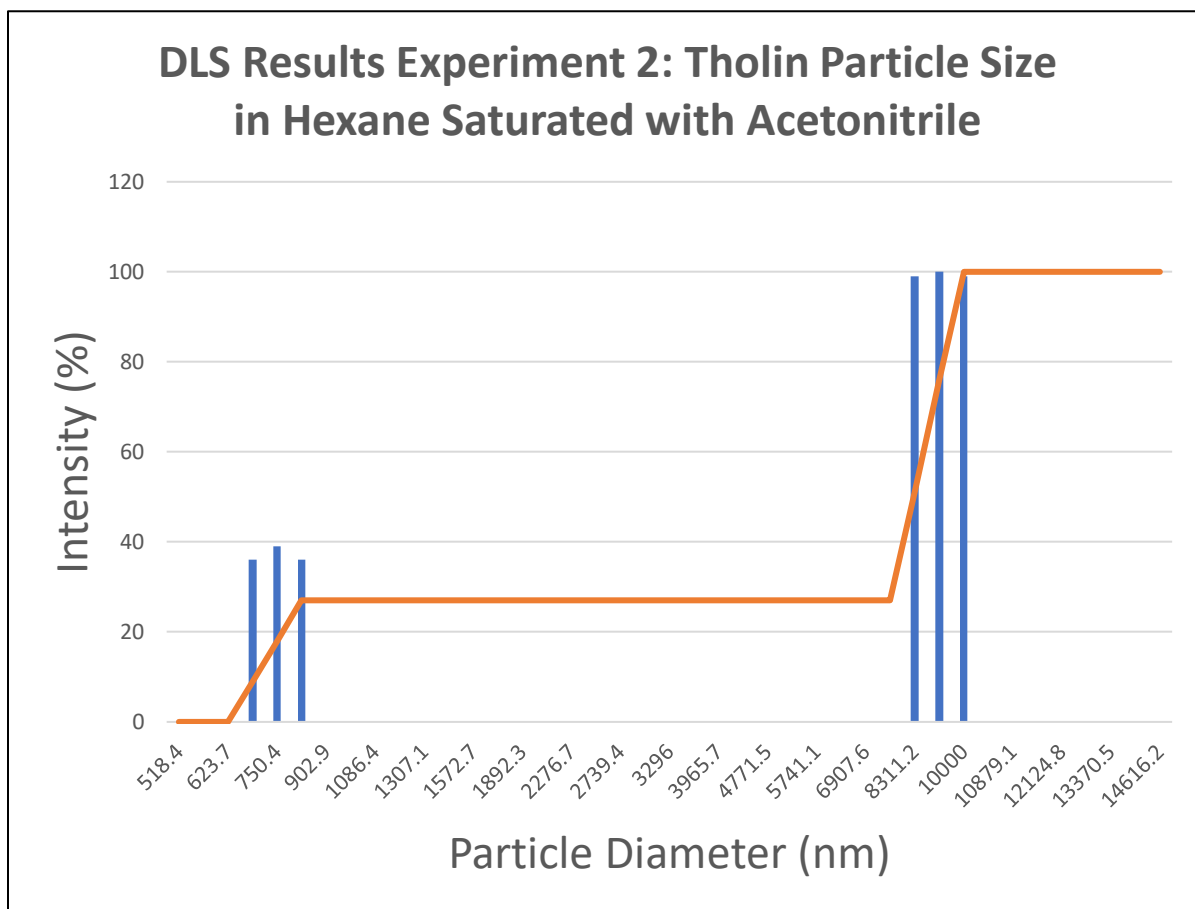


Figure 13: Multimodal size distribution results from DLS Measurements from experiment 2 of tholins in hexane saturated with acetonitrile. A peak was observed in this sample around ~600nm - ~900nm that was not able to be repeated in any other experiment and was not included in the averaged peak data. We attribute this as a residual peak from the solvent.

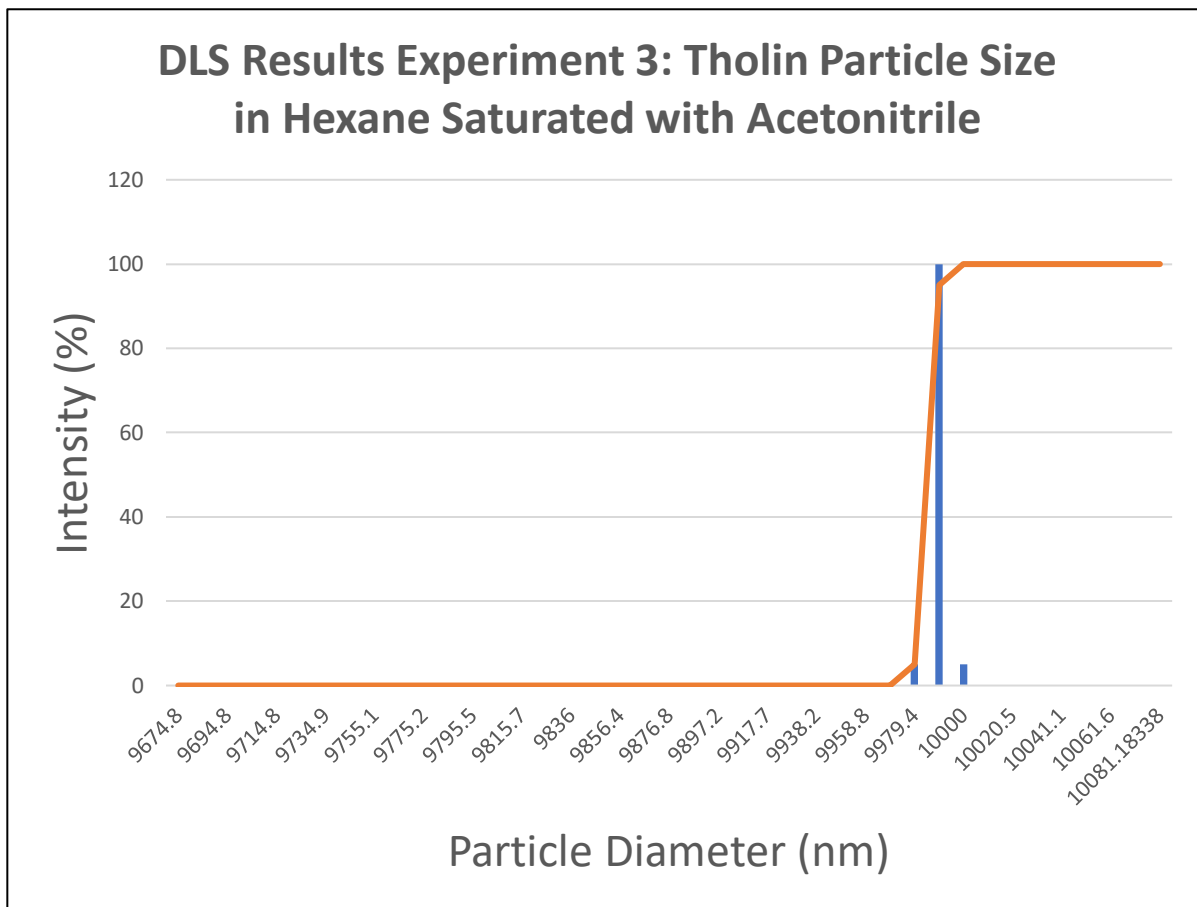


Figure 14: Multimodal size distribution results from DLS Measurements from experiment 3 of tholins in hexane saturated with acetonitrile.

Experiment 1: Raw Data from Tholin Particles in Hexane			
Run	Eff diameter (nm)	Polydispersity	
	1	66614.4	0.005
	2	78021.6	0.645
	3	35970.7	0.525
	4	20854.8	0.005
	5	123110.9	0.476
Mean		64914.5	0.331
Std Error		17805.2	0.136
Combined		56657.5	0.012

Table 3: Raw data from experiment 1 of the hexane and tholin sample.

Experiment 2: Raw Data from Tholin Particles in Hexane			
Run	Eff diameter	Polydispersity	
	1	130612.2	0.199
	2	58870.7	0.653
	3	167186.3	0.005
	4	134842.3	0.482
	5	218547.0	0.553
Mean		142011.7	0.378
Std Error		26062.6	0.340
Combined		123035.8	0.34

Table 4: Raw data from experiment 2 of the hexane and tholin sample.

Experiment 3: Raw Data from Tholin Particles in Hexane			
Run	Eff diameter		Polydispersity
	1	100567.0	0.561
	2	4244.1	0.748
	3	100399.3	0.669
	4	51244.0	0.540
	5	5690.2	0.490
Mean		52428.9	0.602
Std Error		21361.3	0.047
Combined		79303	0.598

Table 5: Raw data from experiment 3 of the hexane and tholin sample.

Experiment 1: Raw Data from Tholin Particles in Acetonitrile			
Run	Eff diameter	Polydispersity	
	1	100025.9	0.699
	2	62667.3	0.716
	3	157780.3	0.878
	4	118691.4	0.484
	5	3223.9	0.375
Mean		88477.8	0.630
Std Error		26252.1	0.090
Combined		111506.1	0.509

Table 6: Raw data from experiment 1 of the acetonitrile and tholin sample.

Experiment 2: Raw Data from Tholin Particles in Acetonitrile			
Run	Eff diameter	Polydispersity	
	1	2234.0	0.005
	2	2130.8	0.187
	3	2109.9	0.179
	4	2034.7	0.130
	5	2001.8	0.173
Mean		2102.2	0.135
Std Error		40.5	0.034
Combined		2115.0	0.103

Table 7: Raw data from experiment 2 of the acetonitrile and tholin sample.

Experiment 3: Raw Data from Tholin Particles in Acetonitrile			
Run	Eff diameter	Polydispersity	
	1	164652.1	0.796
	2	17333.4	0.470
	3	4492.5	0.428
	4	4470.3	0.304
	5	3460.3	0.363
Mean		38881.7	0.472
Std Error		31546.8	0.086
Combined		95332.9	0.721

Table 8: Raw data from experiment 3 of the acetonitrile and tholin sample.

Experiment 1: Raw Data from Tholin Particles in Hexane Saturated with Acetonitrile			
Run	Eff diameter	Polydispersity	
	1	115199.5	0.714
	2	127641.8	0.865
	3	290716.8	0.005
	4	134363.2	0.636
	5	139914.0	0.818
Mean		161567.1	0.608
Std Error		32549.5	0.156
Combined		148699.3	0.519

Table 9: Raw data from experiment 1 of the hexane saturated with acetonitrile and tholin sample.

Experiment 2: Raw Data from Tholin Particles in Hexane Saturated with Acetonitrile			
Run		Hydrodynamic diameter	Polydispersity
	1	10746.7	0.415
	2	5499.9	0.420
	3	7108.9	0.450
	4	12665.0	0.397
	5	12547.2	0.541
Mean		9713.5	0.445
Std Error		1455.1	0.385
Combined		8468.9	0.385

Table 10: Raw data from experiment 2 of the hexane saturated with acetonitrile and tholin sample.

Experiment 3: Raw Data from Tholin Particles in Hexane Saturated with Acetonitrile			
Run	Eff diameter		Polydispersity
	1	293379.0	0.136
	2	91677.6	0.700
	3	172865.0	0.804
	4	189710.2	0.607
	5	265018.3	0.005
Mean		202530.0	0.450
Std Error		35700.8	0.160
Combined		196730.2	0.569

Table 11: Raw data from experiment 3 of the hexane saturated with acetonitrile and tholin sample.

Chapter 3: CH₄/N₂ Tholins and their Solubility in Liquid Hydrocarbons

Introduction

Organic molecules are produced via methane photolysis in Titan's upper atmosphere (Hanel et al., 1981). Another interesting feature of Titan is the lakes present in the polar regions of the moon. Other than Earth, Titan is the only planetary body in the solar system where liquids are present on the surface. However, unlike Earth, Titan's liquids are comprised primarily of methane-ethane-nitrogen-dominated lakes and seas (Raulin et al., 2010; Stofan et al., 2007; Tan et al., 2013). Within these largely nonpolar lakes, small amounts of polar compounds, such as acetonitrile and acrylonitrile might also be present (Cordier et al., 2009, 2012; Corrales et al., 2017).

Interactions between the organics created in Titan's atmosphere and the nonpolar lakes are likely, and this liquid environment could facilitate more interesting reactions. Many studies have investigated the solubility of laboratory analogs to these organics called "tholins". Previous studies have found that reactions between these tholins and solvents such as acetonitrile and methanol have the potential to create compounds of astrobiological relevance, such as nitriles and amino acids (such as glycine, alanine in certain solvents; He & Smith, 2014; Kawai et al., 2013).

Previous Laboratory Studies on Titan Tholin Solubility

On Titan, these organics fall to the surface and may interact with liquid hydrocarbons, including methane rain (Barnes et al., 2013; Turtle et al., 2011) and the hydrocarbon lakes. Preliminary studies have shown that tholins are only weakly soluble in non-polar solvents such as methane and ethane, and will increase in solubility as the polarity of the solvent increase (Carrasco et al., 2009; Coll et al., 1999; McKay, 1996; Sarker et al., 2003).

Carrasco et al. (2009) observed that 35% of the tholins by mass used during their study were soluble in methanol, leaving 65% insoluble. With this large of a difference in solubility percentage between the soluble and insoluble tholin fractions, not all tholins created will be soluble in polar solvents. For this reason, our investigation will observe the liquid and solid fractions of the sample solutions.

While many investigations have observed tholins in singular solvents and produced various biologically important compounds such as glycine, alanine, and hydrogen cyanide, to the best of our knowledge, none have studied tholin reactions in a mixture of polar and nonpolar liquids (Carrasco et al., 2009; Coll et al., 2001; He & Smith, 2014; Kawai et al., 2013; McKay, 1996; Sarker et al., 2003). While methane and ethane are the abundant liquids on Titan, both are in the gas phase under room temperature and pressure. Therefore, hexane (liquid at ambient conditions) was used as a non-polar alkane solvent instead. Furthermore, the solubility of acetonitrile in hexane is quite low, with previous studies finding a mole fraction of 0.035 (Nagata, 1987; Rusling et al., 1969). To this end, we plan to investigate any chemical changes in Titan tholins once they have been added to solutions of liquid hydrocarbons using acetonitrile and hexane mixtures.

Methods

A Technics Hummer II sputtering chamber triggered a DC cold plasma discharge at 30mA that ionized the Titan-simulated 90% N₂/ 10% CH₄ gas mixture which produced tholins after several days. Chamber conditions of 3.00 Torr were maintained at room temperature for 3 days with a continuous flow <5 STCM of the N₂-CH₄ gas mixture. Glass slides inside the chamber collected tholin deposits. Approximately 50-100 mg of tholins were produced using this process.

HPLC grade (>99.8%) acetonitrile, hexane, and a mixture of the acetonitrile in hexane were used as solvents. We prepared a saturated solution of acetonitrile in hexane, which based on previous literature is equivalent to a mole fraction of acetonitrile of 0.035 (Nagata 1987; Rusling et al. 1969) and a solution of hexane in acetonitrile for comparison which has a mole fraction of 0.970 (Nagata 1987; Rusling et al. 1969.) One half milligram of tholins was added to 1 mL of solvent. After the addition of tholins to these solvents, the samples were mixed and allowed to interact for 1, 12, 24, and 72 hours at room temperature to observe changes occurring over various time scales. Once liquid solvents were added to the solid tholin samples, the resulting solution was kept under argon gas within a desiccator along with Drierite desiccant to absorb any ambient moisture.

Liquid and solid fractions were separated by filtration with a .2 μ m PTFE filter. While this will lead to measuring tholins in an environment that is not entirely like Titan lakes (as the organics will not go through an filtering on Titan), it will allow an better look into the individual chemistry of both the soluble and insoluble fraction of tholins. The resulting liquid was place on sample plates and allowed to dry so that any organics within the liquid fraction could be analyzed. The resulting residue was analyzed using a Bruker Ultraflex II matrix-assisted laser desorption/ionization – time of flight- mass spectrometer (MALDI-TOF-MS) with a 337 nm Nitrogen and 355 nm YAG laser. During analysis an α -Cyano-4-hydroxycinnamic acid (HCCA) matrix was used. This technique was incorporated as it provides compounds' molecular weights.

The solid powder left behind after dissolution was analyzed with Fourier-transform infrared spectroscopy (FTIR) using a Shimadzu IRAffinity-1S FTIR with an IR source, KBr beamsplitter, and a DLATGS detector. As is typical with ATR utilization, the exact amounts of

the solid residue placed on the plate varied each time. This was selected to investigate bonds and functional group differences between each sample.

Results

After tholins were added into each liquid beaker, visual differences were observed between the pure solvents and the hexane / acetonitrile mixture. Figure 1 shows tholins dispersed into the liquid as they were added to acetonitrile and hexane. However, when tholins were added to the solution of hexane saturated with acetonitrile, they sank to the bottom of the beaker within seconds, where they appear as larger particle aggregates compared to the other liquid samples.

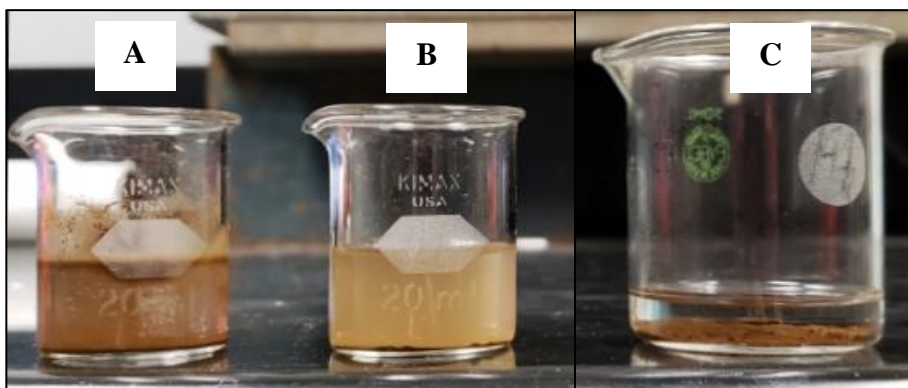


Figure 1: Solvents after 5mg of tholins were added in immediate succession. Hexane (A) and acetonitrile (B) all had similar results; the tholins slowly dispersed into the liquids, and with time, settled at the bottom. Hexane saturated with acetonitrile (C) shows tholins resting at the bottom after they immediately sank, which left behind a clear solvent in contrast to the other samples.

Solid Fraction

FTIR measurements were taken from each solid sample and were compared by solvent. Spectra from duplicate experiments are shown in the appendix. Figure 2 displays samples that were allowed to interact for approximately 12 hours, with spectra from samples in hexane (red),

acetonitrile (navy), and hexane saturated with acetonitrile (pink). These spectra were compared to untreated tholins (green). Spectra were similar and showed no large variations. Some peaks were observed at 2900 cm^{-1} that we are attributing to $-\text{CH}_3$ and CH_2 functional groups. All spectra also showed similar bands at $\sim 3300\text{cm}^{-1}$, $\sim 2900\text{ cm}^{-1}$, and various peaks in the fingerprint region ($1700\text{-}1000\text{ cm}^{-1}$ regions). We are correlating the 2900 cm^{-1} peaks to $-\text{CH}_2$ and $-\text{CH}_3$ groups and the 3300cm^{-1} peaks to amine groups.

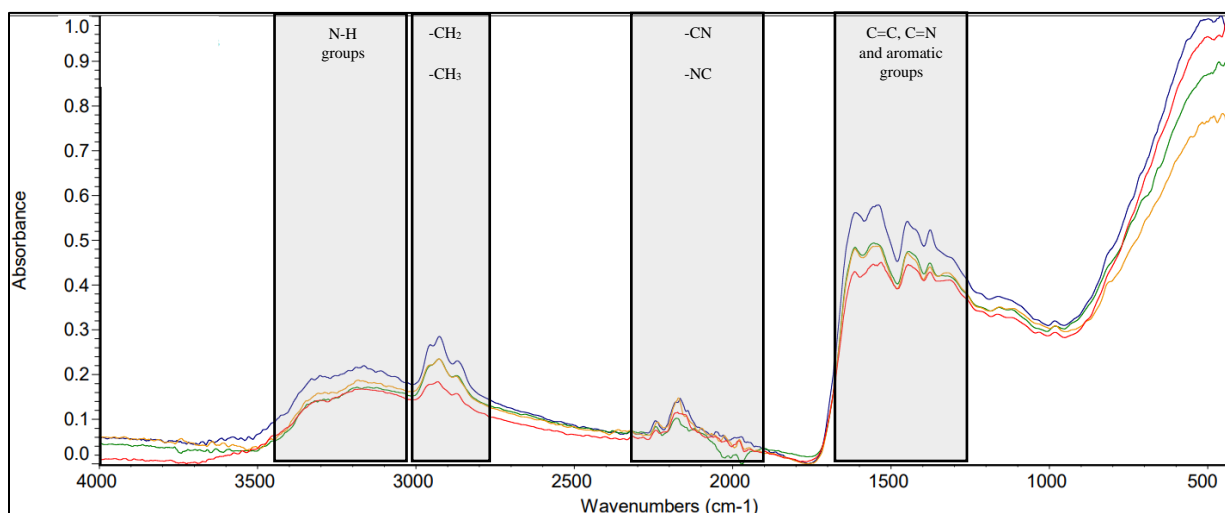


Figure 2: Normalized FTIR spectra from tholins dissolved for 12 hours in hexane (yellow), acetonitrile (red), and hexane saturated with acetonitrile (blue) at room temperature. An unreacted tholin spectrum is also shown (green). Spectra in each solvent sample were similar, with peaks observed in the sample in the 1700 cm^{-1} range (corresponding and $\text{C}=\text{C}$, $\text{C}=\text{N}$ and aromatic groups) and the 2300 cm^{-1} range (corresponding to $-\text{NC}/-\text{CN}$ groups), respectively. Peaks in the 2900 cm^{-1} range we are corresponding to $-\text{CH}_2$ and $-\text{CH}_3$ groups. Additionally, a large arching peak was observed from $3300\text{-}3000$ that we are corresponding to amine (N-H) groups.

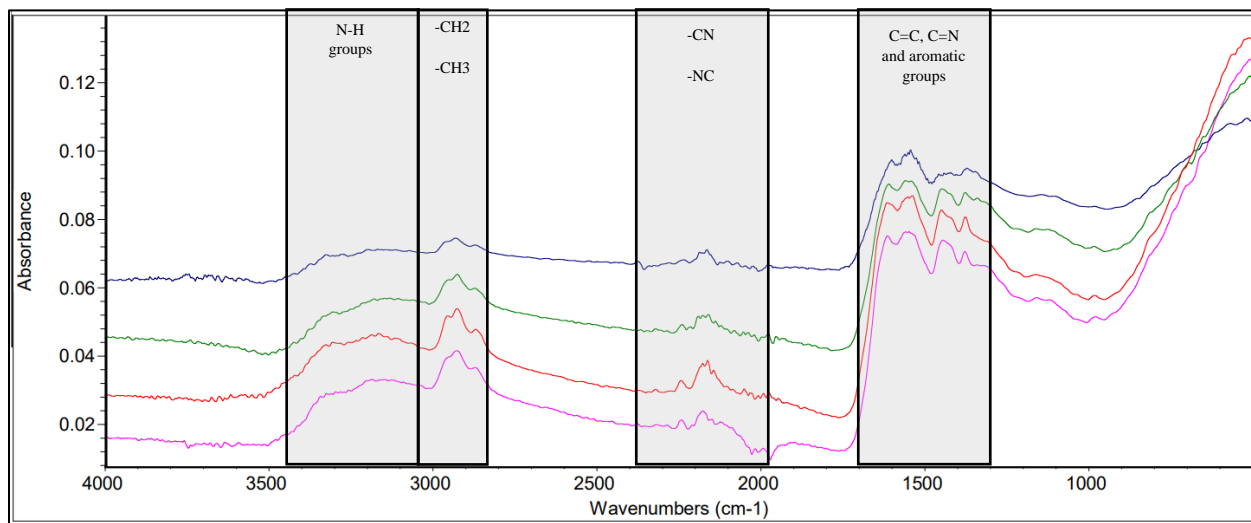


Figure 3: Absorbance spectra of tholins with hexane saturated with acetonitrile for 12 hours (red), 24 hours (green), and 72 hours (blue), at room temperature. Original tholins that were not added to any solvent (pink) were also included as a reference. Peaks observed in the sample in the 1700 cm^{-1} range (corresponding to C=C, C=N and aromatic groups) and the 2300 cm^{-1} range (corresponding to -NC/-CN groups), respectively. Peaks in the 2900 cm^{-1} range we are corresponding to -CH₂ and -CH₃ groups. Additionally, a large arching peak was observed from 3300-3000 that we are corresponding to amine (N-H) groups. Spectra offset for clarity.

Samples of tholins reacted with hexane saturated with acetonitrile were analyzed for spectral changes at various time scales. Timescales of 72, 24, and 12 hours were measured (Figure 3) and compared to study any effect's different reaction times might have on the solid fraction. We interpret spectral peaks in the 1600 cm^{-1} region to correspond NH_2 and R-C=N groups, while 1500 cm^{-1} and fingerprint region corresponding to C-CH_3 , C-CH_4 , $=\text{CH}_2$ groups. No major changes between samples were observed in this wavelength range.

Liquid fraction

To determine variations between the samples, MALDI analysis was utilized on each of the liquid fractions. Results from repeated experiments are shown in the appendix. Figure 4 shows results from 24-hour hexane, acetonitrile, and hexane saturated with acetonitrile. Spectrum A in Figure 4 shows results taken from the liquid fractions from the sample of tholins reacted in pure hexane. In the spectrum, background peaks that are consistently observed in each spectrum are attributed to the HCCA matrix and are not utilized in our analysis.

Spectrum B in Figure 4 shows results taken from the liquid fractions from the acetonitrile and tholin sample. In the spectrum, large gaussian distributions of peaks within the sample are observed. Each peak observed in the distributions will correlate to a molecule's molecular weight indicating that a compound was detected at every molecular weight. A majority of these gaussian distributions were observed under 300m/z with their highest peaks at $\sim 80\text{m/z}$, $\sim 115\text{m/z}$, $\sim 123\text{m/z}$, $\sim 137\text{m/z}$, $\sim 150\text{m/z}$, $\sim 172\text{m/z}$, $\sim 190\text{m/z}$, $\sim 207\text{m/z}$, $\sim 218\text{m/z}$, $\sim 235\text{m/z}$, $\sim 245\text{m/z}$, $\sim 250\text{m/z}$, $\sim 260\text{m/z}$, $\sim 272\text{m/z}$, and $\sim 285\text{m/z}$, $\sim 299\text{m/z}$, $\sim 313\text{m/z}$. This is consistent with the previous literature of tholins being soluble in polar solvents.

Spectrum C in Figure 4 shows results taken from the liquid fractions from the hexane saturated with acetonitrile and tholin sample. In the spectrum, only peaks from the background of

HCCA were observed. Overall, large molecular compounds were mostly observed in the pure polar compound (acetonitrile).

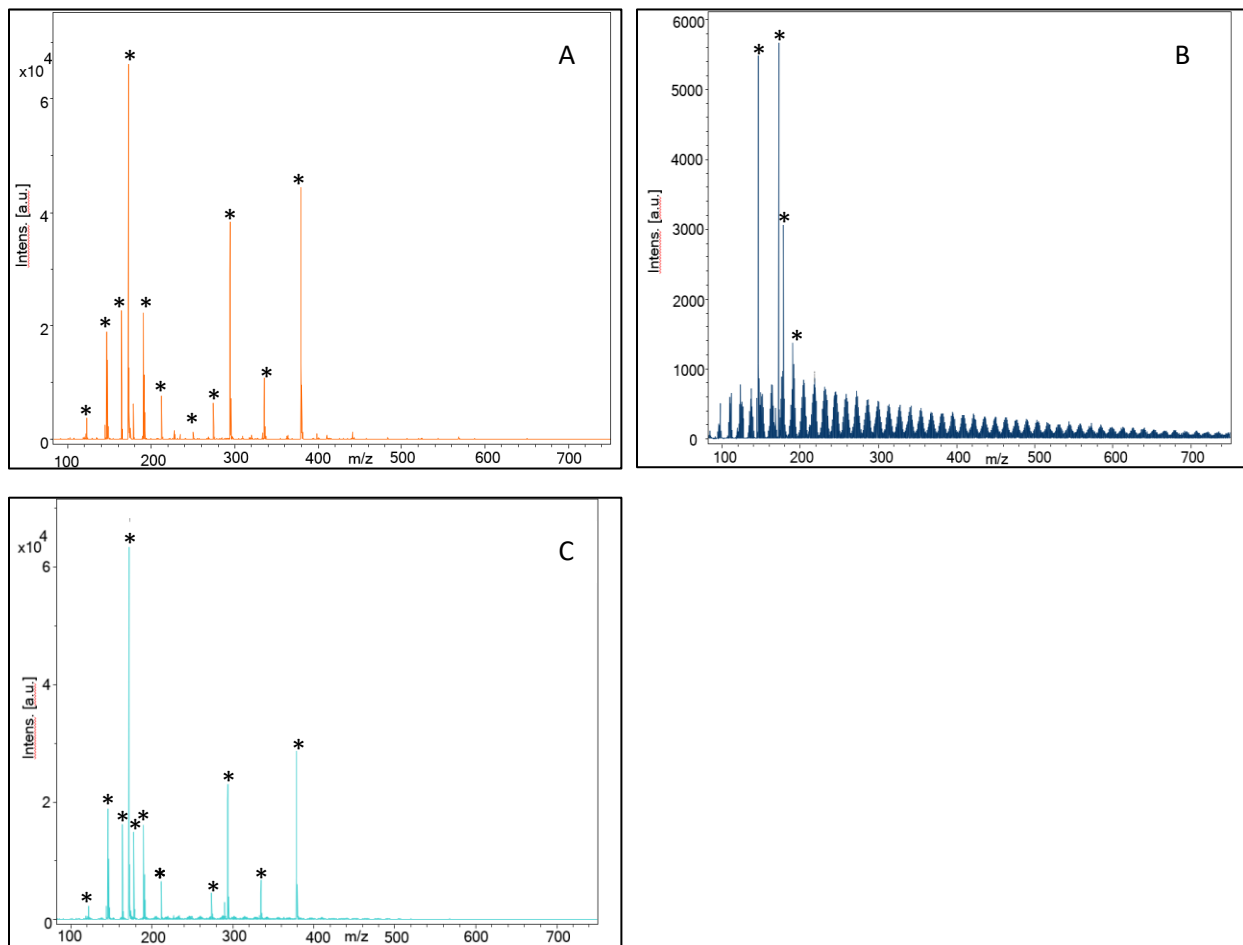


Figure 4: MALDI spectra taken from the liquid fraction of hexane (A), acetonitrile (B), and hexane saturated with acetonitrile (C). Large peaks consistently seen in each spectrum are attributed to the HCCA matrix and background noise. These peaks are denoted by a star (*).

RESULTS FROM THESE STUDIES SHOW INSIGHT INTO THE INTERACTIONS OF THAL THOLINS IN SOLVENTS analogous to Titan lakes. Tholins produced in a laboratory setting may be changing on a physical level (Fig. 1) with the organics in the mixture of solvents sinking to the bottom of the liquid as opposed to tholins in the singular solvent diffusing throughout the liquid. FTIR spectra show

minimal difference between the different timescales and solvents. However, it might be possible that differences in peak height could correlate to losing and gaining functional groups.

Previous studies have shown that tholins may react in different fractional quantities based on solvent polarity (Carrasco et al., 2009). The portion of tholins that is soluble in polar compounds could react in lakes of Titan, even with the small amounts of polar compounds found within the lakes. Products from these reactions may contain variable amounts of -CH₃ and -CH₂, and CN/NC, as most Titan organics do. However the quantity of these functional groups within these tholins might change based on their reaction times.

MALDI results do not show large differences between the hexane and the hexane saturated with acetonitrile, indicating that the observations of tholin immediately settling to the bottom of the liquid might be a physical interaction as opposed to a chemical change within the organics. While organics are observed in the acetonitrile sample (as is expected from previous literature (Carrasco et al., 2009; Coll et al., 1999; McKay, 1996; Sarker et al., 2003), no organic products are observed in the hexane or hexane saturated with acetonitrile samples. We interpret these results to indicate that products from any solubility between the primarily non-polar solvents and tholins were not contained in the liquid fraction at large enough volumes to be detected by MALDI. Therefore, from these results we can conclude that tholins would be largely insoluble in the lakes of Titan.

From our visual observations, the solid organics sank toward the bottom of the solvents. Previous studies have noted that organics sinking to the bottom of Titan lakes and organics increasing in particle size could potentially occur (Yu et al., 2017, 2020). Our observations provide experimental evidence of particles potentially sinking to the bottom of Titan lakes.

Further study is needed to identify specific compounds produced and their astrobiological potential. While our methods did provide insight into the general trends of tholin solubility in solvents, additional analytical techniques could provide compositional breakdowns. FTIR spectra will show bonds and functional groups but does not go as far as to provide sample composition. MALDI results did not show any dissolved organics within the liquid samples, however utilizing a technique with better resolution and compositional information (such as liquid chromatography–mass spectrometry (LCMS) could provide more information. Additionally, completing solubility studies with polar and nonpolar solvents at Titan-relevant temperature and pressure might produce different results. Our research provides a foundation for these future studies.

References

- Barnes, J. W., Buratti, B. J., Turtle, E. P., Bow, J., Dalba, P. A., Perry, J., Brown, R. H., Rodriguez, S., Mouélic, S. L., Baines, K. H., Sotin, C., Lorenz, R. D., Malaska, M. J., McCord, T. B., Clark, R. N., Jaumann, R., Hayne, P. O., Nicholson, P. D., Soderblom, J. M., & Soderblom, L. A. (2013). Precipitation-induced surface brightenings seen on Titan by Cassini VIMS and ISS. *Planetary Science*, 2(1), 1. <https://doi.org/10.1186/2191-2521-2-1>
- Carrasco, N., Schmitz-Afonso, I., Bonnet, J.-Y., Quirico, E., Thissen, R., Dutuit, O., Bagag, A., Laprévotte, O., Buch, A., Giuliani, A., Adandé, G., Ouni, F., Hadamcik, E., Szopa, C., & Cernogora, G. (2009). Chemical Characterization of Titan's Tholins: Solubility, Morphology and Molecular Structure Revisited. *The Journal of Physical Chemistry A*, 113(42), 11195–11203. <https://doi.org/10.1021/jp904735q>
- Coll, P., Coscia, D., Smith, N., Gazeau, M.-C., Ramírez, S. I., Cernogora, G., Israël, G., & Raulin, F. (1999). Experimental laboratory simulation of Titan's atmosphere: Aerosols and gas phase. *Planetary and Space Science*, 47(10–11), 1331–1340. [https://doi.org/10.1016/S0032-0633\(99\)00054-9](https://doi.org/10.1016/S0032-0633(99)00054-9)
- Coll, P., Ramirez, S. I., Navarro-Gonzalez, R., & Raulin, F. (2001). Chemical and optical behaviour of tholins, laboratory analogues of Titan aerosols. *Advances in Space Research*, 27(2), 289–297. [https://doi.org/10.1016/S0273-1177\(01\)00060-6](https://doi.org/10.1016/S0273-1177(01)00060-6)
- Cordier, D., Mousis, O., Lunine, J. I., Lavvas, P., & Vuitton, V. (2009). An Estimate of the Chemical Composition of Titan. *The Astrophysical Journal*, 707(2), L128–L131. <https://doi.org/10.1088/0004-637X/707/2/L128>
- Cordier, D., Mousis, O., Lunine, J. I., Lebonnois, S., Rannou, P., Lavvas, P., Lobo, L. Q., & Ferreira, A. G. M. (2012). Titan's lakes chemical composition: Sources of uncertainties and variability. *Planetary and Space Science*, 61(1), 99–107. <https://doi.org/10.1016/j.pss.2011.05.009>
- Corrales, L. R., Yi, T. D., Trumbo, S. K., Shalloway, D., Lunine, J. I., & Usher, D. A. (2017). Acetonitrile cluster solvation in a cryogenic ethane-methane-propane liquid: Implications for Titan lake chemistry. *The Journal of Chemical Physics*, 146(10), 104308. <https://doi.org/10.1063/1.4978395>

- Hanel, R., Conrath, B., Flasar, F. M., Kunde, V., Maguire, W., Pearl, J., Pirraglia, J., Samuelson, Herath, L., Allison, M., Cruikshank, D., Gautier, D., Gierasch, P., Horn, L., Koppany, R., & Ponnampertuma, C. (1981). *Infrared Observations of the Saturnian System from Voyager 1*. 10.
- He, C., & Smith, M. A. (2014). Solubility and stability investigation of Titan aerosol analogs: New insight from NMR analysis. *Icarus*, 232, 54–59.
<https://doi.org/10.1016/j.icarus.2014.01.007>
- Kawai, J., Jagota, S., Kaneko, T., Obayashi, Y., Khare, B. N., McKay, C. P., & Kobayashi, K. (2013). Titan Tholins as Amino Acid Precursors and Their Solubility in Possible Titan Liquidospheres. *Chemistry Letters*, 42(6), 633–635. <https://doi.org/10.1246/cl.130101>
- McKay, C. P. (1996). Elemental composition, solubility, and optical properties of Titan's organic haze. *Planetary and Space Science*, 44(8), 741–747. [https://doi.org/10.1016/0032-0633\(96\)00009-8](https://doi.org/10.1016/0032-0633(96)00009-8)
- Nagata, I. (1987). Liquid-liquid equilibria for the acetonitrile + methanol + saturated hydrocarbon and acetonitrile + 1-butanol + saturated hydrocarbon systems. *Thermochimica Acta*, 114(2), 227–238.
- Raulin, F., Hand, K. P., McKay, C. P., & Viso, M. (2010). Exobiology and Planetary Protection of icy moons. *Space Science Reviews*, 153(1–4), 511–535.
<https://doi.org/10.1007/s11214-009-9610-x>
- Rusling, J. F., Bertsch, R. J., Barford, R. A., & Rothbart, H. L. (1969). Liquid equilibria in the system methyl oleate-methyl palmitate-acetonitrile-hexane. Significance in Craig countercurrent extraction. *Journal of Chemical & Engineering Data*, 14(2), 169–173.
<https://doi.org/10.1021/jc60041a018>
- Sarker, N., Somogyi, A., Lunine, J. I., & Smith, M. A. (2003). Titan aerosol analogues: Analysis of the nonvolatile tholins. *Astrobiology*, 3(4), 719–726.
<https://doi.org/10.1089/153110703322736042>

- Stofan, E. R., Elachi, C., Lunine, J. I., Lorenz, R. D., Stiles, B., Mitchell, K. L., Ostro, S., Soderblom, L., Wood, C., Zebker, H., Wall, S., Janssen, M., Kirk, R., Lopes, R., Paganelli, F., Radebaugh, J., Wye, L., Anderson, Y., Allison, M., ... West, R. (2007). The lakes of Titan. *Nature*, *445*(7123), 61–64. <https://doi.org/10.1038/nature05438>
- Tan, S. P., Kargel, J. S., & Marion, G. M. (2013). Titan’s atmosphere and surface liquid: New calculation using Statistical Associating Fluid Theory. *Icarus*, *222*(1), 53–72. <https://doi.org/10.1016/j.icarus.2012.10.032>
- Turtle, E. P., Perry, J. E., Hayes, A. G., Lorenz, R. D., Barnes, J. W., McEwen, A. S., West, R. A., Del Genio, A. D., Barbara, J. M., Lunine, J. I., Schaller, E. L., Ray, T. L., Lopes, R. M. C., & Stofan, E. R. (2011). Rapid and Extensive Surface Changes Near Titan’s Equator: Evidence of April Showers. *Science*, *331*(6023), 1414–1417. <https://doi.org/10.1126/science.1201063>
- Yu, X., Hörst, S. M., He, C., McGuiggan, P., & Bridges, N. T. (2017). Direct Measurement of Interparticle Forces of Titan Aerosol Analogs (“Tholin”) Using Atomic Force Microscopy. *Journal of Geophysical Research: Planets*, *122*(12), 2610–2622. <https://doi.org/10.1002/2017JE005437>
- Yu, X., Hörst, S. M., He, C., McGuiggan, P., Kristiansen, K., & Zhang, X. (2020). Surface Energy of the Titan Aerosol Analog “Tholin.” *The Astrophysical Journal*, *905*(2), 88. <https://doi.org/10.3847/1538-4357/abc55d>

Appendix

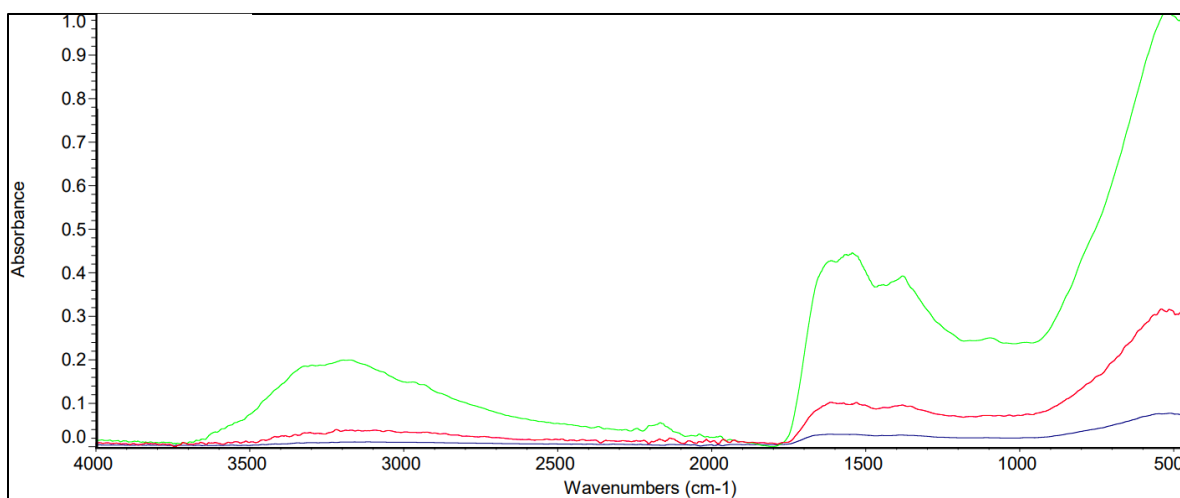


Figure 5: Normalized FTIR spectra from repeated experiment of tholins dissolved in hexane (blue), acetonitrile (red), and hexane saturated with acetonitrile (green) at room temperature.

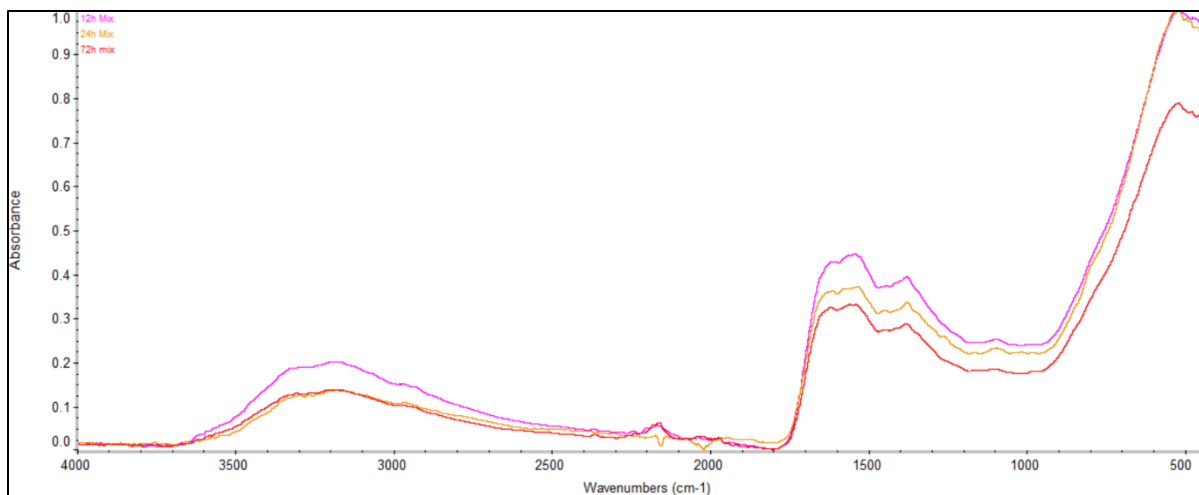


Figure 6: Normalized absorbance spectra of repeated experiments of tholins with hexane saturated with acetonitrile for 12 hours (pink), 24 hours (orange), and 72 hours (red), at room temperature.

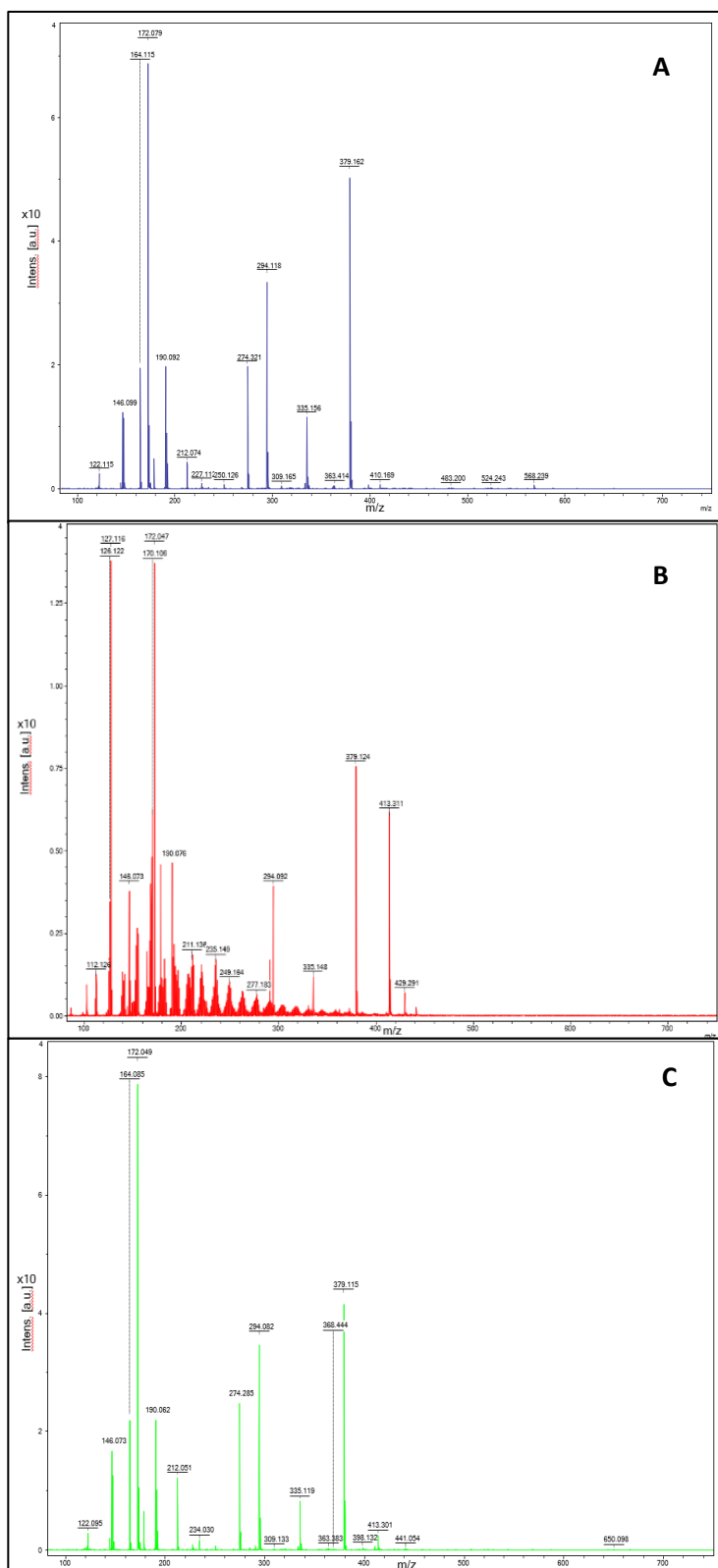


Figure 7: MALDI spectra taken from repeat experiments of the liquid fraction of hexane (A), acetonitrile (B), and hexane saturated with acetonitrile (C). Large peaks consistently seen in each spectrum are attributed to the HCCA matrix and background noise

CH₄/N₂/CO Tholins and their Solubility in Liquid Hydrocarbons: Implications for Titan Habitability

Introduction

Titan holds some of the most complicated organic chemical processes known in our solar system. In Titan's upper atmosphere, UV radiation from the sun interacts with the 94% nitrogen and 6% methane creating complex organic aerosols (Hanel et al., 1981, 1981; Israël et al., 2005; Vinatier et al., 2010; Yung et al., 1984). These hydrocarbons form in the thermosphere and become more complex as they fall toward the surface (Waite et al., 2007). During Huygens' descent to the surface of Titan, the Aerosol Collector and Pyrolyser (ACP) onboard gathered samples of the atmosphere where GC-MS analysis identified hydrogen cyanide (HCN) and ammonia (NH₃) in the collected specimens (Israël et al., 2005). Laboratory experiments have created analogs for these hydrocarbons and referred to them as 'tholins'.

Titan's atmosphere is primarily made up of methane and nitrogen, and not an insignificant quantity of oxygen containing species is also known to be present (Baines et al., 2006; Coustenis et al., 1998; Lutz et al., 1983; Samuelson et al., 1983). Indeed, the fractions of these oxygen containing compounds will vary within the atmospheric structure and will change over differing altitudes. Previous studies of the experimental, observational, and modeling results of oxygen compounds in Titan's atmosphere and relative abundance show a higher levels of CO, CO₂, and H₂O in the stratosphere where many of the organic compounds are produced (Baines et al., 2006; Coustenis et al., 1998; de Kok et al., 2007; Dobrijevic et al., 2014; Hörst et al., 2008, 2012b; Lutz et al., 1983; Samuelson et al., 1983)

While most of elements in Titan's atmosphere are going to be nitrogen containing hydrocarbons, the CO, CO₂, and H₂O in the atmosphere are going to be incorporated into the

resulting organics. Previous studies have shown that oxygen present can be incorporated into compounds including NO (nitric oxide), HNO (nitrosyl hydride), HNCO (isocyanic acid) and N₂O (nitrous oxide; Dobrijevic et al., 2014). Additionally, the production of amino acids and nucleotide bases in this environment with oxygen has also been reported (Hörst et al., 2012b).

Additionally, Titan is the only other planetary body besides Earth to have liquid present on its surface. Lakes present in the polar regions of Titan have been found to contain nonpolar liquid hydrocarbons, primarily methane and ethane, along with small amounts of polar compounds (Cordier et al., 2009, 2012; Corrales et al., 2017; McKay, 1996; Raulin et al., 2010; Stofan et al., 2007; Tan et al., 2013). It has also been shown that tholins are only weakly soluble in non-polar solvents such as methane and ethane (Carrasco et al., 2009; He et al., 2012; Kawai, Jagota, Kaneko, Obayashi, Khare, Mckay, et al., 2013). However, amino acids, such as glycine, alanine, aspartic acid, valine, and nitriles have been observed to form in certain polar solutions, such as acetonitrile (He & Smith, 2014a; Hörst et al., 2012a; Kawai et al., 2013). While Titan lakes will mostly be comprised of nonpolar methane and ethane, the small amounts of polar compounds (like acetonitrile) present in the lakes present an opportunity for the organics to react with the polar hydrocarbons (Cordier et al., 2009, 2012; Corrales et al., 2017).

Production of many biologically relevant compounds is reliant on the presence of oxygen. Amino acids and nucleosides require oxygen in their structures, along with many other biological processes on Earth. The oxygen present in the atmosphere can be incorporated in the organic structures during production at the high altitudes (Hartle et al., 2006; Hörst et al., 2008). Tholins with and without oxygen have been found in previous solubility studies to create biologically relevant compounds, including amino acids (He & Smith, 2014b, 2014b; Kawai et al., 2013; Sarker et al., 2003).

While numerous solubility experiments have been conducted, to our knowledge there has been no such study with tholins produced with oxygen containing species and mixtures of polar and non-polar solvents analogous to Titan lakes. Our project seeks to investigate the interactions of oxygen containing tholins within mixtures of polar acetonitrile and non-polar hexane solvents to better characterize the Titan lake environment.

Methods

To simulate the lakes on Tian, mixtures of solvents were incorporated to simulate the polar and non-polar hydrocarbons comprising the liquid. While the lakes are primarily comprised of methane and ethane, both are in the gas phase at room temperature. Hexane was selected as the nonpolar analog to methane and ethane for these experiments as they were conducted at room temperature and pressure. Experiments were conducted at room temperature as the warmer temperature will increase reaction rates, and to lay a foundation as to what to expect from these reactions in future studies Acetonitrile was selected as the polar solvent as it known to be present in Titan's atmosphere (Niemann et al. 2005). Previous investigations found acetonitrile and hexane to have a mole fraction of ~ 0.035 (Nagata 1987; Rusling et al. 1969) in primarily hexane solutions at room temperature. This provides an analog solvent to the environment in Titan lakes as the solution will be mostly non-polar (hexane) with low amounts of polar acetonitrile.

Tholins were produced in a Hummer Technics II Sputtering machine's cold plasma discharge at 60 Hz, 115 V and 15 A. A gas mixture of (by volume) 96% nitrogen (N_2), 2% methane (CH_4), and 2% carbon monoxide (CO) was utilized, in line with previous experiments (Hörst et al. 2012b). Additionally, tholins without oxygen containing gas (95% N_2 /5% CH_4) were also produced and used as a comparison. Production continued for ~ 72 hours with the resulting

tholins deposited on VWR Micro Slides (25x75 mm) for easy removal of the organics and limiting the amount of handling the organics encountered during sample preparation.

After production, 5 mg of tholins were added to 1 ml of either hexane, acetonitrile, or hexane saturated with acetonitrile. After tholins were added to their respective solvents, samples were placed in a desiccator under Argon to reduce atmospheric oxygen contamination. DrieRite desiccant was placed at the bottom of the desiccator to absorb any ambient moisture. Once samples had interacted for 72 hours the liquid within the sample vials was extracted and filtered from the solid precipitate with a 0.22 μm filter. The solid precipitate was allowed to dry at room temperature under Ar to ensure all solvent was removed. The solid portion was analyzed with a Shimadzu IRAffinity-1S Fourier transform infrared spectrophotometer (FTIR) with a KBr beamsplitter, an IR source, and a TEC InGaAs 2.6 μm detector to investigate bond and functional groups within the different samples. Variable quantities were used for the ATR analysis.

Matrix-assisted laser desorption/ionization (MALDI) was utilized to observe any differences in the organics' molecular weight between tholins produced with and without oxygen. A 337 nm Nitrogen and 355 nm YAG laser was used during analysis along with an α -Cyano-4-hydroxycinnamic acid (HCCA) matrix. The liquid portion of the samples was dried and analyzed by direct infusion electrospray ionization mass spectrometry (ESI-MS) with the same 337 nm Nitrogen and 355 nm YAG laser utilized in the MALDI analysis. ESI-MS was incorporated as it provides a general overview of the compounds within a sample's molecular weights.

Results

Comparison of Tholins Produced with and without Oxygen

MALDI spectra taken from tholins incorporating oxygen differed slightly from organics produced with a N_2/CH_4 gas mixture (Figures 1 and 2). Gaussian distributions of peaks observed in the two organics spectra are shown. Peaks in the oxygen containing tholins range in height from ~ 50 - 300 au while the N_2/CH_4 tholins have a height ranging from ~ 50 - 250 au. Additionally, the peaks are higher in the spectra of oxygen containing tholins within the 0 - 300 m/z range compared to tholins produced without oxygen (Figure 2).

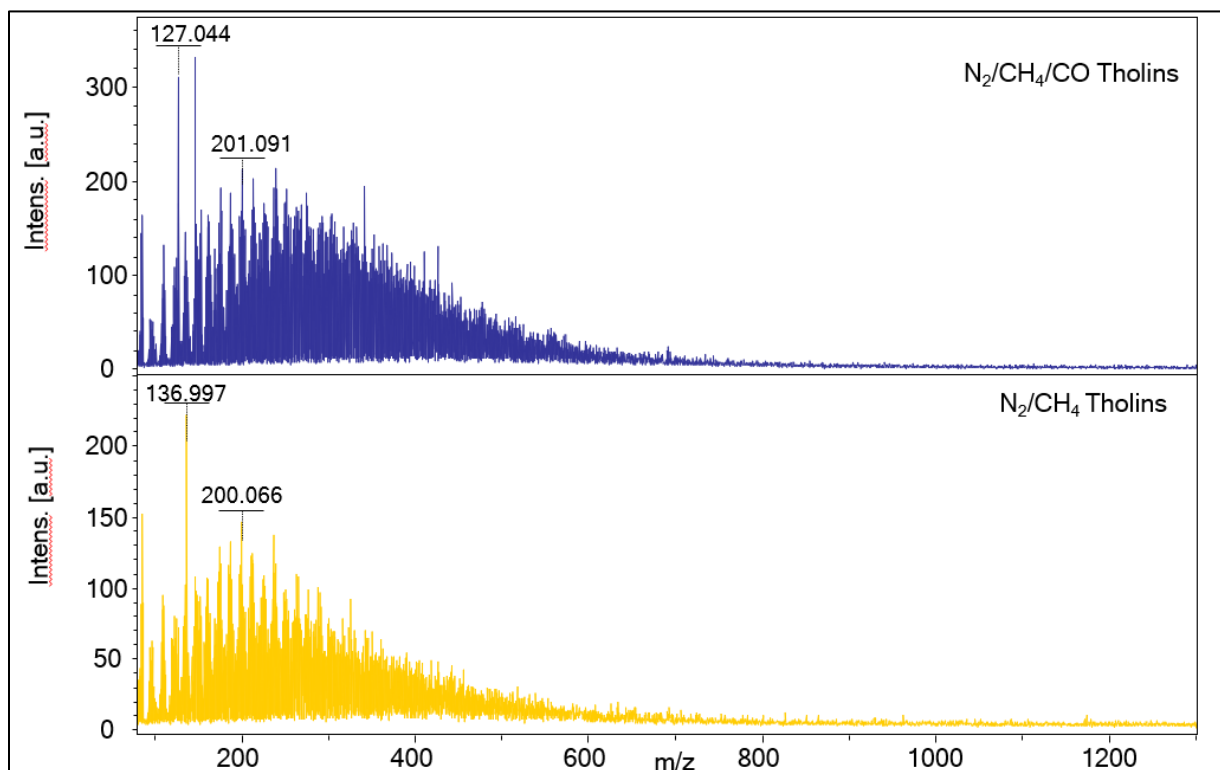


Figure 11: Tholins produced with carbon monoxide (CO; top) and without an oxygen containing species (bottom). Peaks within each sample were similar, however peak heights within the oxygen tholins were slightly higher in the 0 - 300 m/z range. Additionally, peaks above 300 m/z were much higher and more numerous than tholins produced without oxygen.

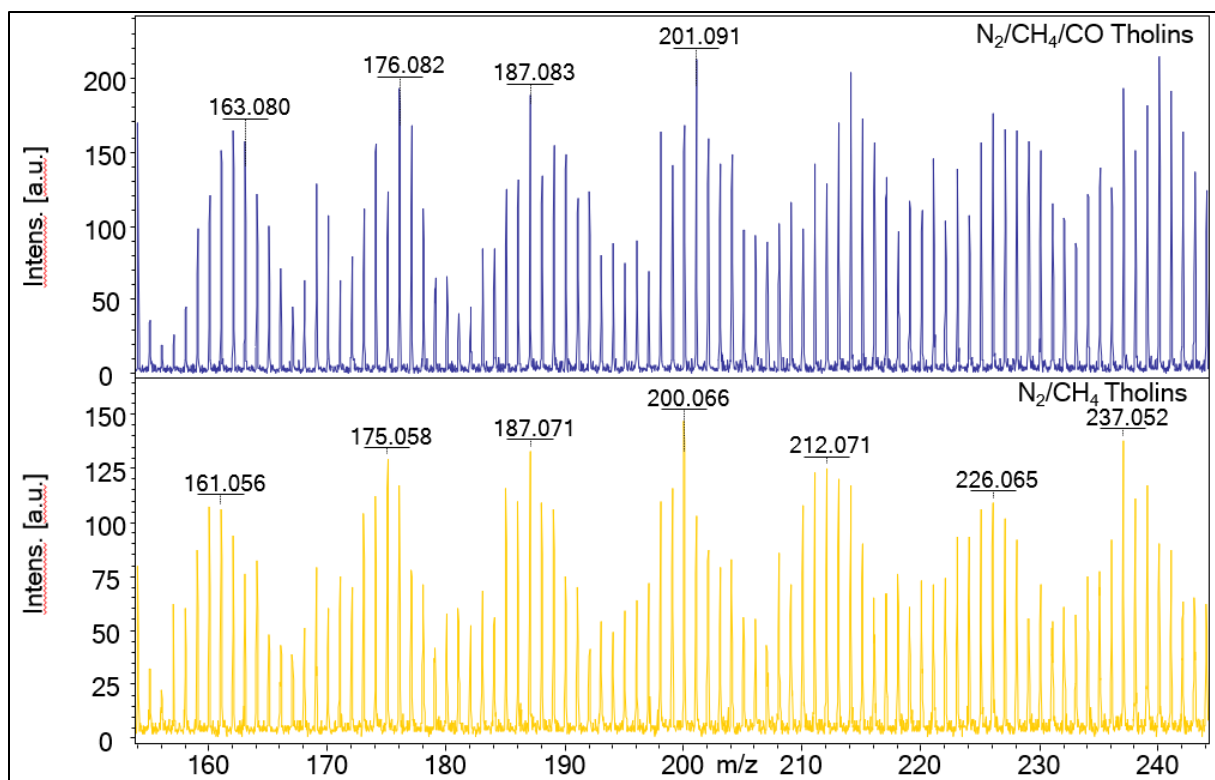


Figure 12: MALDI spectra at the 150-250 m/z range. Tholins produced with oxygen (top) and without oxygen (bottom) are similar with slightly higher peaks observed in tholins with oxygen.

Observing peaks within the 150-250 m/z range of both oxygen and non-oxygen containing tholin show similar peaks, however there are some differences. Tholins containing oxygen show much wider peaks, despite having similar peak heights to tholins produced without oxygen (Figure 2). For example, peaks around (226 m/z) are observed in both samples, however

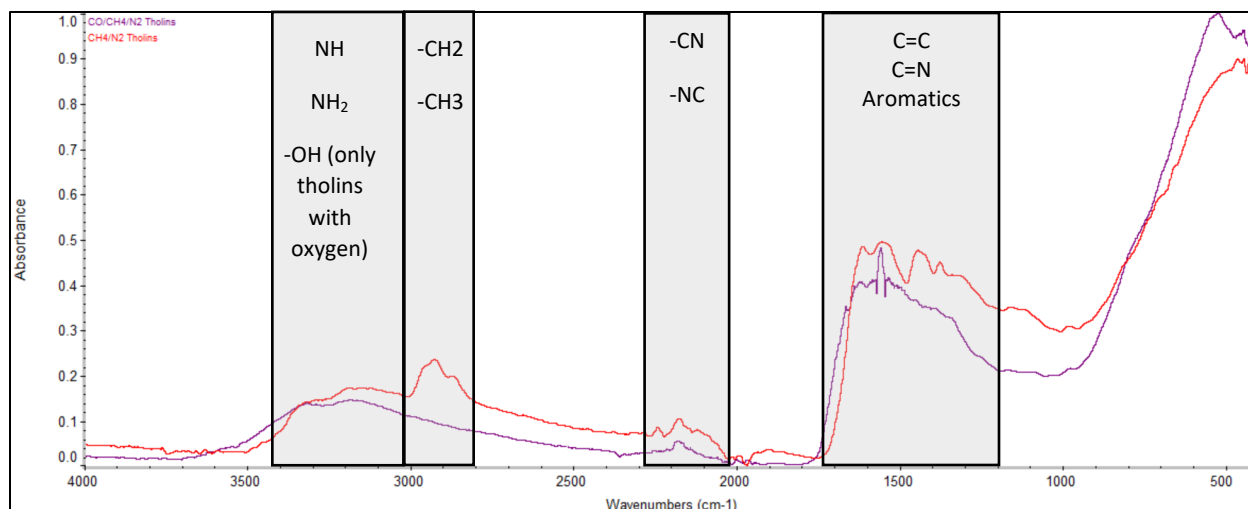


Figure 3: Normalized spectra comparing tholins produced with and without oxygen containing species. While some features are observed in both samples (such as the strong peaks observed in the $\sim 1300\text{ cm}^{-1}$ $\sim 1700\text{ cm}^{-1}$ range), differences are present. For example the absence of the strong peak around 2900 cm^{-1} as well as the broader peak in the 3100 cm^{-1} - 3500 cm^{-1} range in the tholins produced with oxygen are indicative of molecular differences between the two samples.

the troughs of these peaks (around 205 m/z and 220 m/z) have higher peak intensity in the oxygen containing tholins when compared to the non-oxygen containing tholins.

Additionally, FTIR comparison between oxygen and non-oxygen containing tholins does show some differences (Figure 3). Most prominently is the absence of the strong peak observed in the $\sim 2900\text{ cm}^{-1}$ range correlating to $-\text{CH}_2$ and $-\text{CH}_3$ groups being more present in the tholins without oxygen. Additionally, the broader peak in the 3100 cm^{-1} - 3500 cm^{-1} range in the tholins produced with oxygen are indicative not only of changes in the NH/NH_2 incorporation between the two samples, but also the inclusion of $-\text{OH}$ groups in the tholins produced with CO .

Solid Fraction

FTIR spectra show little variation between tholins before and after their reactions within solvents (Figure 4). A range in peak heights within 1250 cm^{-1} - 1700 cm^{-1} was observed.

However, higher range of these spectra correlate to various carbonyl groups, such as ketones and

acids (around $1700\text{cm}^{-1} - 1750\text{cm}^{-1}$). Additionally, peaks were observed in $3000\text{ cm}^{-1} - 3500\text{ cm}^{-1}$ range indicating the incorporation of $-\text{NH}$ and $-\text{NH}_2$ functional groups. However, the higher wavenumbers within this range also correlate to alcohol groups (around $3400\text{cm}^{-1} - 3700\text{cm}^{-1}$).

Interestingly, we do see differences between the solvents. Similar spectra are observed in the pure tholins and the tholins interacting in hexane (as expected as tholins are largely unreactive in non-polar solvents), the functional groups found in the acetonitrile and hexane saturated with acetonitrile are much more prevalent than the pure tholins. Despite the concentration of acetonitrile in the mixture being minimal, the spectra from organics in hexane saturated with acetonitrile lies equidistant in between the spectra from organics in hexane and acetonitrile singularly.

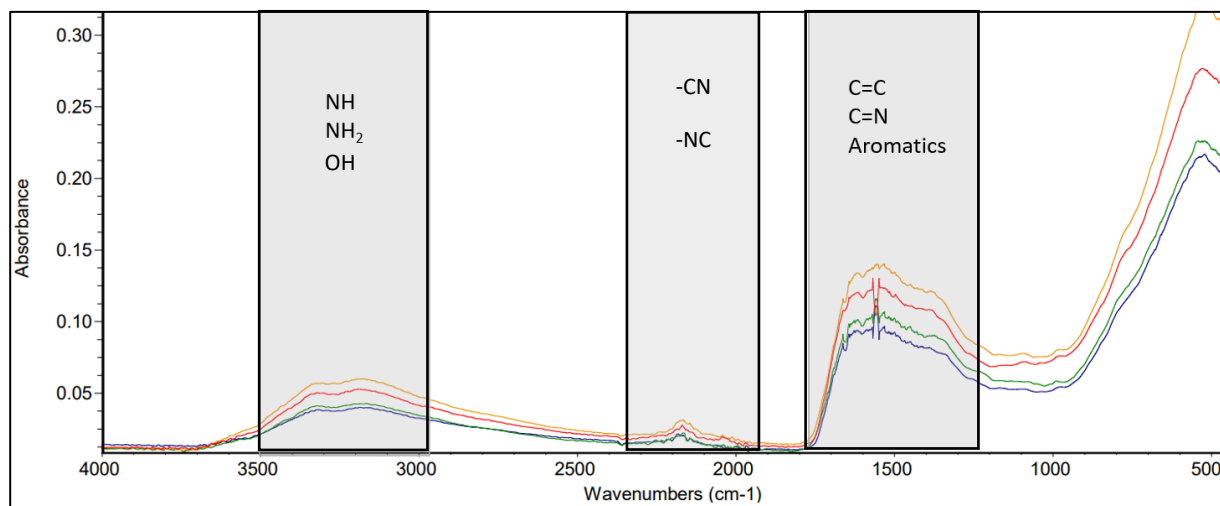


Figure 4: Common scale FTIR spectra from the solid fraction of oxygen containing tholins after interacting with solvents for 24 hours. Tholins that were not added with any solvent (navy), were compared to hexane (green), acetonitrile (yellow), and hexane saturated with acetonitrile (red). Peaks can be observed in the acetonitrile and hexane saturated with acetonitrile samples within the $1250\text{cm}^{-1} - 1700\text{cm}^{-1}$ m/z range and correlate to the presence of pi bonds and aromatic structures. Additionally, peaks in the 2300 cm^{-1} range correlate to $-\text{CN}/-\text{NC}$ groups. Peaks can also be observed in the $3000\text{cm}^{-1} - 3600\text{cm}^{-1}$ range in the acetonitrile and hexane saturated with acetonitrile samples.

Liquid Fraction

Liquid fractions of the tholin/solvent samples were analyzed with ESI-MS and compared to observe any differences in the organics dissolved. Background noise in the ESI-MS spectra was observed in the hexane sample (Figure 5 A) and the hexane saturated with acetonitrile sample (Figure 5 C). This background noise was not included in our analysis. Peaks observed in the acetonitrile sample (Figure 4 B) are to be expected as tholins have been previously observed to be soluble in polar liquids.

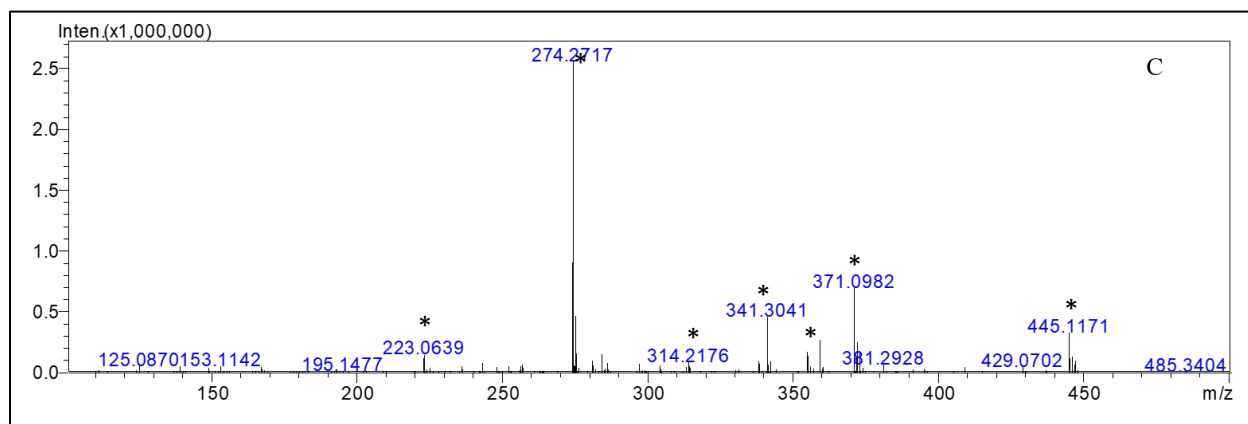
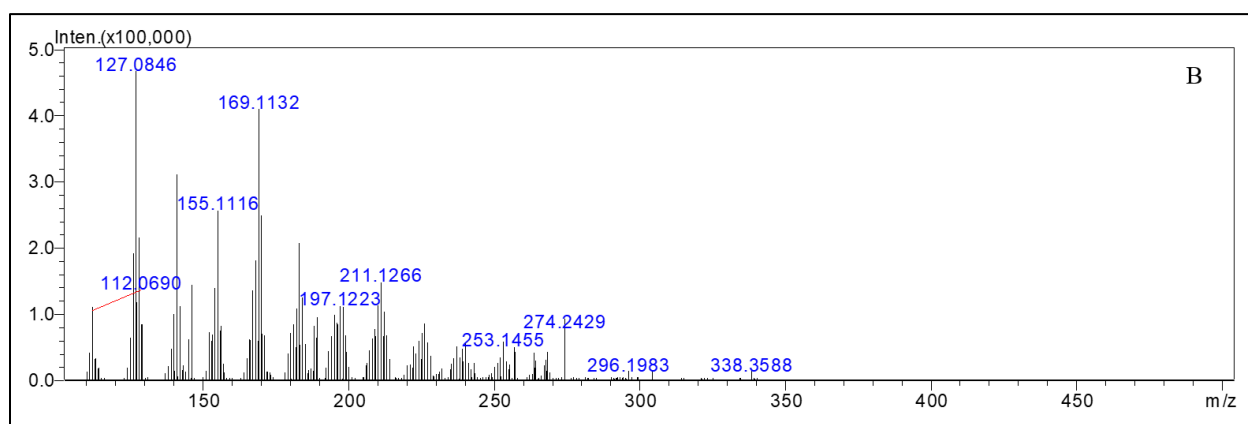
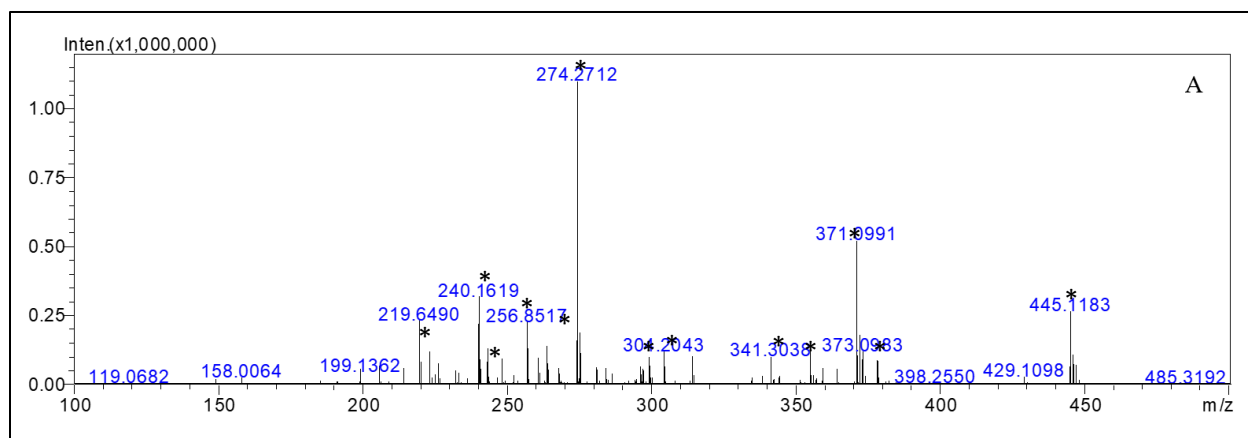


Figure 5: ESI-MS measurements from the liquid fractions of the tholin-solvent samples. Hexane (A), acetonitrile (B), and hexane saturated with acetonitrile (C) were analyzed. Acetonitrile shows compounds present within the liquid. Background noise was seen in the hexane and hexane saturated with acetonitrile samples and was not included in our analysis. These peaks are denoted with a star (*). No other compounds were observed in the primarily nonpolar liquids.

Discussion

Our results show that tholins being produced with an oxygen containing gas have many similarities to N_2/CH_4 tholin in their behavior in mixtures of polar and nonpolar solvents.

MALDI results from oxygen containing tholins may show an increase in the general peak height throughout the spectra and an increase in peak quantity within higher molecular weights (>300 m/z) as compared to non-oxygen containing tholins, however analysis with a higher resolution equipment would be necessary for confirmation.

FTIR spectra show very little difference in bond structures based on solution solvent. However, some small variations might have been observed. Increases in spectral height in the 1250cm^{-1} - 1700cm^{-1} m/z range may show the incorporation of double bonds, and aromatic ring structures in the sample containing polar solvent. Additionally increases in spectral height in the 3000 cm^{-1} – 3600 cm^{-1} m/z may show the incorporation of -NH, -NH₂, and possibly alcohol groups in the sample containing polar solvent. However, overall no large distinct differences were observed between the spectra. This leads to the conclusion that very little to no bond changes were detectable with FTIR in the solid tholin residue after being added to the solvents.

With previous chapters in this dissertation concluding that higher mass ranges correspond to aromatic molecules, it is possible that the oxygen is being incorporated into and is leading to a greater production of, higher molecular weights compounds, many of which will be aromatic rings structures, as they will be the most thermodynamically stable. If occurring, this could have great implications for the habitability of Titan, as many biologically important compounds, such purines, pyrimidines, and certain amino acids, require ring structures that include oxygen. The presence of oxygen containing rings could mean a production of these biologically relevant compounds or their molecular precursors within the lakes of Titan.

Data from ESI-MS measurements do not show any soluble compounds within the hexane, or the mixture of hexane saturated with acetonitrile liquid samples. The samples utilizing acetonitrile were found to have organics dissolved in the solution. However, this was expected based on previous literature. We interpret these results to indicate that tholins in the lakes of Titan will not be soluble with the liquids present in any manner detectable with ESI-MS. While some reactions might be occurring, they might not have a meaningful impact on increasing the biological potential in the lakes of Titan if that are reacting on the levels observed in this study.

While results from this study can provide insight into the overall themes present within oxygen containing tholins and their solubility in different solvents, further investigation is needed. Analytical techniques such as GC/LC-MS on the liquid fractions of these tholin-solvent samples could provide information on the compounds present in the solution. Additionally, studying tholin solubility in hexane saturated with acetonitrile under Titan temperature and pressure could provide more accurate data on the environment in Titan lakes, and its habitability.

References

- Baines, K. H., Drossart, P., Lopez-Valverde, M. A., Atreya, S. K., Sotin, C., Momary, T. W., Brown, R. H., Buratti, B. J., Clark, R. N., & Nicholson, P. D. (2006). On the discovery of CO nighttime emissions on Titan by Cassini/VIMS: Derived stratospheric abundances and geological implications. *First Results on Titan from VIMS Observations Onboard the Cassini/Huygens Mission/Astrobiology: The Detection of Life in the Solar System*, 54(15), 1552–1562. <https://doi.org/10.1016/j.pss.2006.06.020>
- Carrasco, N., Schmitz-Afonso, I., Bonnet, J. Y., Quirico, E., Thissen, R., Dutuit, O., Bagag, A., Laprévotte, O., Buch, A., Giuliani, A., Adandé, G., Ouni, F., Hadamcik, E., Szopa, C., & Cernogora, G. (2009). Chemical characterization of titan's tholins: Solubility, morphology and molecular structure revisited. *Journal of Physical Chemistry A*, 113(42), 11195–11203. <https://doi.org/10.1021/jp904735q>
- Cordier, D., Mousis, O., Lunine, J. I., Lavvas, P., & Vuitton, V. (2009). An Estimate of the Chemical Composition of Titan. *The Astrophysical Journal*, 707(2), L128–L131. <https://doi.org/10.1088/0004-637X/707/2/L128>
- Cordier, D., Mousis, O., Lunine, J. I., Lebonnois, S., Rannou, P., Lavvas, P., Lobo, L. Q., & Ferreira, A. G. M. (2012). Titan's lakes chemical composition: Sources of uncertainties and variability. *Planetary and Space Science*, 61(1), 99–107. <https://doi.org/10.1016/j.pss.2011.05.009>
- Corrales, L. R., Yi, T. D., Trumbo, S. K., Shalloway, D., Lunine, J. I., & Usher, D. A. (2017). Acetonitrile cluster solvation in a cryogenic ethane-methane-propane liquid: Implications for Titan lake chemistry. *The Journal of Chemical Physics*, 146(10), 104308. <https://doi.org/10.1063/1.4978395>
- Coustenis, A., Salama, A., Lellouch, E., Encrenaz, Th., Bjoraker, G. L., Samuelson, R. E., de Graauw, Th., Feuchtgruber, H., & Kessler, M. F. (1998). Evidence for water vapor in Titan's atmosphere from ISO/SWS data. *Åp*, 336, L85–L89.
- de Kok, R., Irwin, P. G. J., Teanby, N. A., Lellouch, E., Bézard, B., Vinatier, S., Nixon, C. A., Fletcher, L., Howett, C., Calcutt, S. B., Bowles, N. E., Flasar, F. M., & Taylor, F. W. (2007). Oxygen compounds in Titan's stratosphere as observed by Cassini CIRS. *Icarus*, 186(2), 354–363. <https://doi.org/10.1016/j.icarus.2006.09.016>

- Dobrijevic, M., Hébrard, E., Loison, J. C., & Hickson, K. M. (2014). Coupling of oxygen, nitrogen, and hydrocarbon species in the photochemistry of Titan's atmosphere. *Icarus*, 228, 324–346. <https://doi.org/10.1016/j.icarus.2013.10.015>
- Hanel, R., Conrath, B., Flasar, F. M., Kunde, V., Maguire, W., Pearl, J., Pirraglia, J., Samuelson, Herath, L., Allison, M., Cruikshank, D., Gautier, D., Gierasch, P., Horn, L., Koppany, R., & Ponnampuruma, C. (1981). *Infrared Observations of the Saturnian System from Voyager 1*. 10.
- Hartle, R. E., Sittler Jr., E. C., Neubauer, F. M., Johnson, R. E., Smith, H. T., Crary, F., McComas, D. J., Young, D. T., Coates, A. J., Simpson, D., Bolton, S., Reisenfeld, D., Szego, K., Berthelier, J. J., Rymer, A., Vilppola, J., Steinberg, J. T., & Andre, N. (2006). Preliminary interpretation of Titan plasma interaction as observed by the Cassini Plasma Spectrometer: Comparisons with Voyager 1. *Geophysical Research Letters*, 33(8). <https://doi.org/10.1029/2005GL024817>
- He, C., Lin, G., Upton, K. T., Imanaka, H., & Smith, M. A. (2012). Structural investigation of Titan tholins by solution-state ¹H, ¹³C, and ¹⁵N NMR: One-Dimensional and Decoupling Experiments. *Journal of Physical Chemistry A*, 116(19), 4760–4767. <https://doi.org/10.1021/jp3016062>
- He, C., & Smith, M. A. (2014a). Solubility and stability investigation of Titan aerosol analogs: New insight from NMR analysis. *Icarus*, 232, 54–59. <https://doi.org/10.1016/j.icarus.2014.01.007>
- He, C., & Smith, M. A. (2014b). Solubility and stability investigation of Titan aerosol analogs: New insight from NMR analysis. *Icarus*, 232, 54–59. <https://doi.org/10.1016/j.icarus.2014.01.007>
- Hörst, S. M., Vuitton, V., & Yelle, R. V. (2008). Origin of oxygen species in Titan's atmosphere. *Journal of Geophysical Research: Planets*, 113(E10). <https://doi.org/10.1029/2008JE003135>
- Hörst, S. M., Yelle, R. V., Buch, A., Carrasco, N., Cernogora, G., Dutuit, O., Quirico, E., Sciamma-O'Brien, E., Smith, M. A., Somogyi, Á., Szopa, C., Thissen, R., & Vuitton, V. (2012a). Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere

Simulation Experiment. *Astrobiology*, 12(9), 809–817.
<https://doi.org/10.1089/ast.2011.0623>

Hörst, S. M., Yelle, R. V., Buch, A., Carrasco, N., Cernogora, G., Dutuit, O., Quirico, E., Sciamma-O'Brien, E., Smith, M. A., Somogyi, Á., Szopa, C., Thissen, R., & Vuitton, V. (2012b). Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere Simulation Experiment. *Astrobiology*, 12(9), 809–817.
<https://doi.org/10.1089/ast.2011.0623>

Israël, G., Szopa, C., Raulin, F., Cabane, M., Niemann, H. B., Atreya, S. K., Bauer, S. J., Brun, J.-F., Chassefière, E., Coll, P., Condé, E., Coscia, D., Hauchecorne, A., Millian, P., Nguyen, M.-J., Owen, T., Riedler, W., Samuelson, R. E., Siguier, J.-M., ... Vidal-Madjar, C. (2005). Complex organic matter in Titan's atmospheric aerosols from in situ pyrolysis and analysis. *Nature*, 438(7069), 796–799. <https://doi.org/10.1038/nature04349>

Kawai, J., Jagota, S., Kaneko, T., Obayashi, Y., Khare, B. N., McKay, C. P., & Kobayashi, K. (2013). Titan Tholins as Amino Acid Precursors and Their Solubility in Possible Titan Liquidospheres. *Chemistry Letters*, 42(6), 633–635. <https://doi.org/10.1246/cl.130101>

Kawai, J., Jagota, S., Kaneko, T., Obayashi, Y., Khare, B. N., McKay, C. P., & Kobayashi, K. (2013). Titan Tholins as Amino Acid Precursors and Their Solubility in Possible Titan Liquidospheres. *Chemistry Letters*, 42(6), 633–635. <https://doi.org/10.1246/cl.130101>

Lutz, B. L., de Bergh, C., & Owen, T. (1983). Titan: Discovery of Carbon Monoxide in Its Atmosphere. *Science*, 220(4604), 1374–1375.
<https://doi.org/10.1126/science.220.4604.1374>

McKay, C. P. (1996). Elemental composition, solubility, and optical properties of Titan's organic haze. *Planetary and Space Science*, 44(8), 741–747. [https://doi.org/10.1016/0032-0633\(96\)00009-8](https://doi.org/10.1016/0032-0633(96)00009-8)

Nagata, I. (1987). Liquid-liquid equilibria for the acetonitrile + methanol + saturated hydrocarbon and acetonitrile + 1-butanol + saturated hydrocarbon systems. *Thermochimica Acta*, 114(2), 227–238.

- Niemann, H. B., Atreya, S. K., Bauer, S. J., Carignan, G. R., Demick, J. E., Frost, R. L., Gautier, D., Haberman, J. A., Harpold, D. N., Hunten, D. M., Israel, G., Lunine, J. I., Kasprzak, W. T., Owen, T. C., Paulkovich, M., Raulin, F., Raaen, E., & Way, S. H. (2005). The abundances of constituents of Titan's atmosphere from the GCMS instrument on the Huygens probe. *Nature*, *438*(7069), 779–784. <https://doi.org/10.1038/nature04122>
- Raulin, F., Hand, K. P., McKay, C. P., & Viso, M. (2010). Exobiology and Planetary Protection of icy moons. *Space Science Reviews*, *153*(1–4), 511–535. <https://doi.org/10.1007/s11214-009-9610-x>
- Rusling, J. F., Bertsch, R. J., Barford, R. A., & Rothbart, H. L. (1969). Liquid equilibria in the system methyl oleate-methyl palmitate-acetonitrile-hexane. Significance in Craig countercurrent extraction. *Journal of Chemical & Engineering Data*, *14*(2), 169–173. <https://doi.org/10.1021/je60041a018>
- Samuelson, R. E., Maguire, W. C., Hanel, R. A., Kunde, V. G., Jennings, D. E., Yung, Y. L., & Aikin, A. C. (1983). CO₂ on Titan. *Journal of Geophysical Research: Space Physics*, *88*(A11), 8709–8715. <https://doi.org/10.1029/JA088iA11p08709>
- Sarker, N., Somogyi, A., Lunine, J. I., & Smith, M. A. (2003). Titan aerosol analogues: Analysis of the nonvolatile tholins. *Astrobiology*, *3*(4), 719–726. <https://doi.org/10.1089/153110703322736042>
- Stofan, E. R., Elachi, C., Lunine, J. I., Lorenz, R. D., Stiles, B., Mitchell, K. L., Ostro, S., Soderblom, L., Wood, C., Zebker, H., Wall, S., Janssen, M., Kirk, R., Lopes, R., Paganelli, F., Radebaugh, J., Wye, L., Anderson, Y., Allison, M., ... West, R. (2007). The lakes of Titan. *Nature*, *445*(7123), 61–64. <https://doi.org/10.1038/nature05438>
- Tan, S. P., Kargel, J. S., & Marion, G. M. (2013). Titan's atmosphere and surface liquid: New calculation using Statistical Associating Fluid Theory. *Icarus*, *222*(1), 53–72. <https://doi.org/10.1016/j.icarus.2012.10.032>
- Vinatier, S., Bézard, B., de Kok, R., Anderson, C. M., Samuelson, R. E., Nixon, C. A., Mamoutkine, A., Carlson, R. C., Jennings, D. E., Guandique, E. A., Bjoraker, G. L., Michael Flasar, F., & Kunde, V. G. (2010). Analysis of Cassini/CIRS limb spectra of Titan acquired during the nominal mission II: Aerosol extinction profiles in the 600–1420

cm⁻¹ spectral range. *Icarus*, 210(2), 852–866.
<https://doi.org/10.1016/j.icarus.2010.06.024>

Waite, J. H., Young, D. T., Cravens, T. E., Coates, A. J., Crary, F. J., Magee, B., & Westlake, J. (2007). The Process of Tholin Formation in Titan's Upper Atmosphere. *Science*, 316(5826), 870–875. <https://doi.org/10.1126/science.1139727>

Yung, Y. L., Allen, M., & Pinto, J. P. (1984). Photochemistry of the atmosphere of Titan—Comparison between model and observations. *The Astrophysical Journal Supplement Series*, 55, 465. <https://doi.org/10.1086/190963>

Appendix

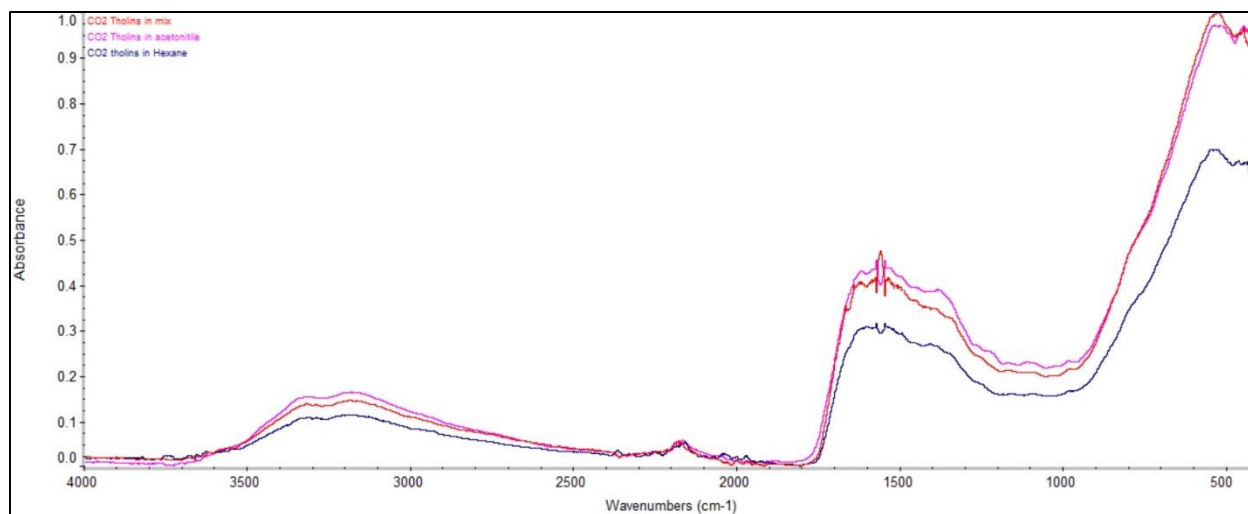


Figure 5: Common scale FTIR spectra from repeat experiment of the solid fraction of oxygen containing tholins after being added to solvents and left for 24 hours. Spectra show tholins after being added to hexane (navy), acetonitrile (pink), and hexane saturated with acetonitrile (red).

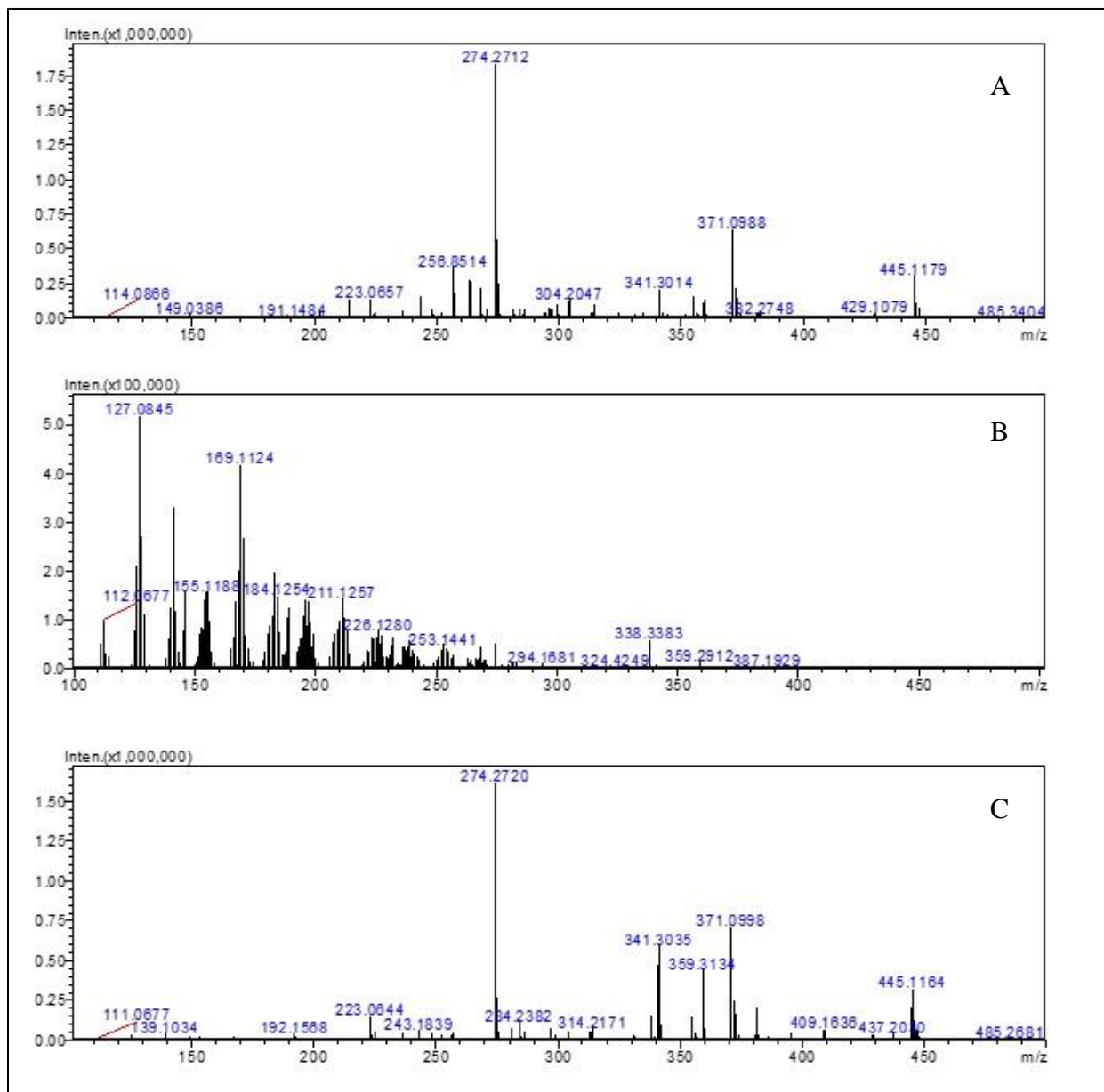


Figure 6: ESI-MS measurements liquid fractions from repeat experiments of the tholin-solvent samples. Hexane (A), acetonitrile (B), and hexane saturated with acetonitrile (C) were analyzed. Acetonitrile shows compounds present within the liquid. Background noise was seen in the hexane and hexane saturated with acetonitrile samples and was not included in our analysis. No other compounds were observed in the primarily nonpolar liquids.

Conclusions and Synthesis

Scientific Motivation

These projects were motivated by our lack of knowledge of the organics on Titan and their ability to be observed in laboratory settings. Previous studies have found low solubility rates within the non-polar solvent, high solubility in polar solvents (Carrasco et al., 2009; Coll et al., 1999; McKay, 1996; Sarker et al., 2003) and smaller amounts of polar compounds (Cordier et al., 2009; Raulin et al., 2010, 2012). Since the lakes present on the surface of Titan are primarily comprised of methane (CH_4) and ethane (C_2H_6), it would seem to reason that the organics on Titan would not react when in contact with these features as these are non-polar compounds.

With the previously observed ability for the tholins to produce biologically relevant compounds (He & Smith, 2014; Kawai et al., 2013; Sarker et al., 2003), it is crucial to understand the solubility of the organics produced on Titan as the presence of polar compounds could have great effects on the habitability of the moon. These projects sought to better understand the solubility of tholins in these environments. By utilizing mixtures of hexane and acetonitrile, we can simulate the low volumes of polar compounds present in the lakes. With the addition of organics, solubility in the samples can inform us about the potential for organics to change in these environments. Additionally, we can provide insight into the potential locations of organics within the lakes as we can observe and quantify physical changes with the tholin samples themselves.

These results are of particular importance due to future missions and investigations planned for Titan (such as DragonFly). Laboratory studies utilizing tholins can make observations from results, however, due to the nature of tholin production, variations in the structure, incorporations of certain groups is to be expected.

Summary of Results and Implications for Titan

Chapter 1 investigated tholins produced at the University of Arkansas Keck Lab and their structural changes between production times of 1 hour, 4 hours, 6 hours, and 12 hours. Direct desorption ionization spectra show very little changes between production times. Interestingly, peaks at every molecular weight, indicating the production of complex organics, were observed at all timescales. Additionally, visual inspection of the MALDI disks show darkening of the organics (indicating a higher production of compounds which is to be expected as production times increase). Based on our results, the chemistry producing these organic compounds might be occurring at very early production stages, possibly occurring in timescales >1 hour.

Chapter 2 investigated the physical reactions of tholins within hexane, acetonitrile, and acetonitrile-saturated hexane. Dispersity values and hydrodynamic diameters of the tholin particles within each solvent were analyzed with DLS. This study provided evidence that the organics produced on Titan will sink toward the bottom with tholins in the mixture of solvent settling at the bottom leaving behind a clear liquid.

Chapter 3 observes the chemical changes occurring in the solvents. Tholins were allowed to interact for 12-, 24-, and 72-hour timescales. Liquid fractions were extracted using a .2 μ m PTFE filter. The liquid was analyzed using FTIR to observe any spectral shifts and bond preferences, while MALDI was used to investigate dissolvable compounds present in each of the liquid samples.

FTIR spectra from this study showed very little difference between the solid tholin residue after being added to each solvent, indicating that there were no changes in the solid fraction that were detectable with FTIR. Additionally, MALDI spectra show no organics in the hexane saturated with acetonitrile. From this result we conclude that there would not be any

chemical changes detectable with MALDI in the lakes of Titan. Observations from tholins within the mixture of hexane saturated with acetonitrile show the particles immediately sinking toward the bottom of the solvent, providing experimental evidence for organics present in the evaporites on Titan.

Chapter 4 investigated how oxygen containing tholins in liquid hydrocarbons will solubilize. FTIR results show very little difference between the solid portion of these samples. We interpret these results to indicate that little to no reactions are occurring in the solid phase of these samples detectable by FTIR. ESI-MS spectra show no organics present in the hexane and hexane saturated with acetonitrile liquid fractions. Organics were only present in the polar solvent, acetonitrile, as to be expected based on previous literature identifying tholins to be partially soluble in polar liquids.

From the results presented in this dissertation, we can conclude that organics produced in laboratory studies will be similar to each other in their molecular weight distributions and will not be dependent on production time. Our results indicate that the only difference between samples will be organic abundance. From a physical perspective, organics might increase in diameter when in a mixture of non-polar and polar solvents. From a chemical perspective, the solubility of tholins in the lakes of Titan are very low. As MALDI and ESI measurements show, tholins in our study did not show organics dissolved in the liquid phase. Therefore, the potential for organics to be solubilizing in the lakes of Titan is low.

Future Research

These studies lay the foundation for future investigations into Titan tholins. While our investigations are observing large structural differences and themes within tholins, a greater investigation into the specific molecular compounds is needed. Gas chromatography-mass

spectrometry (GCMS) analysis of the liquid fractions in the solubility studies can provide great insight, not only into the compounds produced but also if the compounds have any biological relevance.

All experiments conducted were done so at room temperature and pressure. With surface conditions on Titan being much colder (~ 90 K) and higher pressure (1.5 Barr), the solubility rates and properties may differ from our results. Conducting experiments at Titan temperature and pressure will also allow for solubility studies of tholins to be performed with methane and ethane, bettering aligning with the environment in Titan lakes. Future investigations should conduct the solubility experiments at Titan temperature and pressure.

Lastly, with NASA's Dragonfly mission currently planned to investigate the organics of Titan, our results can inform our expected data return. Our results can help predict expected results and tailor reference libraries as the mission develops. While more investigation is needed into specific structures and Titan condition's effects on the chemistry, this research can help further our understanding of Titan tholins.

References

- Barnes, J. W., Lorenz, R. D., Radebaugh, J., Hayes, A. G., Arnold, K., & Chandler, C. (2015). Production and global transport of Titan's sand particles. *Planetary Science*, 4(1), 1. <https://doi.org/10.1186/s13535-015-0004-y>
- Carrasco, N., Schmitz-Afonso, I., Bonnet, J.-Y., Quirico, E., Thissen, R., Dutuit, O., Bagag, A., Laprévotte, O., Buch, A., Giuliani, A., Adandé, G., Ouni, F., Hadamcik, E., Szopa, C., & Cernogora, G. (2009). Chemical Characterization of Titan's Tholins: Solubility, Morphology and Molecular Structure Revisited. *The Journal of Physical Chemistry A*, 113(42), 11195–11203. <https://doi.org/10.1021/jp904735q>
- Coll, P., Coscia, D., Smith, N., Gazeau, M.-C., Ramírez, S. I., Cernogora, G., Israël, G., & Raulin, F. (1999). Experimental laboratory simulation of Titan's atmosphere: Aerosols and gas phase. *Planetary and Space Science*, 47(10–11), 1331–1340. [https://doi.org/10.1016/S0032-0633\(99\)00054-9](https://doi.org/10.1016/S0032-0633(99)00054-9)
- Cordier, D., Mousis, O., Lunine, J. I., Lavvas, P., & Vuitton, V. (2009). An Estimate of the Chemical Composition of Titan. *The Astrophysical Journal*, 707(2), L128–L131. <https://doi.org/10.1088/0004-637X/707/2/L128>
- He, C., & Smith, M. A. (2014). Solubility and stability investigation of Titan aerosol analogs: New insight from NMR analysis. *Icarus*, 232, 54–59. <https://doi.org/10.1016/j.icarus.2014.01.007>
- Kawai, J., Jagota, S., Kaneko, T., Obayashi, Y., Khare, B. N., McKay, C. P., & Kobayashi, K. (2013). Titan Tholins as Amino Acid Precursors and Their Solubility in Possible Titan Liquidospheres. *Chemistry Letters*, 42(6), 633–635. <https://doi.org/10.1246/cl.130101>
- McKay, C. P. (1996). Elemental composition, solubility, and optical properties of Titan's organic haze. *Planetary and Space Science*, 44(8), 741–747. [https://doi.org/10.1016/0032-0633\(96\)00009-8](https://doi.org/10.1016/0032-0633(96)00009-8)
- Raulin, F., Brassé, C., Poch, O., & Coll, P. (2012). Prebiotic-like chemistry on Titan. *Chemical Society Reviews*, 41(16), 5380. <https://doi.org/10.1039/c2cs35014a>

Raulin, F., Hand, K. P., McKay, C. P., & Viso, M. (2010). Exobiology and Planetary Protection of icy moons. *Space Science Reviews*, 153(1–4), 511–535.
<https://doi.org/10.1007/s11214-009-9610-x>

Sarker, N., Somogyi, A., Lunine, J. I., & Smith, M. A. (2003). Titan aerosol analogues: Analysis of the nonvolatile tholins. *Astrobiology*, 3(4), 719–726.
<https://doi.org/10.1089/153110703322736042>