Self-assembly of tholins in environments simulating Titan liquidospheres: Implications for formation of primitive coacervates on Titan

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Abstract

Titan, the largest satellite of Saturn, has a thick atmosphere containing nitrogen and methane. A variety of organic compounds have been detected in the atmosphere, most likely produced when atmospheric gases are exposed to ultraviolet light, electrons captured by the magnetosphere of Saturn, and cosmic rays. The Cassini / Huygens probe showed that the average temperature on the surface of Titan is 93.7 K, with lakes of liquid ethane and methane. Sub-surface mixtures of liquid ammonia and water may also be present. We have synthesized complex organic compounds (tholins) by exposing a mixture of nitrogen and methane to plasma discharges, and investigated their interactions with several different liquids that simulate Titan's liquidosphere. We found that coacervates formed when tholins were extracted in non-polar solvents followed by exposure to aqueous ammonia solutions. The results suggest that coacervates can self-assemble in Titan's liquidosphere which have the potential to undergo further chemical evolution. Similar processes are likely to occur in the early evolution of habitable planets when tholin-like compounds undergo phase separation into microscopic structures dispersed in a suitable aqueous environment.

Key words: Titan, Tholin, self-assembly, coacervates, liquidosphere.

Introduction

Titan is Saturn's largest satellite with a diameter is 5150 km, and is known to have a dense atmosphere in contact with liquids on its surface (Lunine and Atreya, 2008). The atmospheric pressure at the surface is about 1500 hPa at a temperature of 94 K (Samuelson et al., 1981; Lindal et al., 1983). The main component of the atmosphere is nitrogen, with smaller amounts of methane and ethane. Voyager's infrared interferometer spectrometer also detected traces amounts of ethane (C_2H_4), acetylene (C_2H_2), propane (C_3H_8), hydrogen cyanide (HCN), acetonitrile (CH_3CN), cyanoacetylene ($CHCCN$) and other diverse organic compounds in Titan's atmosphere (Hanel et al., 1981; Kunde et al., 1981; Maguire et al., 1981). In 1995, the Cassini spacecraft was launched by NASA and ESA to explore the Saturn system, and continues to return remarkably detailed images of Saturn and its satellites. In 2005, the Huygens probe separated from Cassini and descended through the atmosphere to Titan's surface. During descent, a gas chromatograph-mass spectrometer (GC/MS) analyzed atmospheric gases and detected HCN and NH_3 after pyrolysis of captured mist particles (Israel et al., 2005).

Syntheses of organic material in simulated Titan atmosphere were first performed by Sagan and Khare (1979) who irradiated mixtures of nitrogen and methane with ultraviolet light and electrical discharge. A viscous brown organic polymer formed, which they named *tholin* after the Greek word for 'muddy'. The tholin was pyrolyzed and the products were analyzed with a gas chromatograph-mass spectrometer (Py-GC/MS). A variety of aliphatic and aromatic hydrocarbons, nitriles and nitrogen-containing compounds such as pyrrole and pyridine were detected after pyrolysis at 900°C. It was suggested that tholins were kerogen-like substances having complex structures. The initial observations were followed by later studies in which a variety of gas mixtures were irradiated with different energy sources and the products analyzed (Khare et al., 1986; Sagan et al., 1993; Ehrenfreund et al., 1995; Coll et al., 1999). (See Table 1 for a list of relevant publications.)

Hydrocarbon lakes on the surface on Titan were first predicted by a photochemical model (Lorenz, 1994). The Huygens probe revealed that Titan's surface temperature was 93.7K (Fulchignoni et al., 2005), at which ethane and methane can remain liquid, and the Cassini radar observations showed that there are lakes composed of hydrocarbons (mainly liquid ethane and methane) at both high and low latitudes (Stofan et al, 2007; Brown et al., 2008; Griffith et al., 2012). Furthermore, if water ice on the surface of Titan is melted by a meteorite strike, as suggested by computer simulations, a liquid water lake would remain for $10^2 \sim 10^4$ years (O'Brien, et al., 2005). Over the 10^8 years history of Titan, it is estimated that 10^{22} kg of carbonaceous chondrites have delivered organic compounds to its surface, including possible lakes (Jones and Lewis 1987). Biologically important elements such as P, S, K and Na are contained in C1 chondrites (Anders and Grevesse 1989), and would also be supplied to Titan's liquidospheres.

Ammonia frost has been observed on the surface of Titan (Nelson et al, 2009), and the existence of sub-surface ammonia-water solutions was suggested by simulation models (Grasset et al., 2000;

Tobie et al., 2005). The pH of an ammonia-water solution with 15 wt% ammonia is ~11.4 (Engel et al., 1994, Fortes, 2000) but the pH is expected to decrease at high pressure to a value closer to ~10.5 (Krauskopf and Bird 1995). Given that both tholins and a liquidsphere are present on Titan, what happens when tholins come into contact with liquids like ammonia-water? There have been several reports on the solubility of tholins. McKay (1996) synthesized tholins by spark discharges in a 9:1 mixture of N₂ and CH₄ and found that they could be dissolved in water, methanol and ethanol. Coll et al. (1999) examined the solubility of tholins formed by direct current (DC) glow discharges in a gas mixture of N₂/CH₄ (98:2), and found that they were soluble in nitriles such as acetonitrile, isobutyronitrile, propionitrile and benzonitrile. When a mixture of CH₄ and N₂ was exposed to an alternating current (AC) electrical discharge at 195 K, the products were soluble in a 1:1 methanol/acetonitrile mixture which was used for ESI-MS and FT-ICR-MS measurements in order to measure their molecular weights and elemental compositions (Sarker et al., 2003; Smogyi et al., 2005). He et al. (2012a, b) synthesized tholins by a similar procedure but with ¹³C and ¹⁵N isotopic labeling, then dissolved them in DMSO to investigate structures of tholins by nuclear magnetic resonance (NMR) spectroscopy.

The presence of a liquidsphere on Titan containing an admixture of tholins suggests that it would be worth investigating how tholins behave in aqueous dispersions. Trainer et al. (2006) irradiated a gas mixture composed of nitrogen and methane, and observed spherical particles several nm in diameter by transmission electron microscopy. However, there have been no studies of tholins exposed to conditions simulating an ammonia-water ocean. The purpose of the present study is to examine self-assembly properties of tholins under these conditions. Both hydrophilic and hydrophobic liquids are presumed to be present on Titan, so we investigated solubility of tholins in non-polar solvents and possible self-assembly properties in a polar ammonia-water solvent. Hexane and chloroform were used in place of methane and ethane because they can exist as liquid at a room temperature. Solutions of tholins in these solvents were analyzed by FT-IR and fluorescence spectrometry. The tholin fractions that dissolved in organic solvents were exposed to an ammonia-water solvent system and were observed to self-assemble into coacervates.

Materials and Methods

Chemicals

Deionized water was further purified with Milli-Q to remove both inorganic ions and organic contaminants. A gas mixture of CH₄/N₂ (10/90) was purchased from Matheson Tri-Gas / Aeris (USA). All other chemicals used were analytical grade. Glassware and a metal rod were heated in high-temperature oven at 500°C to eliminate possible organic contaminants.

Synthesis of Tholins

Tholins was synthesized by plasma discharge into the gas mixture at 1 Torr (133 Pa) for 72 hours.

The discharge device at NASA Ames was RFX-600 with a glass slide attached to the lower electrode. The chamber was flushed with oxygen to clean it before the gas mixture was injected. After plasma discharge for 72 hours, the products on the plate were scraped off with a metallic rod. The collected tholins were dissolved in either non-polar solvents such as hexane and chloroform, or dispersed in an ammonia-water solution, followed by analysis.

FT-IR

FT-IR spectra of tholin samples in chloroform (1 mg mL^{-1}) were obtained with a Nicolet Nexus 670 spectrometer over the range of 4000 to 500 cm^{-1} with a resolution of 1 cm^{-1} . After filtration through a PTFE membrane filter (pore size; $2 \text{ }\mu\text{m}$, Varian Inc.), the filtrate was completely dried, then $50 \text{ }\mu\text{L}$ of chloroform was added. Twenty μL of the solution was dried on a KBr window (PIKE Technologies). The FT-IR sample chamber was constantly purged with dry air to remove moisture.

Fluorescence Spectrometry

Tholin samples were dispersed in $10 \text{ mM NH}_4\text{OH}$ at a concentration of 0.67 mg mL^{-1} . The dispersion was filtered through cellulose acetate filter (pore size; $0.2 \text{ }\mu\text{m}$, ADVANTEC), and the filtrate was put in a quartz cell (Scientific Ltd.). Three-dimensional fluorescence spectra were measured with a JASCO FP-6300 fluorescence spectrometer. Excitation and emission were scanned over the range of 220 - 600 nm at 1 nm intervals with the excitation slit set at 20 nm .

Fluorescence Microscopy

A Zeiss Axiovert 135M fluorescence microscope was used to monitor possible self-assembly properties. Tholin samples dissolved either in chloroform or hexane were dried on a clean glass slide, followed by addition of $10 \text{ }\mu\text{L NH}_4\text{OH}$ and a cover slip. The initial drying process was designed to allow self-assembly of tholins into aggregates which could then be visualized. The same field was observed by differential interference contrast (DIC) mode and in fluorescence mode with a filter (excitation: 365BP25) and a dichroic mirror (370). Images were taken with a digital camera (Canon EOS Kiss X3).

Results

Solubility of tholins

The samples described here were synthesized in gas mixtures either at high pressure (1 Torr , 133 Pa) or low pressure (0.2 Torr , 26 Pa). Hereafter the former product is referred to as HP tholins and the latter as LP tholins. Tholins could be partially dissolved in chloroform to produce a pale yellow-orange solution, but solubility of tholins in hexane was quite low.

FT-IR

Infrared spectra of chloroform soluble fractions of HP and LP tholins are shown in Fig. 1a and b.

The bands at 3324–3366 cm^{-1} (Fig. 1a) and at 3355 cm^{-1} (Fig. 1b) were assigned to stretching of N-H groups. Other bands at 3019, 2959, 2918 and 2849 cm^{-1} (Fig. 1a) and at 3019, 2962, 2918 and 2849 cm^{-1} (Fig. 1b) were assigned to stretching of C-H groups. In addition, tholins gave peaks of unsaturated bonds such as C=C and C=N bonds (1600–1670 cm^{-1}) and C $\equiv$ N bond (2200–2260 cm^{-1}). LP tholins gave stronger peaks of –NH and –CN groups compared the chloroform-soluble fraction of HP tholins.

Fluorescence Spectrometry

Three-dimensional fluorescence spectra of ammonia-water extracted of HP tholins and LP tholins are similar (Fig. 2a and b). Maximum excitation and emission wavelengths of the HP tholins were 346 nm and 426 nm, respectively, and those of LP tholins were 345 nm and 428 nm.

Microscopy

We used DIC microscopy and fluorescence microscopy to investigate the behavior of tholins exposed to aqueous ammonia solutions. After tholin samples were dried from chloroform or hexane solutions onto glass slides, the HP tholins adhered to the slide as brown particles, whereas LP tholins were black. Following addition of 10 mM NH_4OH , the tholins dried from chloroform solutions assembled into spherical structures in the range of 1–15 μm diameters. Tholins dried from hexane solutions produced somewhat larger and more complex globular structures (Fig. 3). Because chloroform is more polar than hexane, the chloroform extract of tholins contained higher concentrations of polar molecules, while hexane extracts contained more non-polar molecules. The difference in polarity probably accounts for the observed variations in the self-assembled structures.

Discussion

In order to understand the self-assembly properties of tholins, it is essential to have general knowledge of their synthesis and composition. Possible energy sources available on Titan include ultraviolet light, electrical discharge and cosmic rays. Ultraviolet light is not absorbed by molecular nitrogen gas and therefore does not photochemically activate a nitrogen-methane mixture (Yung et al., 1984), so synthesis of organic compounds containing nitrogen requires higher energy sources such as electrical discharges.

A variety of two to four ring polycyclic aromatic hydrocarbons (PAHs) were identified by two step laser mass spectrometry (L^2MS) in tholins that were formed from a 90% N_2 /10% CH_4 gas mixture by direct current (DC) discharges (Sagan et al., 1993). Ehrenfreund et al. (1995) synthesized tholins by plasma discharge of a mixture of 90% nitrogen and 10% methane at 0.2 hPa, and characterized the products by Py-GC/MS. They reported that tholins were composed of hydrogen cyanide, acetonitrile, long-chained unsaturated nitriles, aromatic compounds such as benzene and toluene, and nitrogen-containing heteroaromatics such as pyridine and pyrrole. The tholins had peaks at 14 m/z intervals, suggesting that a major component of tholins were methylene groups ($-\text{CH}_2-$). Because

Py-GC/MS analysis provides such rich information about chemical composition, the Huygens probe loaded an Aerosol Collector and Pyrolyser (ACP) for *in situ* characterization of aerosols in Titan's atmosphere.

Imanaka et al. (2004) analyzed the tholins synthesized by plasma discharge in a mixture of methane (10%) and nitrogen (90%) at varying pressures (13 – 2300 Pa), and reported that nitrogen-containing polycyclic aromatic compounds more easily formed at the lower pressure. Somogyi et al. (2005) analyzed tholins synthesized by an alternating current (AC) electrical discharge in a gas mixture of 95% nitrogen and 5% methane, and the resulting tholins were analyzed by high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). These authors reported that tholins were complex molecules composed of carbon, hydrogen and nitrogen, with molecular weights in the range of several hundred daltons. Szopa et al. (2006) analyzed atoms and radicals in the discharged plasma with optical emission spectroscopy (OES). The emission spectra showed that atoms and radicals produced by discharges were growing into particles in the plasma. McGuigan et al. (2006) analyzed the tholins by pyrolysis-two-dimensional gas chromatography - time of flight mass spectrometry (GC $\times$ GC-TOF-MS). They identified several organic compounds of nitriles, pyrroles, hydrocarbons, benzenes and PAH compounds. Polycyclic aromatic hydrocarbons (PAHs) may also have a significant role in the synthesis of tholins (Wilson and Atreya, 2003).

Tholins have also been characterized with several techniques including estimation of their molecular weights. Gas mixtures of CH₄ and N₂ (1 mbar, 2: 98 or 10: 90 v/v) were irradiated with a radio frequency plasma discharge. The products were dissolved in methanol or toluene, and identified by atmospheric pressure photoionization (APPI) - hybrid quadrupole time-of-flight mass spectrometry (QTOFMS) with a tandem collision induced dissociation (CID) method. The spectra showed peak clusters with 13-14 *m/z* intervals in 50 -800 *m/z* area (Carrasco et al., 2009). Elementary analysis of tholins produced by RF discharge from 13 - 2300 Pa gas mixtures of CH₄ and N₂ (10: 90 v/v) showed that the C/N ratio was 1.45 - 2.76 (Imanaka et al., 2004). These previous analytical results suggested that Titan tholins we synthesized would have hydrophobic groups that were large enough to undergo self-assembly.

Finally, it is significant that biomolecules used by terrestrial life have also been detected in tholins synthesized when a gas mixture of 10% methane and 90% nitrogen at 0.2 mbar was exposed to direct current (DC) discharge. The products were hydrolyzed with 6 M HCl, then derivatized with N-trifluoroacetyl isopropyl esters, and 16 amino acids were identified (Khare et al., 1986). In more recent studies, tholins were synthesized by radio frequency (RF) discharge in a gas mixture (N₂:CH₄ = 98:2), hydrolyzed in 2 M HCl, and derivatized with N-tert-butyl-dimethyl-silyl-N-methyltrifluoroacetamide (MTBSTFA) for GC/MS. A variety of organic compounds including amino acids and carboxylic acids were identified (Nguyen et al., 2008). Neish et al. (2008, 2009, 2010) also synthesized tholins by a procedure similar to that reported by Sarker et al. (2003) and Smogyi et al. (2005), and dissolved the tholins in ammonia solution at 253 K or 293 K. They identified almost all amino acids except sulfur-containing species such as cysteine and methionine and nucleobases such as adenine, guanine,

thymine, cytosine and uracil in the solution without acid-hydrolysis. [Horst et al. \(2012\)](#) also identified various organic compounds including amino acids and nucleotide bases without hydrolysis for tholins synthesized according to [Szopa et al. \(2006\)](#), [Nguyen et al. \(2008\)](#) and [Carrasco et al. \(2009\)](#).

Fluorescence properties of tholins

[Hodyss et al. \(2004\)](#) synthesized tholins by alternating current (AC) electrical discharge in gas mixtures of CH₄ and N₂ (1 mbar, 2: 98 or 10: 90 v/v) at 195 K. When the tholins were dissolved in acetonitrile, the fluorescence excitation spectra showed a peak at 410 nm, with an emission peak at 471 nm. When water was used, tholins showed complex fluorescence spectra with multiple peaks. The effects of solvents, temperature and synthesis conditions had only minor effects on the excitation and emission wavelengths. From the Py-GC/MS results, it was suggested that tholins synthesized by direct current (DC) and radio frequency (RF) discharge contain aromatic compounds such as benzene, toluene and heterocyclic compounds like pyrrole and pyridine ([Ehrenfreund et al., 1995](#); [McGuigan et al., 2006](#)). It seems likely that the fluorescence characteristics of our tholins were mainly due to the presence of aromatic hydrocarbon groups and heterocyclic groups. Since the tholins had fluorescence, it was possible to image self-assembled tholin structures by fluorescence microscopy.

Mechanism of self-assembly of amphiphilic molecules

Tholins are composed of C (carbon), N (nitrogen), H (hydrogen) present in the original gas mixture, but lack O (oxygen). Most hydrophilic organic compounds have O-containing groups such as hydroxyl (-OH) and carboxyl groups (-COOH), but they cannot be expected to be present in our tholins. For this reason amino or imino groups are likely to be hydrophilic groups in Titan tholins. In fact, FT-IR spectra of chloroform solutions of HP and LP tholins revealed the presence of both hydrophilic amine groups and hydrophobic alkyl groups. In preliminary experiments, we confirmed amphiphilic properties of tholins by spreading a few microliters of a chloroform solution of HP tholin on the air-water interface of a Langmuir trough. The tholin readily spread to form a film at the interface which produced a measureable surface pressure. When the film was compressed it went through a transition to a solid phase. This result clearly indicates that tholins have surface active properties expected of an amphiphile and are likely to undergo self-assembly into more complex structures.

Self-assembly of ordinary amphiphilic compounds such as fatty acids and phospholipids is related to the length of carbon chains in the molecule. Amphiphiles with hydrophobic chains more than eight carbons in length form micelles can assemble into vesicles bounded by bilayer membranes ([Apel et al., 2002](#)). Long alkyl chains with 13-23 carbons were detected in CM2 chondrites by gas chromatography ([Studier et al. 1968](#); [Smith and Kaplan, 1970](#); [Ander et al. 1973](#)). FT-IR spectra showed that the extracted molecules had both hydrophilic groups such as carboxyl and hydroxyl groups and hydrophobic group such as alkyl groups. If sufficiently long amphiphilic molecules are present, it is likely that they will undergo self-assembly into more complex structures. For instance, [Deamer and Pashley \(1989\)](#) demonstrated that amphiphilic components in chloroform-methanol extracts from the

Murchison meteorite produced membranous vesicles and coacervates.

However, because tholins are polymers of varying length and do not have long hydrocarbon chains, self-assembly into true membranes cannot occur. Instead the self-assembled structures are better described as coacervates. These could be observed as self-assembled microspheres when an ammonia solution was added to tholin films dried from chloroform solutions onto glass slides. If hexane-soluble tholins were used instead, the tholins assembled into more complex aggregates with small fluorescent particles on their surfaces (Fig.3). The differences are most likely due to differential solubility of tholin components in solvents having more or less polar character. For instance, the solubility of tholins is higher in polar solvent such as ethanol ($\epsilon = 24$) than that in non-polar solvents such as hexane ($\epsilon = 1.9$), toluene ($\epsilon = 2.4$) and benzene ($\epsilon = 2.3$). In other less polar solvents, chloroform has a larger dielectric constant ($\epsilon = 4.8$) than hexane, and tholins are more soluble in chloroform than in non-polar solvents such as hexane (McKay, 1996; Carrasco et al., 2009).

Table 1 summarizes characteristics of organic solvents, and Table 2 shows characteristics of molecules found in Titan environments. If Titan lakes are composed of liquid methane and ethane, our results suggest that only small amounts of tholins could dissolve in them. However, if Titan lakes have more polar molecules as minor constituents, solubility of tholins would be markedly increased. Some of the compounds shown in Table 3 do exist in Titan atmosphere, and those with oxygen atoms would increase dielectric constant of Titan's lakes if they are components. It would be interesting to examine the solubility and behavior of Titan tholins in actual low temperature environments like liquid methane (boiling point -161.6°C , melting point -182.5°C) and ethane (boiling point -89°C , melting point -172°C) lakes.

Coacervates

Formation of self-assembled structures is an important first step of chemical evolution which separates an internal molecular system from the external environment. Oparin (1957, 1976) discussed the relevance of coacervates to the origin of life. Coacervates are defined as aggregates formed by colloidal macromolecules in aqueous solvent systems. In his pioneering studies, Oparin had little knowledge of the actual macromolecular composition of cytoplasm, so he used polymers such as gum arabic and gelatin to produce coacervates. More recent studies employed polymers of amino acids, such as the proteinoid microspheres described by Fox et al. (1963). Egami (1974) proposed that transition metal ions in seawater may have played a significant role such as catalysis during chemical evolution. Coacervates called marigranules can be produced when a mixture of amino acid is maintained in seawater at 105°C for 4 weeks (Yanagawa and Egami, 1981). The sea water was modified by addition of transition metal ions to concentrations simulating those estimated to be present in early oceans.

The self-assembled structures formed from the Titan tholins described here are best defined as coacervates. The fact that they are fluorescent when activated by near-UV wavelengths is significant, because it is possible that the energy absorbed from UV photons could drive further chemical evolution by photochemical processes. Future studies directed toward this possibility are likely to be fruitful.

The significance of the results for astrobiology

The results reported here show that tholins synthesized in simulated upper atmosphere of Titan could undergo self-assembly in ammonia-water solutions to form coacervates. In our simulation experiments, the synthesized tholins were extracted with hexane or chloroform and then $\text{NH}_3/\text{H}_2\text{O}$ solution was added. A possible scenario of self-assembly processes on Titan is as follows: complex organic compounds (tholins) are formed in Titan's atmosphere by energy sources such as high-energy electrons, the equivalent of the plasma discharge used in our simulations. Then Titan tholins are accumulated onto the Titan surface, and then can be processed with methane rain, or liquid methane and ethane in lakes. The dissolved moiety of tholins then comes into contact with ammonia-water jets associated with cryovolcanic activity and assemble into the coacervates as found in our simulations.

Assuming that the coacervates represent the first steps in evolution toward living systems, is it possible that microbial organisms could emerge and thrive in Titan's environment? For instance, [Fortes \(2000\)](#) estimated that Titan's underground sea is composed of 15% ammonia aqueous solution, with a pH of 11.5. Some terrestrial microbes can in fact survive in such highly alkaline solutions. Alkaliphiles isolated from water samples in a gold mine near Carletonville, South Africa can grow at pH ~12.5 ([Takai et al., 2001](#)), so the pH of subsurface water in Titan does not prohibit the emergence of life. Another interesting question is whether life might be able to adapt to non-polar hydrocarbon solvents. In this regard, it is interesting that microbial populations have been found in the Pith Lake of Trinidad and Tobago, where the liquid mostly consists of asphaltenes ([Schulze-Makuch et al., 2011](#)). It would be interesting to determine whether such microorganisms could use tholins as a source of carbon, fixed nitrogen and chemical energy, thereby relating the requirements of terrestrial life to possible life in the Titan liquidosphere. There is in fact a precedent for such a study, because [Mautner et al. \(1995\)](#) demonstrated that aqueous extracts of the Murchison meteorite provided a sufficient nutrient source for a variety of microorganisms.

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Fig. 1. FT-IR Spectra of HP (a) and LP (b) Tholin (Chloroform fraction) on KBr.

Fig. 2. Two-dimensional fluorescence spectra of HP (a) and LP (b) tholins (ammonia-water fraction).

Fig. 3. (a) The DIC image when 10 mM ammonia solution was added to tholins dried from a chloroform solution onto slide glass ($\times 400$). (b) Fluorescence image of the same field.

(c) The DIC image when 10 mM ammonia solution was added to the tholins dried from a hexane solution onto slide glass ($\times 400$). (d) Fluorescence image of the same field.

Table 1. Parameters of tholin synthesized by an electrical discharge

Reference	Pressure	Gas mixture	Discharge	Temperature	System	Experiments	Solvents
Khare et al (1986)	200Pa	10% CH ₄ , 90% N ₂	DC glow	300K	Flow	GC/MS	HCl
Sagan (1993)	200Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	μL2MS	
Ehrenfreund (1995)	20Pa	10% CH ₄ , 90% N ₂	DC glow	300K	Flow	Pyr-GC/MS	
McKay (1996)	10000Pa	10% CH ₄ , 90% N ₂	Spark	300K	Flow	Elementray analysis	water, methanol, ethanol, ethylene glycol, DMSO, hexane. benzene
Coll (1999)	~200Pa	10% CH ₄ , 90% N ₂	DC glow	300K	Flow	GC/MS, FT-IR, SEM	Nitriles
Sarker (2003)	1000Pa	2% CH ₄ , 98% N ₂	AC glow	195K	Flow	ESI FT-ICR	methanol : acetonitrile 1:1
Hodyss (2004)	1000Pa	2% CH ₄ , 98% N ₂	AC glow	195K	Flow	FS	water, acetonitrile
Imanaka (2004)	13Pa-2300Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	FT-IR, Elementary analysis, Raman, UV-Vis, μL2MS	
Smogyi (2005)	1000Pa	5% CH ₄ , 95% N ₂	AC glow	196K	Flow	FT-ICR, MALDI-TOF,	methanol : acetonitrile 1:1
Szopa (2006)	100Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	OES, SEM, Pyr GC/MS	
McGuigan(2006)	160Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	GC×GC	
Nguyen (2008)	100Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	FT-ICR	HCl
Carrasco (2009)	100Pa	2,10% CH ₄ , 98, 90% N ₂	RF glow	300K	Flow	SEM, FT-IR, APPI-TOFMS, CID	Methanol , toluene
Neish (2008,2009,2010)	880Pa	2% CH ₄ , 98% N ₂	AC glow	300K	Flow	FT-ICR	ammonia, methanol : acetonitrile 1:1
Horst (2012)	90Pa	2% CH ₄ , 96.2% N ₂ , 1.8%CO 5% CH ₄ , 93.2% N ₂ , 1.8%CO	RF glow	330K	Flow	GC/MS	Methanol
He (2012)	850Pa	5% CH ₄ , 95% N ₂	AC glow	195K	Flow	NMR	DMSO
This research	133Pa, 26Pa	10% CH ₄ , 90% N ₂	RF glow	300K	Flow	FM, FS, FT-IR	chloroform, hexane

Table 2.

Boling point, melting point and dielectric constant of organic solvents

Compound	Formula	Freezing Point (K)	Boling Point(K)	Dielectric Constant(D)
Alkanes				
1-Pentene	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$	107.95	303.12	2.02
Isopentane	$\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	113.25	301	1.84
Isohexane	$(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{CH}_3$	119.45	333.42	1.88
1-Hexene	$\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}_2$	133.35	336.64	2.05
Organohalogen compound				
2-Chlorobutane	$\text{CH}_3\text{CH}_2\text{CHClCH}_3$	132.65	341.4	7.09
Chloroethane	$\text{CH}_3\text{CH}_2\text{Cl}$	136.75	285.42	9.45
3-Chloropropene	$\text{CH}_2=\text{CHCH}_2\text{Cl}$	138.65	318.25	8.2
1-Chloro-2-methylpropane	$(\text{CH}_3)_2\text{CHCH}_2\text{Cl}$	142.85	342	6.49
1-Chlorobutane	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$	150.05	351.59	7.39
1-Chloropropane	$\text{CH}_3\text{CH}_2\text{CH}_2\text{Cl}$	150.35	319.75	9.45
2-Chloropropane	$\text{CH}_3\text{CHClCH}_3$	155.97	308.89	9.82
Alcohol				
1-Propanol	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$	146.95	370.35	20.33
Ether				
Ethyl ether	$(\text{C}_2\text{H}_5)_2\text{O}$	149.35	307.7	4.34
Propyl ether	$(\text{CH}_3\text{CH}_2\text{CH}_2)_2\text{O}$	149.95	362.79	3.39
Isobutyl formate	$\text{HCOOCH}_2\text{CH}(\text{CH}_3)_2$	178.65	371.55	6.41
Propyl formate	$\text{HCOOCH}_2\text{CH}_2\text{CH}_3$	180.25	354	7.72
Propyl acetate	$\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}_3$	180.65	374.7	6
Sulfur Compounds				
Ethyl Sulfide	$(\text{CH}_3\text{CH}_2)_2\text{S}$	169.22	365.252	5.72
Methyl sulfide	$(\text{CH}_3)_2\text{S}$	174.88	310.49	6.2

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Table 3. Composition of Titan Atmosphere and their Boiling point and Freezing point
(<http://sci.esa.int/science-e/www/object/index.cfm?fobjectid=31187>)

	Symbol	Amount	Freezing Point	Boiling Point
Major Constituents		(%)	(K)	(K)
Nitrogen	N ₂	87-99	63.1	77.3
Argon	Ar	0-6	83.8	87.3
Methane	CH ₄	1-6	90.6	111
Minor Constituents		(ppm)	(K)	(K)
Hydrogen	H ₂	2000	14.0	20.3
Hydrocarbons				
Ethane	C ₂ H ₆	20	89.2	185
Acetylene	C ₂ H ₂	4	190	191
Ethylene	C ₂ H ₄	1	104	169
Propane	C ₃ H ₈	1	85.5	231
Methylacetylene	C ₃ H ₄	0.03	170	250
Diacetylene	C ₄ H ₂	0.02	268	284
Nitrogen Compounds				
Hydrogen Cyanide	HCN	1	260	299
Cyanogen	C ₂ N ₂	0.02	245	252
Cyanoacetylene	HC ₃ N	0.03	278	316
Acetonitrile	CH ₃ CN	0.003	228	355
Oxygen Compounds				
Carbon Monoxide	CO	50	68.1	81.6
Carbon Dioxide	CO ₂	0.01	195	217