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New insights into the structure and chemistry of Titan's tholins via $^{13}$C and $^{15}$N solid state nuclear magnetic resonance spectroscopy

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Tholins are complex C,N-containing organic compounds produced in the laboratory. They are considered to provide materials that are analogous to those responsible for the haze observed in Titan's atmosphere. These compounds present an astrobiological interest due to their ability to release amino acids upon hydrolysis. Their chemical structure has been investigated using a large number of techniques. However, to date no detailed nuclear magnetic resonance (NMR) study has been performed on these materials despite the high potential of this technique for investigating the environment of given nuclei. Here $^{13}$C and $^{15}$N solid state NMR spectroscopy was applied to obtain new insights into the chemical structure of tholins produced through plasma discharge in gaseous $\text{N}_2$–$\text{CH}_4$ mixtures designed to simulate the atmosphere of Titan. Due to the low natural abundance of these isotopes, a $^{13}$C and $^{15}$N-enriched tholin sample was synthesized using isotopically enriched gas precursors. Various pulse sequences including $^{13}$C and $^{15}$N single pulse, $^1$H–$^{13}$C and $^1$H–$^{15}$N cross-polarisation and $^1$H–$^{13}$N–$^{15}$C double cross-polarisation were used. These techniques allowed complete characterisation of the chemical and structural environments of the carbon and nitrogen atoms. The NMR assignments were confirmed by $\text{ab initio}$ electronic structure calculations for model structures and molecular fragments.

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1. Introduction

Titan, the largest moon of Saturn, is characterized by a dense atmosphere, mainly composed of $\text{N}_2$ (ca. 97%) and $\text{CH}_4$ (ca. 2%). In the upper atmosphere, methane and nitrogen molecules undergo dissociation under the influence of solar UV radiation and electron impacts, followed by recombination reactions leading to a large variety of organic molecules (Waite et al., 2007). Some of these compounds form a thick, orange-coloured haze composed of solid organic aerosols that subsequently fall to the surface or remain in suspension in the atmosphere.

To gain insight into the chemical composition and structural nature of these complex organic compounds, analogous materials are produced in the laboratory, in particular using plasma dis-
to infrared absorption, visible, UV and near-IR Raman and luminescence spectroscopy (Sagan et al., 1993; McDonald et al., 1994; Khare et al., 2002; Imanaka et al., 2004; Bernard et al., 2006; Quirico et al., 2008; Ruiz-Bermejo et al., 2008, 2009; Carrasco et al., 2009), X-ray diffraction and high resolution transmission electron microscopy (Quirico et al., 2008). The fraction of the tholins that was soluble in normal or deuteronated solvents, or in the presence of crown ethers in certain cases, has been studied using electrospray ionization mass spectrometry (Sarker et al., 2003; Somogyi et al., 2005; Hodyss, 2005; Carrasco et al., 2009; Pernot et al., 2010) and gas chromatography coupled to mass spectrometry (Hodyss, 2005).

All these studies have provided a wealth of information about potential functional groups and structural building blocks present within the tholin samples. Taken together, the results converge on a structure based on a C,H,N, chemistry, that can contain various oligomeric and polymeric chain or ring units, and containing a variety of C-C, C-N, N-H, etc. single or multiple bonds. It is now necessary to build on that information to refine the chemical and structural models for the Titan model tholins. Here we investigate these complex materials using solid state nuclear magnetic resonance (NMR) techniques.

NMR is a powerful tool for structure determination of complex organic molecules and solid state species, providing detailed element-specific information about local structure and chemical environments. In the present study, we used solid state NMR techniques to investigate the carbon and nitrogen bonding environments in a 13C- and 15N-enriched sample recovered from the plasma discharge synthesis experiment. Solid state NMR was preferred to solution state due to the low solubility of the studied sample (Carrasco et al., 2009). A preliminary solid state 13C NMR study of Titan tholins was performed by Sagan et al. (1993) but no spectrum was published and it only reported that 25% of the carbon atoms were unsaturated. We used techniques of isotopic enrichment and cross-polarisation (CP) to enhance the signal intensity combined with magic angle spinning (MAS) to reduce the broadening of the NMR peaks. We also compare the results with selected data from a parallel study of graphitic carbon nitride materials that are being developed for photocatalysis and other optoelectronic applications (Bojdys et al., 2008; Wang et al., 2009; McMillan et al., 2009).

Quantitative NMR data related to structure can be obtained by measuring relative peak intensities obtained through direct polarisation using a single pulse (SP) sequence. However, due to the low natural abundance and NMR sensitivity of the main isotopes of interest, especially 15N, such SP spectra often have a low signal to noise ratio. To overcome this, tholins were synthesised from isotopically enriched samples recovered from the plasma discharge synthesis experiment (Schafer and Stejskal, 1976). These experiments involve excitation of the most abundant and NMR sensitive spins within the sample (i.e., 1H nuclei in the case of the C,N,H, tholins) and magnetisation is transferred towards less abundant spins of interest (i.e., 13C or 15N). During the transfer process characterized by the contact time, the 13C or 15N magnetisation increases exponentially; however, the protons are simultaneously relaxing during this period, resulting in a decrease in the overall magnetisation. The magnetisation transfer and relaxation rates depend on the local 13C or 15N environments and are influenced by the proximity to as well as the number of the excited protons, along with any molecular motions present in the system. Here we present CP spectra obtained as a function of the contact time. The analysis of these results provides new information on the spatial distribution of C, N and H nuclei within the materials.

Linkages between carbon and nitrogen atoms were also investigated by NMR, using the double 1H-15N-13C CP technique, in which 1H magnetisation is first transferred to 15N nuclei which are then allowed to evolve and their magnetisation is further transferred to 13C (Schafer et al., 1979). As a result, using the double CP sequence, only the 13C signals of those carbons that are closely coupled to 15N nuclei are observable and the intensity of the signal varies according to the strength of their coupling. The key parameters here are therefore the 1H-15N and 15N-13C contact times. One- and two-dimensional NMR experiments can be conducted, providing additional information on the C,N,H arrangements within the sample (Fujiwara et al., 1995). Double CP spectra were recorded using various 1H-15N and 15N-13C contact times on tholins.

One main question to be addressed concerns the structural assignment of 13C and 15N NMR shifts obtained from the SP and CP experiments. Usually these attributions are carried out based on knowledge of functional groups within the existing NMR large data base for organic compounds. These provide useful structural analysis tools but do not always provide reliable ways to interpret the potentially unusual C-N-H bonding or molecular fragments present within tholins produced in the laboratory. Here we complemented our experimental study by carrying out ab initio predictions of 13C and 15N NMR shifts for molecular models and fragments using electronic structure methods in order to support and aid in the structural interpretation of our experimental data.

2. Experimental

2.1. Samples

The tholin sample for the solid state NMR study was produced within the PAMPRE experiment using a N2—CH4 gaseous mixture submitted to a plasma discharge (Szopa et al., 2006). The gaseous mixture contained 2.00 ± 0.06% of pure 13C-labelled methane (Eurisotope, Saclay, France) in N2 isotopically enriched with 30% of 15N2 nitrogen (Eurisotope, Fr.). That enrichment procedure enabled us to obtain single pulse (SP) 13C and 15N NMR spectra in the present study. The operating conditions were 0.9 mbar total pressure, ambient laboratory temperature, and a 30 W injected radio-frequency power. The elemental composition had H/C and N/C atomic ratios of 1.1 and 0.77, respectively (Quirico et al., 2008).

2.2. NMR experiments and first principles calculations

The 13C and 15N-enriched tholin material prepared by PAMPRE was examined by solid state NMR using a Bruker AVANCE III 700 spectrometer at B0 = 16.4 T with ν0(13C) = 176.07 MHz and ν0(15N) = 70.95 MHz, with a 3.2 mm triple resonance Bruker MAS probe, spinning at 22 kHz. Samples were spun at the magic angle using ZrO2 rotors. 13C and 15N NMR chemical shifts were calibrated to glycine enriched in 13C and 15N and referenced with respect to TMS and nitromethane respectively. Full experimental details (e.g., number of scans (NS), recycle time (RD), etc.) are shown in the figure captions. Decomposition of spectra was performed using the dmf0t software (Massiot et al., 2002).

Electronic structure calculations were carried out to support and confirm the NMR assignments. Because many of the candidate structures appear within solid state crystalline or nanocrystalline materials we chose to use the CASTEP code (version 5.5), which uses plane wave basis functions to model the electronic wavefunction in the Kohn–Sham formulation of density functional theory (DFT) (Clark et al., 2005). CASTEP enables a consistent calculation of NMR shifts for molecules and solid-state systems and provides a way to discriminate between the individual atom environments predicted to occur within the tholins at various C:N:H ratios. To model NMR shifts for certain species, individual molecules or
molecular fragments were also examined using CASTEP. In these cases a large unit cell was constructed containing a single molecular fragment that was sufficiently distant from any neighbours to eliminate possible interaction effects. We have recently tested such an approach comparing CASTEP with molecular NMR shift calculations (using the GAUSSIAN 09 code: Frisch et al., 2009) (Aliev et al., 2011). All calculations used the PBE exchange correlation functional (Perdew et al., 1999) with ultrasoft pseudopotentials for the atoms involved (Vanderbilt, 1990). Prior to the calculation of NMR shielding parameters, full geometry optimizations were carried out, with all atoms and unit cell parameters allowed to relax according to the determined crystalline structures. Molecular systems simulated using periodic boundary conditions were fully optimised within a fixed unit cell of sufficient size to ensure that interactions between the periodic images of the molecule were negligible. A k-point spacing of 0.04 Å⁻¹ and cut-off energy of 700 eV provided suitable convergence for the various C—N—H structures considered, including pyridine, pyrimidine etc. and substituted triazines and their derivatives such as melon and melem (Jurgens et al., 2003; Bojdys et al., 2008). The optimized geometries were then used in NMR shielding calculations using the “gauge including projector augmented-wave” (GIPAW) method (Pickard and Mauri, 2001; Yates et al., 2007). Our calculations for the NMR reference standards tetramethylsilane (TMS) and nitromethane (CH₃NO₂) gave the following isotropic shielding parameters (Δσiso) for ¹³C and ¹⁵N: TMS (¹³C) Δσiso = 178.2 ppm and nitromethane (¹⁵N) Δσiso = −165.5 ppm. For the C—N—H structures investigated, the calculated isotropic shielding parameters (Δσiso) were subtracted from the reference values in order to convert them into chemical shifts (Δδiso) in ppm units relative to TMS and nitromethane for direct comparison with experiment (Table 1).

3. Results and discussion

3.1. Characterisation of carbon chemical environments using ¹³C NMR

The solid state ¹³C MAS single pulse (SP) spectrum of the tholin shows a dominant peak at 140–180 ppm that contains at least two contributions corresponding to sp²-bonded carbon atoms (Fig. 1a). This region can contain substantial contributions from heterocyclic aromatic species containing triazine (CN₃) rings and/or heptazine (C₇N₇) units, as well as carbons involved in imino groups. These possibilities are discussed below. There is also a broad signal extending between 5 and 100 ppm due to aliphatic carbons, and a well-resolved peak at 121 ppm that is characteristic of nitrile species (C≡N) (Fig. 1a). Those assignments are supported by our first principles calculations (Table 1). Indeed, the DFT calculation results indicate values for ¹³C shifts for the nitrile carbon in H—C≡N and CH₃C≡N to be 120 and 124 ppm, respectively (Table 1). The effect of substitution of H by other functional groups in R—C≡N systems including an aromatic moiety was also investigated, and it resulted in a relatively small variation of the chemical shift (Table 1). We also investigated the effects of substituting the H substituents within melamine and triazine derivatives, where the ¹³C chemical shifts for the attached carbons were predicted to occur at 126 ppm and 124 ppm respectively. The aromatic carbons occurred in the region of 120–129 ppm for melamine and 129–133 ppm for triazine. We also investigated the effect of adding an amine function attached to the nitrile group (N=C—NH₂). Here we expect that the chemical shift would lie at approximately 118 ppm (Table 1).

To assess the relative contributions of the different types of carbon species, the SP spectrum was decomposed into contributions using a Gaussian fitting procedure (Fig. 1a). A minimum number of lines were used to obtain a satisfactory fit, taking into account the spinning side bands. This decomposition indicates that 60% of the carbons are sp², 11% correspond to nitrile species and 29% to aliphatic carbons.

In order to gain further insight into the detailed peak assignments, variable contact time (τCP) ¹H—¹³C cross-polarisation (CP) experiments were performed (Fig. 1b). The spectra were initially recorded at spinning speeds of 14 and 22 kHz to identify possible contributions from spinning side bands. None were observable in the 22 kHz spectrum and so this spinning rate was chosen for all subsequent experiments.

When compared with the SP spectrum, we observe an increase in intensity of the 0–100 ppm signal at short contact time (τCP = 0.5 ms) indicating that the aliphatic carbons are mainly protonated. When the contact time is increased from 0.5 to 5 ms, the relative abundance of this signal decreases rapidly relative to that of the 140–200 ppm signal, indicating a lower degree of protonation for the sp² carbons than for the aliphatic sp³ sites.

The aliphatic range is characterized by an asymmetric feature centered at around 44 ppm, with main shoulders occurring at 26 and 31 ppm (Fig. 1). When the contact time is varied between 0.5 and 5 ms, a regular increase in the intensity of the shoulder at 26 ppm and to a lesser extent that at 31 ppm is observed, compared with the overall maximum near 44 ppm, indicating differences in the CP dynamics (Fig. 1b). Based on their chemical shift values, the most deshielded carbons are most likely linked to nitrogen, as found in amine groups (C—N). The most shielded carbons most probably correspond to methyl groups (CH₃) as it is known that their pseudo-free rotation partially averages the CP process and the chemical shifts of methyl groups substituting C atoms adjacent to N in pyrimidine III or triazine II occur in the 20–27 ppm range (Stothers, 1972). Values in the same range were obtained for the chemical shift of methyl groups in various molecules as the result of our DFT calculations (Table 1).

Within the envelope of the 140–200 ppm signal, two peaks are clearly distinguished at 160 and 168 ppm. Carbon atoms responsible for the 168 ppm peak are evidently more protonated than those responsible for the 160 ppm signal based on the variable contact time (τCP) experiments. Based on their chemical shift, these sp² carbons may be linked to nitrogen as found in imine groups or in aromatic heterocyclic units. Indeed, C adjacent to N in six-membered heterocycles including triazine and its derivatives have chemical shifts ranging between 140 and 170 ppm and the resonances become more deshielded when the C atoms are substituted by aliphatic or amino groups (Stothers, 1972). For example, C atoms adjacent to N in pyridine III and pyrimidine I exhibit chemical shifts of 151 and 158 ppm, respectively, and these resonances shift to 160 and 167 when the heterocycle is substituted by a methyl or an amino group. C in triazine II and in its triamino derivative (i.e., melamine) is characterized by a chemical shift of 167 ppm. This is further supported by our theoretical calculations (Table 1). Likewise, the ¹³C NMR spectrum of melem (triaminoheptazine) IV shows a peak at 165 ppm for the C atom bearing the amino group, compared with 155 ppm for the other ¹³C resonances in the structure (Jurgens et al., 2003; Table 1). The central carbon atoms in dicyandiamide (C₂N₄H₈; NH₂—C(=N)H—NH—C(=N)H) exhibit a ¹³C chemical shift at 163 ppm (Cardamone et al., 2006). The theoretical results combined with experimental observations indicate that it is not possible to discriminate between imine carbons (C=N) occurring in straight chains vs heterocyclic environments using ¹³C NMR spectroscopy alone, however.

¹³C resonances in the 155–170 ppm range have been recorded for a nanocrystalline graphitic carbon nitride material obtained by reaction between melamine and cyanuric chloride under high pressure-high temperature conditions (Zhang et al., 2001). The resulting solid had a composition C₆N₉H₃Cl (or [C₆N₉H₄]⁺Cl⁻) with Cl atoms or Cl⁻ ions included in the structure derived from
| Functional group | Experimental chemical shifts (ppm) | Occurrence in tholin sample | Justification from experimental data | Structures simulated theoretically | Theoretical chemical shifts (ppm) |
|------------------|-----------------------------------|-----------------------------|--------------------------------------|----------------------------------|----------------------------------|
| Nitriles –C≡N    | 121 -134                          | Present                     | $^{13}$C and $^{15}$N chemical shift, CP behaviour | H–C≡N                            | 120 -144                        |
|                  |                                   |                             |                                      | H$_2$C–C≡N                        | 124 -131                        |
|                  |                                   |                             |                                      | H$_2$N–C≡N                        | 118 -134                        |
|                  |                                   |                             |                                      | Melamine –C≡N ^                   | 134 -111                        |
|                  |                                   |                             |                                      | Triazine–C≡N ^                    | 126 -137                        |
| Amines –C–N      | 44 −300 to −330                   | Present, most likely linked to sp$^2$-bonded C possibly in heterocyclic structures | $^{13}$C and $^{15}$N chemical shift, 1D double CP behaviour | Melamine (C–NH$_2$)               | 165 -329                        |
|                  |                                   |                             |                                      | Melem IV (C–NH$_2$)               | 162 -313                        |
|                  |                                   |                             |                                      | Molecule resulting from the fusion of 3 heptazines | 161–163 -304                    |
|                  |                                   |                             |                                      | H$_2$C–NH$_2$                     | 30 -388                         |
| Methyl –CH$_3$   | 26 –                              | Present                     | $^{13}$C chemical shift, CP behaviour | H$_2$C–NH$_2$                     | 30 –                            |
|                  |                                   |                             |                                      | H$_2$C–C≡N                        | 2 –                             |
|                  |                                   |                             |                                      | H$_2$C–N=C–N−CH$_3$               | 34 –                            |
|                  |                                   |                             |                                      | Hexamethylbenzene                 | 18 –                            |
| sp$^2$ carbons, imines C=C and/or C=N | 140–180 −185 to −285          | Present                     | $^{13}$C and $^{15}$N chemical shift | Triazine                         | 168 -95                         |
|                  |                                   |                             |                                      | Melamine                          | 165 -219                        |
| Isocyano –N=C   | 160 −200                          | Possible but minor          | $^{13}$C and $^{15}$N chemical shift | H−N=C                            | 175 -240                        |
|                  |                                   |                             |                                      | H$_2$C−N=C                        | 165 -227                        |
|                  |                                   |                             |                                      | Melamine–N=C ^                    | 161 -188                        |
|                  |                                   |                             |                                      | Triazine–N=C ^                    | 150 -193                        |
| Heterocyclic amine | − −356                       | Possible                    | $^{15}$N chemical shift               | Hexahydrotriazine                 | 61 -334                         |
|                  |                                   |                             |                                      | Hexahydropyridine                 | 29–51 -336                      |
|                  |                                   |                             |                                      | Hexahydropyrimidine               | 35–67 -327                      |
| NH$_4^+$, NH$_2$−NH$_3^+$ | − −356                        | Possible                    | $^{15}$N chemical shift               | NH$_4^+$Cl                      | − -350                         |
| Carboximide –N=C=N− | 140 −280 to −300        | Possible but minor          | $^{15}$N chemical shift               | H$^+$N−C=N–H                      | 145 -324                        |
|                  |                                   |                             |                                      | H$_2$C–N=C–N−CH$_3$               | 130 -319                        |
|                  |                                   |                             |                                      | H$_2$C$^+$N$^+$–C$^+$=N$^+$ ^     | 180^a -66^a                     |
|                  |                                   |                             |                                      | Hexaamidobenzene ^                | 122^b -90^b                     |
| C=N−C=N=C=N     | − −356                           | Possible                    | Double CP experiment                 | Hexaamidobenzene                  | 154 -238                        |
|                  |                                   |                             |                                      | 3CN (central N)                  | 154 -198                        |
| Heptazine [see structure IV] | ca.160 −185 and −240                 | Possible                    | $^{13}$C and $^{15}$N chemical shift | H$_2$N−C 3CN (outer N)           | 162 -313                        |
|                  |                                   |                             |                                      | 3CN (central N)                  | 154 -198                        |
|                  |                                   |                             |                                      | 3CN (central N)                  | 154 -238                        |
| Aromatic hydrocarbons | 100–140 −                        | Possible but minor and mostly non-protonated | $^{13}$C chemical shift, CP behaviour | Benzene                           | 128 –                            |
|                  |                                   |                             |                                      | Hexamethylbenzene                 | 131 –                            |
| Hydrazines R$_1$R$_2$ N−N R$_3$R$_4$ | 150 −275 to −330      | Possible but minor          | $^{13}$C and $^{15}$N chemical shift | –                                 | –                               |
| Hydrazones R$_1$R$_2$N=N=CR$_3$R$_4$ | −20 −                          | Absent                      | $^{15}$N chemical shift               | –                                 | –                               |
| Diazo compounds R$_1$R$_2$C=N=N | −5 to −65                        | Absent                      | $^{15}$N chemical shift               | –                                 | –                               |

^a^ H substitution with C=N.

^b^ NH$_2$ substitution with N=C.

^c^ H substitution with N=C.
contains triazine units linked by bridging imido/amido groups to the synthesis along with additional NCl species (Zhang et al., 2001; McMillan et al., 2009). Two main resonances were observed at 166 and 159 ppm. The higher ppm value was attributed to C atoms involved in the triazine (C3N3) rings, as on resonances were observed at 166 and 159 ppm. The higher ppm value was attributed to C atoms involved in the triazine (C3N3) rings, as found in melamine and other structures. The lower ppm signal was attributed to C atoms linked to bridging –NH– units. Those assignments agree with results of our first principles calculations (Table 1). Recently we have also studied other graphitic C,N materials produced by ionothermal/solvothermal methods that are thought to contain heptazine units (i.e., three fused triazine rings sharing a central N atom) (Bojdys et al., 2008; Wang et al., 2009). These materials also exhibit the main 166 ppm 13C resonance due to triazine rings accompanied by a second feature at approximately 155 ppm that appears for those C atoms linked to the central triply-bridging N atom (Rahman et al., in preparation).

Our first principles calculations support these assignments for various species that are possibly or probably present within the tholin sample. For melamine, the isotropic 13C NMR shift occurs at 165 ppm in the molecular solid, and in an extended graphitic melamine framework modelled in this study the chemical shift is calculated to occur at 162 ppm. For molecular melem units, there are two C environments occurring in a 1:1 ratio, that are attached to two nitrogen atoms and amino group, and those that are attached to three N atoms including the central atom providing the bonding between triazine units, respectively. The C atom bearing the amino group gives a calculated shift at 162 ppm, whereas the other C atomic environment results in a shift at 154 ppm (Table 1). Within an extended graphitic system based on polymerised melem units, these values occur at 162 and 156 ppm respectively. In these structural units, an additional third C environment attached to the imido bridging group also gives rise to a chemical shift occurring at 164 ppm, so that it is impossible for the 13C NMR results to distinguish between the two.

Other potential contributions to the 140–180 ppm peak result from the possible presence of isocyano groups (–N=C–) and carbodiimide moieties (–N=C≡N–). These alternative assignments would be consistent with the low CP dynamics revealed by the variable contact time experiments. They are further discussed and elucidated with reference to the 1H–15N cross-polarisation experiments described below.

The broad signal between 100 and 140 ppm likely indicates a small contribution from other aromatic carbon species present in the sample. DFT calculation results indicate 13C values around 130 ppm for benzene and its hexamethyl homologue (Table 1). The behaviour of the 100–140 ppm signal in the variable contact time experiment is similar to that of the nitrile peak, suggesting that these C sites are mainly non-protonated.

To summarise, these 13C NMR experiments combined with the first principles theory predictions reveal the presence of mainly protonated aliphatic carbons present within the tholin sample. Some of the aliphatic carbons are linked to N incorporated within amine functional groups and others are clearly involved in methyl species. The occurrence of nitrile groups within the tholin sample is also evident. The dominant 13C NMR signal can be assigned to sp2 carbons linked to N within heterocyclic units or imine functions although a contribution from isocyanate or carbodiimide moieties cannot be excluded at this stage of the analysis. A minor contribution of non-protonated aromatic carbons could also be present.

3.2. Characterisation of nitrogen chemical environments using 15N NMR

The single pulse solid state 15N MAS data recorded for the tholin sample presents a quite complex spectrum (Fig. 2a). A main broad peak occurs between –230 and –340 ppm with maxima at –272 ppm and –304 ppm, along with two shoulders at –330 and –256 ppm. An additional broad signal occurs throughout the –160 to –230 ppm range with maxima near –185 ppm and –170 ppm, as well as an additional well-resolved peak at –134 ppm (Fig. 2a). The peak at –134 ppm corresponds to positions usually assigned to nitrile species, whereas –330 ppm is a typical chemical shift for amines. Both of these species were indicated to be present in the tholin sample from 13C SP and CP MAS NMR spectroscopy discussed above. Their assignment is supported by first principles calculations (Table 1).
There is also a sharp peak at ~356 ppm. This falls within an unusual range for 15N chemical shifts. Heterocyclic amines might account for this observation as supported by literature data (Martin et al., 1981) although the present DFT calculations result in slightly higher chemical shifts (Table 1). However, the corresponding 13C chemical shifts fall in the 30–70 ppm range, reported above for aliphatic carbons. Ammonium or hydrazinium ions located close to polynitrogen chains such as azides have also been reported to exhibit such anomalously negative chemical shift values (Klapötke and Stierstorfer, 2009; Klapötke et al., 2009). The low efficiency of CP dynamics for the peak at ~356 ppm observed in this study may be explained by the presence of NH3 groups in the sample combined with their expected rotational motion. That assignment is supported by the theoretical results that predict a 15N shift for ammonium species at ~350 ppm (Table 1).

Based on 13C chemical shift data, contributions from six-membered heterocycles were considered to be present. This might initially seem at variance with the observed 13C chemical shifts, as values of ~63 ppm, ~85 ppm and ~103 ppm have been reported for pyridine III, pyrimidine I and triazine II, respectively. However, it has also been observed that substitution by amino groups induces a strong shift towards more negative values (~190 and ~207 ppm for 2,4,6-trimino-pyrimidine and -triazine, respectively) and that the chemical shift for the amino group in such compounds occurs at around ~300 ppm (Martin et al., 1981). This is supported by our first principles calculations which indicate that the NH3 groups in melamine give rise to a chemical shift at ~329 ppm whereas the aromatic N species incorporated in the triazine rings leads to a signal at ~219 ppm (Table 1). Similar shifts were reported for 2-exo-cyanomethylene substituted quinazolines VI and their cyanoimino analogues VII where values of ~274 and ~285 ppm were recorded for the N atoms (Benassi et al., 2000). Interestingly, the carbon atom located between the two N atoms in the heterocycle exhibited 13C shifts of 156–158 ppm, similar to the values observed for the tholin sample (Fig. 1).

15N chemical shifts at around ~280 ppm have also been reported to occur for N atoms contained within carbodiimide structures (~N=N=C=N~), that become shifted to ~297 ppm when an additional amino group is present on the side chain (Martin et al., 1981). However, in such structures the corresponding chemical shift of the central C atom occurs at ca.140 ppm. These chemical shifts are supported by first principles calculations (Table 1). The low abundance of the signal in 13C CP MAS points to, at most, a minor contribution of carbodiimide functions.

From the 13C NMR spectrum, a contribution of isocyanogroups could be considered. We therefore investigated the corresponding 15N chemical shifts. A value of ~204 ppm was experimentally obtained for disocyanobenzene and first principles calculations led to values ranging from ~188 to ~240 ppm for 15N and 161–180 ppm for 13C. Isocyanogroups may therefore contribute to a part of the NMR signal.

13C chemical shifts reported for melem IV containing triazine and heptazine units were similar to those observed for the tholin sample (Jurgens et al., 2003), and so we examined the corresponding 15N shifts for molecular units associated with these species. The N atoms located at the periphery of the condensed rings give rise to an intense signal at ~200 ppm, whereas the central N is observed to resonate at ~234 ppm (Jurgens et al., 2003). Amino groups from local groups such as those existing in melem are also detected at ~267 and ~281 ppm (Jurgens et al., 2003). In the present work, the simulated 15N chemical shifts for the heptazine (melem) molecular unit yielded three values, with the amine group occurring at ~313 ppm, the central N at ~238 ppm and outer N atoms at ~198 ppm (Table 1). These results are consistent with the idea that heptazine units are present within the tholin sample. It must be noted that similar values are obtained when the fusion of three heptazine moieties is considered (Table 1).

15N CP MAS experiments were performed at variable contact times (tCP = 0.5–10 ms) (Fig. 2b). The results indicate the most efficient CP dynamics for 15N resonances occurring in the ~270 to ~295 ppm range, compared with the resonances in the ~134 to ~198 ppm range that showed a rather slow polarisation transfer. This is consistent with the assignments of the ~134 ppm peak to nitrile groups and of at least a part of the ~200 ppm peak to isocyano groups.

These 15N NMR experiments confirm the presence of nitrile and amine groups in the tholin sample. The occurrence of isocyano groups is consistent with the 13C NMR data, along with a low contribution of carbodiimide entities to the NMR spectra. The occurrence of amino-substituted six-membered heterocycles is supported by resonances both in the range of the amino substituents and of the imino functions from the rings, although other types of substituents (such as some bearing cyano groups) might also be present.

Decomposition of the SP spectrum into Gaussian components indicates that ca. 56% of the 15N signal is observed between ~230 and ~340 ppm and 29% between ~160 and ~230 ppm. These resonances can be mostly assigned to amino groups (the more shielded area) located on imino groups (the less shielded area) possibly in heterocycles (triazine- or heptazine-like environments). The signal at ~134 ppm, assigned to nitrile groups, accounts for 9% of the 15N signal. This nitrile contribution is consistent with that (11%) derived from the 13C NMR spectrum (Fig. 1a), when taking into account the N/C atomic ratio of the tholin sample. Finally, the neat signal at ~356 ppm that we assign to the presence of NH3 species or heterocyclic amines represents 6% of the N atoms.

3.3. N–C interactions studied via 1H–15N–13C double CP–MAS NMR

3.3.1. One-dimensional 1H–15N–13C double CP experiments

To specify further the linkages occurring between N and C centres in the tholin sample, 1H–15N–13C double CP experiments were performed. Based on our previous experience obtained with the 15N CP MAS spectra above, we selected 1H–15N contact times at 0.5 and 5 ms. With the lower tC value, the spectrum is chiefly composed of the main signal between ~220 and ~310 ppm, whereas the full spectral signal is observed at 5 ms. A variable 15N–13C contact time experiment (Fig. 3) was then performed to highlight the N–C coupling characteristics and to select the most appropriate values for the 2D NMR experiments.

For 1H–15N contact (tCP1) experiments carried out at 0.5 ms, three values were selected for 15N–13C contact times (tCP2), namely 1, 5 and 20 ms (Fig. 3a). At the lowest 15N–13C contact time, the peak at 160 ppm is already most intense, indicating a stronger 15N–13C coupling than for the C resonating at 168 ppm. Consistent with that result, the intensity of the 13C peak at 168 ppm is higher than the 160 ppm one for a longer 15N–13C contact time (Fig. 3a). As expected, a weak resonance is observed between 5 and 100 ppm corresponding to aliphatic carbons located far from the N atoms. The nitrile carbons resonating at 121 ppm are clearly detected at longer contact time.

When using 5 ms as 1H–15N contact time (tCP1), we observed the same relative behaviour of the peak at 160 ppm with respect to that at 168 ppm (Fig. 3b). A broad signal is observed at 60–80 ppm using 5 ms as 1H–15N contact time, but this is not detected with 0.5 ms contact time. This is consistent with the assignment of this signal to amine groups, as N from amines is generally difficult to detect in 15N CP MAS with short contact time conditions.
3.3.2. Two-dimensional $^1$H-$^{15}$N-$^{13}$C double CP experiments

Following on from the double CP experiments described above, we ran two-dimensional spectra with two $^{1}$H-$^{15}$N, $^{15}$N-$^{13}$C couples of contact times, namely (5, 5) and (0.5, 5) ms (Figs. 4 and 5). For the (0.5, 5) couple we ran spectra using recycle times of 3 and 5 s. It is clearly apparent that not all the C atoms are detected with the shorter recycle time and that using 3 s strongly enhances the contribution from aliphatic carbons. The short delay time was then used to analyse the coupling with this type of C atoms.

The 2D spectrum recorded with $t_{CP1} = 5$ ms and $t_{CP2} = 5$ ms and a recycle time of 3 s (Fig. 4) clearly shows the coupling between the C atoms resonating at 168 ppm with the N atoms at $-280$ ppm and to a lesser extent with N at $-174$ ppm. In contrast, C centres at 160 ppm are only correlated with the N contributions from the main $^{15}$N peak at $-282$ ppm. When the aliphatic carbons are considered, two types are distinguished at 31 and 44 ppm, and both are coupled with the broad N signal around $-278$ ppm.

A higher resolution of the aliphatic area is obtained with the spectrum recorded with a recycle time of 3 s and 0.5 and 5 ms as $^{1}$H-$^{15}$N and $^{15}$N-$^{13}$C contact times, clearly showing that the 31 ppm C are coupled with N at $-280$ ppm whereas the N which are coupled with C at $-244$ ppm are shifted towards $-278$ ppm (Fig. 5). It also clearly shows a correlation between C at 26 ppm and N at $-278$ ppm. A small correlation signal can be seen on the $^{13}$C axis projection at 120 ppm (Fig. 5, arrow). When decreasing the gain factors within the map, a very low intensity spot can be detected, revealing a coupling between these carbons (involved in nitrile groups) and N atoms at $-280$ ppm, hence the possible presence of a C-N=C=O moiety. A similar low intensity signal was also detected when using a recycle time of 3 s and 5 ms for both $^{1}$H-$^{15}$N and $^{15}$N-$^{13}$C contact times. Such a low level of detection for this coupling can be partly explained by the slow polarisation of the nitrile group due to its lack of bonded H atoms.

It must be noted that C atoms that are more shielded than 20 ppm are never detected in these double CP experiments, consistent with aliphatic C not linked to N atoms. When the most deshielded aliphatic carbons are considered in the analysis, their behaviour in 1-D CP NMR (Fig. 3b) suggests a coupling with N but no corresponding spot can be seen in 2-D experiments. Similarly, no correlation is observed with the N at $-330$ ppm, although this is assumed to correspond to amino groups. This may be the result of both the low abundance of this type of N containing centres and the low $^{15}$N-$^{13}$C coupling constant. Similarly, no correlation is observable between carbon atoms and N resonating at $-356$ ppm, that could indicate either that these N atoms are involved in heterocyclic structures or that they are isolated within N-rich structures. Ammonium or hydrazinium compensat-
ing negatively charged polynitrogen moieties might be good candidates. The free rotation of the NH$_4^+$ group results in a poor efficiency of the $^{1}H-^{15}N$ magnetisation transfer. It should also be noted that nitriles are not clearly visible in these 2D spectra because their detection requires longer $t_{CP}$ ($\geq 10$ ms) than those used in the 2D experiments.

3.4. Discussion

This comprehensive NMR study has allowed the elucidation and confirmation of structural units and functional groups that have been suggested to be present within tholins from other techniques, especially the presence of nitriles. Characteristic nitrile (C$\equiv$N) stretching vibrations were observed by FTIR (Khare et al., 2002; McDonald et al., 1994; Sarker et al., 2003; Imanaka et al., 2004; Quirico et al., 2008) and Raman spectroscopy (Imanaka et al., 2004; Bernard et al., 2006; Szopa et al., 2006; Quirico et al., 2008). Various compounds and molecular fragments containing a nitrile group were released upon pyrolysis (Ehrenfreund et al., 1995; Coll et al., 1999; Hodyss, 2005; Szopa et al., 2006; McGuigan et al., 2006). Mass spectrometry results on the tholin soluble fraction report losses of HCN, indicative of the presence of nitrile functions (Sarker et al., 2003; Hodyss, 2005). Various oligomeric aminonitrile units (CN$\cdots$(CH$_2$)$_x$$\cdots$NH$_2$) were proposed to constitute structural units of tholins based on mass spectrometric studies (Hodyss, 2005; Pernot et al., 2010).

The contribution of aminonitriles implies the presence of amino groups that have also been detected by FTIR spectra (Khare et al., 2002; McDonald et al., 1994; Sarker et al., 2003; Imanaka et al., 2004; Quirico et al., 2008). However, the precise nature (primary or secondary) of the species cannot be specified. D–H exchange experiments in solution also support the presence of amino (or imino) functions (Somogyi et al., 2005). From the present study, several types of amino groups may contribute to the chemical structure of tholins. No direct evidence for amino groups linked to aliphatic carbons can be derived from the 2D NMR spectra; however, their occurrence is strongly suggested by the observations in the 1D double CP experiments. This might at first appear in variance with the discussion and conclusions of Quirico et al. (2008). However, the amino group in arylcyanoguanidines VIII has a chemical shift around $\sim 300$ ppm and it is linked to an imino carbon which resonates at 160 ppm in $^{13}$C NMR (Cunningham and Wan, 1996). Guanidine IX and biguanide X have been proposed as “chemical roots” for the methanol soluble fraction of tholin based on ESI analyses (Pernot et al., 2010). The present double CP experiments suggest the coupling of nitrogen resonating at $-280$ ppm with a cyano group. In addition, a correlation is observed between $^{15}$N at $-280$ ppm and $^{13}$C at 168 ppm. Based on their chemical shifts, these N and C species may belong to an imino group suggesting the occurrence of a C$\equiv$N$\cdots$C$\equiv$N moiety as in VIII. The presence of imino groups has already been inferred from FTIR spectroscopy (McDonald et al., 1994; Sarker et al., 2003).

Another type of amino group that cannot be excluded is the case of amino groups attached to C atoms contained within heterocyclic structures. Their chemical shift values occur at around $-300$ ppm and they are linked to C atoms for which the shifts occur in the 160–170 ppm range. The adjacent N atoms show chemical shifts at around $-180$ ppm due to amino substitution of the carbon atoms. Our double CP experiment clearly shows a correlation between $^{13}$C at 168 ppm and $^{15}$N at $-280$ and $-174$ ppm, in agreement with such a sub-structure. The presence of heterocycles in tholins was suggested by pyrolysis studies as well as by UV Raman spectroscopy and FTIR (Ehrenfreund et al., 1995; Pietrogrande et al., 2001; Khare et al., 2002; Imanaka et al., 2004; Hodyss, 2005; McGuigan et al., 2006; Quirico et al., 2008). Interestingly, the C$\equiv$N$\cdots$C$\equiv$N moiety can be related to such a heterocyclic structure, as dicyandiamide XI is known to be readily converted into melamine and melem by heating (Belsky et al., 1997).

As far as we know, ammonium and hydrazinium ions have not been considered so far in elucidating the chemical structure of tholins. However, the present NMR results suggest their existence close to a negatively charged nitrogen-rich moiety in the model tholin material studied here.

Aromatic hydrocarbon moieties were also proposed from pyrolysis studies to contribute to the chemical structure of tholins (Ehrenfreund et al., 1995; Pietrogrande et al., 2001; Coll et al., 1999; Szopa et al., 2006; McGuigan et al., 2006). The low intensity of the signal around 130 ppm in $^{13}$C NMR and its behaviour in the variable contact time experiment points to a lack of protonated aromatic carbons in agreement with previous FTIR observations (Quirico et al., 2008).

Occurrence of isocyno groups in tholins was suggested from FTIR data (Imanaka et al., 2004; Quirico et al., 2008). However, the isocyano groups are characterized by resonances at 160 ppm for $^{13}$C and $-200$ ppm for $^{15}$N. Signals are observed in these regions in each CP/MAS spectrum but double CP experiments could not reveal any correlation between these two signals. The isocyano contribution must therefore be considered to be minor in the tholin.

Several other species and structural groups have also been proposed to be present in tholins according to FTIR (Imanaka et al., 2004) but the present study has shown that if they are present, it can only be in minimal quantities. For example, carbondiimide moieties (N$\equiv$C$\equiv$N) could give rise to $^{13}$N chemical shifts at around $-280$ ppm, but $^{13}$C in these structures would resonate at $140$ ppm, where no obvious NMR signal is observed. Hydrazines (R$_1$R$_2$N$\equiv$NR$_3$R$_4$) are characterized by $^{15}$N and $^{13}$C chemical shifts at $-275$ to $-330$ ppm and $150$ ppm respectively. The low abundance of the $^{13}$C signal in this latter range rules out a major contribution from these species. Contribution of hydrazones (R$_1$R$_2$N$\equiv$N$\equiv$CR$_3$R$_4$) was previously excluded from the analysis of the FTIR spectrum (Quirico et al., 2008) and this is confirmed by the present NMR study because the chemical shift of the N atoms involved in the imino bond would occur at ca. $-20$ ppm. Similarly, diazo compounds (R$_1$R$_2$C$\equiv$N$\equiv$N) can be excluded because the terminal nitrogen is strongly deshielded ($-5$ to $-65$ ppm).

In conclusion, the present $^{13}$C and $^{15}$N NMR study combined with the first principles electronic structure calculation results indicate that the model tholin structure is mainly based on unsaturated C$\equiv$N bonded units, contained within sp$^2$ bonded species such as imines or aromatic triazine or heptazine units, or nitrile (C$\equiv$N$\cdots$C$\equiv$N) groups. Amino groups (NH$_2$) are also present, most likely linked to sp$^3$-bonded C as are some methyl (–CH$_3$) groups. Along with previous mass spectrometry and FTIR, UV–visible and FT–Raman investigations, this places additional and new constraints on the likely structures and compositions of the Titan’s tholins.

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Appendix A

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