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► **To cite this version:**

Julien Maillard, Nathalie Carrasco, Isabelle Schmitz-Afonso, Thomas Gautier, Carlos Afonso. Comparison of soluble and insoluble organic matter in analogues of Titan's aerosols. *Earth and Planetary Science Letters*, 2018, 495, pp.185-191. 10.1016/j.epsl.2018.05.014 . insu-01802505

HAL Id: insu-01802505

<https://insu.hal.science/insu-01802505v1>

Submitted on 22 Jun 2018

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1 Comparison of soluble and insoluble
2 organic matter in analogues of Titan's
3 aerosols

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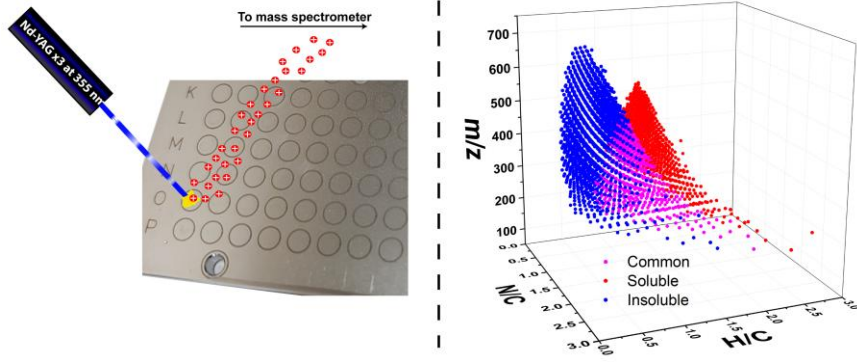
15

16 **Abstract**

17 Titan, the biggest moon of Saturn, has a thick atmosphere which presents similarities with the
18 one thought to be on Earth at its beginning. The study of Titan's photochemical haze is thus a
19 precious tool in gaining knowledge of the primitive atmosphere of Earth. The chemistry
20 occurring in Titan's atmosphere and the exact processes that act in the formation of the hazes
21 remain largely unknown. The production of analog samples on Earth has proved to be a
22 useful tool to improve our knowledge of the aerosols formation on Titan. Such solid organic
23 analog samples, named tholins, were produced with the PAMPRE experiment (French
24 acronym for Aerosols Microgravity Production by Reactive Plasma). PAMPRE tholins were
25 found to be mostly insoluble, with only one-third of the bulk sample that can be dissolved in
26 methanol. This partial solubility limited the previous studies in mass spectrometry, which
27 were done only on the soluble fraction. The goal of the present study is to compare the two
28 fractions of PAMPRE's tholins (insoluble and soluble) using a ultra-high resolution Fourier
29 transform ion cyclotron resonance mass spectrometer (FTICR) equipped with a laser
30 desorption/ionization source. Using modified Van Krevelen diagrams, we compare the global
31 distribution of the molecules within the samples according to their Hydrogen/Carbon ratio and
32 Nitrogen/Carbon ratio. Major differences are observed in the molecular composition of the
33 soluble and the insoluble fraction. The soluble fraction of tholins was previously identified as
34 a set of polymers of average formula $(C_2H_3N)_n$. In this work we observe that the insoluble
35 fraction of tholins is comprised of a significantly different set of polymers with an average
36 composition of $(C_4H_3N_2)_n$.

37 **Keywords:** Titan's atmosphere, Tholins, Organic matter, mass spectrometry, Ion cyclotron
38 resonance, Van Krevelen diagram

39 **Graphical abstract**



40

41 **Introduction**

42 Titan, the largest satellite of Saturn, is surrounded by a thick atmosphere rich in nitrogen with
43 a small percentage of methane. The chemistry in this atmosphere leads to the formation of a
44 complex smog due to the radicalization and recombination of the two gases upon interaction
45 with solar UV photons and charged particles from Saturn's magnetosphere. The observations
46 of the Cassini mission and the information obtained during the landing of the Huygens probe
47 provided partial information about the composition of the aerosols. Pyrolysis of the aerosols
48 by the ACP experiment aboard Huygens released mainly NH₃ and HCN but was not sufficient
49 to further elaborate on the structural composition of the aerosols because of the detection limit
50 of the instrument (Fulchignoni et al., 2005; Israel et al., 2005; Niemann et al., 2005).

51 Titan's aerosols also represent an interest for astrobiology (Sagan et al., 2002) due to their
52 peculiar formation and their organic complexity. Some analogs, called tholins, were produced
53 on Earth to investigate the formation processes and the structure of Titan's aerosols (Cable et
54 al., 2012; Imanaka and Smith, 2010; Somogyi et al., 2005; Szopa et al., 2006; Toupance et al.,
55 1975).

56 Infrared (IR) spectroscopy analyses have demonstrated that tholins structure includes nitrile,
57 amine, hydrocarbon and unsaturated functions (Cable et al., 2014; Coll et al., 1999; Gautier et
58 al., 2012; Imanaka et al., 2004; Sciamma-O'Brien et al., 2017). Primary amines were also
59 identified in tholins using laser-induced fluorescence with nonaqueous capillary
60 electrophoresis (Cable et al., 2014). Additional analyses were performed on the soluble
61 fraction of the tholins by mass spectrometry showing a polymeric structure (Bonnet et al.,
62 2013; Gautier et al., 2016; Vuitton et al., 2010). First analyses with ultra-high resolution mass
63 spectrometry also brought to light repetition patterns (CH₂ and HCN) in the tholins' soluble
64 fraction (Anicich et al., 2006; He et al., 2012; Somogyi et al., 2005).

65 However, some tholins are not completely soluble in solvents such as methanol (Carrasco et
66 al., 2009). This is the case of the tholins synthesized with the PAMPRE experiment, described
67 below (Szopa et al., 2006). The soluble fraction of tholins obtained from this experiment
68 represents only one third of the total material. Only this soluble fraction was analyzed with
69 mass spectrometry so far, using electrospray ionization and photoionization (Carrasco et al.,
70 2009). The soluble fraction provided numerous information about the general structure of
71 these samples. Several studies compared various tholins to a set of polymeric structures based
72 on a CH₂-HCN repeating unit, spreading from m/z 100 to m/z 800, as emphasized by the
73 Kendrick mass defect diagram using HCN or CH₂ normalization (Hughey et al., 2001;
74 Kendrick, 1963). This explained the periodic nature of the mass spectra of tholins showing
75 repeating ion clusters (Gautier et al. 2014, Pernot et al. 2010).

76 A previous study emphasized that IR spectrum of the soluble and non-soluble fractions of
77 tholins were nearly identical (Carrasco et al., 2009). As a consequence, it has been postulated
78 that both fractions had a similar molecular content, and that the difference of solubility was
79 only related to the difference of mass of the molecules composing tholins, as larger molecules
80 are expected to be less soluble. In the present study, we reexamine this insoluble fraction at
81 the molecular level by using ultra-high resolution mass spectrometry.

82

83 **Experimental**

84 *Sample production*

85 Tholins were produced using the PAMPRE experiment and following the procedure detailed
86 in previous publications (Gautier et al., 2011; Szopa et al., 2006). In this setup, the reactor is
87 composed of a stainless steel cylindrical reactor in which the Radio Frequency – Capacity
88 Coupled Plasma (RF-CCP) discharge is established by an RF 13.56 MHz frequency
89 generator. The gas mixture, containing nitrogen and 5% of methane, is injected in the
90 chamber as a continuous flow through polarized electrodes and then extracted by a primary
91 vacuum pump to ensure that gases are homogeneously distributed. The plasma discharge is
92 maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature.

93

94 *Sample treatment for mass spectrometry analysis*

95 In order to separate soluble and insoluble fractions, 4 mg of tholins were dissolved in 1 mL of
96 methanol in a vial under ambient atmosphere. The vial was vigorously stirred for 3 minutes to
97 dissolve the maximum amount of species. The brown mixture was then filtered using a 0.2
98 μm polytetrafluoroethylene (PTFE) membrane filter on a filter holder. Of the filtered solution,
99 the soluble fraction, was transferred in a glass vial. Half dilution with a 50/50 water/methanol
100 mixture was performed just before analysis in order to be analyzed using similar conditions as
101 previous studies by electrospray ionization. The PTFE membrane was then recovered, placed
102 in a vial and left open under a neutral atmosphere of Nitrogen to evaporate the remaining
103 methanol and avoid contamination. The insoluble fraction, recovered as a black powder from
104 the membrane, was then analyzed by mass spectrometry. The global sample was directly
105 analyzed avoiding any contact with solvents.

106

107 *Mass spectrometry analyses*

108 Different ionization methods can be used for the analysis of molecules in their solid state,
109 especially Laser Desorption Ionization (LDI). LDI-MS has already been used for the analysis
110 of tholins (Gautier et al., 2017; Imanaka et al., 2004; Mahjoub et al., 2016; Sagan et al., 1993;
111 Somogyi et al., 2012). It has also been used for the analysis of asphaltenes, a fraction of
112 petroleum non-soluble in pentane or heptane (Pereira et al., 2014; Sagan et al., 1993; Tanaka
113 et al., 2004). In this work a LDI source is coupled with a Fourier transform ion cyclotron
114 resonance mass spectrometer (FTICR) to compare the soluble and non-soluble fractions of
115 tholins at the molecular level. A comparison between electrospray and LDI sources using the
116 same analyzer parameters can be found in the supporting information (Figures S1 and S2). In
117 addition, LDI-FTICR was compared to LDI-TOF analyses (Figure S1).

118 Previous works have demonstrated the significant differences in the molecules detected in
119 tholins depending on the ionization mode used (Carrasco et al., 2009; Somogyi et al., 2012).
120 For this work we chose to investigate only positive ionization as this is the one most widely
121 used in the community for tholins analysis.

122 All analyses were performed on a FTICR Solarix XR from Bruker equipped with a 12 Tesla
123 superconducting magnet and a laser desorption ionization source (laser NdYAg 355 nm). The
124 mass spectrometer was externally calibrated with a solution of sodium trifluoroacetate. Mass
125 spectra were afterwards internally calibrated with confidently assigned signals yielding a mass
126 accuracy below 300 ppb in the considered mass range. The soluble fraction was deposited on
127 a LDI plate using the dry droplet method (5 x 1 μ L to ensure a good concentration of the
128 sample). Insoluble fraction and the global sample were deposited using a solvent-free method,
129 following a previously published procedure (Barrere et al., 2012). Mass spectra were recorded
130 in positive mode at 8 million points with a sum of 500 scans, yielding a resolution of 1 500
131 000 at m/z 150 and 500 000 at m/z 500. The following instrumental parameters were

132 implemented for the insoluble and total fractions: Plate offset 100 V, Deflector plate 210 V,
133 Laser power 19 %, Laser shots 40, Frequency of laser shots 1000 Hz, Funnel 1 at 150 V,
134 Skimmer 1 at 25 V. The same parameters were used for the soluble fraction, except the laser
135 power that was raised to 29% and the number of shots increased to 150. Fraction responses to
136 the laser ionization were different for each fraction of the sample due to the molecular
137 differences between each fraction, especially the hydrogen/carbon ratio. To obtain sufficient
138 signal for the soluble fraction, it was necessary to increase the laser power compared to the
139 parameter used for the non-soluble fraction spectra. A comparison between the global
140 intensity of the spectra and the laser power used for the ionization is given in the
141 supplementary material (Figure S3). We then defined a laser power value for each fraction in
142 order to obtain similar signal intensities. We fixed the value at 5% of laser power above the
143 determined threshold – 19 % and 29% for the insoluble and soluble fraction respectively.
144 Peak picking was done with a signal/noise ratio of 5 and 0.01% of intensity. Molecular
145 formulas were obtained using the SmartFormula tool from the Bruker Data Analysis 4.4
146 software with the following parameters: molecular formula $C_{0-x}H_{0-y}O_{0-2}$, even and odd
147 electron configuration allowed, 0.5 ppm error tolerance.

148 It should be noted that while we try to limit oxygen contamination, tholins are known to be
149 prone to oxidation when exposed to oxygen. Numerous discussions can be found in the
150 literature on this point (see for example (Carrasco et al., 2016) for a discussion on PAMPRE
151 tholins). In our case, oxidation principally happens when tholins are exposed to the air during
152 their collection after production. This exposition is intrinsic to the experimental setup used.
153 Studies on plasma produced organic materials have shown that this oxidation happens within
154 seconds of exposition of the sample to ambient air (Hörst et al., 2018; McKay, 1996; Swaraj
155 et al., 2007; Tran et al., 2003). In the case of the sample analysed here, the amount of oxygen
156 contamination can be estimated through the detection of oxygenated molecules in the samples

157 from the LDI-FTICR measurement. These measurements show the presence of ~20% of
158 oxygenated signals (Table 1). This part is non negligible principally because the material is
159 composed of thousands of molecules in low abundance and so, thousands of molecules start to
160 pick up oxygen after the exposition to ambient atmosphere. Nevertheless, especially because
161 oxygenated species are produced from the bulk material and contain one or two oxygen
162 atoms, no modification was observed between these oxygenated and non-oxygenated species
163 concerning the number of unsaturation or the H/C ratio.

164 The formation of such oxygenated species was observed previously upon the addition of
165 ammonium hydroxide and they were depicted as hydrolysis products (Neish et al., 2009; Poch
166 et al., 2012). We could expect some changes in composition due to exposure to water for our
167 soluble fraction. However, the soluble fraction is prepared with water a few minutes before
168 the analysis. This is much shorter than the hydrolysis condition found in the literature for
169 tholins hydrolysis (10 weeks for Poch et al. 2012, 1 year for Neish et al. 2010). Neish et al.
170 2009 showed that tholins hydrolysis rate in pure water was significantly slower than in the
171 presence of ammonia, only yielding a total oxidation of ~2% of the tholins after 53h exposure
172 to water. To first order we can therefore consider hydrolysis negligible considering neutral pH
173 of our solution and the hydrolysis kinetics. All molecular formulas were attributed thanks to
174 the high precision m/z measures. The oxygenated species were then excluded from the results
175 presented here by filtering the CSV data file using Excel 2016 spreadsheet, keeping only
176 formulas containing ^{12}C , ^1H and ^{14}N . Table 1 summarizes all attributed formulas by the
177 SmartFormula tool.

178

179

180

**Table 1 (1 column) : Classification of detected species
by their families**

	%	Attributed formulas
Total ions	100	16952
Non-oxygenated	33	5538
Oxygenated	20	3472
¹³ C containing species	21	3614
¹⁵ N containing species	16	2652

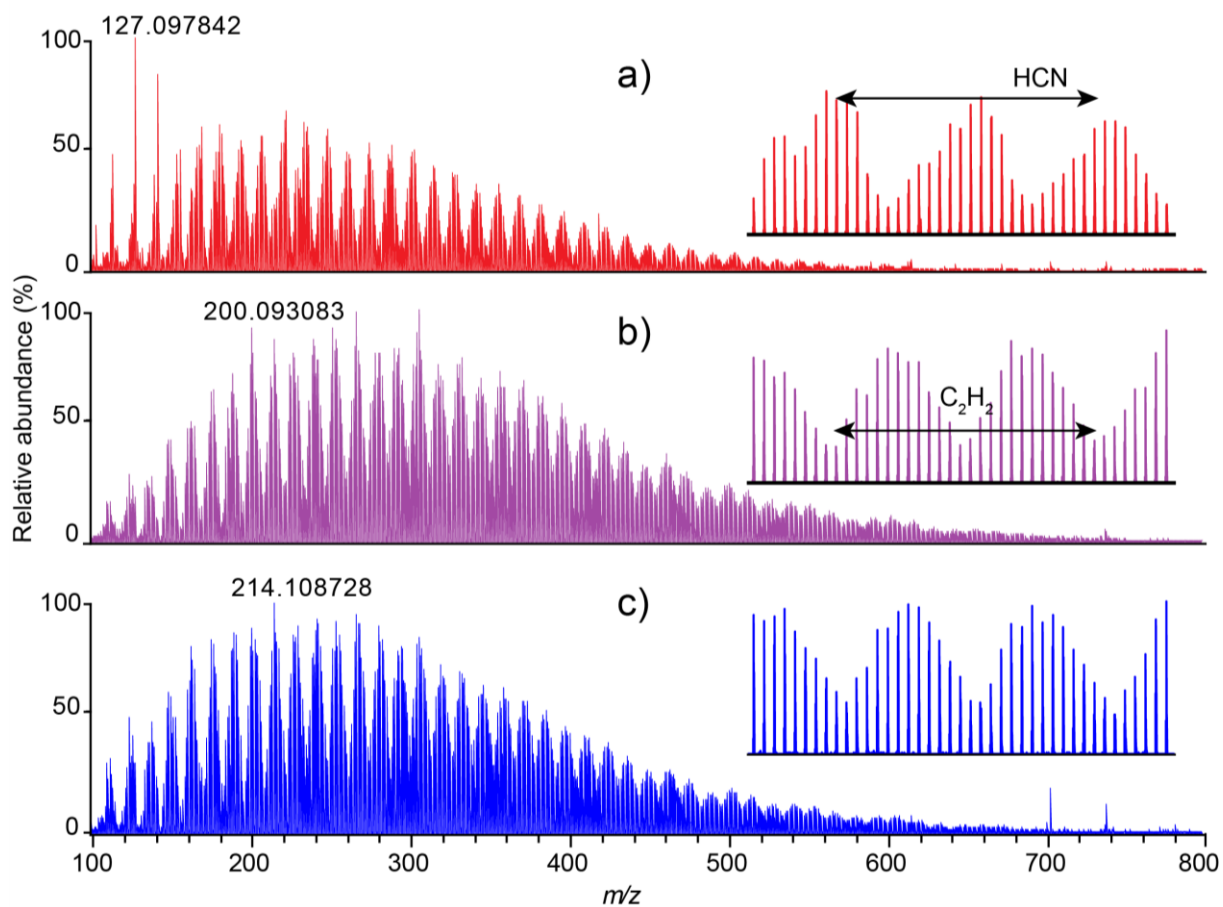
182 **Results and discussions**

183 *LDI-FTICR analyses*

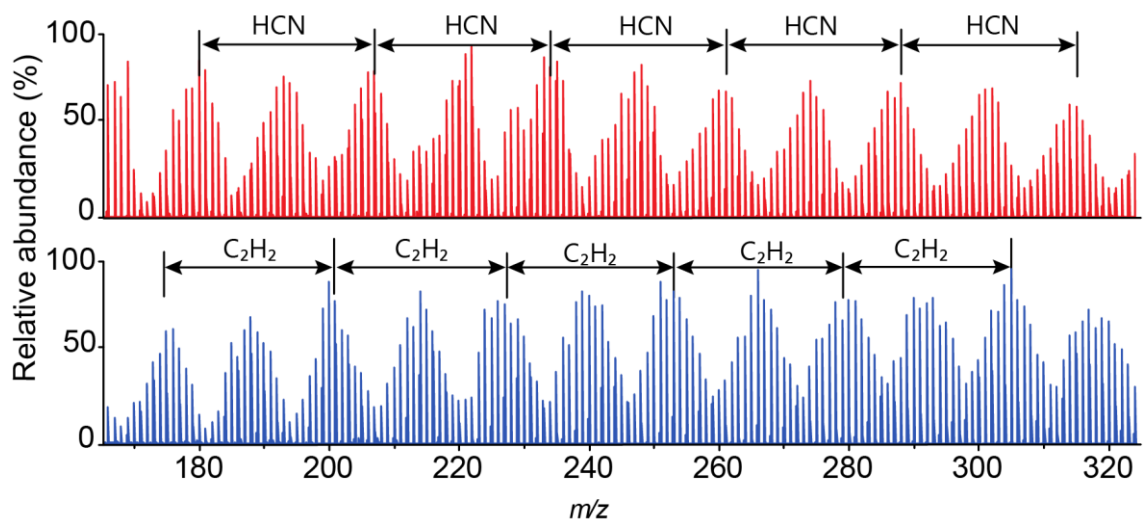
184 Figure 1 presents a general view of the recorded mass spectra for the two different fractions
185 (soluble and insoluble) and for the global sample (soluble + insoluble). It can be seen that the
186 base peaks are different in each fraction. For the soluble fraction, m/z 127.09784 is the base
187 peak while it is m/z 200.09308 for the insoluble fraction and 214.10872 for the global sample.
188 On the other hand, the ion distribution is relatively similar for all fractions but we observe that
189 low m/z species are more abundant in the soluble fraction than in the other samples. The three
190 mass spectra present a periodic profile with patterns consistent with the expected repeating
191 unit of tholins $\text{CH}_2 - \text{HCN}$ (Pernot et al., 2010).

192 The enlargement in the inset of Figure 1 highlights that for the insoluble fraction and for the
193 global sample, the formed repetition patterns are similar, while they appear shifted in the
194 soluble fraction. This phenomenon is better illustrated on Figure 2 by zooming in on several
195 periods. We can observe that the soluble fraction has a period (two repetition patterns) of m/z
196 27.0109, which corresponds exactly to the HCN formula. In comparison, insoluble and the
197 global sample have a period of m/z 26.0156, which corresponds exactly to the C_2H_2 formula.
198 This explains why in some parts of the spectra, the waves of the soluble fraction present their
199 lowest intensity and their highest intensity for the two different fractions. These results
200 highlight that significant differences exist between the soluble and non-soluble samples in the
201 growth structure of the tholins. However, these two specific periods are not the only one
202 detected in each fraction. In addition to this C_2H_2 growth pattern, there are actually numerous
203 ions series present in the samples. Previous work from Gautier et al. 2014 showed that one
204 would need at least 8 different growth series to represent the samples, many of them bearing
205 nitrogen thus explaining the presence of N-containing compounds at relatively high molecular

206 masses. We consider that presenting a full description for all ion series goes beyond the scope
207 of the present paper and chose to focus solely the C_2H_2 as an illustration of the phenomenon.
208 The obtained results are also consistent with those recorded in previous studies on tholins
209 produced on another experimental setup according to the spectrum appearance (He et al.,
210 2012; Somogyi et al., 2005).



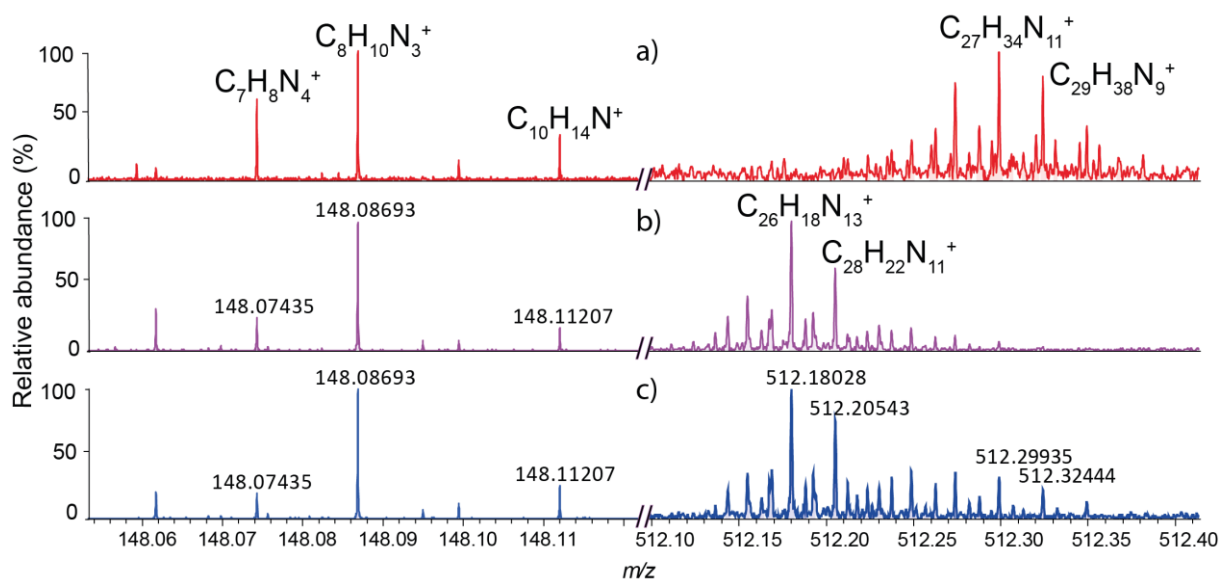
211
212 Figure 1 (2 columns): Laser desorption/ionization FTICR MS spectra of a) soluble fraction, b)
213 non-soluble fraction, c) global sample



214

215 Figure 2 (2 columns): Enlargement on the soluble (in red) and non-soluble fraction
 216 to highlight the difference between the periodic phenomenon on each fraction

217 Figure 3 shows different sections of the mass spectra centered at m/z 148 and m/z 512, for the
 218 two different fraction and the global sample. These two parts of the spectrum were chosen to
 219 highlight the differences for the low and high m/z values. The ultra-high resolution of the
 220 FTICR instrument allows the separation of the isobaric ions even for highly complex ion
 221 distributions evidenced at the higher mass range. Interestingly, the three spectra are almost
 222 identical in the low mass range but differences can be observed for the higher mass range,
 223 with the detection of ions present in the soluble fraction but not in non-soluble fraction. As
 224 one can see on the enlargement of Figure 3 on m/z 512, the signal for the global sample seems
 225 to correspond to the sum of the signals present in the soluble and non-soluble fraction. The
 226 obtained ion relative abundance shows a higher amount of species specific to the non-soluble
 227 fraction in the total fraction. This is consistent with the amount of soluble species, which
 228 corresponds to only 35% of the total tholins (Carrasco et al., 2009).



229
 230 Figure 3 (2 columns): Enlargement of the m/z 148 and m/z 512 ions for a) soluble fraction, b)
 231 non-soluble fraction, c) global sample
 232
 233

234 In order to obtain a broader picture on the detected species, molecular formulas were
235 determined for each ion. This was done by considering only species presenting C, H and N
236 elements. Due to the mass accuracy of our measurement (<300 ppb), only one molecular
237 formula could fit with each experimental mass. Looking into details at the molecular formula
238 (see for example m/z 512 on Figure 3), the ions specifically detected in the global sample and
239 non-soluble fractions present a low mass defect, consistent with less hydrogenated species.
240 This comparison emphasizes once again important differences between the soluble and the
241 non-soluble fractions: not on the mass of the molecules as initially thought, but rather in the
242 type of molecules present in each sample.

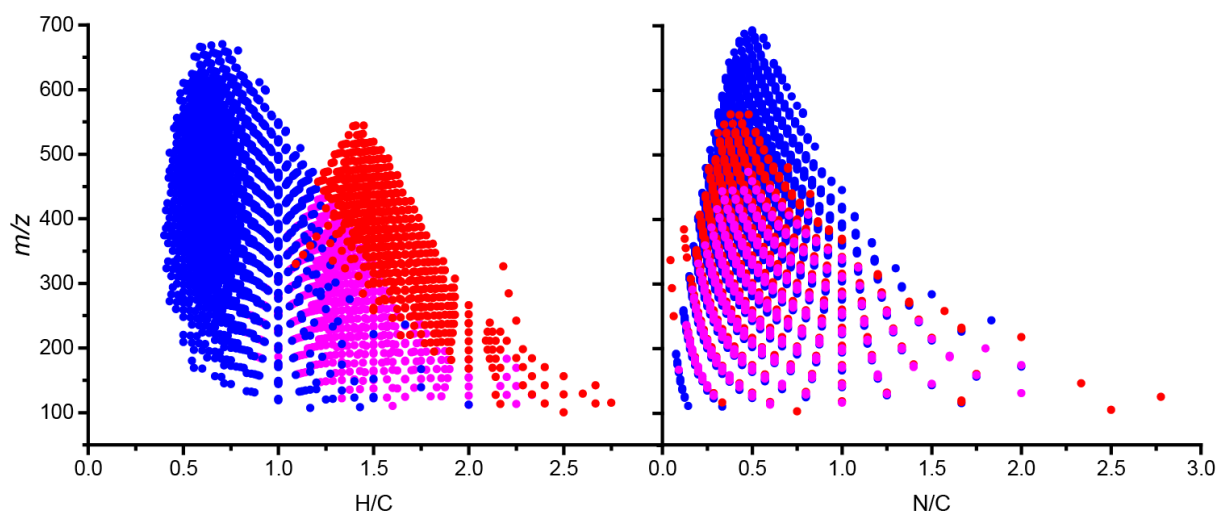
243

244 *Data treatment using modified Van Krevelen diagrams*

245 Van Krevelen diagrams were originally proposed for displaying the molecular complexity of
246 petroleum or coal with bidimensional graphics (Kim et al., 2003; Van Krevelen, 1950). Such
247 diagrams were used more recently for other research fields including global ‘omics’ analysis
248 of metabolites but also tholins (Pernot et al., 2010). In typical Van Krevelen diagrams O/C are
249 plotted as a function of H/C ratio. These ratios are readily obtained from the molecular
250 formulas. These diagrams particularly show evidence of unsaturation based on the H/C ratio
251 and the number of hetero-elements with the O/C ratio. Previous studies in tholinomics
252 demonstrated the interest of these diagrams by switching the x-axis that commonly represents
253 the oxygen/carbon ratio with the nitrogen/carbon ratio (Gautier et al., 2014; Imanaka and
254 Smith, 2010; Somogyi et al., 2005). Van Krevelen diagrams are limited because they do not
255 consider the m/z dimension and ions with very different m/z ratio can yield the same H/C or
256 N/C ratio. The m/z dimension can be added to the Van Krevelen plot to generate 3D plots
257 (Gautier et al., 2014; Pernot et al., 2010). Specific 2D plots H/C vs m/z or N/C vs m/z have
258 also been used (Imanaka and Smith, 2010).

259 A comparison was done to choose the most appropriate axis to be used to represent our
260 samples. Soluble and insoluble fractions were found quite equal in their nitrogen/carbon ratio
261 as visible in the Figure 4-right. The nitrogen/carbon ratio bearing little information, the most
262 interesting representation for our samples was determined to be the m/z value versus the ratio
263 hydrogen/carbon (Figure 4 left). 3D Van Krevelen to highlight the interest for the first global
264 view on the sample based on the data presented in the Figure 4 can be found in supporting
265 information (Figure S2).

266 Figure 4 shows the H/C versus m/z plot and the N/C versus m/z plots obtained from the
267 soluble (in red) and the insoluble fractions (in blue). We focused our study on the major
268 contributors to the sample. Thus only species with an intensity above 5% of the base peak
269 (corresponding to 81% of all species detected in the samples according to the sum of all peaks
270 intensities) are displayed. In this illustration, we observe for the soluble fraction (including the
271 common part between the different samples in purple) a triangular distribution that converges
272 toward a unique peak. This distribution has been observed in a previous work (Pernot et al.,
273 2010). This structure starts at a low m/z value with a large base and then becomes thinner with
274 the increase of the m/z value. This shape is in agreement with a set of copolymers involving
275 the $-\text{CH}_2\text{-HCN-}$ repeating unit with an addition of some carbon in the structure that enlarges
276 the distribution at low m/z values. This confirms the previous results on the soluble fraction
277 described in Pernot et al., 2010, a previous study realized with an OrbitrapTM analyzer with a
278 resolution of 200 000 at m/z 150 and 100 000 at m/z 450.



279

280 Figure 4 (2 columns): Van Krevelen diagram of the comparison between soluble and
 281 insoluble fraction with a filter at 5% of intensity. Soluble is in red, insoluble in blue and
 282 common part in purple.

283

284 Figure 4 also presents the data from the non-soluble fraction. As evidenced above, both
 285 soluble and non-soluble fractions present very different molecular structures. For the lower
 286 mass range numerous ions are present in both fractions but the difference between soluble and
 287 non-soluble fractions increases for m/z values larger than m/z 200, yielding to two distinct
 288 convergence points at high masses. The soluble fraction ions converge around H/C 1.5 and
 289 N/C 0.5 whereas the non-soluble species converge around H/C 0.75 and N/C 0.5. As
 290 discussed above, the two distribution have similar N/C ratio but they differ on the H/C ratio.
 291 The non-soluble fraction is composed of, relatively, the same amount of nitrogen as the
 292 soluble fraction, but is much less hydrogenated and so, presents a higher amount of
 293 unsaturation.

294 The distribution representative of the insoluble fraction tends to show that there is another set
 295 of polymeric structures in tholins that has not be identified before from the analysis of the
 296 soluble fraction only.

297 Previous postulate, based on IR spectroscopy, considered that soluble species became
298 insoluble when they grew in mass and so that soluble fraction was representative of the global
299 sample at low m/z value. With the results presented here, we identify a very different partition
300 in tholins. Soluble and insoluble fractions while emerging from the same set of molecules at
301 low m/z actually represent two families of compounds that are totally uncorrelated at higher
302 m/z values.

**Table 2 (1 column) : Weight of each fraction according
to LDI source response**

Fraction	Average weight (%)	Convergence (H/C)
Soluble	16.7	1.5
Common	33.3	x
Insoluble	50.0	0.75

303
304 Table 2 summarizes the relative weight of each fraction in the global sample according to LDI
305 source response and also the convergence point of each fraction for H/C ratio. The relative
306 weight was calculated by doing the sum of all intensities of species for each fraction. We can
307 see that the common part represents a third of all the species, the insoluble a half and the
308 soluble less than a quarter. This emphasizes that the global sample is mainly composed of the
309 insoluble part, and that the soluble part, the one mainly analyzed in previous mass
310 spectrometry study of tholins, is actually a minor component of tholins.

311

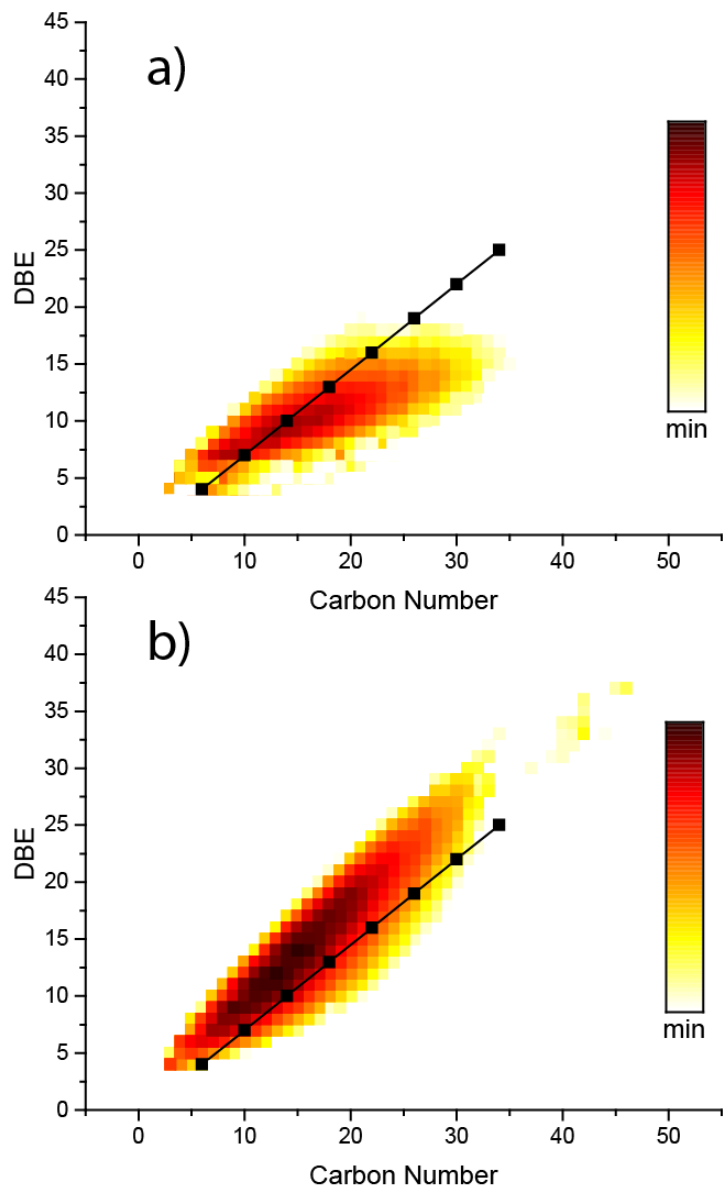
312 *Comparison between soluble and non-soluble species using DBE vs Carbon number plots*

313 Double Bond Equivalent (DBE) vs carbon number plots are generally used in petroleomic to
314 observe the number of unsaturation in a family of species and to compare different oils
315 (McKenna et al., 2010). In Figure 5 we adapted this graphic representation to highlight the
316 differences between soluble species and non-soluble tholins species.

317 A specific range of molecules was selected to create these graphics as the use of the all data
318 set lead to the overlap of multiple species. The Figure 5 presents the DBE vs Carbon atoms
319 number plot for species containing between 6 and 9 nitrogen atoms for the soluble and non-
320 soluble fractions. We added the Polycyclic Aromatic Hydrocarbon (PAH) line, which
321 represents the most unsaturated family of species containing only carbon and hydrogen as
322 shown previously (Cho et al., 2011). Soluble species are principally under the PAH line
323 whereas non-soluble species are mainly above this reference line. This highlights the fact that
324 the non-soluble species in the tholins are much more unsaturated than the soluble species.
325 This result is also consistent with what was observed on the Van Krevelen plot. It should be
326 pointed out that this behavior presents several analogies with asphaltenes, which correspond
327 to the fraction of crude oil non-soluble in pentane or heptane. The molecules present in the
328 asphaltene fraction are mainly polyaromatic, which explain the specific properties of this
329 fraction.

330 Sample treatment with methanol, which efficiently solubilized polar molecules, could explain
331 these observations: we recover in the soluble sample several families that are solubilized but
332 which do not necessarily have the same properties. Using different steps of washing on the
333 PAMPRE tholins sample could be, for future studies, a good approach to isolate new families
334 of compounds among the soluble fraction.

335



336

337

338

339

Figure 5 (1 column): a) Soluble species containing 6 to 9 Nitrogen, b) Non-soluble species containing 6 to 9 Nitrogen

340 **Conclusions**

341 With our FTICR mass spectrometer equipped with a laser desorption/ionization source it was
342 possible to analyze all the fractions of a tholins sample produced with the PAMPRE
343 experiment (soluble in methanol, non-soluble and total) using solvent-free deposition method.
344 The high accuracy of mass measurement coupled with the ultra-high resolution of the FTICR
345 instrument allowed us to assign molecular formula to all species contained in the tholins.
346 Modified Van Krevelen diagrams enabled us to easily compare the different fractions of the
347 tholins sample. Based on infrared absorption spectroscopy signatures, it was previously
348 postulated that soluble fraction was representative of the global sample and the only
349 difference between soluble and insoluble species would be the m/z values of the molecules
350 composing the material. In the present work, we observed significant differences between the
351 soluble and non-soluble fractions of this sample, and showed that soluble fraction was mainly
352 not correlated to the insoluble fraction. We highlighted that these fractions are mostly
353 different at the molecular level when m/z increases and that the non-soluble fraction is much
354 less hydrogenated than the soluble fraction. We also confirmed that the soluble fraction does
355 not represent the major part of the sample as expected before, but the ionization yield is
356 similar for each fraction according to their respective proportions observed with the laser
357 ionization. Furthermore, this work demonstrated the presence of a second set of copolymeric
358 structures within the tholins that had not been identified yet and which are less saturated than
359 the one present in the soluble fraction.

360 This work highlights the importance to study of all the material and not only the soluble
361 fraction. First, the non-soluble part represents 65% of the global mass sample and is then
362 more representative of the bulk sample. Thus Titan's tholins have been found less
363 hydrogenated than previously thought, based on the analysis of the soluble fraction. This high
364 unsaturation level can be explained either by reticulation (interconnections of atoms to form a

365 network rich in cycles and single bounds, see figure S5 for example), or by the presence of
366 double and triple bonds. Yet the two structures involve different reactivities, which could
367 impact our understanding of aerosols/gas interactions in Titan's atmosphere.

368 In addition, we have shown the importance of the fractionation between the soluble and
369 insoluble part of Titan's aerosols analogues. This phenomenon could also occur at the surface
370 of Titan at the interface between the atmosphere and the lakes and/or cryovolcanos. This
371 would have strong implications on the organic content of Titan's lakes.

372 This work also confirmed the potential of the Laser Desorption Ionization source for the study
373 of organic matter in planetary environment (Gautier et al., 2017), as being currently
374 implemented for the analysis of Martian organic matter in the ESA/ExoMars mission
375 (Arevalo et al., 2015). We also showed, on the case of analogs of Titan aerosol analogues, that
376 this ionization method would be a compelling asset if coupled with high resolution mass
377 spectrometry, as currently developed for future space missions (Briois et al., 2016).

378 Finally, our results on Titan's tholins can be compared to studies on the soluble (SOM) and
379 insoluble (IOM) organic matter found in meteorites. The insoluble matter is known to be
380 constituted of highly branched aliphatics and substituted aromatics (Derenne and Robert
381 2010), whereas the soluble matter is comprised of more saturated molecules (Schmitt-Koplin,
382 2010). The origin of both IOM and SOM and their relation with each other is highly debated.
383 They could originate from different reservoirs. One could be the result of the processing of
384 the other, or they could be both produced by the same starting point, and then differ when
385 they continue to grow as we showed in this study (Derenne and Robert, 2010). Our work on a
386 laboratory complex organic material shows that two fractions with different properties and
387 structures can actually originate from the same formation processes.

388

389 **Acknowledgments**

390 N.C. thanks the European Research Council for funding via the ERC PrimChem project (grant
391 agreement No. 636829.).

392 Financial support from the National FT-ICR network (FR 3624 CNRS) for conducting the
393 research is also gratefully acknowledged.

394 This work was supported at COBRA laboratory by the European Regional Development Fund
395 (ERDF) N°31708, the Région Normandie, and the Labex SynOrg (ANR-11-LABX-0029).

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