Very high resolution mass spectrometry of HCN polymers and tholins

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HCN polymers are complex organic solids resulting from the polymerization of hydrogen cyanide (HCN) molecules. They have been suspected to contribute to the refractory carbonaceous component of comets as well as the distributed CN sources in cometary atmospheres. Titan's tholins are also organic compounds produced in a laboratory setting but result from the complex chemistry between N_2 and CH_4 induced by UV radiation or electric discharges. Some of these compounds have optical properties in the visible range fairly similar to those of Titan's aerosols or those of the reddish surfaces of many icy satellites and small bodies. It has been proposed that HCN polymers are constituents of tholins but this statement has never received any clear demonstration. We report here on the comparative analysis of tholins and HCN polymers in order to definitely establish if the molecules identified in the HCN polymers are present in the tholins as well. First, we present a global comparison of HCN polymers with three kinds of tholins, using elemental analysis measurements, infrared spectroscopy and very high resolution mass spectrometry of their soluble fraction. We show that the chemical composition of the HCN polymers is definitely simpler than that of any of the tholins studied. Second, we focus on six ions representative of the composition of HCN polymers and using mass spectrometry (HRMS and MS/HRMS), we determine that these tholins contain at best a minor fraction of this kind of HCN polymers.

Introduction

Tholins and HCN polymers are complex polymeric organic solids mostly composed of H, C and N atoms. The term tholins designates solid products formed by organic chemistry in gas mixtures exposed to electrical discharges or ultraviolet radiation.[1,2] Since the pioneering experiments by Urey and Miller,[3] which were focused on the origin of life on Earth, numerous studies have been prompted by new fields of interest: formation of cometary refractory organics,[4,5] formation of Titan's aerosols[6] and composition of reddish icy surfaces of satellites in the outer solar system.[7] The so-called Titan's tholins are formed from N_2–CH_4 gas mixtures, in the general

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framework of simulating the chemistry in Titan's atmosphere. They have received considerable attention for the last 30 years, as some of these compounds have optical properties in the visible range fairly similar to those of Titan's aerosols.[8] Titan's tholins have also been extensively used to simulate the reddish properties of the surfaces of many icy satellites and small bodies, like centaurs and trans-Neptunian objects.[7] Their global albedo and spectral variations in the visible range have been reproduced by mixtures of dark amorphous carbon and tholins.[9]

Titan's tholins (termed simply tholins below) are generally produced in cold plasma conditions, using either Direct Current (DC) or RadioFrequency (RF) discharges. Sophisticated experimental setups have been developed worldwide, allowing reproducible experiments and systematic studies. Tholins are recovered either as thin films deposited onto substrate windows or wall reactors,[6,10] or as fine-grained powders formed and grown in levitation (PAMPRE experiment[11]). Systematic studies have described the effect of pressure and gas composition on the composition of tholins, on the relative distribution of the major functional groups and on the optical properties. The control of the chemical composition and structure on optical properties has also been investigated, emphasizing in particular the role of nitrogen abundance on the absorption strength in the visible range.[10,12]

Tholins are complex combinations of C–N–H molecules, with m/z ranging from a few u up to macromolecules insoluble in organic solvents. A major issue lies in the elucidation of their chemical composition and structure, which are still poorly known. Tholins solubility in standard polar solvents ranges between ~20–35%, depending on the N abundance.[13] The soluble fraction analyzed by High Resolution Mass Spectrometry (HRMS), appears as a highly complex mixture, with at least one peak at every m/z and m/z that extend up to 800 u.[13–15] The molecules detected can be classified along families, consisting of the addition of CH_2 functional groups on an initial structure with a fixed number of nitrogen atoms. Periodicity is observed between patterns formed by different families, pointing to a polymeric structure. In all molecules detected, unsaturation involves nitrogen atoms.

The tholins' insoluble macromolecular fraction is more complicated to investigate. Non-destructive spectroscopic techniques like infrared spectroscopy (IR) and Nuclear Magnetic Resonance (NMR) have revealed major chemical groups, mostly terminal ones: CH_2/CH_3, NH/NH_2, –CN and possibly –NC, and imines $C{=}N$, which are similar to those observed in the soluble fraction.[12,13,16] These techniques also exclude the presence of double bonded carbons as polyaromatic species and olefinic bonds. This observation suggests that the key role of nitrogen in the unsaturation of the soluble fraction also applies to the insoluble macromolecular fraction.

HCN polymers are also complex organic solids. They result from the polymerization of HCN molecules under various conditions, generally in the presence of a catalyst.[17] HCN polymers exhibit various solubilities, chemical structures and compositions. They form a broad family of compounds, which makes the term "HCN polymer" misleading. The control of the experimental conditions is definitely more complex than in the case of tholins, and a major issue lies in the reproducibility of the synthesis experiments.

HCN polymers are also, like tholins, very reactive compounds and are easily hydrolyzed, forming amino acids and other prebiotic molecules.[17–19] For this reason, they are considered as compounds of high exobiological interest. HCN polymers have also been suspected to contribute to the refractory carbonaceous component of comets. Their detection in cometary grains analyzed in the laboratory by secondary ion mass spectrometry has been claimed, but there is to date no chemical evidence of their presence.[20,21] They are also suspected to be a contributor to the distributed CN sources in cometary atmospheres.[22] Unlike tholins, HCN polymers have never been analyzed by HRMS. IR spectroscopy and NMR show that both kinds of samples exhibit similar chemical groups, but spectra appear simpler in the case of HCN polymers.[12,16,21] HCN polymers have been proposed as constituents of tholins,[23] however this statement has never received a clear demonstration.

In this publication, we present a comparison of the soluble fraction of tholins and of HCN polymers. The tholin samples were produced with the PAMPRE experiment at LATMOS (Guyancourt – France), as fine-grained powders grown in levitation. Previous studies have shown that tholins generated in the PAMPRE experiment are spherical in shape and 0.1 to 2.5 μm in diameter depending on the experimental conditions (CH_4 concentration, gas flow, RF power, plasma duration[24]). This size range is in agreement with the size of Titan's aerosols as deduced from the DISR observational data,[25,26] *i.e.* aggregates of thousands of 0.1 μm diameter monomers. The HCN polymers are one of a series synthesized at LISA (Créteil – France), containing a negligible oxygen amount and being almost completely soluble in methanol.

We report here on the comparative analysis of tholins and HCN polymers in order to establish if the molecules identified in the HCN polymers are present in tholins as well. First, we use elemental analysis, infrared spectroscopy and very high resolution mass spectrometry to present a global comparison of HCN polymers with three kinds of tholins. These tholins have been synthesized with a range of relative abundances of CH_4 in the initial gas mixture representative of those used for the simulation of Titan's atmosphere. Second, we focus on six ions representative of the composition of HCN polymers. Using HRMS, we determine if these ions are present in the tholins studied and with tandem mass spectrometry (MS/HRMS) techniques, we compare the structure of the ions present in both HCN polymers and tholins.

Experimental synthesis

HCN polymers

The HCN polymers are synthesized by hydrogen cyanide polymerization. As HCN is not a commercial product, this synthesis includes two steps: first HCN is synthesized and purified, and then it is polymerized.

HCN is produced in a vacuum manifold by reaction of NaCN (cyanide sodium, Aldrich, 98%) and $CH_3(CH_2)_{16}COOH$ (stearic acid, Merck, 97%) powders in an equimolar amount. The reagents are placed under vacuum ($<10^{-5}$ mbar) for a few hours in order to remove any water adsorbed on the powder mixture. They are subsequently heated to 350 K as the melting of the stearic acid drives the reaction. All gases produced during this reaction are trapped into a flask immersed into a liquid nitrogen bath. Then, the flask is very slowly heated at about 140 K to sublimate all the ices except the HCN that remains frozen in the flask. The purity of the remaining sample is checked by infrared spectroscopy and the main contaminant is found to be HCOOH. Any CO_2 produced from the thermal decomposition of stearic acid is eliminated during distillation.

Many methods have been proposed to synthesize HCN polymers.[19,27–33] The protocols, which propose a direct polymerization of HCN, differ by the nature of the catalyst. As NH_3 is detected in numerous astrophysical media and particularly in comets, we choose to catalyze the HCN polymerization with ammonia. We can note that Matthews and Moser[19,28] have already used ammonia to catalyze HCN polymerization.

HCN and NH_3 (Air Liquide, 99.995%) are mixed in an Erlenmeyer (10 : 1, HCN–NH_3). After the introduction of the reactants, the Erlenmeyer is closed with a glass cap and put in a water bath at room temperature in order to prevent high temperatures due to the exothermicity of the polymerization reaction. At room temperature, HCN is liquid while ammonia stays in the gaseous state in the Erlenmeyer. The liquid HCN darkens and some solids appear slowly. In a few hours, it turns to yellow, orange, brown and finally black. To reach the most complete polymerization as possible, we wait 51 days before stopping the reaction and collecting the resulting solids.

Tholins

The tholins used in this study were generated in the PAMPRE experiment. This experimental setup uses an RF capacitively coupled plasma (RF CCP) discharge as the energy source to induce chemistry in a N_2–CH_4 gas mixture. The RF CCP discharge is produced between two electrodes in the shape of a grid cage, thus confining the plasma.[11] The tholins generated are charged solid particles that are formed and grow in suspension in the plasma. They are maintained in levitation by the electrostatic force between the electrodes until they are large enough for other forces (ion and neutral drag, gravity) to compensate the latter. The tholins are then ejected from the plasma through the grid anode and deposited onto a glass crystallizer surrounding the plasma grid cage. After a production run, tholins are collected under atmospheric conditions and stored in plastic vials at room temperature for further *ex situ* analysis.

For the study presented here, tholins were produced using a 30 W, 13.56 MHz RF discharge at a pressure of 1 mbar, and at ambient temperature (neutral gas temperature $\sim$ 70 °C). Before producing tholins, the chamber was baked out at 110 °C for several hours, while being pumped down to 6×10^{-5} mbar, in order to remove H_2O adsorbed on the chamber walls and the electrodes. After this baking procedure, an additional cleaning of the electrodes was done by running an argon plasma discharge. Tholins were produced in three different N_2–CH_4 gas mixtures with 2% (SA98), 5% (SA95) and 10% (SA90) CH_4 concentrations, for 8 h each. These gas mixtures were chosen in order to compare tholins with known different chemical compositions. The production rates obtained for tholins produced at 2%, 5% and 10% were 32 mg h^{-1}, 29 mg h^{-1} and 3.3 mg h^{-1}, respectively.

Analytical techniques

Elemental analysis

An elemental composition analysis of the tholins produced in PAMPRE has been achieved at the Institut de Chimie des Substances Naturelles (ICSN, France) in order to measure the C, H, N, and O mass percentages in the tholins. For C, H and N, the analysis is done using a CHN 2400 Perkin Elmer analyzer which uses combustion under helium flow. A precisely weighed 1.5 mg sample is placed in the analyzer chamber which is first flushed with pure helium. The sample is then burnt at 940 °C with a 2-s long pure oxygen injection. The gases released, *i.e.* CO_2, H_2O and N_2, are mixed and analyzed by gas chromatography. The oxygen quantification is obtained *via* a Vario EL III Elementar analyzer which uses carbon pyrolysis at 1150 °C under helium flow. CO is then measured using a thermal conductivity detector.

Infrared spectroscopy

Fourier Transform Infrared measurements were performed using a Bruker Hyperion micro-spectrometer operating in the spectral range 4000–600 cm^{-1} and equipped with a MCT detector. The spectral resolution was 4 cm^{-1}. Samples were measured as crushed grains onto a ZnS window. The infrared spectra of the tholin samples were acquired under ultra-vacuum ($\sim 10^{-7}$ mbar) in order to minimize the spectral contribution of adsorbed water, while the spectrum of the HCN polymers was measured at ambient conditions.

Very high resolution mass spectrometry

While HCN polymers are almost completely soluble in methanol, tholins are not[13] and therefore a strict protocol of solubilisation of the samples was used. HCN polymers and tholins were dissolved in pure methanol (1 mg mL^{-1}), followed by 30 min sonication and 3 min centrifugation at 9000 g in order to collect the clear solution. Solubility of the tholins is estimated to be up to 30% in these conditions.[13] In order to check for possible hydrolysis of the sample before analysis, the same protocol of solubilisation was performed with acetonitrile instead of methanol. No significant difference could be observed in the spectra although the ionisation efficiency was systematically poorer.

An LTQ-Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with the standard IonMax electrospray ionization system (ESI) was operated in the positive ionization mode with a spray voltage of 3.5 kV. Sheath gas was set to 6 arbitrary units and the ion transfer tube was maintained at 275 °C. Tube lens potential was kept at 50 V. Transfer capillary was changed and thoroughly washed between samples. Samples were introduced into the mass spectrometer by flow infusion at 3 μL min^{-1}. The Orbitrap mass analyzer was calibrated daily according to the manufacturer's directions using a mixture of caffeine, MRFA peptide, and Ultramark. No internal calibration was used in these measurements.

Full scan profile MS data (50–300 u window and 150–1000 u window) were acquired in the Orbitrap with resolving power setting of 100 000. The injection time was from 3 to 150 ms with a target of 100 000 ions in the Orbitrap adjusted by automatic gain control (AGC). All MS^n measurements were performed in the LTQ ion trap with a normalized collision energy of 30%. The activation time was set at 30 ms with the activation parameter $q = 0.25$. The isolation window was set to 1.2 u. The data presented here were all obtained with the FT-Orbitrap as the final mass analyser (MS/HRMS).

Data acquisition and processing were performed using the Xcalibur software (version 2.0.7; Thermo Fisher Scientific, San Jose, CA, USA). Elemental formula assignments were made by comparison of the observed m/z with calculated elemental formulas using H, C, N and O. We assumed that the elemental formula whose calculated m/z was closest to the observed m/z was the correct formula. In our tholins' spectra, low intensity peaks are often attributed to oxygen-bearing ions while this is seldom the case for HCN polymers. This observation is consistent with the lower C/O ratio in tholins than HCN polymers as determined from their elemental analysis (see next section).

Results and discussion

Global analysis of the samples

Elemental analysis. From an elemental analysis of the HCN polymers and tholins,[34] we calculated the C/H, C/N and C/O elemental ratios, as shown in Table 1. The oxygen content originates both from residual oxygen present during the synthesis and from oxidation/adsorption of water vapor during sample collection. Thermogravimetry experiments as well as IR spectra suggested that a non-trivial fraction of the tholins' H content originates from water adsorbed on the samples.[34] This fraction is probably similar in all tholins as the O content does not vary much from one sample to the other. The lower O fraction in HCN polymers indicates

Table 1 Elemental analysis of HCN polymers and tholins.[34] Relative errors are typically of 1%

14	C/H	C/N	C/O
SA90	0.74	2.4	6.9
SA95	0.84	1.7	5.8
SA98	0.95	1.2	5.9
HCN polymers	0.83	0.95	10

that the quantity of water adsorbed on HCN polymers is smaller than on tholins. These observations imply that the relative trend in the C/H ratio in tholins is real but that the C/H ratio of tholins cannot be directly compared to the C/H ratio of HCN polymers.

The results presented in Table 1 indicate that the tholins composition depends on the CH_4 content in the initial gas mixture. Tholins become more hydrogen rich and nitrogen poor when the amount of CH_4 increases. The C/N ratio is lower in HCN polymers than in any of the tholins, indicating higher nitrogen content. This shows that the global composition of the HCN polymers is different from the composition of all the tholin samples although some specific molecules constituting the HCN polymers might be present in tholins as well.

Infrared spectroscopy. The infrared spectra in Fig. 1 highlight the main chemical groups present in the samples. The broad feature in the spectral region 3500–3000 cm^{-1} shows evidence of the presence of primary and likely secondary amines. In SA98 and SA95, aliphatic groups CH_2 and CH_3 exhibit features around 2950, 1450 and 1380 cm^{-1}. In the four samples, cyanides and possibly isocyanides are identified by a group of features at $\sim$2200 cm^{-1}. Finally, the peak at $\sim$1650 cm^{-1} is attributed to the C=N bond (imine). Nevertheless, the spectral region 1650–1000 cm^{-1} is very congested pointing at a complex spectral mixture of different components. Beside the identification of these chemical groups, we observe that the spectra of tholins are smooth and composed of broad features, while the spectrum of HCN polymer is simpler, exhibiting narrow features. This suggests that the HCN polymer is chemically simpler than the tholins. A detailed assignment of the HCN polymer spectrum is beyond the scope of this paper. Note that the spectral contribution of adsorbed water, either in tholins and HCN polymers, does not significantly alter their spectral signatures.

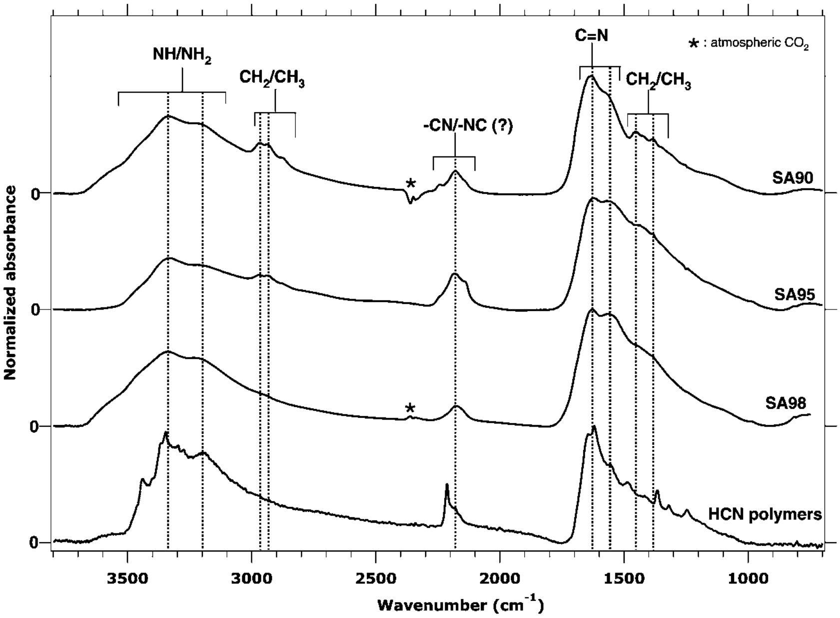

Fig. 1 Infrared spectra in the 3800–700 cm^{-1} region of the three tholin samples and of the HCN polymers.

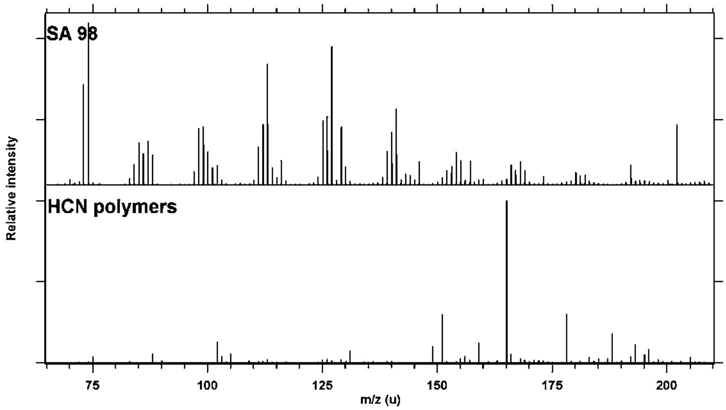

Fig. 2 Positive mode ESI Orbitrap spectrum in the *m*/*z* 65–210 region. (a) SA98, (b) HCN polymers.

Very high resolution mass spectrometry. ESI-HRMS allows the analysis of highly complex mixtures of organic compounds by direct infusion without prior separation, and therefore provides a snapshot of the thousands of molecules that can ionize under selected experimental conditions. ESI is considered to be a "cold" ionization method, which strips molecules from solution predominantly as protonated (or deprotonated) species into the gas phase. In general, no significant fragmentation of the protonated molecules occurs during the ESI process.

We compare ESI-Orbitrap spectra of HCN polymers and SA98 on Fig. 2. The global Orbitrap spectra of SA95 and SA90 are not reproduced here as their general shape and structure are similar to that of SA98. This is consistent with previous observations that every tholin spectrum is identical to the first order, not depending on the synthesis process.[14,15,35] They all show simple and regular patterns in the molecular weight distribution of the ions, *i.e.*, regularly spaced clusters separated by 13–14 u.[13] However, complex statistical representations based on Kendrick and van Krevelen diagrams reveal that the SA90 sample is richer in CH_2 than the SA98 sample.[15] This is consistent with their respective elemental analysis, showing a lower C/H ratio and a higher C/N ratio in SA90 than in SA98[34] (see Table 1). The general aspect of the HRMS spectrum of the HCN polymers is very different, with peaks extending to higher *m*/*z* and no obvious structure. However, several families of molecules could be identified, as discussed in the next section.

In order to better compare the general aspect of the mass spectra of HCN polymers and the three tholin samples, we focus in Fig. 3 on the *m*/*z* 150–185 region. This *m*/*z* region corresponds to three characteristic clusters of about 13 ions in the tholin samples while the HCN polymers spectrum is much simpler, with only a few ions in the same *m*/*z* range. Moreover, the representative close-up on *m*/*z* 165 in the inset shows that while a single ion is present at a given nominal *m*/*z* in the HCN polymers, at least three ions with the same nominal mass are observed in the tholins. Very high resolution and exact mass measurements in the Orbitrap allow the identification of these ions: $C_6H_9N_6^+$ (measured at +1.8 ppm of its exact *m*/*z*), $C_8H_{13}N_4^+$ (measured at +0.12 ppm of its exact *m*/*z*) and $C_{10}H_{17}N_2^+$ (measured at −0.24 ppm of its exact *m*/*z*). Although $C_6H_9N_6^+$, the ion identified in the HCN polymers, is also present in SA98, its relative abundance decreases in SA95 and it is almost absent from SA90. At the same time, the relative abundance of $C_8H_{13}N_4^+$ and $C_{10}H_{17}N_2^+$ increases from SA98 to SA90. This observation is consistent with the results of the elemental analysis, showing a lower C/N ratio in the HCN polymers than in any of the tholins and an increasing C/N ratio from SA98 to SA90.

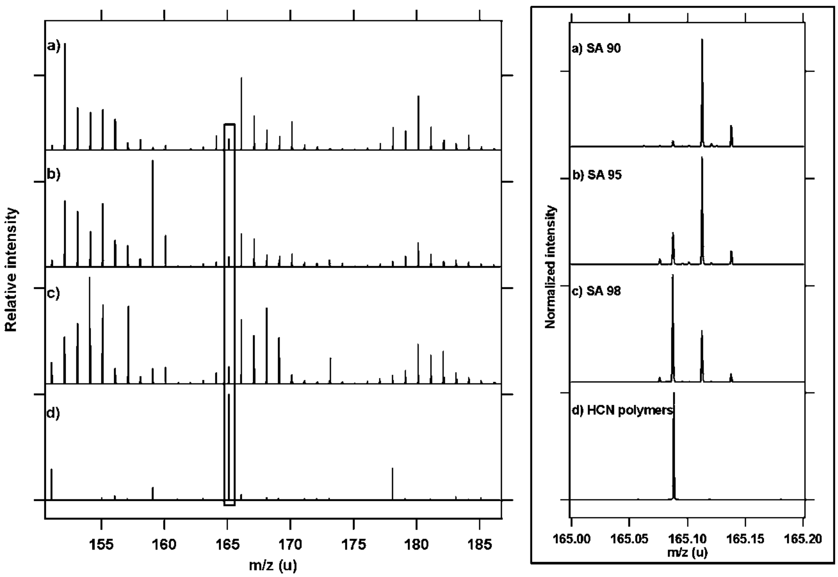

Fig. 3 Positive mode ESI Orbitrap spectrum in the 150–185 *m*/*z* region. (a) SA90, (b) SA95, (c) SA98, (d) HCN polymers. The inset gives a close-up on *m*/*z* 165 with intensities normalized for better visualization.

The elemental analysis results, infrared spectra and very high resolution mass spectra clearly indicate that the HCN polymers have a much simpler composition than the three tholins studied. At first, their composition appears closer to the composition of SA98 than of SA95 or SA90. In order to better determine if some of the molecules identified in the HCN polymers are present as well in the tholins, we next focus on a few specific ions representative of the molecules present in the HCN polymers. We first determine if ions with the same chemical composition are present in the tholin samples and then we perform MS/HRMS measurements in order to show if these ions have the same chemical structure.

Comparison of the HCN polymers and tholins

Fig. 4 presents a very high resolution mass spectrum of HCN polymers in the mass range *m*/*z* 150–300. From these data, we have identified several families of molecules in the HCN polymers. All molecules of a same family present an offset of *m*/*z* 27, the mass of HCN. For instance we have identified pure $(HCN)_n$ polymers from *m*/*z* 54 to *m*/*z* 891 (*i.e.* from $n = 2$ to $n = 33$). In order to compare tholins and HCN polymers, we have selected six molecules (*m*/*z* 109, 165, 178, 195, 244 and 261) from five distinct families. Three of these families are highlighted in Fig. 4. The *m*/*z* 109 ion ($(HCN)_4$ molecule) was selected because of its importance in HCN polymerization processes evoked in the literature.[17,36] The *m*/*z* 244 ion ($(HCN)_9$ molecule) was selected because it is the base peak in the mass spectrum of HCN polymers. The other molecules were selected because they are the most intense of their respective family, which are in turn the most intense families after the pure HCN one. The *m*/*z* 165 ion belongs to the family identified as $(HCN)_xH_2$ ($x = 6$), the *m*/*z* 178 ion to the $(HCN)_xNH$ ($x = 6$) family, the *m*/*z* 195 ion to the $(HCN)_xN_2H_4$ ($x = 6$) family, and finally the *m*/*z* 261 ion to the $(HCN)_xNH_3$ ($x = 9$) family. The different ions and their respective families are summarized in Table 2.

A summary of the (non)-detection of six selected HCN polymer ions in the three tholin samples is presented in Table 2. The Table shows that the trends presented

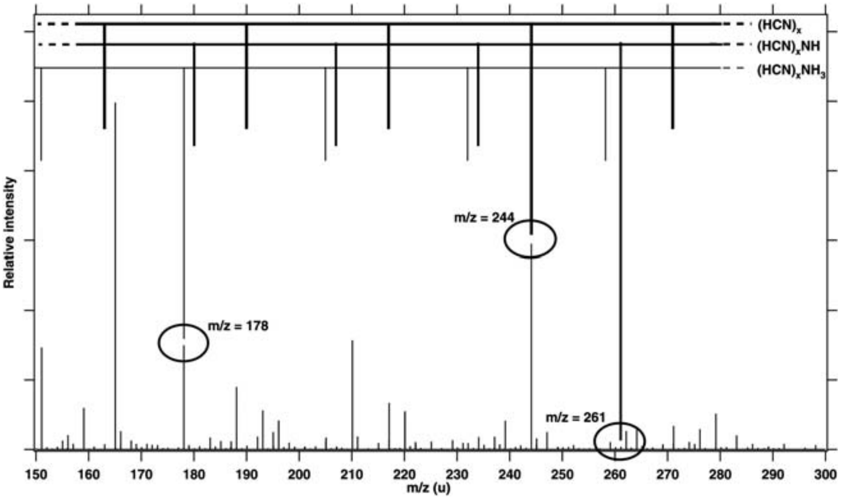

Fig. 4 Positive mode ESI Orbitrap spectrum of HCN polymers in the 150–300 *m*/*z* region. Three different families are highlighted along with the specific ions studied here (Table 2).

Table 2 Chemical formula, measured and theoretical *m*/*z* and Δppm as well as their HCN polymers family of the six ions studied here. The (non)-detection of these ions in the three tholin samples is also given. "++": major ion at its nominal *m*/*z*, "+": minor ion at its nominal *m*/*z*, "-": non-detected

HCN polymers family	Main ion in the HCN polymer sample				Tholins		
	Chemical formula	Measured *m*/*z*	Theoretical *m*/*z*	Δppm	SA98	SA95	SA90
$(HCN)_x$	$C_4H_5N_4^+$	109.0506	109.0509	−2.6	++	+	—
$(HCN)_xH_2$	$C_6H_9N_6^+$	165.0882	165.0883	−0.58	++	++	+
$(HCN)_xNH$	$C_6H_8N_7^+$	178.0833	178.0836	−1.2	++	+	—
$(HCN)_xN_2H_4$	$C_6H_{11}N_8^+$	195.1096	195.1101	−2.7	+	+	—
$(HCN)_x$	$C_9H_{10}N_9^+$	244.1055	244.1054	+0.35	++	+	—
$(HCN)_xNH_3$	$C_9H_{13}N_{10}^+$	261.1320	261.1319	+1.3	—	+	—

above for $C_6H_9N_6^+$ can be generalized to the other HCN polymer ions: they are detected in SA98 and to a lesser extent in SA95 but are mostly absent from SA90. The HCN polymer ions are systematically the ions containing the most N atoms at a given nominal *m*/*z* in the tholins. This is again consistent with the lower C/N ratio in HCN polymers than in any of the tholins and the increasing C/N ratio from SA98 to SA90 (Table 1). Only $C_9H_{13}N_{10}^+$ has a slightly different behavior: it is present in SA95 but is absent from both other samples.

Although HCN polymer ions are present in SA98, their relative intensity in a given ion cluster is fairly low, as illustrated in Fig. 3 for $C_6H_9N_6^+$ at *m*/*z* 162. However, the relative intensity of the peaks in the mass spectra is not directly correlated with the molecules concentration in the samples as their solubility and ionization efficiency may be different. Our HCN polymers are almost entirely soluble in methanol suggesting that those present in the tholins must have been extracted during the solubilization protocol. Ionization efficiency with an electrospray in the positive mode mostly depends on the proton affinity of the molecules, which is difficult to determine without knowing their exact structure. However, the ionization efficiency of the HCN

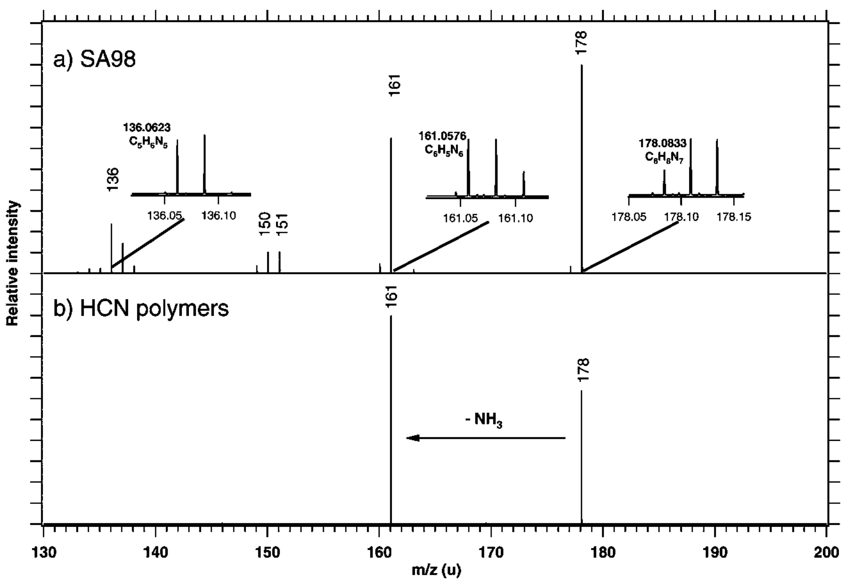

Fig. 5 Positive mode ESI Orbitrap MS^2 spectrum of *m*/*z* 178. (a) SA98, the inset gives a close-up on *m*/*z* 178, *m*/*z* 161 and *m*/*z* 136, (b) HCN polymers.

polymers was excellent and it is difficult to believe that their ionization efficiency is systematically lower than that of the tholins. To summarize, the low intensity of the HCN polymer ions in the tholins most likely reflects a real low abundance of these molecules in the tholin samples.

While the very high resolution mass spectra provide unambiguously the atomic composition of the molecules, they do not give much information on their structure as only general trends can be derived. In order to determine whether the same isomers are present in both HCN polymers and tholins, representative ions have been selected and used as precursor ions in MS/HRMS experiments. This technique has already been used on tholins and supported the presence of amino and nitrile functionalities in the samples studied.[35] Fig. 5 shows a characteristic MS^2 spectrum of *m*/*z* 178 for HCN polymers and SA98. The results of MS^2 experiments for the four major HCN polymer ions identified in SA98 (Table 2) are compared to the results obtained for the HCN polymer sample in Fig. 6. The low intensity of the HCN polymer ions in the tholin samples precludes from performing any further MS^n.

We first note that the loss of NH_3 is the main fragment for *m*/*z* 165, 178 and 244 in HCN polymers. This indicates that NH_2 represents a significant fraction of the HCN polymers, in agreement with the IR and NMR spectra. The two ions at *m*/*z* 109 and 244 are from the same family ($(HCN)_x$ with $x = 4$ and 9, respectively) and it came as a surprise that these two ions exhibit a very different fragmentation pattern. The ion at *m*/*z* 109 loses HCN exclusively, while the ion at *m*/*z* 244 loses mainly NH_3, a small fraction of HCN and NH_2CN. This implies a non-trivial structural evolution inside a same family, probably invalidating the process of linear polymerization.

While the analysis of the fragmentation patterns of HCN polymers is straightforward, those of the tholins must be interpreted with caution. First, for instrumental reasons, the selection window for the precursor ion cannot be narrower than 1 u and a mixture of ions with a different chemical composition is actually selected in the case of the tholin samples (Fig. 3). This implies that most of the fragment ions can originate from more than one parent ion. As shown for example in Fig. 5, for the MS^2 of *m*/*z* 178 in SA98, the fragment at *m*/*z* 136 $C_5H_6N_5^+$ can be attributed to a loss of NH_2CN from $C_6H_8N_7^+$ as well as a loss of C_3H_6 from $C_8H_{12}N_5^+$.

Fig. 6 Graphical representation of the MS^2 data for *m*/*z* 109, *m*/*z* 165, *m*/*z* 178 and *m*/*z* 244 from SA98 and HCN polymers. MS^2 data for *m*/*z* 165 from SA95 are included as well.

Only the loss of NH_3 and HCN can be systematically traced back to $C_6H_8N_7^+$ as the single parent ion. Next, tholins are obviously complex mixtures, and it may be possible that at a given same exact mass isomeric molecules are present, which will each exhibit their specific fragmentation signatures in the MS/HRMS. MS^2 experiments were obtained for *m*/*z* 165 in SA95 as well as in SA98 and in both cases major fragments include the loss of NH_3, HCN and N_3H, although the relative intensity of the loss of HCN and N_3H are reversed. This comparison illustrates the typical variability in the fragments intensity for two different samples with a same group of ions having different relative intensities.

Taking all these considerations into account, it is remarkable to see how similar are the tholins' and HCN polymers' dissociation patterns for the same nominal *m*/*z*. First, the percentage of fragmentation (intensity of the parent ion *vs.* sum of the intensity of the fragments) of the four parent ions is similar in SA98 and HCN polymers, with increasing fragmentation from *m*/*z* 109 to 165 to 178 to 244. Next, in both samples, the loss of NH_3 is by far the main fragment for the three heaviest ions, while for *m*/*z* 109 the only major fragment corresponds to the loss of HCN. Other major fragments in SA98 (loss of N_2 and NH_2CN for *m*/*z* 178, loss of NH_2CN for *m*/*z* 244) observed with very low intensities in HCN polymers may be explained by the further presence of other ions/isomers at this same nominal *m*/*z* in the tholin sample.

MS^2 fragments from SA98 are hence compatible with the fragments from HCN polymers, suggesting that the ions (or at least the major isomer) have a similar structure. This shows the very detailed description of complex mixtures that can be obtained with our LTQ-Orbitrap instrument. However, the interpretation of the data is rather difficult *a priori* and further work is needed in order to totally confirm our results. For example, further studies using pure compounds of known structure are necessary to gain understanding on the fragmentation pathways of molecules.

Conclusion

Mass spectrometry confirmed the relative molecular simplicity of HCN polymers compared to tholins already suggested by IR and NMR spectroscopy. Very high resolution and exact mass measurements coupled to MS/HRMS techniques revealed that HCN polymers are at best a minor component of tholins. This result indicates that the similar appearance of the IR spectrum of tholins and HCN polymers is only due to a similar distribution of functional groups rather than a detailed structural identity. An in-depth study of the composition of HCN polymers from their very high resolution mass spectrometry and MS^n will be presented in a forthcoming work.[37]

Titan's tholins are currently produced in a wide variety of experimental setups that employ different gas mixtures, energy sources, temperatures, and pressures.[1,2,6,10,11,35] However, the refractive index of only one kind of tholins has been characterized over a broad wavelength range (0.01–1000 μm).[1] As a consequence, these tholins have been used as a standard when retrieving the optical properties of Titan.[8] It appears that their albedo resembles that of Titan's actual aerosols in the visible range but that there are significant differences in the infrared.[26] For example, the VIMS results from occultation data show a lack of the 3 and 4.6 μm tholin features in the Titan aerosols.[38] The characterization of the optical properties of the tholins generated in the PAMPRE experiment is currently underway[39] and it will be interesting to compare the Titan albedos to these measurements.

MS/HRMS experiments on tholins proved to be difficult to interpret because of the high number of peaks at each nominal m/z making the assignment of a single parent to each fragment almost impossible. Therefore, it is challenging to retrieve some information on the structure of each ion individually. However, a broad picture of the tholins' composition in terms of chemical functionality could be obtained by performing systematic analyses of a large number of ions. For example, $C_2N_3^-$ has been identified as a major ion as well as an ubiquitous fragment in APPI-TOF spectra of SA98, suggesting that it is an important building brick in the growth of this tholins.[13] The most common fragments will be compared to a database of MS^n fragments of known standards in both positive and negative ionization mode. Another approach could be to simplify the spectra by running the sample through an HPLC column before injecting it into the mass spectrometer.

These results highlight the importance and necessity of very high mass resolution, accurate mass measurements and MS^n experiments for a more coherent and in-depth characterization of complex organic samples. Development of very high resolution ($m/\Delta m > 10^5$) mass spectrometers for spaceflight capable of *in situ* sampling of the atmosphere and the surface of Solar System bodies is mandatory.

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