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Composition chimique du brouillard de Titan par mobilité ionique couplée à la spectrométrie de masse haute résolution

Julien Maillard

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Composition chimique du brouillard de Titan par mobilité ionique couplée à la spectrométrie de masse haute résolution

Thèse de doctorat de l'université Paris-Saclay

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Spécialité de doctorat : Structure et évolution de la terre et des autres planètes

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Thèse présentée et soutenue à Rouen, le 1^{er} Juillet 2020, par

Julien MAILLARD

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Chapitre 1

Titan – Caractéristiques et Intérêts

J'introduirai dans ce premier chapitre le sujet principal de cette thèse : le satellite Titan et la complexité de sa chimie atmosphérique. Trois parties seront détaillées. Dans la première partie, j'introduirai une présentation du satellite glacé ainsi que des différentes missions spatiales qui l'ont survolé. Dans la seconde partie, je discuterai l'intérêt des reproductions expérimentales de l'atmosphère de Titan en laboratoire, ainsi que des méthodologies employées par les différentes équipes de recherche travaillant sur ce sujet. Enfin, je présenterai dans la troisième partie les résultats reportés dans la littérature concernant la caractérisation en laboratoire des analogues d'aérosols de Titan.

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Partie 1 : Titan et ses missions spatiales

Partie 2 : Les reproductions en laboratoire de l'atmosphère de Titan

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Partie 1 : Titan et ses missions spatiales

1.1. Caractéristiques générales

Bien qu'observée pour la première fois en 1655 par Christian Huygens, la plus grosse des 62 lunes de Saturne ne fut nommée qu'en 1847. John Herschel lui attribua alors le nom de Titan du fait de sa grande taille. En effet, c'est le plus gros satellite du système solaire après Ganymède avec son rayon moyen de 2575 km. En comparaison celui de la Terre est de 6 371 km.

C'est aussi le seul à posséder une atmosphère épaisse contrairement aux autres satellites du système solaire ([Khare 1973 \[1\]](#)). Cette atmosphère a été suggérée pour la première fois en 1908 par Comas i Sola. Cette thèse a été renforcée, plus tard, grâce aux études concernant la conservation des espèces volatiles sur Titan du fait de sa basse température (James Jeans, 1931). Mais c'est en 1944 que cette hypothèse a été définitivement affirmée par la découverte de méthane autour de Titan grâce aux mesures infrarouges réalisées sur Terre par Gérard Kuiper. L'atmosphère de Titan est majoritairement composée d'azote et de méthane (98,4-1,6 % à la surface) avec des traces de plusieurs autres gaz comme le dioxyde de carbone, et l'argon ([Niemann, et al. 2005 \[2\]](#)). Cette atmosphère est épaisse de plus de 1400 km, ce qui est supérieur à celle de la Terre qui elle, n'est épaisse que de 800 km. La pression à la surface de Titan est 1.5 fois celle de la Terre et la gravité y est environ 7 fois plus faible ([Fulchignoni, et al. 2005 \[3\]](#)). Du fait que cette lune soit très froide, avec une température de 90 K à la surface, il y a liquéfaction des hydrocarbures présents comme le méthane et l'éthane. Par analogie au cycle de l'eau sur Terre, on retrouve sur Titan le cycle du méthane, avec ses nuages, ses précipitations, ses rivières et ses mers.

Une autre grande découverte fut la présence d'aérosols produit par photochimie dans l'atmosphère de Titan. La présence d'un tel brouillard organique a suscité beaucoup d'intérêt dans la communauté scientifique.

En effet, Titan possède ainsi les caractéristiques idéales pour l'étude de la chimie prébiotique. On entend par chimie prébiotique la chimie organique complexe présente lors des périodes primitives antérieures à l'apparition de la vie sur Terre. L'hypothèse de la présence d'aérosols organiques sur la Terre primitive est largement acceptée aujourd'hui, et on suppose que les conditions de formation de ces poussières prébiotiques sont proches de celles observées pour les aérosols de Titan. C'est donc, poussées par cet intérêt principalement, que des missions spatiales ont eu pour point de passage la lune rousse.

1.2. Les missions d'explorations

La grande majorité des informations connues sur Titan aujourd'hui proviennent des différentes missions spatiales qui ont survolé la lune glacée. En effet, les informations récupérées grâce aux télescopes terrestres de l'époque ont tout d'abord essentiellement permis de soulever des hypothèses concernant les vraies propriétés de la lune ainsi que de supputer de la présence d'aérosols organiques dans son atmosphère. Ainsi, deux premières missions spatiales ont été conçues et envoyées. Elles portent le nom de Voyager 1 et Voyager 2 et ont été lancées dans les années 1970. Ces deux sondes lancées plus précisément en 1977 ont, à 9 mois d'intervalles, récupérées de précieuses informations sur Titan avant de continuer leur route vers les confins de notre système solaire. Elles ont, entre autres, confirmé la composition moléculaire de l'atmosphère.

De plus, la présence d'un épais brouillard marron masquant la surface a été aussi révélé. Comme énoncé dans la partie précédente, ce brouillard avait déjà été suspecté par Carl Sagan en 1974 ([Sagan and Salpeter 1976 \[4\]](#) ,[Sagan 1974 \[5\]](#)). Ces aérosols ont été la plus grande découverte faite par ces missions.

L'intérêt de la communauté scientifique pour Titan a explosé et certaines personnes ont même proposé la présence d'une forme de vie à la surface de la lune.

Basée sur ces nouvelles informations, une troisième mission spatiale a vu le jour avec pour but de percer les mystères de Titan et de répondre aux spéculations.

Cassini-Huygens a donc été schématisée en 1988. La sonde se composait de deux parties distinctes : Cassini, l'orbiteur et Huygens, l'atterrisseur. Cassini avait pour objectif d'étudier le système Saturnien ainsi que d'imager Titan et les autres satellites. Cet orbiteur était équipé de douze instruments dont :

- VIMS (*Visual and Infrared Mapping Spectrometer*) chargé d'étudier les compositions chimiques des lunes ainsi que leurs températures.
- CAPS (*Cassini Plasma Spectrometer*) chargé de caractériser les plasmas rencontrés lors de la mission
- ISS (*Imaging Science Subsystem*) chargé de photographier les différentes lunes ainsi que Saturne et ces anneaux
- CIRS (*Composite Infrared Spectrometer*) chargé d'étudier les rayonnements infrarouges émis et ainsi obtenir des informations structurales
- INMS (*Ion and Neutral Mass Spectrometer*) chargé de procéder à la première analyse chimique des molécules et des ions présents dans l'atmosphère haute de Titan ainsi que dans les anneaux de Saturne

Par ailleurs, l'atterrisseur Huygens était équipé de 6 instruments d'analyses afin de percer les mystères de l'atmosphère et de la surface de la lune Titan :

- GCMS (*Gas chromatograph and Mass Spectrometer*) permettant l'analyse de l'atmosphère lors de la descente de la sonde
- ACP (*Aerosols Collector and Pyrolyser*) chargé de réaliser la première analyse chimique des aérosols de la lune glacée
- DISR (*Descent Imager and Spectral Radiometer*) chargé de la prise de clichés de la surface de Titan et de l'analyse de la structure des nuages
- SSP (*Surface Sciences Package*), un ensemble de capteur pour étudier les caractéristiques physiques de la surface lors de l'atterrissage
- DWE (*Doppler Wind Experiment*) afin d'étudier les vents régnants sur la lune
- HASI (*Huygens Atmospheric Structure Instrument*) chargé d'étudier les propriétés physiques de l'atmosphère

Après plusieurs années de conception et de tests, la sonde a été finalement lancée le 15 Octobre 1997. Après un voyage de 7 ans, la sonde Cassini-Huygens effectua son premier survol de Titan le 26 Octobre 2004. Les premières découvertes ne se firent pas attendre. Une surface peu accidentée dépourvue de cratères est révélée grâce au radar embarqué ([Elachi, et al. 2005 \[6\]](#)). Un relief riche mais peu élevé est mis en lumière. Des sillons, des canaux sont observés et laissent suggérer des rivières. On y suggère même la présence de cryovolcans ([Sotin, et al. 2005 \[7\]](#)). L'absence de champ magnétique autour de la lune rousse est observée ([Backes, et al. 2005 \[8\]](#)).

Lors de ses survols, Cassini a pénétré dans la haute atmosphère de Titan, jusqu'à environ 900 km d'altitude. Elle a donc traversé la thermosphère qui s'étend jusqu'à une altitude de 1400 km et ainsi réalisé les premières caractérisations. Un profil atmosphérique de Titan est illustré par la **Figure 1**.

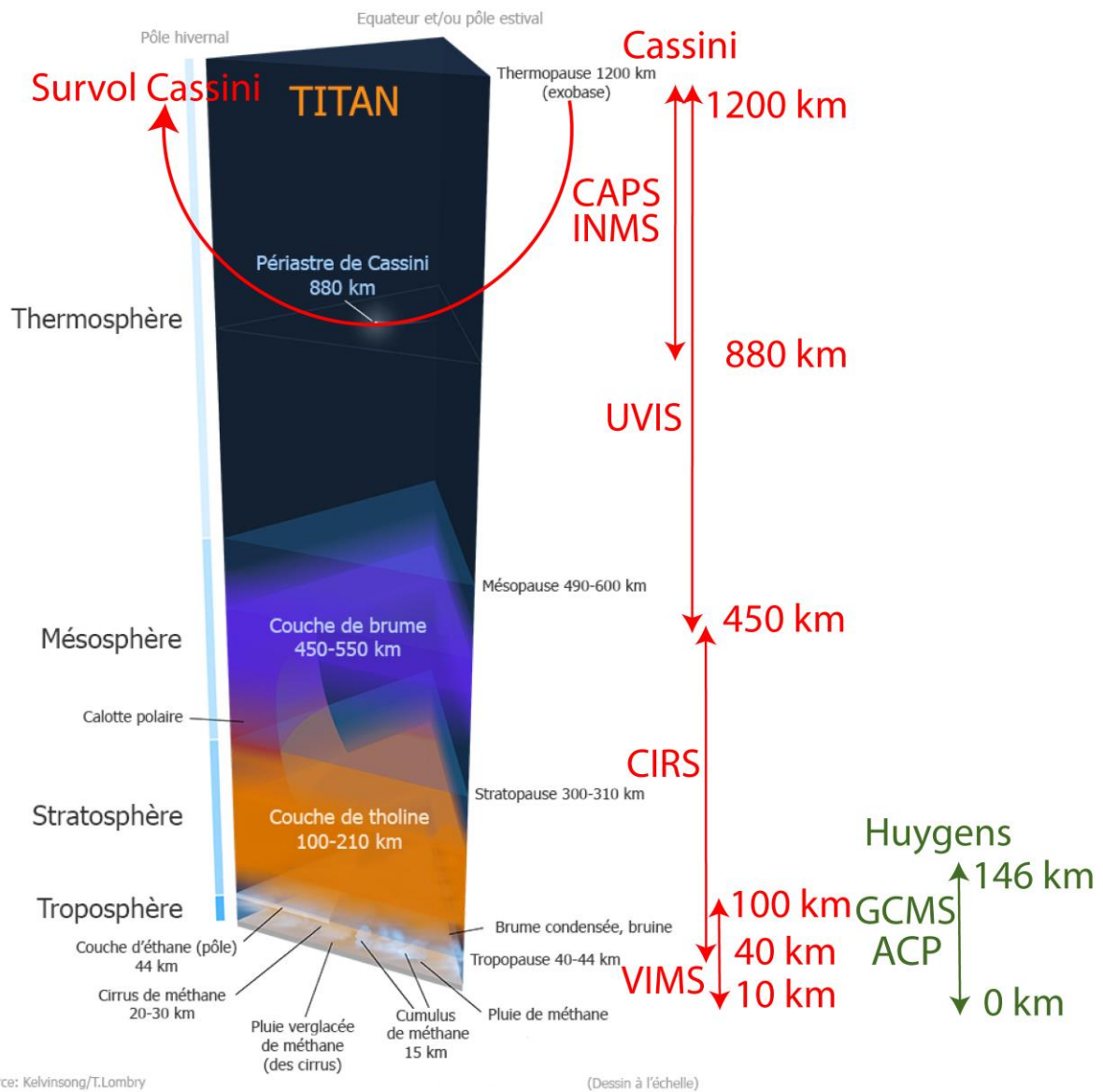


Figure 1: Profil atmosphérique de Titan (ESA/JPL). Sur la droite du schéma sont représentées les zones d'étude des différents instruments ayant sondé Titan. En vert les instruments embarqués à bord de l'atterrisseur Huygens et en rouge ceux embarqués à bord de Cassini.

Grâce à l'instrument INMS, la première caractérisation des espèces neutres et des ions présents dans l'atmosphère a été faite (Waite, et al. 2007 [9], Waite, et al. 2005 [10]). Une forte diversité moléculaire est révélée lors de l'analyse des ions mais aussi lors de l'analyse des neutres. La **Figure 2** montre les spectres de masse obtenus pour la détection des ions et des neutres lors du survol de Titan entre les masses 0 et 100. La première découverte fut la présence d'espèces à chaque masse nominale dans le spectre des ions.

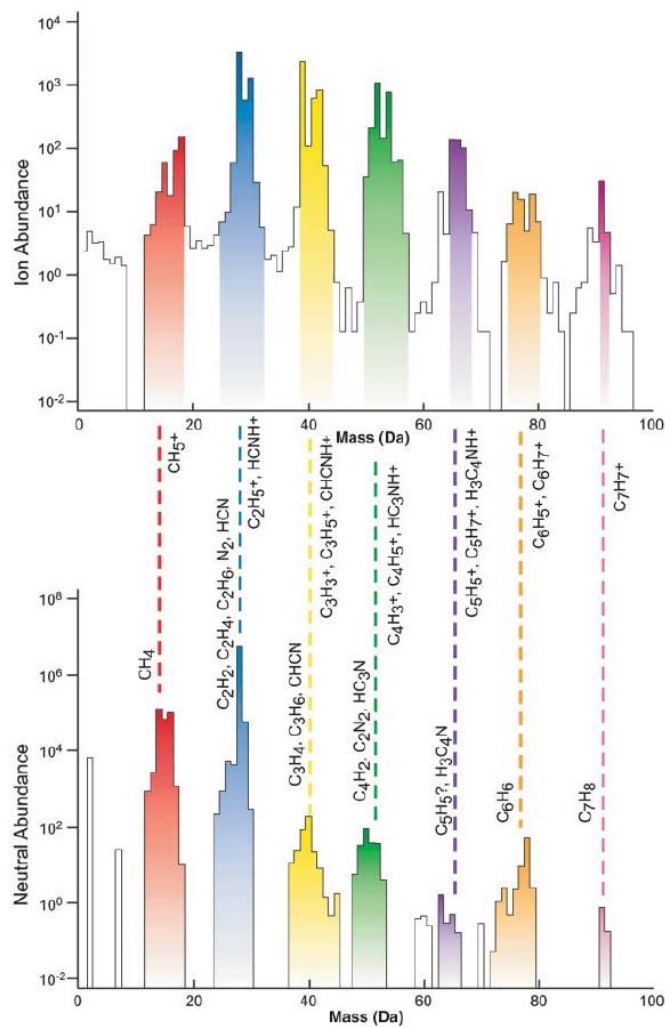


Figure 2 : Spectre d'ions obtenu par INMS lors de son survol de Titan (en haut), spectre des neutres (en bas). Image reprise de la publication de Waite, *et al.* 2007.

La comparaison entre les deux spectres a permis de montrer un lien direct entre l'abondance des espèces neutres et de leurs homologues ionisés. La présence d'azote dans les structures a aussi été hypothétisée mais la précision de l'instrument n'a pas permis de le vérifier dû au manque de résolution. Les pics majoritaires ont été observés tous les 12 Da, montrant l'importance du carbone dans la chimie atmosphérique ([Cravens, et al. 2006 \[11\]](#)). Ainsi INMS a montré que, contrairement à ce qui était supposé, la chimie des ions dans l'ionosphère était très efficace pour générer des ions de masse élevée.

Limitée par les capacités instrumentales d'INMS, l'attribution des espèces détectées a nécessité des études approfondies passant par la création de modèles couplés de chimie ion-neutre et notamment celui réalisé par Vuitton *et al.*, ([Vuitton, et al. 2006 \[12\]](#)). Dans ce modèle, l'attribution des pics d'INMS a été faite en étudiant, notamment, les affinités protoniques des différentes possibilités de combinaisons d'atomes. Par exemple pour m/z 18, deux ions possibles pourraient être attribués : NH_4^+ et/ou H_2O^+ . Cependant, NH_4^+ est privilégié car NH_3 a une affinité protonique très importante. De plus, H_2O^+ est moins probable car l'autre ion issu de l'eau, H_3O^+ , est absent du spectre d'INMS.

De même, les mesures ioniques d'INMS ont confirmé la présence de l'ion méthanimine protonné à m/z 30, CH_2NH_2^+ , ce qui a permis de prédire la présence de méthanimine neutre CH_2NH dans l'ionosphère de Titan de par son affinité protonique importante.

Cette étude a permis d'identifier les espèces détectées dans la gamme de masse 0 – 60 Da et aussi de montrer que la densité d'ions $[\text{M}+\text{H}]^+$ dans la haute atmosphère est directement liée à la densité de leurs molécules neutres. Une seconde étude a permis d'élargir la gamme étudiée, allant jusqu'à m/z 100 ([Vuitton, et al. 2007 \[13\]](#)).

Par la suite, ce modèle a permis de comprendre la formation d'espèces organiques de plus en plus complexes notamment grâce à l'étude de la photochimie du benzène et de sa radicalisation en $C_6H_5^+$ qui va venir se recombinaison avec d'autres espèces neutres ([Waite, et al. 2007 \[9\]](#), [Vuitton, et al. 2008 \[14\]](#), [Vuitton, et al. 2019 \[15\]](#)).

Dans un même temps, l'instrument CAPS a fait la mesure des ions positifs et négatifs de l'ionosphère de Titan avec une plus grande gamme de masse, allant de 0 à 10 000 Da ([Waite, et al. 2007 \[9\]](#), [Westlake, et al. 2014 \[16\]](#), [Vuitton, et al. 2009 \[17\]](#)). La **Figure 3** présente les spectres positifs et négatifs obtenus. Le spectre positif a permis de confirmer les résultats obtenus par INMS en montrant un continuum d'espèces allant jusqu'à 350 Da. Le spectre négatif permis d'observer la présence de très grosses espèces chargées négativement allant jusqu'à 10 000 Da. À cette masse, les espèces sont déjà assez grosses pour s'agréger et former des nanoparticules. Cette observation a montré que la formation des aérosols présents dans la stratosphère débutait grâce à la chimie couplée ions-neutres très efficace qui règne dans l'ionosphère (cf **Figure 1**).

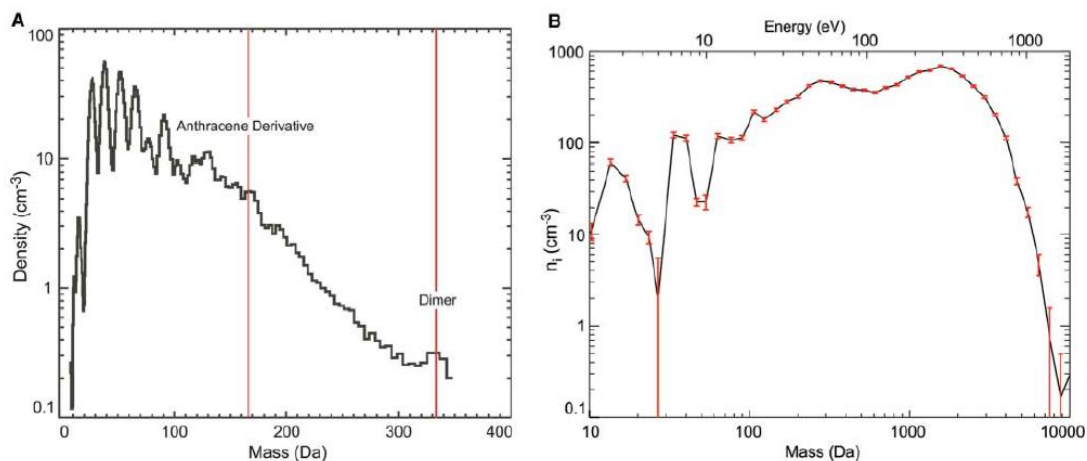


Figure 3 : (gauche) Spectre d'ion positif obtenu par CAPS lors de son survol de Titan, (droite) spectre d'ions négatif. Image reprise de la publication de Waite, *et al.* 2007.

Ce premier survol a aussi permis de préparer l'atterrissage de la sonde Huygens lors d'un prochain passage.

Après une rotation autour de Saturne, un deuxième survol de Titan est effectué le 16 Décembre 2004. La stratosphère est ainsi sondée et sa température, ses vents et sa composition étudiés grâce à l'instrument CIRS ([Flasar, et al. 2005 \[18\]](#)) en Infrarouge. Cette couche est située entre 40 et 310 km d'altitude (voir **Figure 1**). Elle est assez dense, avec des pressions diminuant de 200 mBar jusqu'à 0.1 mBar et abrite aussi les principales couches de brumes d'aérosols.

CIRS a ainsi permis de réajuster l'altitude de la stratopause à 310 kilomètres et sa température à 186 Kelvin. De forts vents ont été mesurés dans l'hémisphère nord de la lune avec des vitesses allant jusqu'à 570 km/h dans la stratosphère. La composition molaire en méthane et en CO₂ a été mesurée à $1,6 \pm 0,5 \times 10^{-2}$ et $4,5 \pm 1,5 \times 10^{-5}$ respectivement. De plus, cet instrument a aussi permis d'identifier des fonction -CH dans les aérosols grâce à la détection de bande d'élongation en infrarouge à 1320, 1380 et 1450 cm⁻¹ ([Vinatier, et al. 2012 \[19\]](#)). Le spectre infrarouge correspondant est donné en **Figure 4**.

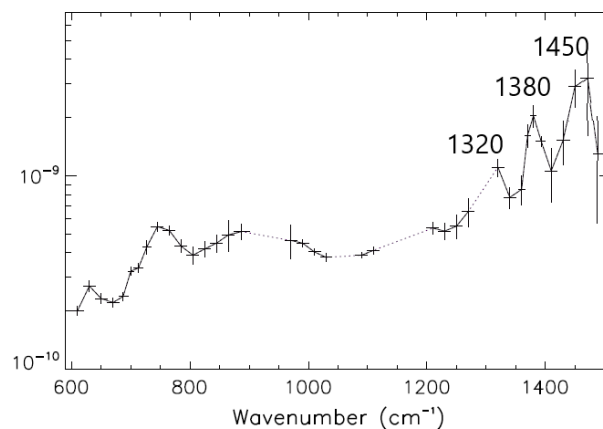


Figure 4 : Spectre infrarouge de l'atmosphère de Titan reconstruit à partir des données de CIRS. Image récupérée de l'article de Vinatier *et al.*, 2012.

Les nuages sur Titan ont été caractérisés grâce à l'instrument VIMS ([Griffith, et al. 2005 \[20\]](#)). Cette étude s'est intéressée à l'analyse des longueurs d'onde allant de 2 à 2.5 μ m, ce qui correspond à des altitudes allant de 10 à 100 km. Il a été observé que les nuages apparaissaient puis disparaissaient sur des périodes courtes de quelques heures. Il a été remarqué que leur disparition était liée aux précipitations. VIMS a aussi permis de confirmer les observations de CIRS concernant la présence de fonction -CH dans les aérosols de Titan.

L'instrument UVIS a permis de mesurer l'abondance de six espèces organiques présentes entre 450 et 1600 kilomètres d'altitude ([Shemansky, et al. 2005 \[21\]](#)) : Le méthane, l'acétylène, l'éthylène, l'éthane, le diacétylène et le cyanure d'hydrogènes. Cela a permis de mieux comprendre la répartition de ces éléments dans une couche d'altitude étendue et continue de l'atmosphère de Titan, recouvrant les mesures *in situ* à haute altitude des instruments INMS et CAPS (>1000 km) et les mesures spectroscopiques IR de CIRS dans la stratosphère (< 500 km).

Enfin, une fois les derniers préparatifs effectués, l'atterrisseur Huygens se détache de son orbiteur le 25 Décembre 2004. Lors de sa descente qui dura 22 jours, la sonde a réalisé de nombreuses mesures. Elle a confirmé les premières données obtenues par Cassini concernant les vents présents dans la stratosphère ([Bird, et al. 2005 \[22\]](#), [Tomasko, et al. 2005 \[23\]](#)) ainsi que les différentes mesures physiques proposées précédemment ([Fulchignoni, et al. 2005 \[3\]](#)).

Lors de la descente de la sonde, l'instrument GCMS a mis en évidence les variations des fractions molaires des constituants présents dans l'atmosphère et notamment le méthane. Il a été découvert que la quantité relative de méthane présente dans l'atmosphère augmente au fur et à mesure que l'altitude diminue : de 1.41% à 5% entre 140 km d'altitude et 10 km d'altitude. Cette observation est reportée par la **Figure 5**.

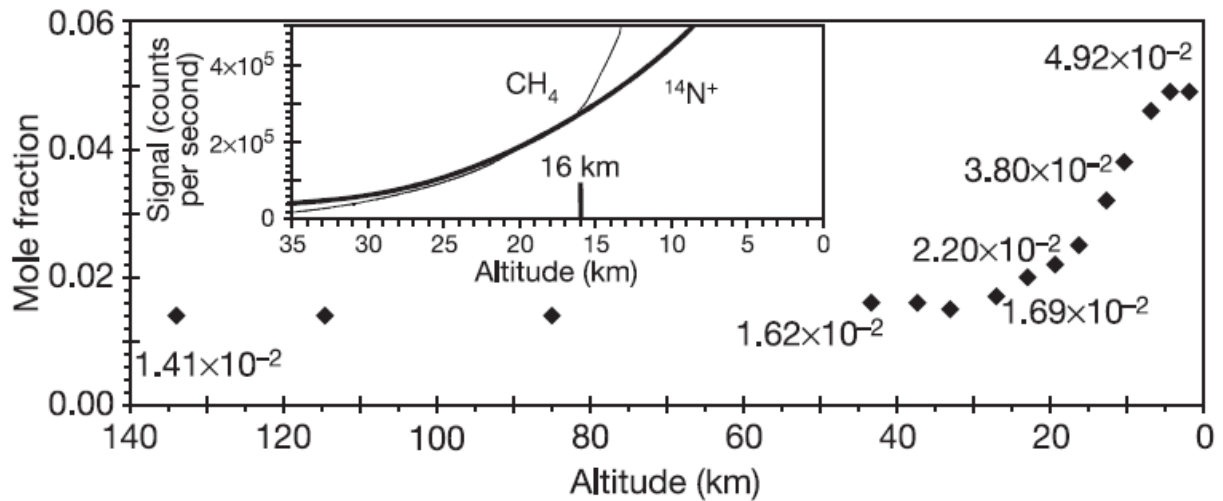


Figure 5 : Analyse du rapport méthane sur azote en fonction de l'altitude lors de la descente de Huygens. Image provenant de ([Niemann, et al. 2005 \[2\]](#)).

Dans le même temps, ACP a effectué la pyrolyse de deux échantillons d'aérosols. Cet instrument a identifié trois résidus principaux : HCN, NH₃ et C₂H₂, ce qui a permis de conclure sur la présence d'azote dans les molécules organiques composants les aérosols.

De plus, toute la descente a été imagée et une photo de la surface du satellite obtenue ([Lebreton, et al. 2005 \[24\]](#)). Sur cette incroyable image reportée en **Figure 6** nous pouvons voir, dans un premier temps, le brouillard de particules présent dans l'atmosphère qui donne cette couleur orangée au ciel de Titan. De plus, une couche de matière organique recouvre la totalité de son sol.

Des cailloux sont aussi visibles. Ils furent beaucoup discutés car leur forme est ronde, ce qui signifie qu'ils ont subi de l'érosion provenant vraisemblablement de rivières qui ont disparu avec le temps.



Figure 6 : Surface de Titan prise par la sonde Huygens. (Crédit : NASA)

Toutes ces informations récupérées par Cassini-Huygens ont permis de faire avancer de façon drastique nos connaissances de Titan. L'action combiné d'INMS et de CAPS a montré que la formation des aérosols commençait à une altitude bien plus haute que celle supposée précédemment (> 1000 km) grâce à une chimie ions-neutres efficace qui forme les premiers ions positifs et négatifs lourds ($m/z > 50$) (Waite et al. 2007)[43]. Ces petites espèces croissent ensuite grâce à des réactions cations – anions jusqu'à devenir des nanoparticules puis, plus tard, des aérosols présents dans la stratosphère (Lavvas et al. 2013)[96].

Concernant l'analyse des aérosols organiques, leur étude fut cependant très limitée étant donné les limitations techniques des instruments embarqués. En effet, la capacité résolutive de GCMS et d'ACP n'a pas permis de déconvoluer les espèces de ce mélange.

Mais, le potentiel prébiotique de ces aérosols a été mis en lumière. En effet, ces deux instruments ont confirmé que les aérosols étaient entièrement organiques. De plus, ACP a montré la présence de résidus de pyrolyse contenant de l'azote et donc, vraisemblablement, des fonctions cyanides et nitriles qui sont de très bons précurseurs d'acides aminés.

C'est grâce à ces découvertes et aux nouvelles questions qui se sont posées que des simulations de l'atmosphère de Titan ont vu le jour sur Terre dans le but de mieux comprendre le satellite glacé.

Partie 2 : Les simulations en laboratoire de l'atmosphère de Titan

La découverte et la première analyse des aérosols présents dans l'atmosphère de Titan ont suscité beaucoup d'intérêt chez les planétologues. Cependant, les analyses chimiques des aérosols furent limitées à cause des contraintes techniques des instruments embarqués. Afin de passer outre cette barrière, plusieurs équipes ont réalisé des reproductions de l'atmosphère de Titan afin de générer artificiellement des aérosols, analogues de ceux présents dans l'atmosphère du satellite.

2.1. Historique

Afin de mieux comprendre ce genre d'expériences, il est important d'en faire un historique. Il faut, dans un premier temps, énoncer la première expérience de ce genre, celle de Stanley L. Miller réalisée en 1953 ([Miller 1953 \[25\]](#)). Cette expérience est schématisée par la **Figure 7**.

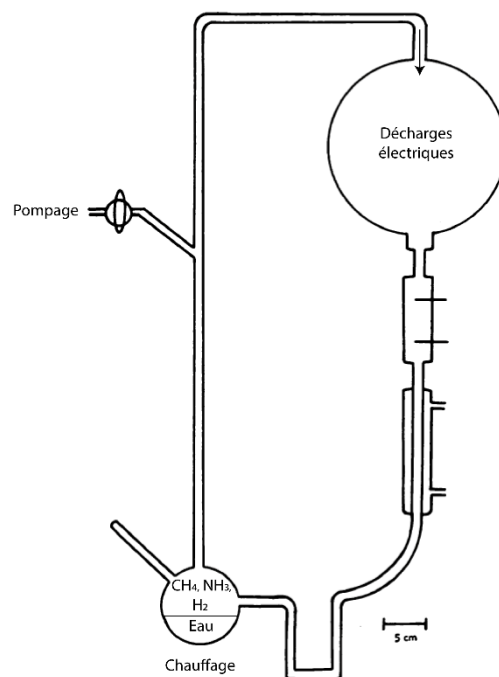


Figure 7 : Schéma de l'expérience de Miller ([Miller 1953 \[25\]](#)).

Miller eut dans l'idée de démontrer la formation des premières espèces organiques à l'origine de la vie sur Terre en laboratoire (e.g. acides aminés). Pour cela, il créa une enceinte contenant du méthane, de l'ammoniac, de l'eau ainsi que de l'hydrogène dans laquelle des décharges électriques sont appliquées afin de simuler l'effet de l'orage dans l'atmosphère de la Terre primitive. Cette enceinte est connectée à un ballon rempli d'eau simulant le couplage de l'atmosphère avec l'océan. Après plusieurs jours, il observa une coloration rose dans l'eau de son enceinte, cette couleur était due à la présence d'acides aminés.

Cette première expérience a ensuite été reprise en 1973 par G. Toupance *et al* ([Toupance, et al. 1975 \[26\]](#)), qui a réalisé un système en flux couplé à une chromatographie en phase gazeuse, de l'infrarouge et des analyses chimiques. Ils ont identifié la formation d'hydrocarbures, des cyanures ainsi que des nitriles.

2.2. Titan

Quelques années plus tard, Bishun Khare et Carl Sagan, ont repris pour la première fois ces méthodes de reproduction afin de simuler l'atmosphère de Titan ([Khare, et al. 1981 \[27\]](#)). En se basant sur les observations faites par Voyager 1, ils ont produit des aérosols à partir d'un mélange N_2/CH_4 ionisé grâce à des décharges électriques. Ces poudres furent nommées *Tholins* (Du grec Tar pour goudron).

Plusieurs équipes se sont mise à produire de différentes manières des Tholins par la suite. Leur objectif était d'être le plus représentatif des conditions réelles régnant sur Titan. Le principal point de différences entre ces expériences est la technique d'apport énergétique utilisée. Cinq méthodes sont aujourd'hui répertoriées ([Cable, et al. 2012 \[28\]](#)).

Les deux premières sont l'ionisation par flux d'électrons ([Gupta, et al. 1981 \[29\]](#)) ainsi que celle par irradiation γ et X ([Ramirez, et al. 2001 \[30\]](#)).

Ces deux méthodes sont peu utilisées car elles sont jugées trop éloignées des conditions sur Titan notamment du fait de l'absorption de ces ondes par l'atmosphère haute et donc de leur incapacité à atteindre l'azote et le méthane.

On retrouve ensuite l'ionisation UV ([Imanaka and Smith 2009 \[31\]](#)) produite grâce à la lumière synchrotron. Cette méthode est la plus représentative en terme d'énergie d'ionisation car c'est la source d'énergie présente sur Titan.

Cependant, ces expériences sont difficiles à mettre en place à cause de l'indisponibilité et le temps de faisceau restreint des équipements synchrotron. Enfin, les deux dernières méthodes sont celles dites de *Hot Plasma* ([Scattergood, et al. 1989 \[32\]](#)) et *Cold Plasma* ([Coll, et al. 1997 \[33\]](#)). Elles sont les deux méthodes les plus utilisées car elles permettent une production d'aérosols 10^4 supérieure à l'ionisation γ et X tout en étant représentative en terme d'énergie d'ionisation.

2.3. L'expérience PAMPRE

L'expérience PAMPRE (Production d'Aérosols en Microgravité par Plasma REactifs) a été développée pour préparer l'analyse des données récoltées par Huygens-ACP sur Titan ([Szopa, et al. 2006 \[34\]](#)).

CAPS et INMS ont montré que la formation des aérosols débutait très haut dans l'ionosphère (>1000 km) grâce aux réactions ions–neutres entre l'azote et le méthane. En effet, les photons UV et les particules chargées provenant de la magnétosphère de Saturne viennent dissocier et ioniser partiellement ces deux gaz. Les radicaux et ions induits se recombinent pour former des espèces de plus en plus lourdes (> 10 000 Da). L'expérience PAMPRE est cohérente avec ces processus de formation d'aérosols par voies ioniques dans la haute atmosphère de Titan.

Cette expérience *Cold Plasma* consiste donc en la formation d'un plasma dans un mélange d'azote et de méthane. Ce plasma permet de reproduire les réactions chimiques observées dans l'ionosphère de Titan. Ainsi, les ions et radicaux issus de N_2 et CH_4 vont se recombinaer et former des molécules de plus en plus grandes jusqu'à former des aérosols au sein du réacteur.

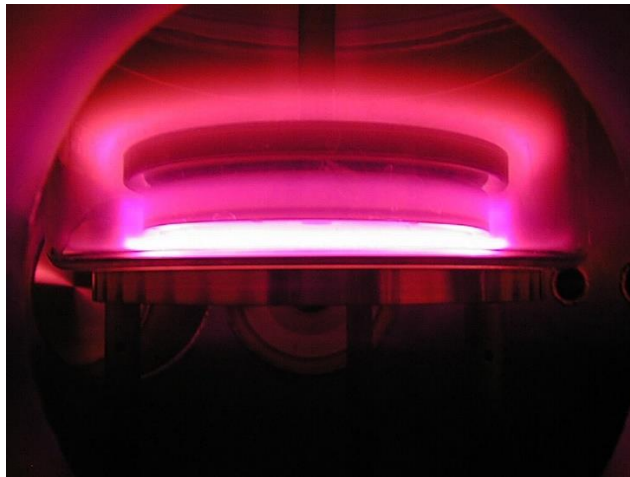


Figure 8 : Vue de l'intérieur du réacteur de l'expérience PAMPRE.

Ces grains d'environ 300 nm de diamètre se chargent électriquement et se maintiennent en suspension dans le réacteur par répulsion électrostatique dans le champ électrique jusqu'à ce que la gravité l'emporte et qu'ils soient expulsés de l'enceinte. Ces poudres peuvent être récoltées après quelques jours par centaines de milligrammes. Ce plasma permet la formation de Tholins en grande quantité et leur croissance en lévitation, sans effets de parois.

Je me suis intéressé dans cette thèse à la caractérisation moléculaire et structurale d'un échantillon produit à partir d'un mélange 95% N_2 et 5% CH_4 . Ce mélange est en effet représentatif de la composition moyenne dans l'ionosphère de Titan.

En effet, l'homopause de Titan se situe à 1200 km d'altitude, ce qui signifie que au-dessus, la diffusion des gaz est responsable d'un transport en altitude dépendant de la masse des espèces ([Waite, et al. 2005 \[10\]](#)). Les proportions de N_2 - CH_4 sont ainsi d'environ de 98-2% en bas de l'ionosphère, et évoluent jusqu'à un maximum de 10% en haut de l'ionosphère par diffusion plus efficace du méthane. La **Figure 8** présente l'intérieur du réacteur, on y voit le plasma rose d'Azote.

Partie 3 : Caractérisation des Tholins sur Terre

Des caractérisations physiques et chimiques des Tholins produits durant les différentes expériences énoncées précédemment ont été réalisées en utilisant plusieurs méthodes. Dans cette partie, je ne citerai que les observations obtenues concernant les caractérisations chimiques et structurales des Tholins.

3.1. Caractérisation chimique

La première caractérisation effectuée sur les Tholins après leur production a été l'analyse élémentaire ([McKay 1996 \[35\]](#), [Coll, et al. 1995 \[36\]](#), [Sciamma-O'Brien, et al. 2010 \[37\]](#), [Sagan and Reid Thompson 1984 \[38\]](#)). Cette analyse permet de mesurer la quantité de chaque atome présent dans un échantillon (Généralement C, H, N, O, S). Les résultats obtenus par quatre différentes équipes sont reportés dans le **Tableau 1**.

Tableau 1 : Composition élémentaire de différents échantillons de Tholins

Reference	Stœchiométrie	Ratio C/N
Sciamma-O'Brien et al (2010)	$C_6H_7N_4$	1.5
Sagan et al (1984)	$C_8H_{13}N_4$	2
McKay et al (1996)	$C_{11}H_{11}N_2$	5.5
Coll et al (1995)	$C_{11}H_{11}N$	11

Des compositions élémentaires différentes ont ainsi été relevées dans les Tholins, avec un ratio carbone sur azote qui varie de 1.5 pour la première à 11 pour la dernière. Ces différences dépendent du montage utilisé, notamment de l'énergie apportée au réacteur pendant l'expérience, mais aussi des conditions expérimentales choisies (par exemple la teneur initiale en méthane injecté).

En effet, si l'énergie n'est pas suffisante, l'azote qui possède des seuils d'énergies pour la dissociation et l'ionisation supérieures à celles du méthane (de l'ordre de 15.58 eV pour N₂ et 12.61 eV pour le méthane pour l'ionisation) ne sera pas ionisé et/ou dissocié et sera par conséquent beaucoup moins incorporé dans les Tholins.

Malgré ces compositions élémentaires très différentes observées entre Tholins, leur analyse en infrarouge a révélé des fonctions chimiques globalement identiques ([Cable, et al. 2012 \[28\]](#)). Par exemple, des fonctions nitriles ont été observées dans toutes les expériences précédemment citées ([Carrasco, et al. 2009 \[39\]](#), [Neish, et al. 2008 \[40\]](#), [Navarro-Gonzalez, et al. 2001 \[41\]](#), [Khare, et al. 2002 \[42\]](#)). Il en est de même pour les fonctions alcènes et alcynes ainsi que pour les cycles aromatiques. Ces trois fonctions avaient été identifiées sur Titan grâce à Cassini-Huygens. Dans le détail, certaines fonctions n'ont été identifiées que dans certaines expériences, comme les cycles contenant de l'azote (pyridine, pyrrole...) ([Carrasco, et al. 2009 \[39\]](#), [Coll, et al. 2001 \[43\]](#)). Mais la faible sélectivité en infrarouge n'a pas permis d'approfondir l'analyse chimique des Tholins et la comparaison entre les différentes expériences s'est avérée difficile.

C'est donc naturellement, avec l'apparition de la spectrométrie de masse à ultra haute résolution, que plusieurs études ont été réalisées en Orbitrap® ([Bonnet, et al. 2013 \[44\]](#), [Pernot, et al. 2010 \[45\]](#), [Vuitton, et al. 2010 \[46\]](#)) et en FTICR ([Anicich, et al. 2006 \[47\]](#), [Somogyi, et al. 2005 \[48\]](#), [Somogyi, et al. 2012 \[49\]](#), [Somogyi, et al. 2016 \[50\]](#)) afin de sonder plus profondément la composition chimique des Tholins. La spectrométrie de masse sera introduite en détail dans le **chapitre II**.

La **Figure 9** présente un spectre de masse obtenu par Pernot *et al.* lors de l'analyse d'un échantillon de Tholins à 10% de méthane injecté. Un mélange complexe a été révélé avec plus de 2500 signaux attribués. Des vagues dans le spectre laissent également supposer une structure polymérique des Tholins. Ces vagues et cette complexité ont aussi été observées par d'autres équipes ([Vuitton, et al. 2010 \[46\]](#) ,[Somogyi, et al. 2005 \[48\]](#)).

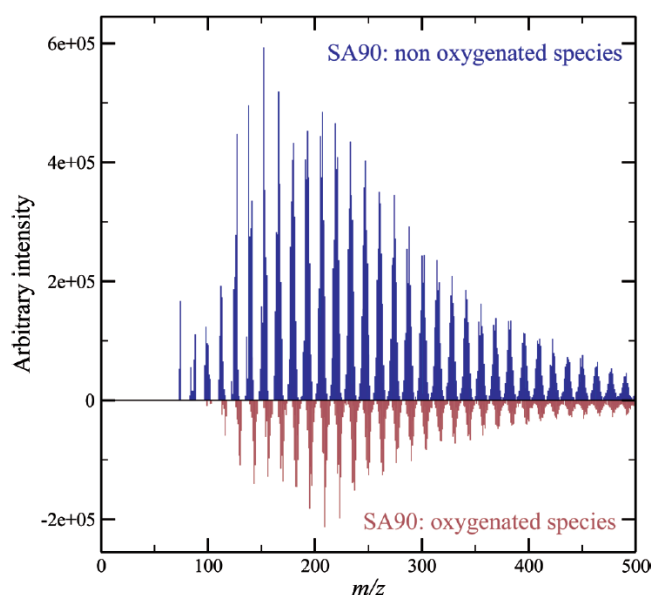


Figure 9 : Spectre de masse Orbitrap[®] d'échantillon de Tholins ([Pernot, et al. 2010 \[45\]](#))

Le diagramme de van Krevelen (présenté dans le **chapitre II**) a aussi été beaucoup utilisé pour améliorer la compréhension des spectres de masse. Un exemple est donné en **Figure 10**. Dans cet exemple, une analyse en mode positif et une analyse en mode négatif ont été superposées. On peut voir que les espèces forment un continuum dans ce graphique, et que les espèces négatives sont plus insaturées que les espèces positives, ce qui pourrait être cohérent avec la présence de multiples fonctions cyanides identifiées précédemment en infrarouge.

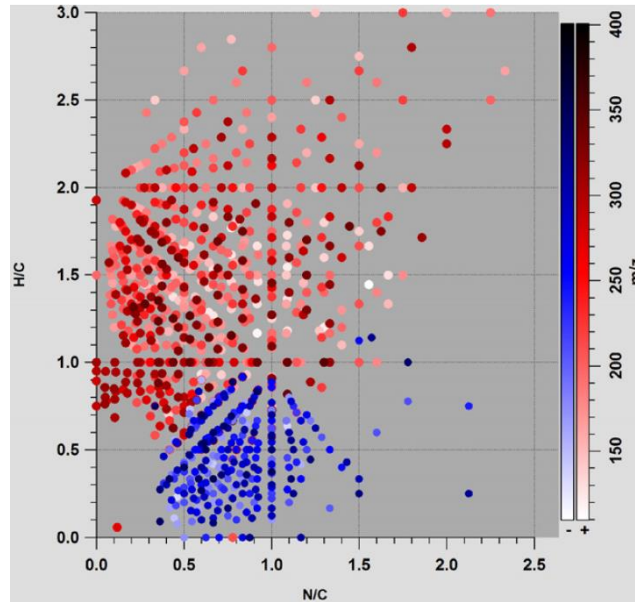


Figure 10 : Diagramme de van Krevelen modifié d'échantillon de Tholins. (Rouge) ions positifs, (bleu) ions négatifs ([Somogyi, et al. 2012 \[49\]](#)).

Il a été aussi observé que certains Tholins, comme ceux produits par l'expérience PAMPRE sont majoritairement insolubles peu importe le solvant. Or toutes les études de spectrométrie de masse à haute résolution sur les Tholins ont été réalisées uniquement sur la fraction soluble. Je me suis donc intéressé à caractériser directement la fraction insoluble. Ce travail sera présenté dans le **chapitre III**. Dans ce chapitre, je présenterai aussi une étude concernant l'impact de la présence d'oxygène sur la composition des Tholins. En effet, ces échantillons une fois oxydés deviennent des analogues que nous avons considérés comme représentatifs de ceux formés sur la Terre primitive. Leur interaction avec l'oxygène libéré lors de l'apparition de la vie représente un fort intérêt prébiotique ([Arney, et al. 2016 \[51\]](#) ,[Haqq-Misra, et al. 2008 \[52\]](#) ,[Trainer, et al. 2006 \[53\]](#) ,[Trainer, et al. 2004 \[54\]](#) ,[Kasting 1993 \[55\]](#) ,[Urey 1952 \[56\]](#)).

3.2. Formation des Tholins par polymérisation ?

En 2006, Matthews *et al.* se sont intéressés au polymère d'HCN en tant que bon candidat pour résoudre la structure inconnue des Tholins ainsi que la matière organique cosmique en générale ([Matthews and Minard 2006 \[57\]](#)). Ils ont montré que la couleur du poly-HCN était la même que celle des Tholins et que la comparaison des spectres infrarouges des deux matériaux ne montrait que peu de différence. Vuitton *et al.* ont par la suite comparé par infrarouge et spectrométrie de masse ce polymère de HCN avec un échantillon de Tholins de l'expérience PAMPRE ([Vuitton, et al. 2010 \[46\]](#)). Ils en ont conclu que les Tholins sont beaucoup plus complexes qu'un simple polymère d'HCN. Ce propos est illustré par les spectres de masse reportés **Figure 11**.

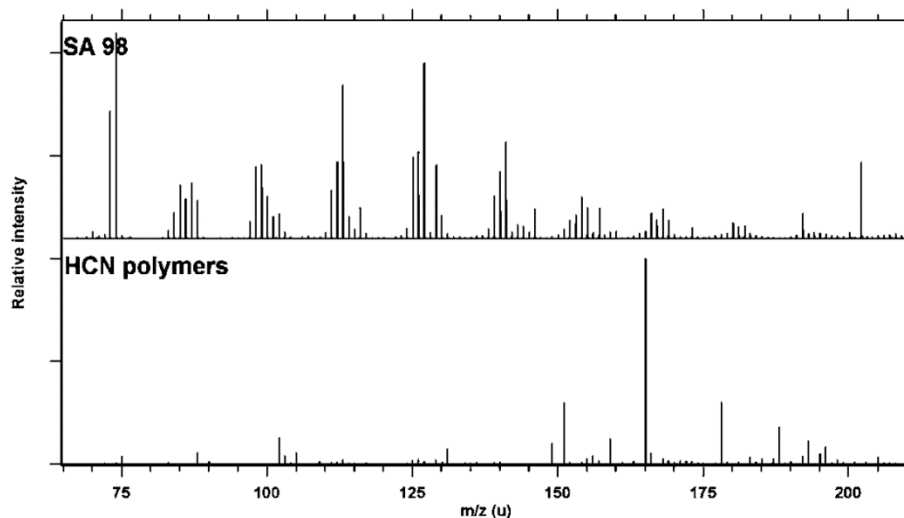


Figure 11 : Comparaison entre un échantillon de Tholins (haut) et un polymère d'HCN (bas) ([Vuitton, et al. 2010 \[46\]](#))

Par la suite, Pernot *et al.* ont mis en évidence des motifs de répétition présents dans les analogues grâce à l'utilisation de diagrammes de Kendrick et de van Krevelen. Un de leur diagramme de Kendrick est reporté en **Figure 12**.

Sur ce type de diagramme, les masses détectées sont normalisées suivant un motif précis (généralement CH₂) afin d'observer la présence ou non de motifs de répétition. Lorsque le motif défini est celui retrouvé principalement dans l'échantillon, le diagramme de Kendrick est centré autour de 0 sur l'axe des ordonnées. Dans l'exemple donné, trois motifs ont été choisis : CH₂, HCN et CH₂-HCN. Avec les motifs CH₂ et HCN les graphiques ne sont pas centrés autour de 0 alors que le motif C₂H₃N l'est. Ils ont pu ainsi proposer un motif polymérique plus complexe que le poly-HCN et défini par la stœchiométrie (CH₂)_n-(HCN)_m.

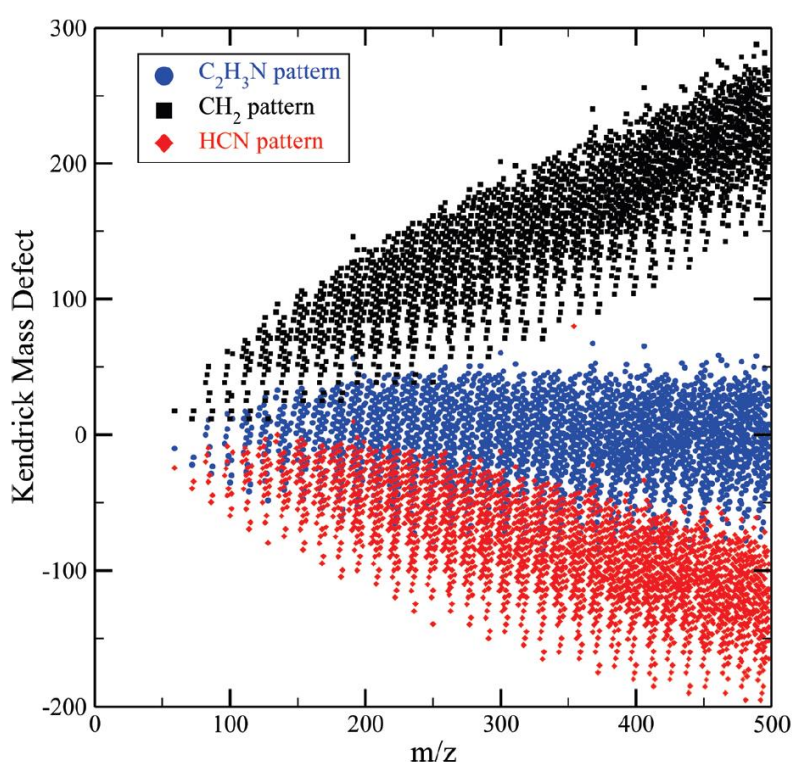


Figure 12 : Diagramme de Kendrick d'un échantillon de Tholins suivant plusieurs normalisations ([Pernot, et al. 2010 \[45\]](#)).

Afin d'étudier plus en profondeur ces supposés polymères, quelques études en spectrométrie de masse en tandem ont pu être réalisées ([Somogyi, et al. 2005 \[48\]](#), [Somogyi, et al. 2012 \[49\]](#)).

La fragmentation de petits ions ($< m/z$ 200) a montré la présence de motifs nitriles et cyanides dans ces structures, en accord avec la spectroscopie IR. Cependant, la piste d'une structure polymérique reste une hypothèse encore aujourd'hui.

Dans le but d'avancer sur ce point, j'ai mené un projet sur la fragmentation des Tholins, qui est reporté dans le **chapitre IV**.

3.3. Structure développée

Le but recherché par l'analyse de ces Tholins reste sans aucun doute l'obtention des formules développées des molécules détectées dans ce mélange. En effet, cela permettrait de mieux comprendre la réactivité de ces aérosols ainsi que leurs propriétés physiques. Du fait de la complexité des Tholins, étudier les structures développées dans leur globalité est un problème qui s'avère non-résolu aujourd'hui. Plusieurs équipes ont tenté d'obtenir des informations sur les fonctions chimiques composant la structure des Tholins, notamment grâce à des études en infrarouge ([Carrasco, et al. 2009 \[39\]](#), [Gautier, et al. 2012 \[58\]](#), [Tran, et al. 2003 \[59\]](#)). Ces études ont montré la présence de nombreuses fonctions chimiques, comme des nitriles, des imines ou encore des cyanides mais l'agencement de ces fonctions entre elles n'est pas connu. Dans le même temps, des études en Résonance Magnétique Nucléaire ont permis de confirmer la présence de petites molécules ([He and Smith 2015 \[60\]](#), [He and Smith 2014 \[61\]](#), [He and Smith 2014 \[62\]](#), [He, et al. 2012 \[63\]](#)).

Des petites molécules ont aussi été identifiées grâce à des analyses en chromatographie liquide haute performance ([Gautier, et al. 2016 \[64\]](#)). Quatre exemples sont donnés en **Figure 13**. Des triazines ont été identifiées ainsi que des triazoles, de la cyanoguanidine et de la mélamine. Certaines de ces petites molécules avaient déjà été identifiées en RMN par He *et al* et possèdent de forts pouvoirs de polymérisation. Cela a permis d'émettre l'hypothèse que ces molécules sont des résidus de précurseurs de polymérisation et que l'azote joue un rôle majeur dans la production de ces analogues d'aérosols.

Cependant, cette hypothèse reste impossible à vérifier et la structure globale reste aujourd'hui inconnue. Je me suis donc également penché sur cette question pendant ma thèse et les résultats obtenus sont reportés dans le **chapitre V**.

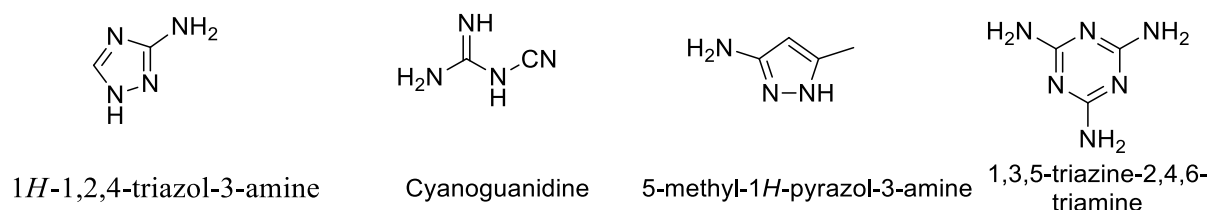


Figure 13 : Exemple de molécules identifiées en HPLC-MS ([Gautier, et al. 2016 \[64\]](#)).

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Chapitre 2

Matériels et méthodes

Dans ce second chapitre, j'introduirai les différents instruments et méthodes utilisés durant cette thèse. La première partie constituera une introduction à la spectrométrie de masse. Le principe général de cette technique d'analyse sera énoncé, suivi de la présentation des différentes sources d'ionisation utilisées durant cette thèse. Ensuite, trois analyseurs seront définis : Le quadripole, le temps de vol et l'analyseur à résonance cyclotronique des ions.

Une seconde partie s'intéressera à la spectrométrie de mobilité ionique et à son couplage avec la spectrométrie de masse. Cette technique, utilisée pendant la thèse, sera présentée en détail ainsi que les instruments qui en sont équipés. Pour cela, trois types de cellule de mobilité ionique seront décrites : le Drift Tube (DTIMS), le Traveling Waves (TWIMS) et le Trapped Ion (TIMS).

Enfin, une dernière partie présentera le traitement de données utilisé dans les parties résultats. Il y sera décrit notamment les étapes d'étalonnage, d'attribution de formules brutes, le diagramme de van Krevelen, le DBE vs #C ainsi qu'un graphique de mobilité ionique.

Sommaire

[Partie 1 : Spectrométrie de masse](#)

[Partie 2 : Spectrométrie de mobilité ionique](#)

[Partie 3 : Traitement de données](#)

Partie 1 : Spectrométrie de masse

1.1 Principe

La spectrométrie de masse (MS) est basée sur la séparation de molécules ionisées en fonction de leur rapport masse sur charge (m/z). Le premier spectromètre de masse a été construit en 1912 par Sir J.J Thomson. Elle est aujourd'hui une technique analytique ubiquitaire, utilisée dans un très grand nombre de domaines notamment grâce à ses nombreux avantages. En opposition aux autres analyses comme l'infrarouge, l'UV ou la fluorescence, c'est une analyse très sélective et universelle, car toutes les molécules ont une masse et peuvent ainsi être séparées. La masse d'une molécule est aussi une propriété intrinsèque et est très facilement prédictible à partir de la formule brute. De même lorsque qu'une masse est mesurée suffisamment précisément, il est possible de remonter à la formule brute de la molécule analysée. De plus, cette analyse est très rapide (de quelques centaines de microsecondes à quelques secondes) mais aussi très sensible et sélective. En effet, des concentrations quelques zeptomoles en analyte ont pu être détectés (10^{-20} moles) ([Belov, et al. 2001 \[1\]](#)). On la retrouve ainsi dans tous les domaines que ce soit dans la pétrochimie ([Rodgers and McKenna 2011 \[2\]](#)), l'agroalimentaire ([Cody, et al. 2005 \[3\]](#)), l'analyse environnemental ([Hertzog, et al. 2018 \[4\]](#)), le biomédical, l'imagerie médicale ([Caprioli, et al. 1997 \[5\]](#)) ou embarquée dans des sondes pour l'étude spatiale ([Israel, et al. 2005 \[6\]](#)). La spectrométrie de masse peut être également vue comme une méthode séparative permettant d'avoir une grande sélectivité et ainsi permettre l'analyse de mélanges complexes et/ou la détection d'analytes à l'état de trace dans un milieu complexe.

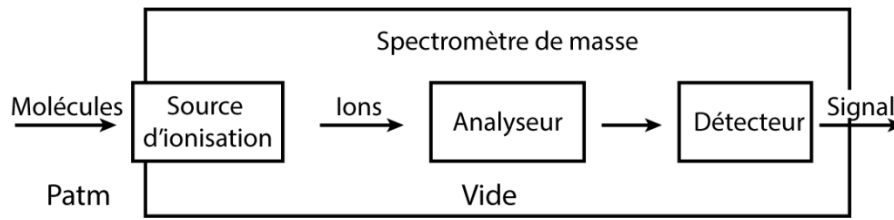


Figure 14 : Schéma simplifié d'un spectromètre de masse

Un spectromètre de masse est toujours composé de trois parties indissociables. Afin d'illustrer cela, la **Figure 14** présente un schéma simplifié d'un instrument. La première partie est l'ionisation, qui permet de charger positivement ou négativement les analytes qui sont ensuite guidés à travers l'instrument par des champs électriques et/ou magnétiques (Voir **section 1.2**).

Dans cette partie seront présentés les principes des sources d'ionisation *electrospray*, de photoionisation à pression atmosphérique et enfin d'ionisation par désorption laser.

La deuxième partie est opérée par l'analyseur et permet de séparer les ions en fonction de leur rapport masse sur charge (m/z). Plusieurs types d'analyseurs sont présentés en **section 1.3**. Dans cette partie sera énoncé le principe du quadripole, de l'analyseur temps de vol et enfin de l'analyse par résonance cyclotronique des ions. Enfin, la dernière partie d'un spectromètre de masse est le détecteur qui va collecter les ions précédemment triés et générer un courant électrique.

1.2. Sources d'ionisation

Dans cette partie, nous nous intéresserons à la première opération systématiquement effectuée en spectrométrie de masse, l'ionisation. De nombreuses sources existent, et chacune d'elles permet une ionisation différente à partir d'échantillon sous forme gazeuse, en solution ou solide. On retrouve ainsi, des ionisations où l'échantillon est introduit en solution tel que l'*electrospray* (ESI) présentée en **section 1.2.1**, l'*atmospheric pressure photoionization* (APPI) présentée en **section 1.2.2** et l'*atmospheric pressure chemical ionization* (APCI). En phase solide on retrouve la source *laser desorption ionization* (LDI ou LI) présenté en **section 1.2.3**, ainsi que la source type *direct introduction probe* ou *atmospheric solid analysis probe* ([McEwen, et al. 2005 \[7\]](#)). Enfin en phase gazeuse on retrouve des sources comme l'ionisation électronique (EI) et l'ionisation chimique (CI). La liste précédente n'est pas exhaustive car il existe de nombreuses sources adaptées des problématiques spécifiques. Le choix de la source d'ionisation dépend en particulier des propriétés physico-chimiques des molécules et du type d'échantillon à étudier. Pour illustrer ces propos, la **Figure 15** met en lumière la sélectivité d'ionisation de chaque source en fonction de la polarité de l'analyte ainsi que de son poids moléculaire.

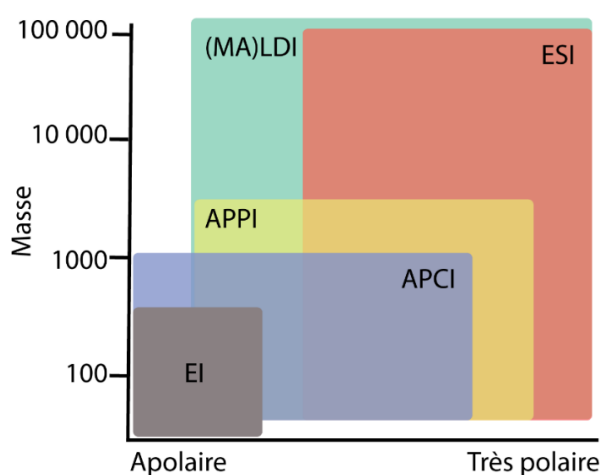


Figure 15 : Capacité d'ionisation des différentes sources citées précédemment en fonction de la masse et de la polarité de l'analyte

1.2.1. Ionisation par electrospray

Cette méthode d'ionisation a été développée dans les années 1980 par John Fenn ([Fenn, et al. 1989 \[8\]](#), [Fenn 1984 \[9\]](#)). Elle ne s'opère qu'en solution dans un solvant le plus souvent protique. Son principe repose sur la production d'ion grâce à l'imposition d'une forte différence de potentiel (de l'ordre de plusieurs kV) entre le capillaire métallique d'introduction d'échantillon et l'entrée du spectromètre de masse. Ce processus peut être assisté par un flux d'azote, on appelle alors cet ensemble (capillaire plus flux d'azote) un nébuliseur ou *sprayer* (voir **Figure 16**). Ce processus conduit à un jet de gouttelettes chargées dont la taille diminue au fur et à mesure que le solvant s'évapore entraînant une augmentation de la répulsion entre les charges jusqu'à atteindre la limite de Rayleigh. Cela conduit à l'explosion coulombienne des gouttelettes. Ces étapes d'évaporation et d'explosions coulombiennes se poursuivent jusqu'à l'obtention d'ions en phase gazeuse essentiellement libres de solvant.

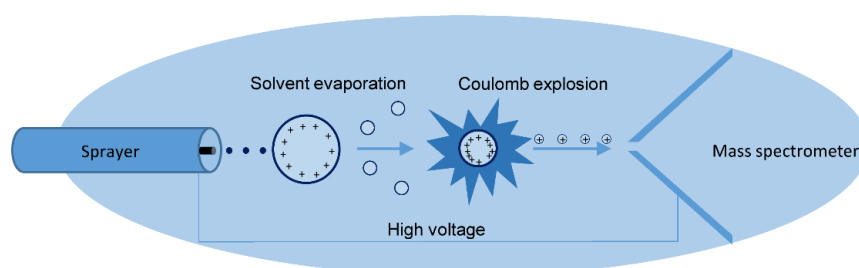


Figure 16 : Schéma de principe de l'ionisation *electrospray*

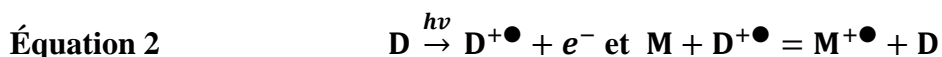
En mode positif, cette ionisation douce permet généralement l'observation d'ions à nombre pair d'électrons intacts qui peuvent être multichargés. Ainsi on observe des molécules protonées $[M+H]^+$ ou multipronées $[M+nH]^{n+}$ pour le mode positif et des molécules déprotonées $[M-H]^-$ et multidéprotonées pour le mode négatif. Des ions adduits tels que $[M+Na]^+$ ou $[M+Cl]^+$ et des formes multimériques telles $[2M+H]^+$ peuvent être également obtenus.

Cela lui vaut d'être une source de choix pour l'analyse des macromolécules polaires comme les protéines mais également pour des petites molécules polaires.

Cependant, cette source implique d'avoir des échantillons en solution et ne permet donc pas l'analyse de molécules insolubles. Cette source est très sélective car elle ne permet que l'ionisation des molécules les plus polaires. Ainsi dans un produit pétrolier en mode positif les molécules présentant des azotes basiques (pyridine, quinoline...) sont ionisées de façon préférentielles même si elles sont présentes de façon minoritaires ([Le Maître, et al. 2019 \[10\]](#)).

1.2.2. Atmospheric pressure photoionisation

La source à photoionisation sous pression atmosphérique (APPI) est une source permettant l'ionisation à partir d'échantillon en solution. Son principe est basé sur l'ionisation de molécules sous forme $M^{+\bullet}$ grâce à une lumière émettant des photons VUV. Généralement, une lampe à décharge de Krypton émettant à 10.6 eV est utilisé ([Robb, et al. 2000 \[11\]](#)). Cette énergie d'ionisation est idéale car elle est suffisante pour ioniser la plupart des molécules organiques et insuffisante pour ioniser la plupart des solvants. Cette photoionisation permet la génération de cations radicaux $M^{+\bullet}$ suivant l'Équation 1.



Pour améliorer l'efficacité du processus d'ionisation il est également possible d'ajouter un dopant pour de faciliter le processus d'ionisation. Ce dernier est le plus souvent du toluène car il possède une énergie d'ionisation de l'ordre de 8.82 eV. Le toluène en grande quantité s'ionise donc en premier, et vient ensuite faire du transfert de charge avec l'échantillon. L'équation d'ionisation peut ainsi être définie par l'**Équation 2**.

Cette source est peu sélective car elle permet d'ioniser toutes les molécules contenant des doubles liaisons comme les espèces aromatiques, mais aussi les alcènes, alcynes... Il est cependant possible d'augmenter sa sélectivité en changeant de lampe UV. On peut par exemple utiliser une lampe à décharge de plasma hydrogène (H₂) émettant à 4.9 eV, cela permettra d'ioniser préférentiellement les espèces aromatiques d'un mélange.

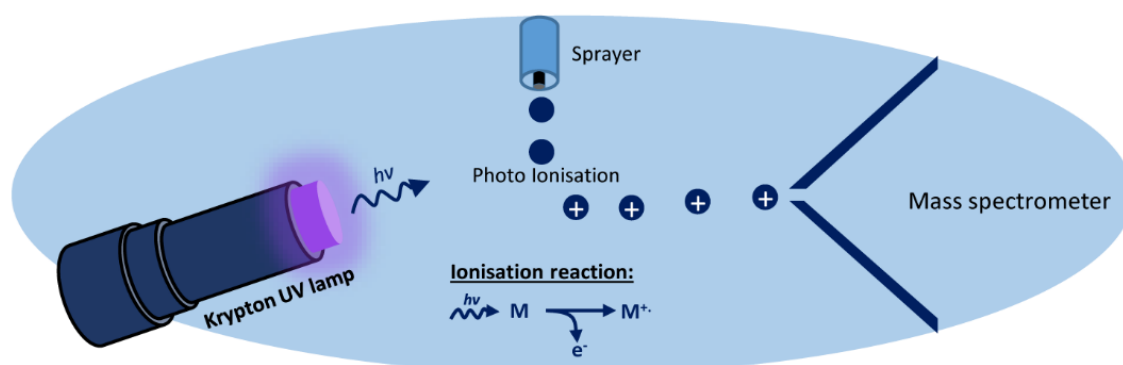


Figure 17 : Schéma de principe de la source à photoionisation

1.2.3. Laser Désorption Ionisation

La source à désorption ionisation laser (LDI) a pour principe d'ioniser un échantillon généralement à l'état solide grâce à un laser UV ou IR ([Tanaka, et al. 2004 \[12\]](#)). On dépose pour cela l'échantillon soit en solution, soit sous forme de poudre sur une surface métallique conductrice. Une fois le solvant évaporé (pour le dépôt liquide), la plaque est introduite dans la source qui est le plus souvent sous vide avant d'être irradié par des tirs de laser. Les photons du laser vont venir arracher de la matière sous forme d'agrégats ce qui conduira ensuite à des ions M^+ et $[M+H]^+$. Cette source d'ionisation est souvent considéré comme douce car elle conduit à peu de fragmentations.

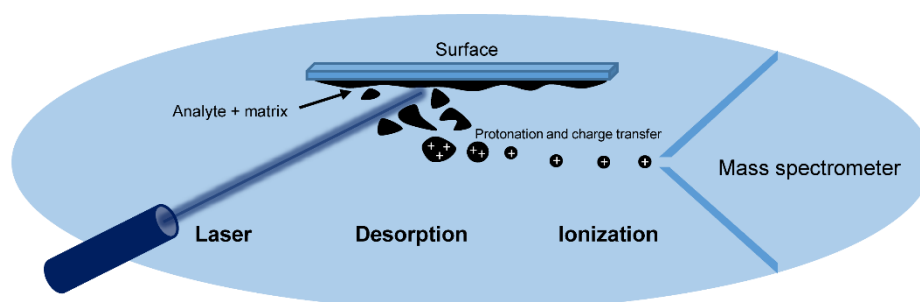


Figure 18 : Schéma de principe de la source à désorption-ionisation laser

Le LDI permet généralement l'ionisation de molécule de faible masse (généralement <2000 u), cependant grance à l'utilisation d'une molécule organique appelé matrice mélangé à l'analyte il est possible comme pour l'electrospray d'analyser des molécules de très grande masse. On parle dans ce cas de MALDI pour *matrix assisted laser desorption ionization*. Le MALDI a été proposé pour la première fois par Hillenkamp et Karas ([Karas, et al. 1987 \[13\]](#) ,[Karas and Hillenkamp 1988 \[14\]](#)). C'est une source particulièrement utile car elle permet, notamment, d'effectué des analyses sans solvants à l'inverse de l'ESI ou de l'APPI. En effet, si l'échantillon

le permet, c'est-à-dire si celui-ci contient quelques espèces aromatiques, il suffit juste de le déposer sans aucuns préparations préalables sur la plaque ([Barrere, et al. 2012 \[15\]](#))

1.3. Analyseurs

1.3.1. Analyseur quadripolaire

L'analyseur quadripolaire fut développer en 1953 par Wolfgang Paul qui eut dans l'idée de crée un spectromètre de masse sans champ magnétique ([Paul and Steinwedel 1953 \[16\]](#)). Il a obtenu, pour cette découverte, le prix Nobel de physique en 1989 ([Paul 1990 \[17\]](#)). Le principe repose sur la séparation d'ions dans un champ électrique radiofréquence.

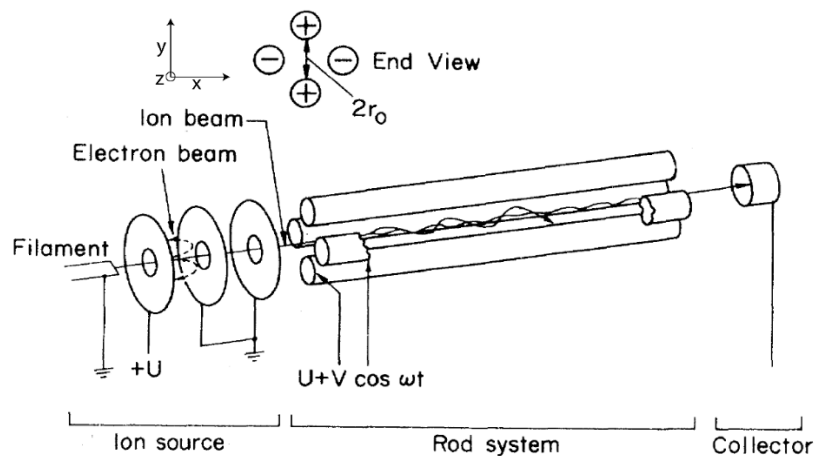


Figure 19 : Schéma d'un analyseur quadripolaire ([Paul 1990 \[17\]](#)).

Afin de détailler le précédent propos, un schéma de principe d'un quadripole et donné en **Figure 19**. Cet analyseur se compose simplement de quatre barres métalliques de sections cylindriques ou hyperboliques reliées deux à deux. On vient appliquer un potentiel Φ sur chaque paire de barres. Ce potentiel est composé d'une composante continue notée U et d'une composante alternative d'amplitude V . La définition du potentiel Φ est donnée par l'**Équation 3**.

Équation 3

$$\Phi = U - V \cos(\omega t)$$

Cette équation montre qu'à un temps t , les paires d'électrodes possèdent des potentiels opposés et à un temps $t + 1$ ce potentiel sera inversé. Un nuage de particules chargées positivement va donc être attiré vers une paire et repoussé par l'autre pair à un temps t selon la **Figure 20**. Ce mouvement sera inverse au temps $t + 1$.

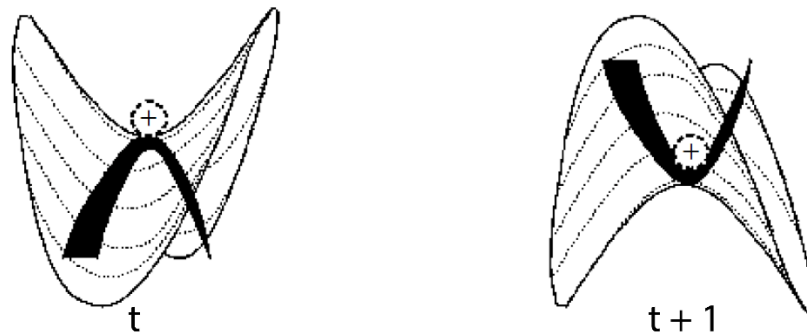


Figure 20 : Puit de potentiel subit par une particule chargée positivement dans un quadripole à un temps t et à un temps $t + 1$ ([Hoffman and Stroobant 2007 \[18\]](#)).

Ce mouvement est régi par l'équation de Mathieu :

Équation 4
$$\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0$$

Avec $\xi = \omega t/2$, $a_u = 8zeU/mr_0^2$, $q_u = 4zeV/mr_0^2\omega^2$, r_0 le rayon du cercle entre les 4 électrodes et ω la fréquence angulaire telle que $\omega = 2\pi f$ où f est la fréquence du champ alternatif. Cette équation s'applique pour l'axe x et l'axe y du quadripole. Ainsi, il est possible de définir un diagramme de stabilité d'une particule de masse m pour chaque axe représentant a_u en fonction de q_u . Ce diagramme est donné en **Figure 21**.

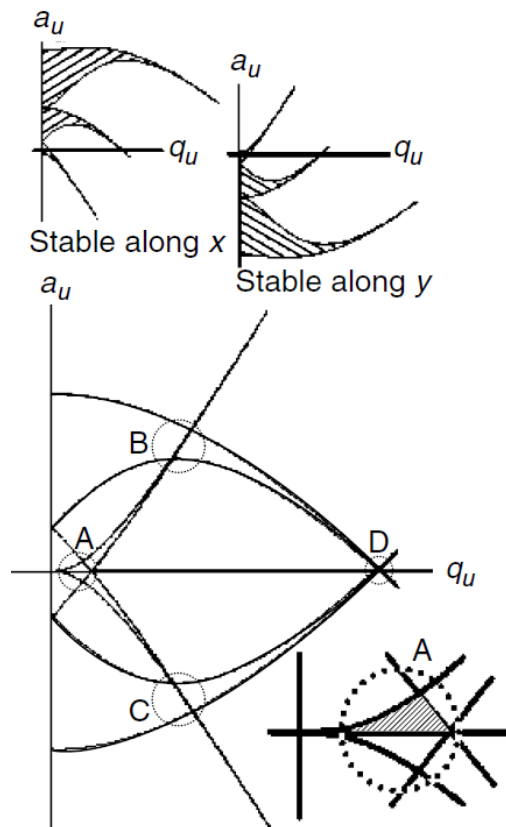


Figure 21 : Diagramme de stabilité d'une particule de masse m suivant les axes x et y ([Dawson 1986 \[19\]](#)).

Dans ce diagramme, l'intersection des deux composantes x et y est noté par les lettres A, B, C et D. C'est dans ces zones particulières que la particule de masse m sera stable et restera piégée dans le quadripole. Cependant, les régions B, C et D sont situés à de trop haut potentiel, seul la zone A représente un intérêt en spectrométrie de masse. C'est pourquoi le diagramme se simplifie en zoomant sur cette zone. Il est possible alors de réaliser un changement de variable afin d'avoir ce diagramme dépendant de U et de V pour plusieurs masses différentes (**Figure 22**).

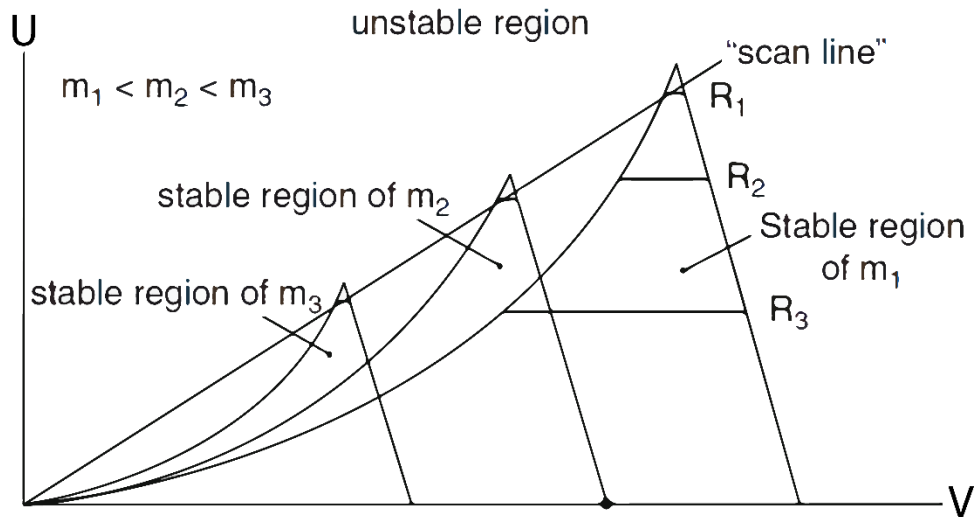


Figure 22 : Diagramme de stabilité simplifié de trois particules de masse m_1 , m_2 et m_3 .

Cette figure décrit les valeurs de potentiel U et V à appliquer au quadripole pour pouvoir analyser des ions. Chaque particule de masse donnée sera stable si elle se trouve dans son diagramme de stabilité. On va pouvoir ainsi, séparer des ions de masse différentes en faisant varier les potentiels afin d'éjecter les masses non désirées. Afin de réaliser un spectre de masse complet, on va donc venir faire un balayage et ainsi isoler chaque unité de masse une à une qui vont ensuite être détectées par un couple photocathode – *Channel photomultiplier* appelé aussi *Channeltron*.

Le quadripole est l'analyseur le plus commun de nos jours car sa fabrication est très peu coûteuse et son entretien très simple. Sa force réside dans sa capacité à faire du *Single Ion Monitoring* (SIM) permettant d'avoir un très bon rapport cyclique (ou *duty cycle*) et donc une sensibilité extrême. C'est aussi un analyseur parfaitement adapté au couplage chromatographique avec des vitesses de balayage suffisamment rapides ($\sim 1\text{Hz}$) ([Schmid 1990 \[20\]](#)). Cependant en mode balayage ou « scan » le *duty cycle* devient très faible ($< 0.1\%$) et la sensibilité diminue.

C'est donc un analyseur parfait pour faire de l'analyse ciblée et du dosage (en mode SIM). Le quadripôle est souvent associé à d'autres analyseurs dans le cas d'instruments en tandem ou hybride. Ainsi il existe des triples quadripôles ou le deuxième quadripôle (souvent un octopôle en pratique) est une cellule de collision pour effectuer des expériences de fragmentations d'ions sélectionnés (MS/MS). Le troisième analyseur peut être un temps de vol, un orbitrap ou un FTICR, on parle alors d'instrument hybride (Qq-TOF, Qq-Orbitrap ou Qq-FTICR). Pour avoir les meilleures sélectivités et sensibilité il est possible d'utiliser un triple quadripôle en mode SRM.

Le quadripôle est un analyseur présentant une résolution relativement faible ($m/\Delta m = 1000 - 3000$). Il est à noter que la largeur des signaux change très peu avec la masse et donc la résolution augmente avec la masse. Lors de cette thèse, des analyseurs quadripolaires ont été utilisés en amont d'analyseurs plus puissants afin de réaliser des étapes de fragmentation ou de sélection d'ion.

1.3.2. Analyseur temps de vol

Le premier analyseur temps de vol (ou *Time-of-flight*, TOF en anglais) a été développé en 1946 par Stephens ([Stephens 1946 \[21\]](#)). Son principe de séparation repose sur le temps de déplacement d'un ion sur une distance précise. Lorsqu'une particule chargée est accélérée grâce à un champ électrique, son énergie cinétique est définie par l'**Équation 5**, avec U est le potentiel appliqué à la particule, q est la charge de celle-ci, m sa masse et v sa vitesse :

Équation 5
$$E_c = qU = \frac{1}{2}mv^2$$

Donc, si l'on définit la vitesse de la particule par le rapport entre la distance parcourue et le temps, nous obtenons l'**Équation 6** avec la masse (m), la distance (d) et le temps (t) :

Équation 6
$$E_c = qU = \frac{1}{2}m \left(\frac{d}{t}\right)^2$$

Par ce fait, il est possible de conclure que si, pour deux particules, U, q et d sont constants mais seul leur masse m varie, leur temps pour parcourir la distance définie sera différent. Ainsi, en lançant des ions de masses différentes dans un champ électrique constant, il est possible de les séparer en fonction de leur temps d'arriver au détecteur. Un schéma est présenté en **Figure 23a**.

Cet analyseur est très simple et couramment répandu dans les laboratoires d'analyses actuel. Son intérêt réside dans sa vitesse d'analyse très rapide, de l'ordre de quelques microsecondes par scans, et dans sa capacité à analyser des ions de très haute masse.

Sa résolution est dépendante de la distance parcourue pour séparer les ions, généralement de quelques milliers pour les TOF linéaire. En 1994, Mamyrin a développer un TOF équipé d'une miroir électrostatique appelé *reflectron* (présenté **Figure 23b**) permettant d'accéder à la haute résolution, de l'ordre de 10000 à 100 000 de nos jours ([Mamyrin 1994 \[22\]](#)). Le *reflectron* permet de compenser les dispersions d'énergies cinétiques des ions de même masse afin de les faires arrivés en même temps au détecteur et ainsi augmenter fortement la résolution par rapport à un TOF linéaire.

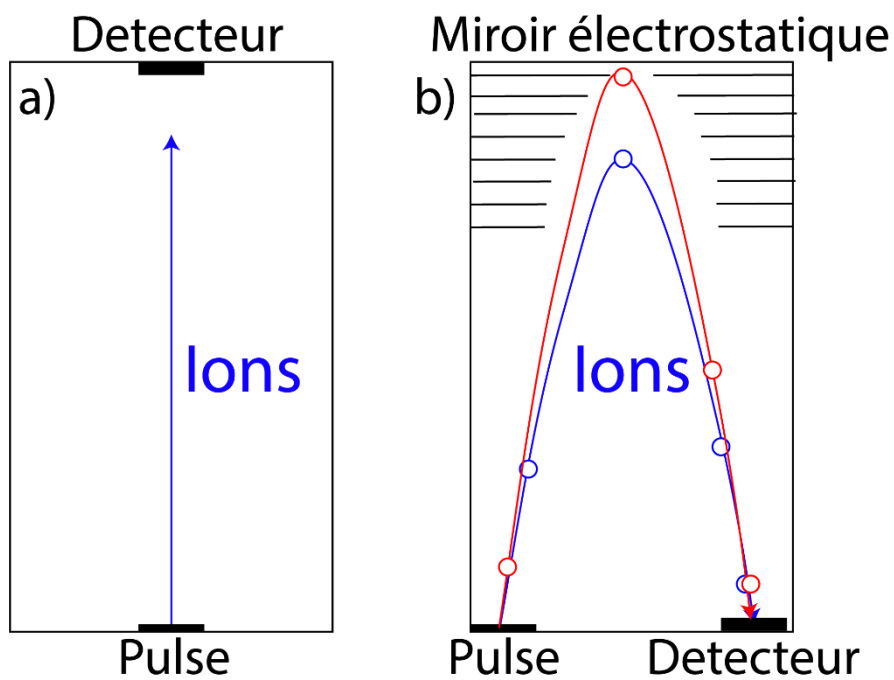


Figure 23 : Schéma d'un analyseur temps de vol de forme a) linéaire et b) *reflectron*

1.3.3. Analyseur à résonance cyclotronique des ions

Le principe

Afin de définir le *Fourier transform ion cyclotron resonance* (FTICR) ou analyseur à résonance cyclotronique des ions, il faut déjà revenir à quelques principes de physique. Pour commencer, il faut reprendre la deuxième loi de Newton qui définit un objet en mouvement et qui est donnée en **Équation 7**.

Dans le cadre d'une particule chargée positivement dans un champ magnétique, les forces s'exerçant sur celle-ci se résument à la force centrifuge et à la force magnétique comme présenté en Figure 24. L'équation qui en découle est donnée celle donnée en **Équation 8**. Ici, la particule ayant un mouvement constant et uniforme, son accélération \mathbf{a} est nulle. L'équation devient donc l'**Équation 9**.

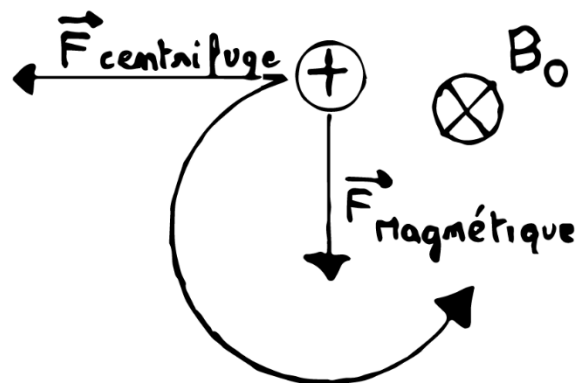


Figure 24 : Représentation des forces s'exerçant sur une particule chargée positivement dans un champ magnétique.

Équation 7

$$m \times a = \sum \vec{F}_i$$

Équation 8

$$m \times a = \vec{F}_{Centrifuge} + \vec{F}_{Magnétique}$$

Équation 9

$$0 = \frac{\vec{F}_{Centrifuge}}{m} + \frac{\vec{F}_{Magnétique}}{m}$$

La force magnétique fut définie par Hendrik Lorentz (Prix Nobel de physique en 1902) par l'Équation 10, qui représente le produit vectoriel entre une particule de charge q et de vitesse \vec{v} et le champ magnétique \vec{B}_0 . La force centrifuge est, elle, donnée en Équation 11. Enfin, la vitesse angulaire ω_c est définie par l'Équation 12 dont on déduit la fréquence cyclotronique f_c tel que $f_c = \omega_c / 2\pi$.

Équation 10
$$\vec{F}_{Magnétique} = q\vec{v} \wedge \vec{B}_0$$

Équation 11
$$\vec{F}_{Centrifuge} = \frac{mv^2}{r}$$

Équation 12
$$\omega_c = \frac{v}{r}$$

Ainsi, on en déduit l'Équation 13:

Équation 13
$$\omega_c = \frac{qB_0}{m} \quad \text{et} \quad f_c = \frac{qB_0}{2\pi m}$$

Cette démonstration montre que, pour une particule ayant une charge dans un champ magnétique constant, la fréquence cyclotronique de celle-ci est uniquement dépendante de son rapport m/z . En d'autre terme, une particule plus lourde aura une fréquence d'oscillation moins importante qu'une particule plus légère. Cette introduction est la base du FTICR.

Le précédent principe a été utilisé, pour la première fois, par Lawrence et Livingston en 1931 dans le but d'accélérer des protons afin de mieux caractériser le noyau ([Lawrence and Livingston 1932 \[23\]](#)). Il a ensuite été repris par Hipple, Sommer et al en 1949 avec l'Omegatron ([Hipple, et al. 1949 \[24\]](#)).

C'est le premier spectromètre de masse dans lequel a été introduit le principe de résonance cyclotronique des ions. Les particules chargées étaient déviées en fonction de leur mouvement cyclotronique dans un champ magnétique et ensuite détectés avec un détecteur fixe. Grâce aux travaux de Baldeschwieler en 1968 ([Baldeschwieler 1968 \[25\]](#)), l'intérêt de cette technique a été confirmé pour l'étude d'ion moléculaire. Mais, c'est par la numérisation du signal analogique et l'utilisation de la transformée de Fourier en 1974 par Comisarow et Marshall, que l'intérêt de cette technique a pris un tout autre tournant ([Comisarow and Marshall 1974 \[26\]](#)). En effet, grâce à cette méthode, les auteurs ont pu montrer les grandes possibilités de ce nouvel analyseur en terme de résolution et de précision de mesure ([Marshall 1985 \[27\]](#)).

Le piégeage d'ions dans une cellule ICR

Une fois cette première introduction faite et le principe général énoncé, nous allons nous intéresser au fonctionnement de cette cellule ICR communément appelé piège à ions ou piège de Penning. Ce piège a été développé par Hans Georg Dehmelt qui lui a valu le prix Nobel de physique en 1989). Les pièges à Penning utilisent un champ magnétique axial homogène puissant pour confiner les particules radialement et un champ électrique pour confiner les particules axialement.

Une fois le paquet d'ions introduit dans la cellule ICR, le voltage des plaques avant et arrière va être augmenté et un champ radiofréquence imposé entre les deux. Le champ magnétique, lui, va pousser les ions de façon radial et ainsi éviter qu'ils rentrent en collision avec les parois de la cellule.

Un mouvement additionnel apparaît dans la cellule appelé mouvement *diocotron* ou de drift. Les ions vont dériver suivant $\mathbf{E} \times \mathbf{B}_0$ et vont ainsi avoir une trajectoire hélicoïdale dans la cellule.

Lorsque ce piégeage est réalisé, les ions peuvent être stockés pendant de longues périodes allant de quelques millisecondes à plusieurs minutes. Le temps de piégeage maximal dépend surtout du vide régnant dans la cellule (généralement 10^{-10} mbar) ainsi que d'autres facteurs minoritaires ([Nikolaev, et al. 2011 \[28\]](#)).

L'excitation des ions

Lorsque les ions sont piégés dans la cellule, ces derniers se trouvent dans un mouvement incohérent avec un petit rayon cyclotronique schématisé par la **Figure 25a**. Ils se trouvent au centre de la cellule et décrivent un cercle où toutes les charges sont diffusées en périphérie. Il faut préciser que se superpose au mouvement cyclotronique un autre mouvement appelé magnétron. Le mouvement magnétron est un mouvement parasite produit quand un champ électrique est associé à un champ magnétique ([Dunbar, et al. 1984 \[29\]](#), [Jertz, et al. 2015 \[30\]](#)). Lorsque les ions sont dans un état non excité, ce mouvement est dominant et le mouvement cyclotronique est minoritaire.

Pour pouvoir détecter les ions, il faut donc rendre le mouvement cyclotronique cohérent et augmenter son rayon. Pour cela, on applique un champ radiofréquence entre les plaques 3 et 4 de la cellule ICR qui va venir exciter le paquet d'ions piégé et ainsi faire augmenter son rayon cyclotronique.

Les ions se retrouvent ainsi sur la trajectoire illustrée par la **Figure 25b** où les charges sont regroupées et où la distance ions-plaques est faible. Cette excitation est généralement réalisée en faisant varier la fréquence du champ RF afin de venir exciter chaque masse de la plage d'étude.

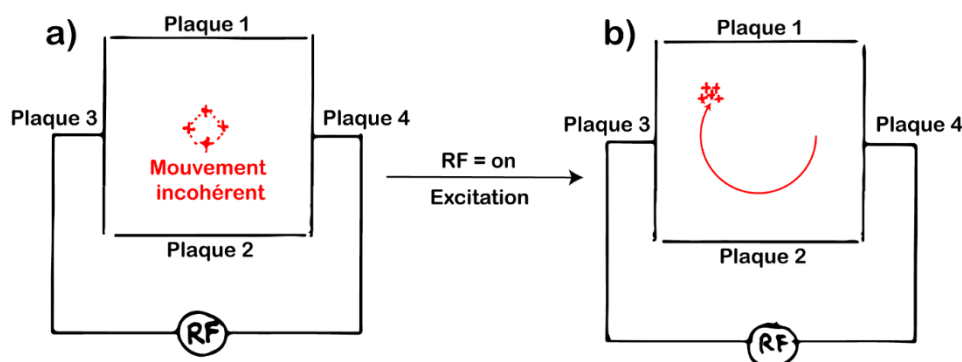


Figure 25 : Schéma de l'excitation des ions. A) Avant l'imposition du champ radiofréquence, b) Après excitation

La détection

Une fois les ions excités, la détection va pouvoir avoir lieu. Un schéma simplifié de cette étape est donné en **Figure 26**. Comme énoncé lors de l'introduction, les ions, de par leur mouvement cyclotronique vont venir osciller entre les deux plaques de détection de façon radiale, ce qui va générer un courant de quelque nano-ampères. Ce courant, une fois amplifié, va donner un signal analogique alternatif qui s'atténue avec le temps appelé *Free Induction Decay* (FID) ou *Transient*. Ce signal est digitalisé puis grâce à une transformée de Fourier convertit en spectre de fréquence puis spectre de masse via l'utilisation de l'équation 2.11 (Page 55) et d'un profil d'étalonnage.

Ainsi, pour chaque analyse FTICR, on retrouve les trois étapes énoncées précédemment : Piégeage, excitation, détection. Une analyse ou « scan » (bien qu'il n'y ai pas en pratique de balayage) prend donc entre quelque milliseconde et plusieurs secondes en fonction du temps de détection choisi.

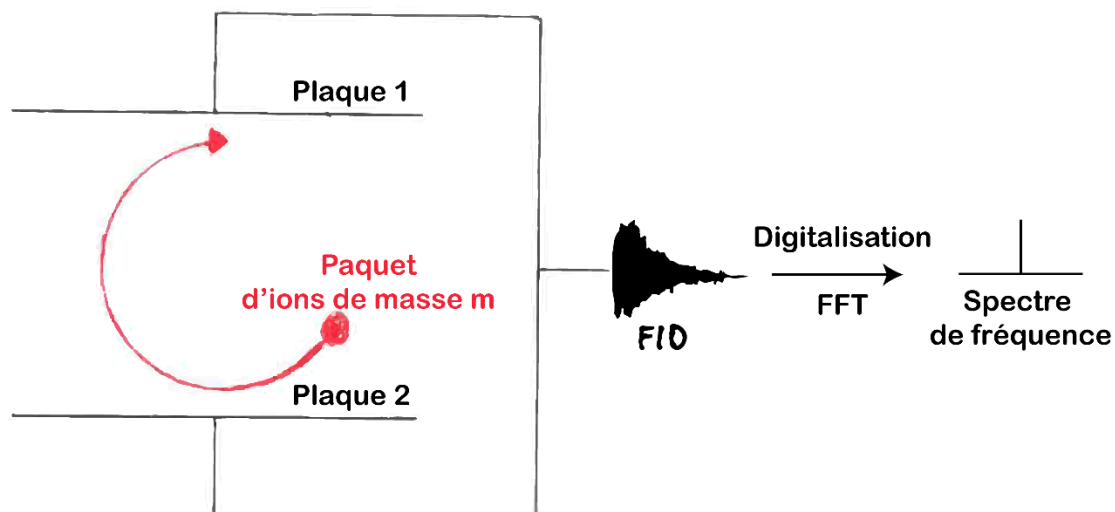


Figure 26 : Schéma de détection des ions dans une cellule ICR.

Les différentes cellules ICR

Lors des débuts du FTICR, deux problèmes majeurs limitaient les caractéristiques des premiers instruments. Le premier était le piégeage des ions. Celui-ci n'était pas parfait et limité souvent la longueur des *transients* et donc la résolution et la sensibilité des appareils. Le deuxième problème était souvent la mauvaise position de piégeage du paquet d'ions. En effet, ce dernier était souvent mal centré dans la cellule et induisait ainsi des artefacts majeurs tel que l'apparition d'harmoniques des pics détectés et aussi une perte de sensibilité. Pour résoudre ces problèmes, de nombreuses géométries de cellules ICR ont été testées et sont référencés dans la littérature. Dans cette partie, six exemples de géométrie de cellules ont été reportés sur la **Figure 27**.

Les différents types de cellules peuvent être triés en deux catégories. Les cellules fermées ou *closed cells* et les cellules ouvertes appelées *open-capp*. La première cellule développée par Comisarow et Marshall était de forme cubique fermé (**Figure 27a**).

Les plaques de piégeage étaient à l'avant et à l'arrière de la cellule et les 4 autres faces du cube servaient à l'excitation et à la détection. C'est le modèle de cellule le plus simple mais ses caractéristiques de piégeage et de détection étaient limitées notamment à cause du champ électrique non optimale qui régnait au sein de l'enceinte de piégeage. Afin d'améliorer le champ électrique et le rendre plus homogène, la première cellule cylindrique fut développée par Comisarow, Marshall et Kofel (**Figure 27b**) ([Kofel, et al. 1986 \[31\]](#)). Elle était composée du même nombre d'électrodes que la cellule cubique, mais, de par sa géométrie, permettait d'améliorer la qualité du champ électrique et ainsi améliorer les caractéristiques techniques. Dans le même temps, une équipe russe dirigée par Gabrielse développa la première cellule compensée (**Figure 27c**) ([Gabrielse and Mackintosh 1984 \[32\]](#), [Gabrielse, et al. 1989 \[33\]](#)). Par l'ajout de deux électrodes circulaires entourant les électrodes de détection et d'excitation, Gabrielse générait un champ électrique hyperbolique, idéal pour le piégeage des ions.

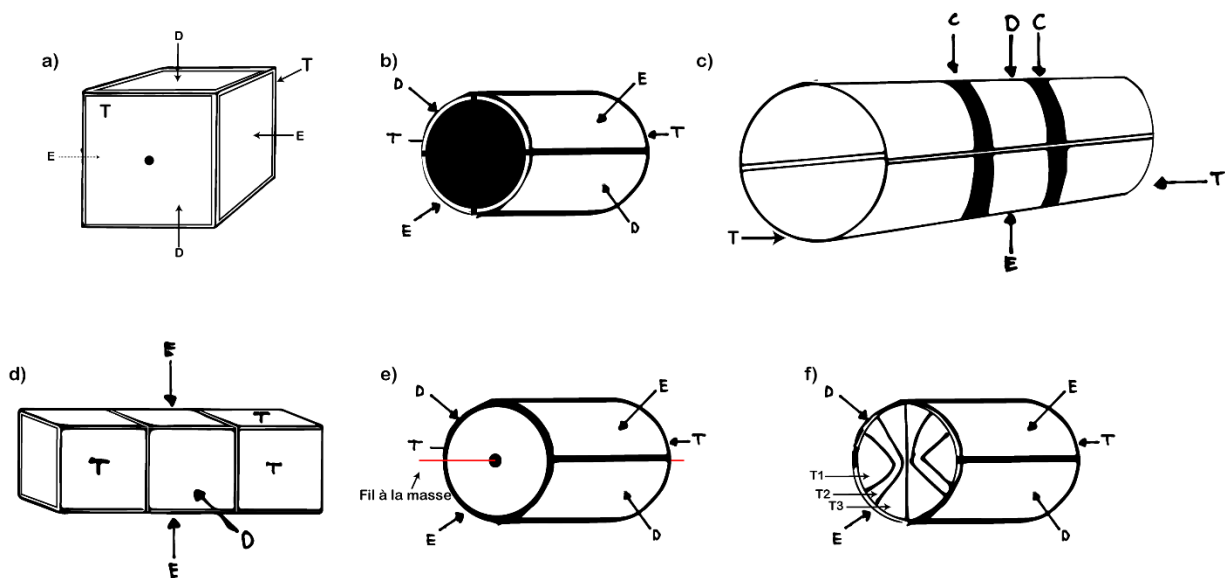


Figure 27 : Schéma de différentes cellules ICR avec T : Trapping, E : Excitation, D : détection. A) Cellule cubique ([Comisarow 1981 \[34\]](#)), B) cellule cylindrique ([Kofel, et al. 1986 \[31\]](#)), C) Cellule orthorhombique à trois sections ([Beu and Laude 1992 \[35\]](#), [Beu and Laude 1992 \[36\]](#)), E) cellule cylindrique avec fil axial ([Solouki, et al. 1994 \[37\]](#)), F) cellule infinie ([Caravatti and Allemann 1991 \[38\]](#)).

Cette cellule permettait, en opposition avec les cellules de géométrie hyperbolique de l'époque (non discutées dans cette partie) d'avoir la même qualité de piégeage tout en laissant libre d'accès le centre de la cellule pour des manipulations extérieures comme de la fragmentation par laser. De plus, cette cellule était beaucoup plus facile à construire que les cellules précédentes. L'ajout de cette compensation a permis d'introduire un nouveau type de cellules dites « harmonisées » qui seront discutées dans la prochaine partie.

Certaines cellules atypiques ont été référencées dans la **Figure 27**. On y retrouve la cellule à trois sections orthogonal *open-capp* de Beu ([Beu and Laude 1992 \[35\]](#) ,[Beu and Laude 1992 \[36\]](#) ,[Beu and Laude 1991 \[39\]](#)) mais aussi la cellule cylindrique fermé avec fil central de Solouki ([Solouki, et al. 1994 \[37\]](#)).

La première cellule permettait, par ces trois sections de taille identique d'améliorer le piégeage des ions. Malheureusement, elle n'a pas été développée davantage.

La seconde cellule permettait d'améliorer le piégeage de biomolécules de très hautes masses de l'ordre de 100-200 kDa) mais son développement n'a pas non plus été poursuivi. En effet, dans le même temps (1990-2000), une cellule beaucoup plus performante a vu le jour, la cellule dite *Infinity Cell* (**Figure 27f**) ([Caravatti and Allemann 1991 \[38\]](#)). Cette cellule, développée par Caravatti et al, a des plaques de piégeages segmentées qui permettent lors de l'excitation des ions d'appliquer un champ radiofréquence qui augmente au fur et à mesure que l'on s'éloigne du centre de la cellule. C'est cette inhomogénéité qui a rendu cette cellule si performante car elle permet de compenser la dispersion suivant l'axe z d'un paquet d'ion de même masse et ainsi améliorer la cohérence de celui-ci. Cela a permis de nettement améliorer les performances d'analyse et notamment la gamme dynamique lors de l'étude de mélanges complexes. Jusqu'en 2012, c'est cette cellule qui a équipé tous les FTICR vendus par la société Bruker.

La Paracell

En 2011, une équipe russe portée par Eugene Nikolaev a mis au point une nouvelle cellule ICR dynamiquement harmonisée, commercialisé par Bruker sous le nom de Paracell®. Née de calculs théoriques poussés concernant le piégeage des ions, cette cellule cylindrique représenté en **Figure 28** possède une structure assez complexe. Celle-ci peut être divisée en quatre segments distincts. Dans chaque segment, 3 types d'électrodes sont présents. Dans un premier temps, les électrodes en forme de feuille dite « Leaf » et en demi feuille « half leaf » permettent l'excitation et la détection, les autres électrodes de forme opposées dite « counter leaf » permettent le piégeage des ions.

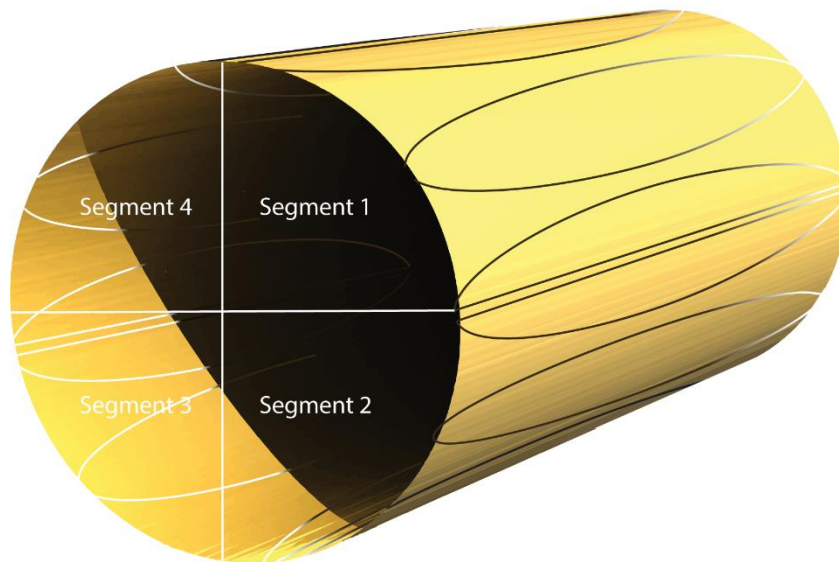


Figure 28 : Représentation schématique de la cellule ICR dynamiquement harmonisée.

Le potentiel appliqué sur ces dernières électrodes peut être modulé afin d'optimiser le champ électrique et ainsi améliorer les performances. Le gros avantage apporté par cette cellule est l'amélioration de compensation des oscillations sur l'axe z des paquets d'ions de même masse par rapport à la cellule infinie ainsi que du piégeage. Cela a encore permis d'accroître énormément son temps de cohérence. En énonçant les performances, ces dernières surpassent les cellules précédentes sur tous les points. En effet, lors de la caractérisation initiale de la cellule, Nikolaev a atteint une résolution de 24 000 000 sur l'ion $[M+H]^+$ de la réserpine à m/z 609 ([Nikolaev, et al. 2011 \[28\]](#)). Cette performance a été possible grâce à l'efficacité de piégeage de la Paracell® qui permet l'obtention de FID extrêmement longs.

La structure du FTICR contemporain

Une fois cette première introduction faite et le principe général énoncé, nous allons nous intéresser à la structure d'un FTICR contemporain. Pour cela, un schéma instrumental est donné en **Figure 29**. Comme tout spectromètre de masse, une source d'ionisation équipe la première partie de l'instrument. On y retrouve la plupart des sources d'ionisation à pression atmosphérique tel que l'ESI, APCI, APPI ainsi qu'une source LDI à pression réduite. La pression régnant dans l'enceinte de la source LDI, qui est la première partie du spectromètre est de l'ordre de 10^{-3} mBar.

Dans une deuxième partie, assure la transmission des ions, présente de nombreuses optiques ioniques afin de faire voyager les ions de la source vers la cellule ICR. En outre, on y retrouve deux *ion funnels*, deux *skimmers*, un quadripole permettant notamment la sélection d'ions ainsi qu'une cellule de collision faisant également le rôle de piège à ion linéaire pour les expériences en spectrométrie de masse en tandem. Le piège linéaire permet de stocker et d'accumuler les ions provenant des sources à pression atmosphérique afin de pouvoir les injecter par paquets dans la cellule ICR. Sauf dans la cellule de collision, la pression régnant dans cette partie est de l'ordre de 10^{-6} mBar.

Enfin, une fois les ions accumulés, ils sont envoyés dans la troisième partie de l'instrument se situant au milieu de l'aimant, la cellule ICR. Comme précisé précédemment, la pression ici est de l'ordre de 10^{-10} mBar ce qui correspond à l'ultravide.

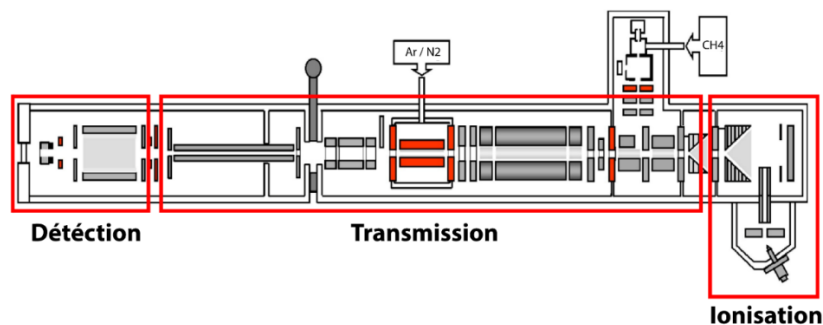


Figure 29 : Schéma structural d'un FTICR commercial (Solarix, Bruker Daltonics®)

Intérêt d'un champ magnétique puissant

De nos jours, les aimants de FTICR vont de 7 T jusqu'à 21 T. Toutes les caractéristiques d'un FTICR s'améliorent avec l'augmentation du champ magnétique. Mais, l'intérêt majeur d'un FTICR équipé d'un aimant à plus haut champ est sa capacité à piéger d'avantage d'ions dans la cellule ICR, ce qui va augmenter fortement la gamme dynamique. En effet, la quantité d'ions piégés augmente de façon quadratique avec le champ magnétique. Simplement, avec un champ magnétique plus élevé, il sera possible de détecter des espèces minoritaires non visible auparavant. C'est un gros avantage lors de l'étude de mélanges très complexes.

Cependant, un champ magnétique plus élevé va conduire à augmenter proportionnellement la fréquence cyclotronique. En conséquence la numérisation de ce signal nécessitera une électronique plus performante. Cela est surtout un problème pour les petits m/z qui correspondent aux plus hautes fréquences. En pratique selon le théorème de Nyquist-Shannon, numériser correctement un signal alternatif avec une fréquence f donnée il faut utiliser un taux d'échantillonnage au double de cette fréquence $2f$. Il faut prendre en compte la fréquence maximale du signal qui correspond au rapport m/z le plus faible. Afin d'illustrer cela, notons par exemple que sur un 7 T, il est possible de détecter des espèces vers m/z 70 alors que sur un 21 Tesla, il est impossible de commencer la détection en dessous de m/z 180.

Conclusion

Durant cette thèse, deux FTICR ont été utilisés. Le premier situé en France (laboratoire COBRA) est équipé d'un aimant 12 Tesla supraconducteur ainsi que d'une instrumentation commerciale Bruker. Le deuxième FTICR est équipé d'un aimant 21 Tesla supraconducteur Bruker ainsi que d'une instrumentation dérivé d'un piège à ion linéaire Thermo au laboratoire des hauts champs magnétiques national (NHMFL) à Tallahassee (Floride).

Partie 2 : Spectrométrie de mobilité ionique

Cette deuxième partie du chapitre 2 s'intéressera à la spectrométrie de mobilité ionique, qui est une technique de séparation d'ions en phase gazeuse utilisée durant deux études reportées dans ce mémoire de thèse. Cette méthode, permet la séparation d'ions en fonction de leur section efficace de collision (CCS) et de leur charge. Dans un premier temps, le principe général sera présenté et les grandes équations régissant la physique de cette technique énoncée. Ensuite, deux types de spectrométries de mobilité ioniques seront présentés. La première technologie est le Travelling Waves Ions Mobility (TWIMS) qui équipe sur un Synapt G2 (Waters) présent au laboratoire COBRA. La deuxième technique présentée sera la Trapped Ions Mobility (TIMS) qui fut utilisées dans une étude, couplée à la spectrométrie de masse à ultra-haute résolution grâce à un FTICR 12 Tesla.

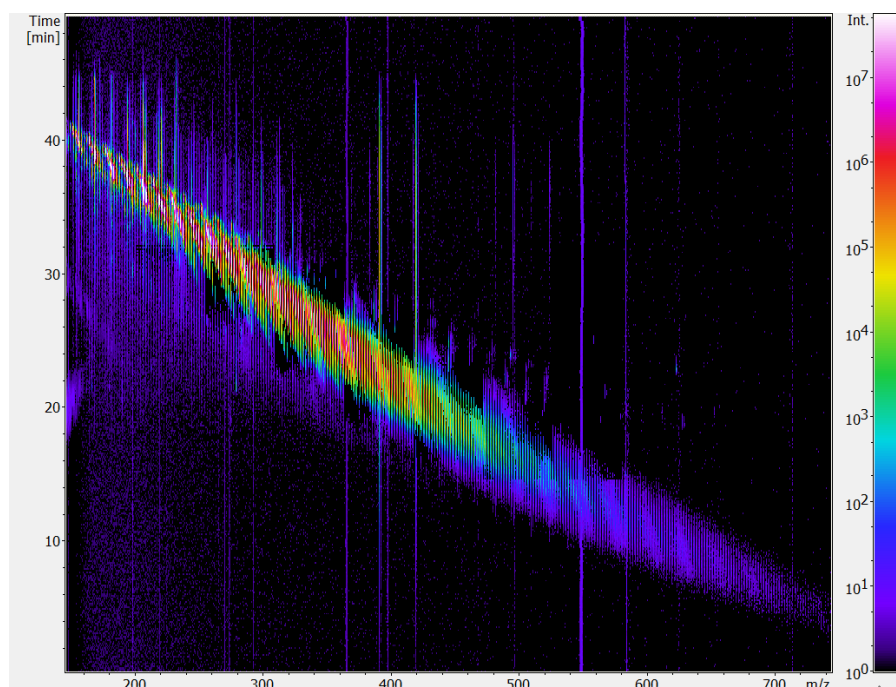


Figure 30 : Carte bidimensionnelle temps en fonction de m/z obtenue à partir d'une analyse de spectrométrie de mobilité ionique couplée à la spectrométrie de masse

2.1. Principe général

Le principe de la mobilité ionique présente des analogies à la chromatographie gazeuse, où les analytes entraînés par un gaz sont séparés en fonction de leur rétention sur une colonne ([Kanu, et al. 2008 \[40\]](#)). Cependant, dans le cas de la spectrométrie de mobilité ionique, l'analyte chargé est entraîné grâce à un champ électrique et retenu par les collisions réalisées avec le gaz tampon. C'est donc une séparation post-ionisation que va venir trier les ions en fonction de leur ombre projetée et de leur charge. En considérant donc deux isomères de même charge, ceux-ci ne seront séparés de par leur section efficace de collision (*Collision Cross Section*, CCS). Cette première explication est illustrée grâce à la **Figure 31**. Dans cette illustration, deux ions de taille différentes entre dans le tube de mobilité ionique sont séparés en fonction de leur collision avec le gaz tampon. L'ion rouge étant plus compact que l'ion vert, celui-ci aura une plus grande mobilité et sera détecté en premier. Il est à remarquer que l'unité présente sur l'axe du spectre de mobilité ionique est le temps de dérive. La CCS énoncé précédemment n'est obtenue qu'après calcul. Cette partie sera présentée dans la suite de ce chapitre.

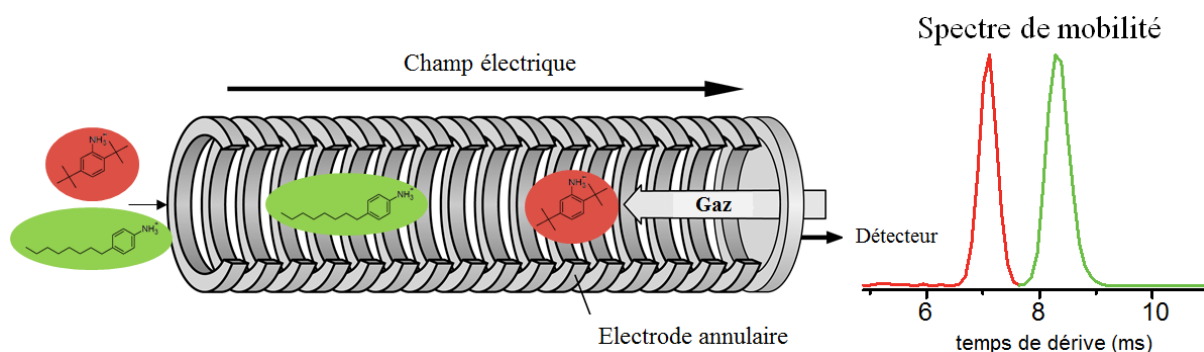


Figure 31 : Principe de fonctionnement d'une cellule de mobilité ionique.

Historiquement les principes de la spectrométrie de mobilité ionique furent décrits par Paul Langevin qui en 1903 émit l'hypothèse que des ions pouvaient être séparés lorsqu'ils rentraient en contact avec un gaz inerte ([Langevin 1903 \[41\]](#)).

C'est en reprenant ce principe en 1958 que Edward Mason et Homer Schamp posèrent les bases théoriques de la spectrométrie de mobilité ionique ([Mason and Schamp 1958 \[42\]](#)).

Afin de mieux comprendre ce principe, il est important de reprendre mathématiquement leur travail. Dans un premier temps, comme précisé dans la partie précédente, la valeur mesurée est le temps de dérive des ions noté t_d . Celui-ci est directement proportionnel au rapport entre la longueur du tube de dérive noté L et la vitesse des ions injectés dans l'instrument, noté v_d . On obtient donc l'**Équation 14** :

Équation 14
$$t_d = \frac{L}{v_d}$$

La vitesse de dérive des ions (v_d) dans un champ électrique (E) est définie par l'**Équation 15**:

Équation 15
$$v_d = K \times E$$

Ainsi, le temps de dérive devient :

Équation 16
$$t_d = L / (K \times E)$$

Ensuite, il faut définir le coefficient de mobilité ionique. Noté K , ce coefficient est lié à la densité du gaz remplissant la cellule de mobilité (N), la pression (p) et la température (T). Dans des conditions d'utilisation normale ($T_0 = 273,15$ K et $P_0 = 760$ Torr), on obtient la constante de mobilité réduite (K_0) définie par l'Équation 17:

Équation 17
$$K_0 = K \times \frac{p \times T_0}{T \times p_0}$$

Cette équation n'est valable que pour les champs électriques inférieurs à 1000 V cm^{-1} .

Finalement, la CCS (noté aussi Ω) est liée à la constante de mobilité K par l'équation de Mason-Schamp ([Mason and Schamp 1958 \[42\]](#)) (Équation 18):

Équation 18
$$K = \frac{3ze}{16N} \times \sqrt{\left(\frac{1}{m} + \frac{1}{M}\right)} \times \sqrt{\frac{2\pi}{kT}} \times \frac{1}{\Omega}$$

Avec z , charge de l'ion, e la charge de l'électron, N est la densité du gaz tampon, m est la masse de la molécule de gaz tampon, M masse de l'ion, k est la constante de Boltzmann et T est la température.

Ainsi, grâce à cette démonstration, il est possible d'accéder à la CCS qui est un descripteur intrinsèque à l'ion (pour un gaz tampon donné) étudié et qui est prédictible au même titre que sa masse. C'est donc un avantage de la spectrométrie de mobilité ionique par rapport aux autres chromatographies.

2.2. Les différentes cellules de mobilité ionique

Dans cette section seront développés les principes de fonctionnement de trois cellules de mobilité ionique différentes :

- Cellule type tube de dérive (*Drift Tube*, DTIMS)
- Cellule *Travelling wave ion mobility spectrometry* (TWIMS)
- Cellule *Trapped ion mobility spectrometry* (TIMS)

Les équations énoncées dans le principe précédent s'appliquent dans le cadre d'une cellule conventionnelle de type tube de dérive.

2.1.1. Le tube de dérive (DTIMS)

La structure d'un tube de dérive est basée sur une succession d'électrode annulaire formant un guide d'ions ([May, et al. 2015 \[43\]](#)). En plus des potentiels présents sur chaque électrode, il est imposé une différence de potentiel entre la première et dernière électrode formant un champ électrique uniforme dans la cellule. Un gaz tampon remplit la cellule, l'amenant à une pression relativement élevée de l'ordre de quelques millibars. La **Figure 32** présente un schéma d'une cellule DTIMS.

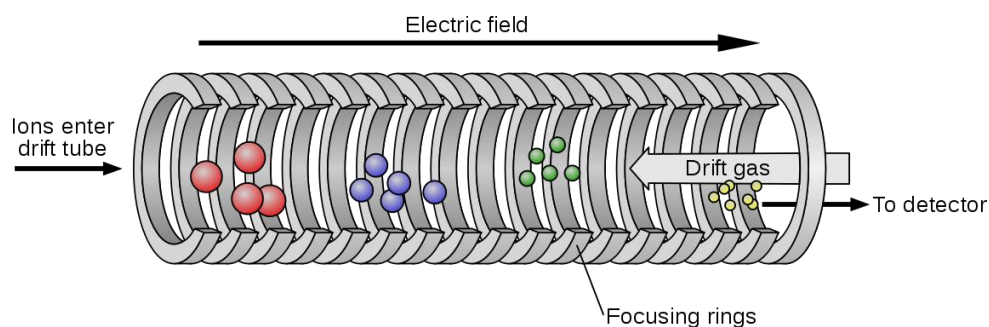


Figure 32 : Schéma d'une cellule de mobilité ionique de type tube de dérive (DTIMS) (Copyright : Jeff Dahl)

Dans la cellule, les ions sont envoyés sous forme de *pulse* par paquet. Grâce au champ électrique uniforme et à la présence de gaz tampon, les espèces ayant des sections efficaces de collision différentes sont séparées et ne sortent pas au même temps du tube de dérive.

Comme énoncé précédemment, le tube de dérive est la seule technologie de mobilité ionique permettant actuellement une mesure directe de la section efficace de collision des espèces mesurées. Cependant cette technologie présente un défaut majeur, son rapport cyclique ([Smith, et al. 2009 \[44\]](#)). En effet, celui-ci est de l'ordre de 1% ce qui limite la détection des espèces de faible intensité. Pour pallier à cela, plusieurs autres technologies de mobilité ionique ont été développées et deux d'entre elles sont présentées dans la suite de cette partie.

2.1.2. Cellule type Travelling wave ion mobility spectrometry (TWIMS)

Développé par la compagnie Waters®, le TWIMS est très proche du tube de dérive en termes de structure. Une série d'électrode annulaire forme un guide d'ion et un gaz tampon remplit la cellule ([Giles, et al. 2010 \[45\]](#)). Cependant, à la différence du DTIMS, un champ radiofréquence est imposé entre chaque électrode ([Giles, et al. 2004 \[46\]](#), [Shvartsburg and Smith 2008 \[47\]](#)). La structure du TWIMS est représentée par la **Figure 33a**. Cette succession de champ radiofréquence va générer des puits de potentiel qui vont venir influencer le mouvement des ions et améliorer la transmission. En effet, lors d'un passage d'un puit à un autre, un ion qui doit franchir la vague de potentiel se voit donc ralentir. Ce ralentissement va s'accroître en fonction de la section efficace de collision de l'ion. La simulation des puits de potentiel est illustrée par la **Figure 33b**. En complément, une oscillation du champ radiofréquence est imposée à la cellule de mobilité ionique, ce qui va venir déplacer les puits de potentiel le long du guide d'ion. Cet effet a donné son nom à cette technologie, le *Travelling Waves*.

Ainsi, le mouvement d'un ion comprend des retours en arrière (*roll over*) plus ou moins intense en fonction de ses collisions avec le gaz tampon et donc de sa CCS.

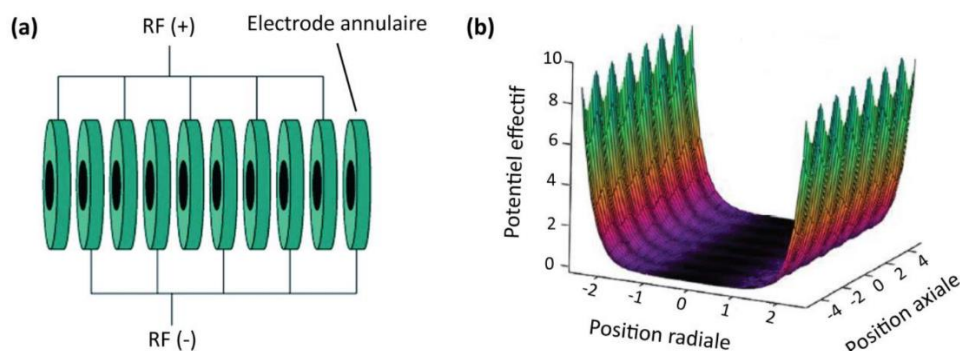


Figure 33 : a) Structure d'une cellule TWIMS, b) Simulation des puits de potentiels régnant dans la cellule ([Giles, et al. 2004 \[46\]](#)).

Afin d'améliorer la séparation dans la cellule TWIMS, il est possible de jouer sur la vitesse de mouvements des puits de potentiel (vitesse de vague) ainsi que sur la profondeur de ceux-ci (hauteur de vague). De plus, comme sur le DTIMS, il est possible d'ajuster la pression de gaz tampon dans l'enceinte de la cellule pour optimiser la séparation.

Cette technologie est utilisée sur les Synapt (Waters®), instrument de type Quadripole-IMS-TOF à haute résolution ([Farenc, et al. 2017 \[48\]](#) ,[Hamilton, et al. 2012 \[49\]](#) ,[Salbo, et al. 2012 \[50\]](#)). La première partie du **chapitre V** présente les résultats obtenus suite à l'analyse de la fraction soluble dans le méthanol des Tholins de PAMPRE par un Synapt G2 en TWIMS-MS.

2.1.3. Cellule de type Trapped ion mobility spectrometry (TIMS)

Dans le but de développer leur propre technologie de mobilité ionique, Bruker® a choisi de se baser sur un *ion funnel* qui est un guide d'ion utilisé dans leurs spectromètres de masse ([Shaffer, et al. 1998 \[51\]](#), [Kelly, et al. 2010 \[52\]](#)). Un *ion funnel* est aussi composé d'une succession d'électrodes annulaires comme le TWIMS, mais le diamètre de ceux-ci diminue et forment un entonnoir comme illustré par la **Figure 34**. Cette structure particulière permet de limiter la diffusion radiale des ions et ainsi améliorer la transmission.

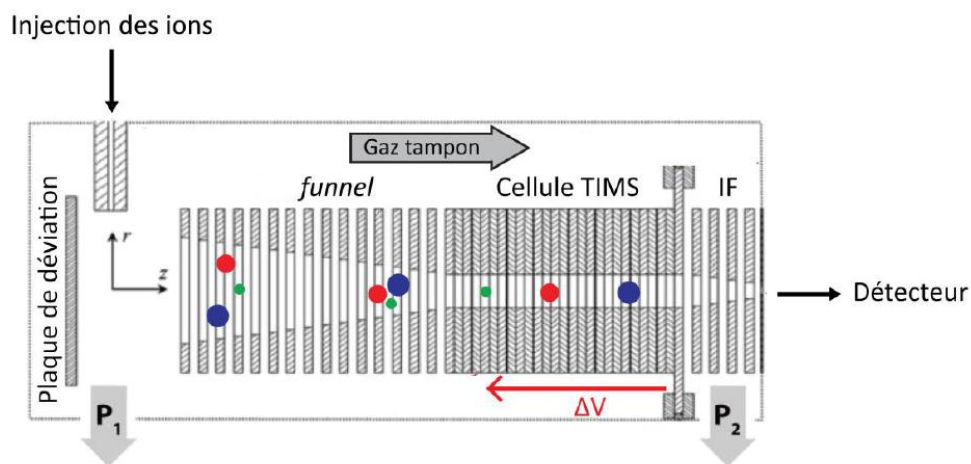


Figure 34 : Structure d'une cellule de mobilité de type TIMS adapté de ([Ridgeway, et al. 2018 \[53\]](#))

En opposition aux deux cellules précédentes, dans le TIMS, le gaz tampon circule dans le sens de mouvement des ions ([Ridgeway, et al. 2018 \[53\]](#)). Généralement, ce courant est créé grâce à la différence de pression régnant entre l'extérieur et l'enceinte de l'instrument.

Le champ électrique, lui, est dans le sens opposé au courant de gaz. Ce champ forme un gradient le long du *funnel* permettant de venir piéger les ions en fonction de leur mobilité ionique c'est-à-dire de leur facilité d'entraînement par le flux de gaz tampon. Pour expliquer plus en détail ce dernier, la **Figure 35** présente la forme qu'adopte le champ électrique le long de la cellule en fonction des étapes de l'analyse.

Dans un premier temps, les ions sont accumulés dans le *funnel* et sont séparés en fonction de leurs collisions avec le gaz tampon jusqu'à être retenu à une valeur de voltage particulière qui correspond à une position particulière dans le *funnel* (**Figure 35a**). Ensuite, une fois le nombre d'ions nécessaires atteint, le voltage des électrodes d'entrée est augmenté afin d'empêcher d'autres ions d'entrer (**Figure 35b**). Enfin, les ions sont élués successivement par diminution de la valeur de la rampe de voltage dans la cellule (**Figure 35c**).

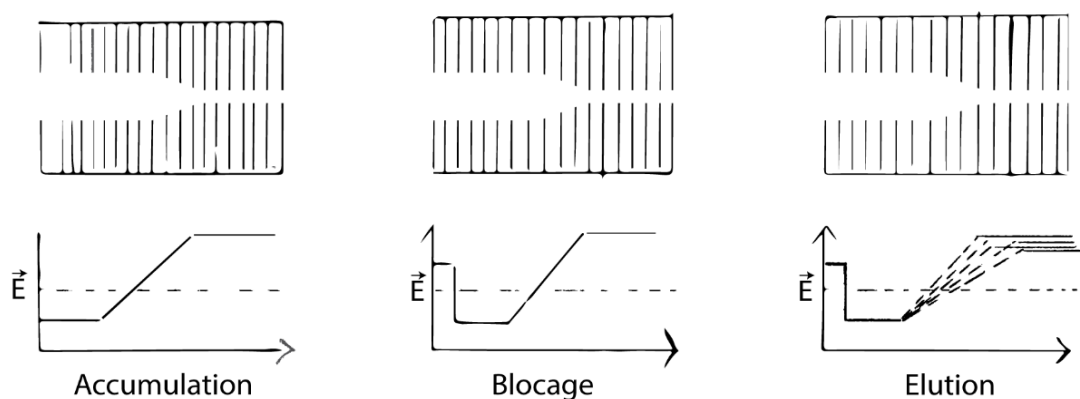


Figure 35 : Représentation du voltage imposé à la cellule TIMS en fonction de la position. Adaptée de ([Ridgeway, et al. 2018 \[53\]](#)).

Le TIMS possède une résolution légèrement plus grande que celle du TWIMS, mais son principal avantage vient du fait de pouvoir piéger les ions pendant une durée voulue, ce qui va permettre de coupler cette technologie à des spectromètres de masse très lents. En effet, un scan de TWIMS prend environ 15 millisecondes, ce qui en fait une technologie facilement couplable à des analyseurs type temps de vol qui ont une fréquence de scan de l'ordre de la microseconde. Cependant, il est impossible de la coupler avec un analyseur à transformée de Fourier qui possède une vitesse de scan beaucoup plus lente, de l'ordre de la seconde.

Ce n'est pas le cas du TIMS qui, comme énoncé précédemment, possède une vitesse de *scan* ajustable et donc qui peut être couplé à un FTICR ([Tose, et al. 2018 \[54\]](#), [Ridgeway, et al. 2016 \[55\]](#)). La deuxième partie du **chapitre V** présente les résultats obtenus de l'analyse de la fraction soluble des Tholins de PAMPRE par un TIMS-FTICR.

Partie 3 : Traitement de données

3.1. Etape du traitement post-acquisition

3.1.1. Etalonnage

Les mélanges organiques complexes telles que les tholines conduisent à des spectres de masses qui peuvent contenir des dizaines de milliers de signaux. Pour déterminer de manière fiable les formules brutes de ces signaux il est nécessaire d'avoir une très haute résolution pour les séparer mais aussi une très grande précision de façon à attribuer de façon fiable une formule brute unique à chaque signal. Cela passe par des étapes d'étalonnage effectué tout d'abord de façon externe puis après l'acquisition des données de façon interne.

L'étape d'étalonnage permet de corriger la déviation liée à la mesure instrumentale et au traitement du signal en prenant comme références des signaux correspondant à des composés connus. En d'autres termes, un composé connu permet à partir de sa formule brute de calculer sa masse exacte permettant de vérifier les erreurs sur les masses mesurées. Lors de l'étalonnage un facteur correctif est appliqué à des masses mesurées. Cela peut être fait en prenant en compte un point ou plusieurs points de façon à appliquer une fonction de correction linéaire ou quadratique.

Généralement, dans un premier temps le spectromètre de masse est étalonné de façon externe avec une solution étalon. Pour l'étalonnage interne, des signaux naturellement présents dans l'échantillon ou dans le bruit de fond peuvent être utilisés ou une faible quantité d'un composé connu est incorporé dans l'échantillon à analyser afin de pouvoir étalonner le spectre. Dans le cadre de mélanges organiques très complexes qui couvrent un large espace moléculaire l'étalonnage interne peut être un mélange de molécules non naturelle telle que des molécules perfluorées qui vont être détectées à un défaut de masse très faible.

Après analyse, une courbe d'étalonnage (linéaire ou quadratique) est créée à partir d'espèces détectées dans l'échantillon et va être appliquée comme correction à l'ensemble des pics (étalonnage interne). Sur un FTICR l'étalonnage interne permet de descendre à des erreurs de mesure sur la globalité du spectre égale à quelques ppb.

La **Figure 36** présente la courbe d'étalonnage d'un spectre de Tholins, avec des espèces allant de m/z 150 à m/z 800. La déviation standard des espèces observées par rapport à la courbe imposé est égale à 26 ppb.

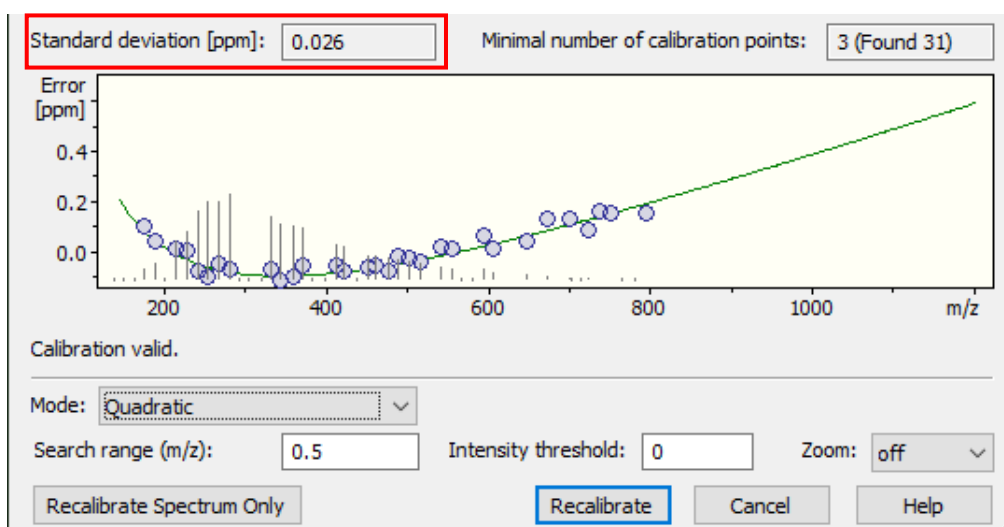


Figure 36 : Résultat de l'étalonnage d'un spectre de Tholins avec l'outil d'étalonnage du logiciel Bruker DataAnalysis 5.0.

3.1.2. Attribution de formules brutes

Une fois le spectre de masse correctement étalonné, l'étape suivante est l'attribution des formules brutes pour chaque pic détecté. La méthode introduite ici est celle utilisée par le logiciel de traitement DataAnalysis de Bruker®.

Pour réaliser l'attribution d'un pic, le logiciel va venir générer toutes les combinaisons d'éléments possibles répondant aux critères imposés par l'utilisateur (rapport H/C, nombre d'insaturations, configuration électronique...). Par défaut, seuls les éléments C, H, N et O sont considérés. L'utilisateur peut définir des seuils inférieurs et supérieurs pour chaque élément ou ajouter d'autres éléments. Cependant, si trop d'éléments sont pris en compte il y a un risque important d'obtenir des erreurs d'attribution car le nombre de combinaisons possible en accord avec une masse donnée augmente très fortement ([Kind and Fiehn 2007 \[56\]](#)).

Ensuite, il va sélectionner les formules brutes qui ne dépassent pas la déviation imposée, qui est généralement inférieure à 1 ppm avec un FTICR. Enfin, si plusieurs formules sont encore possibles, le logiciel va venir comparer le massif isotopique des espèces avec les pics présentes sur le spectre. Celle qui possède le meilleur *fitting* est sélectionnée. Cette étape est réalisée sur chaque pic présent dans le spectre de masse.

Afin de vérifier la qualité des données enregistrées, l'utilisateur peut générer un graphique représentant l'erreur (en ppm) en fonction de la masse pour chaque espèce attribuée. La **Figure 37** présente le diagramme d'erreur pour l'attribution d'un spectre de Tholins. On peut y voir que les erreurs des espèces sont centrées aux alentours de 0 ppm oscillent entre 0.15 et -0.15 ppm ce qui signifie que l'étalonnage est juste et précis. Cela traduit pour la justesse un bon étalonnage et pour la précision des signaux très bien résolus et avec des formes symétriques le long de gamme de masse.

Une fois toutes ces étapes de traitement effectuées et validées, il est possible de visualiser les données grâce à des traitements graphiques, dont une partie est présentée dans la section suivante.

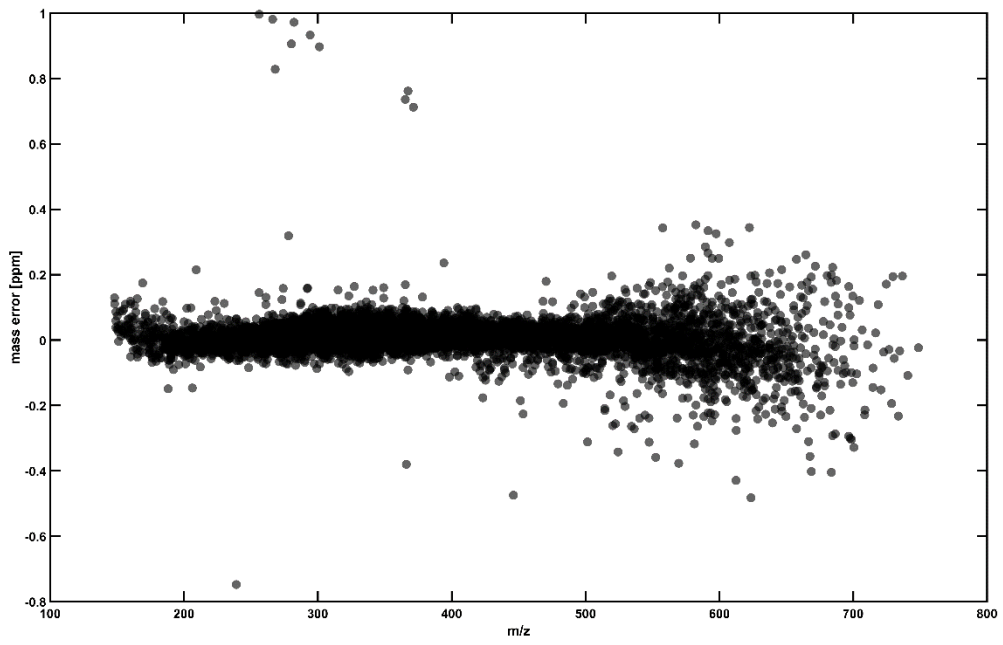


Figure 37 : Diagramme d'erreur d'un échantillon de Tholins.

3.2. Visualisations graphiques

Cette dernière partie du chapitre deux s'intéressera à la présentation des différents traitements graphiques utilisés durant cette thèse. Par traitement graphique, il est entendu figure graphique permettant de récupérer beaucoup plus facilement des informations sur le mélange étudié plutôt que par lecture direct du spectre de masse ou de mobilité ionique. Ce n'est donc pas une analyse en elle-même, et toutes les informations obtenues grâce à ce traitement sont déjà présentes dans les spectres. Cependant, c'est généralement un moyen communément utilisé par les scientifiques pour visualiser sur des graphiques à deux ou trois dimensions des données très complexes. Dans cette partie seront donc présentés dans l'ordre : Le diagramme de Van Krevelen, le diagramme DBE vs #C et enfin le CCS vs m/z .

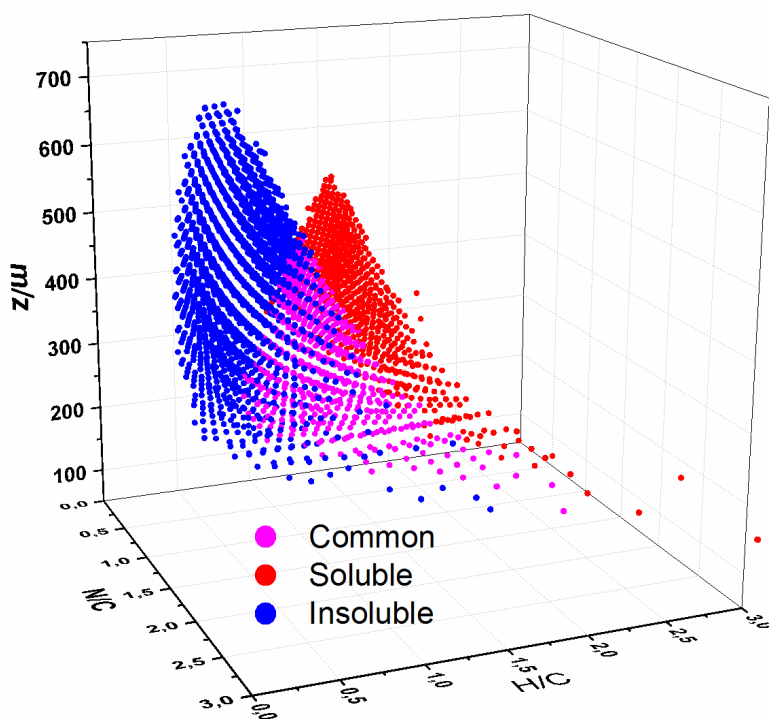


Figure 38 : Diagramme tridimensionnel avec $x = H/C$, $y = N/C$ et $z = m/z$.

3.2.1. Le diagramme de van Krevelen

Proposé la première fois en 1953 par van Krevelen, ce graphique a été développé pour étudier l'avancement de la transformation du bois en charbon ([Van Krevelen 1950 \[57\]](#)). En effet, en regardant le ratio hydrogène sur carbone en fonction du ratio oxygène sur carbone obtenus grâce à une analyse élémentaire, il était possible de voir l'avancement de la transformation comme présenté dans la **Figure 39**.

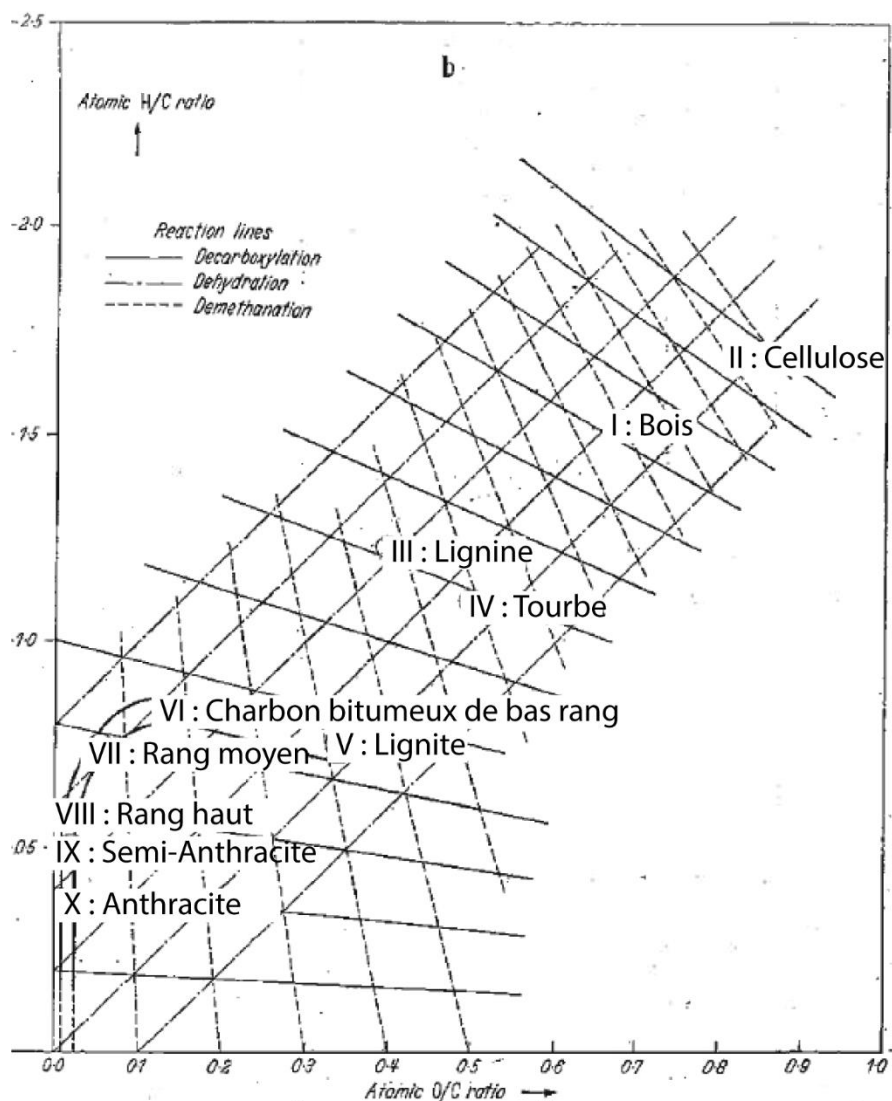


Figure 39 : Premier diagramme de van Krevelen (adaptée de ([Van Krevelen 1950 \[57\]](#)))

Aujourd'hui, ce diagramme est toujours utilisé pour l'analyse de mélanges organiques complexes comme le pétrole. Cependant, il requiert d'avoir au préalable fait l'attribution des formules brutes des espèces détectées dans le spectre de masse. D'autres diagrammes, comme celui de Kendrick permettent de s'affranchir de l'attribution de formules brutes mais ils ne seront pas présentés dans cette partie ([Kendrick 1963 \[58\]](#)).

Les mélanges complexes révélant généralement des milliers de signaux sur un spectre de masse sont difficiles à interpréter directement. Pour remédier à cela, on peut générer un diagramme de van Krevelen. Ce graphique va permettre, dans ce cas, de trier les ions détectés en fonction de leur quantité d'hydrogène et d'oxygène et ainsi pouvoir jauger le mélange. Dans le cas de molécules riches en azote comme les Tholins, une modification de ce graphique est généralement utilisée en remplaçant l'axe des abscisses par le rapport Azote sur Carbone (N/C). C'est ce Van Krevelen modifié qui a été pris pour exemple dans la **Figure 40**.

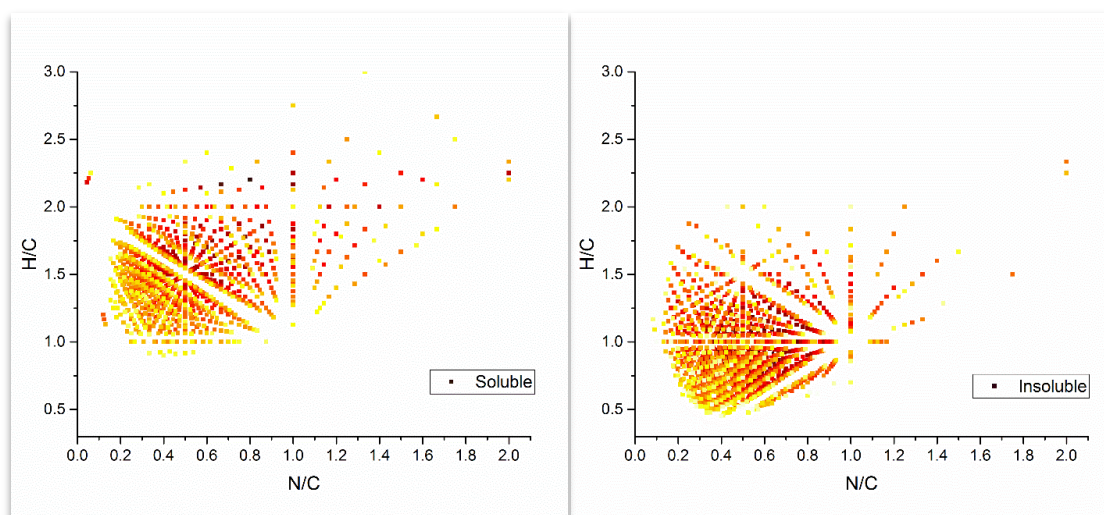


Figure 40 : Diagramme modifié de van Krevelen d'échantillons de Tholins. (droite) fraction soluble, (gauche) fraction insoluble ([Maillard, et al. 2018 \[59\]](#)).

Sur cet exemple, deux diagrammes de van Krevelen modifiés sont présentés. Chacun présentant les espèces d'un échantillon différent. Le code couleur est lié à l'intensité des signaux.

La première chose que l'on peut noter si l'on regarde le rapport N/C, c'est que les deux échantillons sont centrés sur la même position à 0,5. Cette information nous permet de dire que les échantillons semblent contenir le même nombre d'azote.

Si l'on regarde maintenant suivant l'axe de ordonnées (rapport H/C), nous pouvons voir que les espèces de l'échantillon de gauche se trouve plus haut sur l'axe que celles de l'échantillon de droite. Cela signifie que l'échantillon de gauche est plus saturé que l'autre. C'est donc un des points sur lequel ces deux échantillons sont différents.

Ainsi, simplement par lecture graphique montrée par l'exemple précédent, il a été possible de mettre en lumière facilement les différences entre les deux échantillons étudiés.

3.2.2. Le diagramme de DBE versus nombre de carbone

La notion de *Double bond equivalent* ou équivalent en double liaison a été discutée pour la première fois par McLafferty en 1993 ([McLafferty and Turecek 1993 \[60\]](#)). Cette DBE est définie par l'Équation 19:

$$\text{Équation 19} \quad \text{DBE} = c - \frac{h}{2} + \frac{n}{2} + 1$$

Où **C** est égale au nombre de carbone de la formule brute, **N** son nombre d'azote et **H** son nombre d'hydrogène. Par la suite, elle a été adaptée pour la spéciation du soufre dans le pétrole en créant un graphique représentant la DBE en fonction du nombre de carbone pour une famille de composé donné ([Purcell, et al. 2007 \[61\]](#)). Aujourd'hui, ce graphique est couramment utilisé en routine pour comparer des échantillons et jauger leur aromaticité ([Cho, et al. 2011 \[62\]](#), [McKenna, et al. 2010 \[63\]](#), [Marshall and Rodgers 2008 \[64\]](#)). La **Figure 41** présente deux graphiques DBE vs #C dans lequel a été rajouté la famille des hydrocarbures polycyclique (HAP, symbolisé par la ligne rouge). Cette famille est très souvent ajoutée à ce type de graphique car elle représente la famille de composé la plus aromatique ne contenant que du carbone et de l'hydrogène. Dans cette exemple, il est très facile de voir que la famille de composés aromatiques vient se coller à la ligne des HAP alors que celle de composés saturés s'en éloigne. C'est une façon rapide et visuel de comparer deux échantillons.

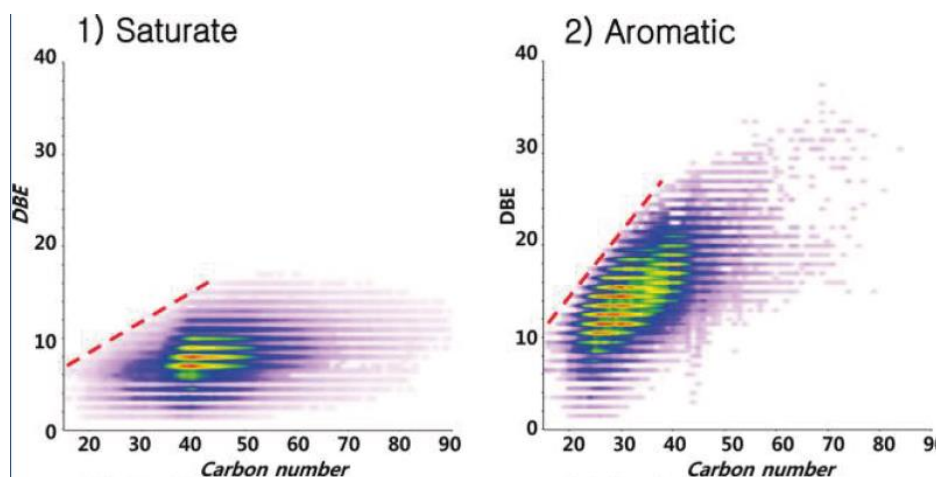


Figure 41 : DBE vs #C d'une famille de composé saturé (1) et aromatique (2) ([Cho, et al. 2011 \[62\]](#)).

3.2.3. Les diagrammes de mobilité ionique

Récemment, avec la popularisation de la mobilité ionique couplée à la spectrométrie de masse, de nouveaux diagrammes ont été imaginés pour répondre aux besoins de visualisation rapide des données. En effet la mobilité ionique permet de récupérer, en plus du rapport m/z , la section efficace de collision pour chaque espèce détecté dans un mélange.

Récemment, deux équipes ont publié des travaux en proposant deux façon de visualiser la donnée IMS en fonction de la masse ([Ponthus and Riches 2013 \[65\]](#), [Lalli, et al. 2015 \[66\]](#)). Cela a permis d'incorporer les isomères dans les visualitions classiques. Deux exemples de graphiques sont illustrés par la **Figure 42**.

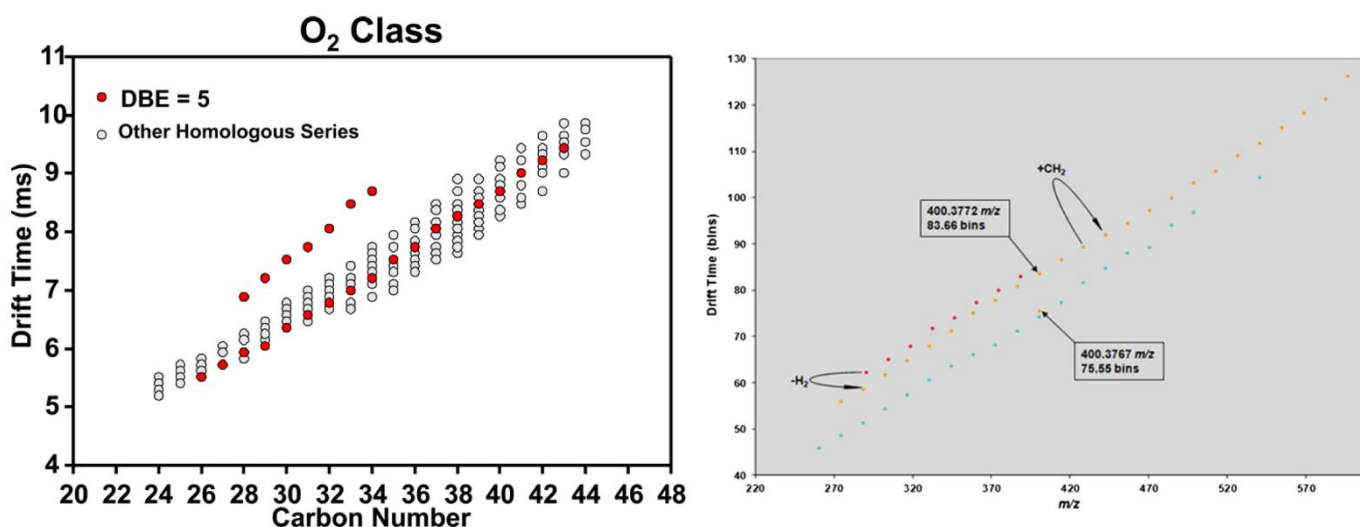


Figure 42 : Temps de dérive en fonction du nombre de carbone ([Lalli, et al. 2015 \[66\]](#)) et temps de dérive en fonction du rapport m/z ([Ponthus and Riches 2013 \[65\]](#))

Une autre façon de visualiser les données d'IMS-MS est de représenter, pour chaque espèce détectée, la CCS en fonction du rapport masse sur charge. Ce diagramme est illustré par la **Figure 43**.

Sur ces graphiques il est possible d'ajouter plusieurs familles de composés dont la CCS est connue afin de situer la conformation préférentielle du mélange étudié.

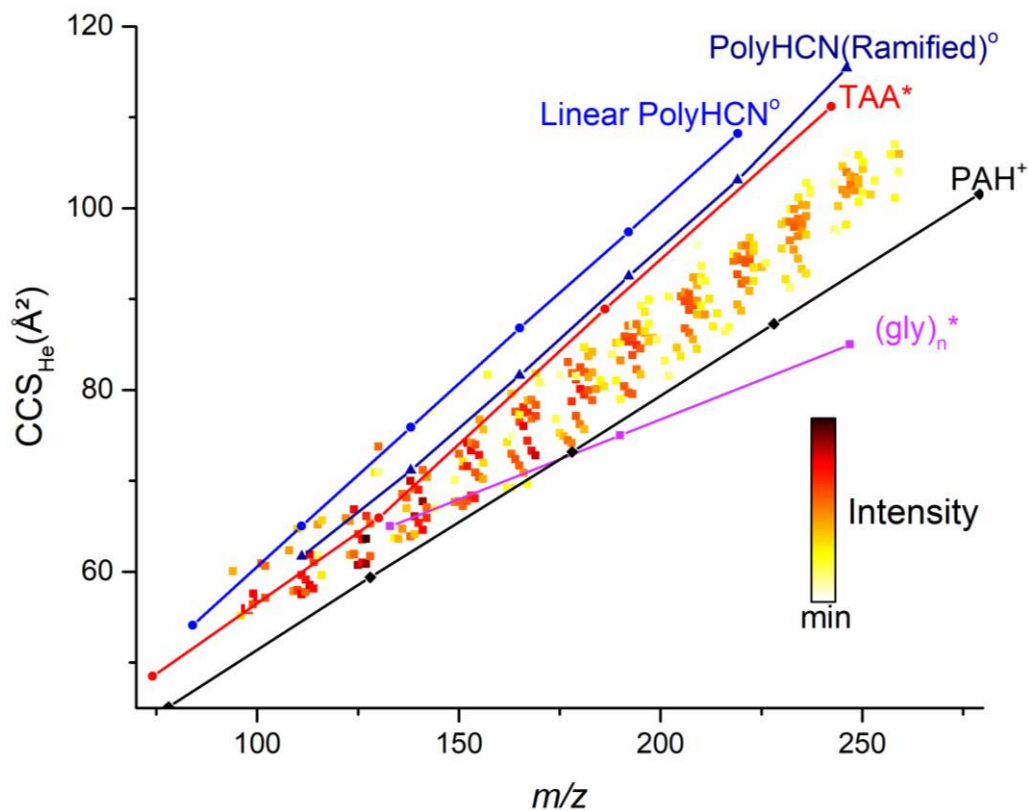


Figure 43 : Diagramme représentant la CCS en fonction du rapport m/z d'un échantillon de Tholins. Les lignes représentent des familles de molécules particulières ajoutées pour faciliter l'interprétation.

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Chapitre 3

Composition chimique globale des Tholins par analyse en spectrométrie de masse à ultra-haute résolution

Sommaire

3.1 : Préparation pour l'analyse : Optimisation de la cellule ICR

3.2 : Oxydation des Tholins

3.3 : Comparaison des fractions insoluble et soluble dans le methanol

3.4 : Conclusions et perspectives

Introduction :

Dans ce chapitre je présenterai les résultats obtenus par l'analyse en spectrométrie de masse à ultra-haute résolution des échantillons de Tholins de PAMPRE. Bien que ces Tholins aient été analysés par spectrométrie de masse dans des études antérieures, comme présenté dans l'introduction (**Chapitre 1**), les développements instrumentaux et méthodologiques récents permettent aujourd'hui d'aller plus loin dans la caractérisation de cette matière organique très complexe. Il nous a semblé pertinent de mettre en œuvre ces nouvelles approches pour mieux appréhender la structure de Tholins. Dans un premier temps, dans le but d'obtenir les meilleures caractéristiques instrumentales, j'ai appliqué une méthodologie d'optimisation au *shimming* de la cellule Paracell du FTICR de Rouen. Ces travaux ont notamment amélioré significativement la gamme dynamique, la résolution et la précision de mesure du FTICR. Cela a permis pour une même analyse l'identification de plus de molécules de faible intensité, notamment à haute masse. Ces résultats sont reportés dans la première partie de ce chapitre par l'**article 1** ([Maillard, et al. 2019 \[1\]](#)).

Grâce aux résultats obtenus par analyse FTICR, il a été possible d'analyser finement les évolutions se produisant dans les Tholins durant leur vieillissement à l'air. En effet, il a pu être observé que pour résoudre les espèces supérieures à m/z 600, une résolution d'au moins 1 500 000 était nécessaire afin de séparer les molécules $C_xH_yN_z$ des isotopes présents dans cette région. J'ai eu l'occasion d'effectuer des expériences sur un FTICR 21 Tesla en Floride en analysant des Tholins oxydésoxydés à l'air libre pendant des durées allant de 1 mois à 3 années. Ce FTICR qui est équipé du plus gros aimant au monde délivre des résolutions supérieures à 2 500 000 à m/z 600 et une gamme dynamique sans précédent. Cela m'a permis d'identifier l'impact de l'oxygène sur ces composés qui pourraient être représentatifs d'aérosols de la terre primitive. Ces résultats sont reportés dans la seconde partie de ce chapitre par l'**article 2** ([Maillard et al. 2020b](#)).

Enfin, le FTICR de Rouen est équipée d'une source LDI (ionisation par désorption/ionisation laser), ce qui permet l'analyse d'échantillons sous forme solide et permet donc l'analyse de molécules insolubles. Cette possibilité d'ionisation m'a ouvert un nouveau champ d'étude sur les Tholins. En effet, seule la fraction soluble des Tholins a été analysée auparavant en utilisant des ionisations de type *electrospray*. Faute de mieux, la composition de la fraction insoluble était donc assimilée à celle de la fraction soluble. Je me suis donc posé la question de savoir ce qui différenciait les fractions soluble et insoluble dans le méthanol des Tholins. J'ai donc réalisé l'analyse des deux fractions soluble et insoluble, ainsi que de la fraction totale par LDI en utilisant une approche sans solvant. J'ai découvert d'importantes différences entre les deux fractions et le fait que la fraction totale était largement dominée par la composition chimique de la fraction insoluble. Ces résultats sont reportés dans la troisième partie de ce chapitre par **l'article 3 (Maillard et al. 2018)**.

3.1. Préparation pour l'analyse : Optimisation de la cellule ICR

Cette première partie présente une méthodologie d'optimisation du *shimming* d'une cellule ICR harmonisée de façon dynamique (Paracell[®]) réalisée lors de la première année de ce doctorat. La volonté d'améliorer les performances du FTICR de Rouen m'a poussé à m'intéresser aux paramètres permettant l'ajustement de la position du nuage d'ions piégé dans la cellule ICR. Les résultats obtenus sont présentés sous la forme d'un article publié en 2019 dans le journal *Rapid communication in mass spectrometry*.

La cellule Paracell est équipée d'électrodes en forme de feuille permettant d'imposer des différences de potentiels entre le haut et le bas de la cellule ainsi qu'entre la gauche et la droite. Ces électrodes ont été ajoutées afin de compenser l'alignement imparfait du spectromètre de masse par rapport au champ magnétique. Cela permet ainsi, en jouant sur ces potentiels, de déplacer le centre du nuage d'ions de quelques millimètres afin que celui soit parfaitement aligné avec le champ magnétique. En pratique, cela permet d'avoir les performances optimales en termes de gamme dynamique, résolution et précision de mesure. Cependant, ce réglage était, jusqu'à aujourd'hui, réalisé à la main. Nous avons donc décidé de réaliser un plan d'expérience assisté par informatique afin de cartographier les effets de chaque potentiel sur le nuage d'ions. Une fois les meilleurs réglages trouvés, nous avons comparé un spectre de Tholins avant, avec optimisation manuelle et après optimisation par plan d'expérience. Les performances analytiques ont été nettement améliorées entre l'optimisation manuelle et celle par plan d'expérience. Ce travail a permis notamment d'obtenir la meilleure résolution parmi les FTICRs européens avec une valeur de 15 000 000 à m/z 560.

Abstract:

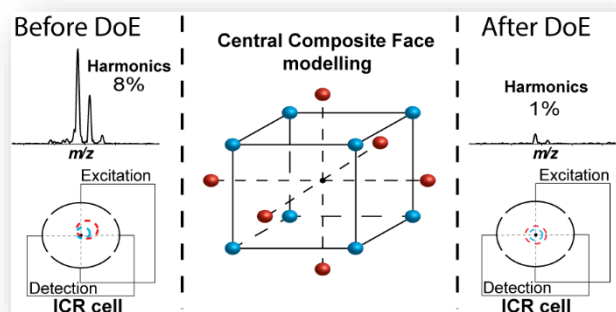
The optimization of the ion trajectories in the ion cyclotron resonance (ICR) cell of a Fourier transform ICR mass spectrometer is a crucial step to obtain the best dynamic range, mass resolution, and mass accuracy.

With the recent introduction of the dynamically harmonized cell, the complexity of tuning expanded drastically,

and a fine-tuning of the DC voltages is required to optimize the ion cloud movement. This adjustment is typically performed manually.

Here, we propose a computational method based on a design of experiments (DoE) strategy to overcome the limits of classical manual tuning. This DoE strategy was exemplarily applied on a 12T FTICR equipped with a dynamically harmonized ICR cell. The chemometric approach, based on a composite central face design (CCF), was first applied on a reference material (sodium trifluoroacetate) allowing for the evaluation of the primary cell parameters. Eight factors were identified related to shimming and gating. The summed intensity of the signal corresponding to the even harmonics was defined as one quality criteria.

Consequently, the DoE response allowed for rapid and complete mapping of cell parameters resulting in an optimized parameter set. The new set of cell parameters was applied to the study of an ultra-complex sample. Here, Tholins, an ultra-complex mixture that mimics the haze present on Titan, was chosen. We observed a substantial improvement in mass spectrometric performance. The sum of signals related to harmonics was decreased by a factor of three (from 4% for conventional tuning to 1.3%). Furthermore, the dynamic range was also increased and lead to an increase of attributed peaks by 13%.



3.1.1. Introduction

Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) is well known to be the most powerful mass spectrometer by reaching unprecedented resolving power, mass accuracy and sensitivity levels([Smith, et al. 1994 \[2\]](#) ,[Nikolaev, et al. 2011 \[3\]](#)). It turned out to be the most appropriate instrument for the characterization of ultra-complex mixtures such as petroleum([McKenna, et al. 2010 \[4\]](#) ,[Smith, et al. 2018 \[5\]](#) ,[Rodgers and McKenna 2011 \[6\]](#) ,[Hughey, et al. 2002 \[7\]](#) ,[Marshall and Rodgers 2008 \[8\]](#)), natural organic matter ([Li, et al. 2019 \[8\]](#))([Li, et al. 2019 \[9\]](#)) or extraterrestrial organic matter ([Schmitt-Kopplin, et al. 2010 \[10\]](#) ,[Schmitt-Kopplin, et al. 2012 \[11\]](#)). [ENREF 1](#) In order to detect the cyclotron motion, FTICR uses the combination of a static electric field which is hyperbolic near the center and a strong homogeneous static magnetic field (up to 21 Tesla – so far). The combination of both fields retains ions in the cell. Ions in the magnetic field travel according to a cyclotron motion defined by **Équation 20**:

$$\omega_0 = \frac{qB_0}{m}$$

Équation 20 : :Cyclotron frequency (ω_0) of a charged particle in a homogeneous magnetic field. q is the charge of the particle, B_0 the strength of the magnetic field and m the mass of the particle.

In addition, the ion packet undergoes a drift of a trajectory equal to $\mathbf{E} \times \mathbf{B}$ where E is the electric field and B is the magnetic field. Both of these movements allow the ion packet to be trapped in the cell([Nikolaev, et al. 2011 \[3\]](#) ,[Jertz, et al. 2015 \[12\]](#)).

The shape of the ICR cell was improved steadily over time from first cubic geometry([Guan and Marshall 1995 \[12\]](#)) to a variety of spherical designs varying in electrode shape and number ([Marshall, et al. 1998 \[13\]](#)).

The shape of the ICR cell was improved steadily over time from first cubic geometry ([Guan and Marshall 1995 \[13\]](#)) to a variety of spherical designs varying in electrode shape and number ([Marshall, et al. 1998 \[14\]](#)).

As an example, the orthorhombic capacitively coupled cell developed by Steven Beu in 1991 ([Beu and Laude 1992 \[14\]](#)) or the infinity cell which was designed by Caravatti et al. in 1991 are cited here ([Caravatti and Allemann 1991 \[15\]](#)), the later remaining the most commonly used nowadays . In recent instruments, the so-called dynamically harmonized cell is frequently deployed ([Nikolaev, et al. 2011 \[2\]](#)). For the purpose of radiofrequency excitation and detection of ions, the cell is composed of several electrodes. In case of the dynamically harmonized cell, these excitation and detection electrodes are specially shaped to compensate the quadrupolar electric field over the entire cell's body. It allows to produce a volume where, over the orbit of the ions, the average field is quadrupolar at all cyclotron radii. Thus, the frequency drifts of ions of the same mass over time along the Z axis are reduced. This makes possible to record transients for a longer time than in the previous cells and thus significantly improve spectral performance. Optimized detection in terms of highest resolving power, sensitivity and dynamic range is obtained when the ion cloud is perfectly centered in the middle of the magnet. However, in

practice, the imperfections of the alignment cause a bias in the position of the ion packets and thus a deviation in the cyclotron motion synchronization. This issue is solved by adding another set of segments to the dynamically harmonized cell in order to smooth imperfections by applying additional DC (Direct Current) voltages ([Jertz, et al. 2015 \[11\]](#)), ([Jertz, et al. 2015 \[12\]](#)). With this approach, it is possible to decrease the abundance of even-harmonic signals and, thus, to minimize magnetron motion. However, the values for the additionally applied DC voltages have to be tuned manually, which requires specially trained personnel and is tedious and time-consuming. In the present work, an experimental design approach is proposed in order to overcome the drawbacks of the manual ICR cell tuning.

The approach seeks to optimize the DC voltages and reach the best set of parameters. Design of experiments (DoE) is a rigorous procedure in which defined modifications are induced based on a given set of variables. The response of the system is recovered to create a mathematical model, which allows a prediction of a set of parameters optimizing the response value ([Gerretzen, et al. 2015 \[17\]](#) ,[Eliasson, et al. 2012 \[18\]](#) ,[Eriksson 2008 \[19\]](#)).

Some example of mass spectrometer's optimization using DoE can be cite, such as the work performed by Lemonakis et al (2016) and Tebani et al (2016) ([Lemonakis, et al. 2016 \[20\]](#) ,[Tebani, et al. 2016 \[21\]](#)). Here, this method is applied on a defined response, the sum of the abundance of the second harmonic of sodium trifluoroacetate cluster.

After optimization, the obtained parameters are applied to the analysis of a complex mixture, Tholins. This synthetic material is used to understand the chemistry occurring in Titan's atmosphere by artificially mimicking the brown haze surrounding the largest moon of Saturn([Khare, et al. 1981 \[22\]](#) ,[Coll, et al. 1999 \[23\]](#) ,[Somogyi, et al. 2005 \[24\]](#) ,[Szopa, et al. 2006 \[25\]](#) ,[Imanaka and Smith 2010 \[26\]](#)). More importantly, Tholins have shown to be ultra-complex mixtures with high isobaric complexity and thousands of elemental compositions

([Maillard, et al. 2018 \[26\]](#)),([Maillard, et al. 2018 \[27\]](#)). Tholins spectra obtained after DoE and after the usual manual method are compared. The potential of the developed method for complex mixtures analysis is highlighted evaluating the mass spectrometric performance, i.e., resolving power, the abundance of harmonics and number of detected signals.

3.1.2. Experimental methods

3.1.2.1. Tholins production

Tholins were produced following the PAMPRE procedure (French acronym for Aerosols Microgravity Production by Reactive Plasmas) presented elsewhere([Szopa, et al. 2006 \[24\]](#) ,[Gautier, et al. 2011 \[27\]](#)),([Szopa, et al. 2006 \[25\]](#) ,[Gautier, et al. 2011 \[28\]](#)). Briefly, in a stainless steel tubular reactor, a continuous gas mixture composed of 95 v-% nitrogen and 5 v-% methane was injected through polarized electrodes and evacuated by a primary vacuum pump system. A Radio Frequency – Capacity Coupled Plasma (RF-CCP) discharge was established in the gas mixture deploying an RF of 13.56 MHz. The pressure in the plasma discharge was maintained at 0.9 ± 0.1 mbar, and the reaction took place at room temperature (293 K).

3.1.2.2. Instrumentation

Fourier transform ion cyclotron resonance mass spectrometer

All analyses were performed on a FTICR Solarix XR from Bruker equipped with a 12T superconducting magnet and a dynamically harmonized ICR cell. The following ion transfer parameters were used for both electrospray (ESI) and laser desorption ionization (LDI) analyses in positive ion mode: capillary exit 150V, deflector plate 200V, funnel1 150V, skimmer1 25V, funnel RF amplitude 60Vpp, octopole frequency 5MHz, octopole RF amplitude 350Vpp, lower cut-off of the quadrupole at m/z 120, time-of-flight 0.7ms, frequency TOF 6MHz, TOF RF amplitude 200Vpp, side kick offset -1V, front and back trapping plate 1.75V.

For ESI the following parameters were used: capillary 4.5kV, spray shield -500V, dry gas flow 2L.min⁻¹, dry gas temperature 180°C, nebulizer gas flow 0.5bar. All analyses were recorded on 20 scans with a quadrupole accumulation time of 0.1s.

Electrospray spectra were recorded with a mass range from m/z 98 to 1,200 and a transient length of 2.2s. Sodium trifluoroacetate (NaTFA, Sigma Aldrich) at 0.1mg.ml⁻¹ in ACN/H₂O (50/50, v/v) was used as a standard for the design of experiments approach.

Complex Tholins mixture analyses was recorded applying LDI. The solid sample was deposited on a MALDI plate following previous work([Barrere, et al. 2012 \[29\]](#)). The third harmonic of a Nd:YAG laser at 355nm delivering a maximum output of 0.5mJ (Smartbeam II, Bruker) was used to ionize the samples with the following parameters: laser power 15%, laser shots per scan 50, shots frequency 1kHz, plate offset 100V. The recorded mass spectra combined 800 scans to achieve high signal-to-noise. LDI spectra were recorded with a mass range from m/z 110 to 1200 and a transient length of 4.3s.

3.1.2.3. Experimental design

Software

DataAnalysis 4.4 (Bruker Daltonics GmbH, Bremen) was used to process all mass spectrometric analyses, including m/z -calibration and response recovering (abundance of the harmonic signals).

MODDE 11 software (Umetrics, Sartorius Stedim Data Analytics AB, Umea, Sweden) was used to perform the consecutive data analyses and modeling. The initial step is the definition of the response (i.e., user observation). Then, in a second step, factors influencing the response are defined. Afterward, several choices of experimental design shapes can be chosen. The software will automatically create the DoE given to the user as a coded matrix (**Table 1**) and a real matrix (**Table S9**).

This matrix defines the experiments to be recorded and from which the response has to be extracted. Then, MODDE propose an automatic fitting method (e.g., partial least squares regression - PLS) allowing the visualization of the quality and the predictability of the DoE. Finally, the optimizer tool was used to predict the set of parameters giving the best response result.

Factorial designs

The tuning of the ICR cell is established with eight principal parameters, four shimming parameters (shimming 0°, shimming 180°, shimming 90° and shimming 270°) and four gating settings (gated 0°, 90°, 180°, and 270°) ([Jertz, et al. 2015 \[11\]](#)) [ENREF 23](#). ([Jertz, et al. 2015 \[12\]](#)) [ENREF 23](#). The set of gating parameters represents the values of DC voltages applied during the injection in the ICR cell in order to correctly trap the ion packets.

The set of shimming parameters represents the values of DC voltages applied for the excitation and the detection of the ions packets in the ICR cell. A simplified description of the ICR cell is given in **Figure 44**. Other parameters of the ICR cell are not included (e.g., back and front plate voltages, sidekick offset) as they are easily and rapidly manually tunable.

This significantly reduces the number of experiments required for the DoE approach, considerably decreasing the optimization time.

Parameters were grouped in pairs leading to four couples of parameters: shimming 0° - 180° (Shi0), shimming 90° - 270° (Shi90), gated 0° - 180° (Gat0) and gated 90° - 270° (Gat90) simplifying the optimization process compared to the conventional manual procedure. The values of these couples change symmetrically, meaning that when the value of one part is increased, the other part decreases about the same value.

Initially, all values are set to 1.5V (default settings). As example, increasing the value of the shimming 0° about 50mV to 1.55V results at a value of the shimming 180° of 1.45V.

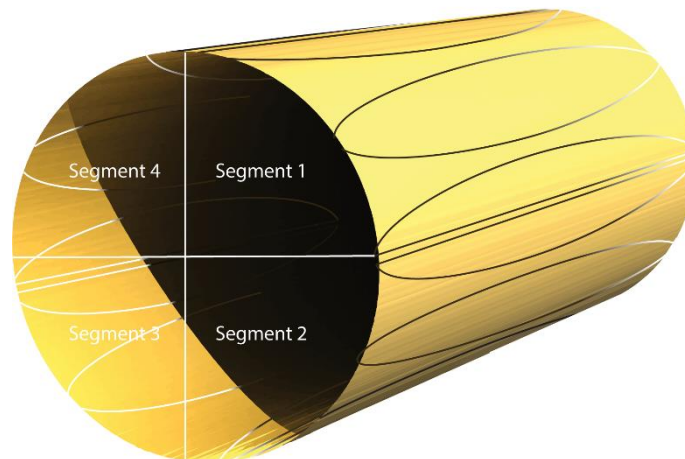


Figure 44 : Schematic representation of a dynamically harmonized ICR cell. The cell is divided into four segments representing each adjustable parameter: Segment 1 (in dashed lines) is for shimming and gating 0° , segment 2 (in solid lines) for shimming and gating 90° , segment 3 for shimming and gating 180° and segment 4 for shimming and gating 270° .

In addition to these four coupled parameters, the sweep excitation power (SEP) was added as a factor parameter for the first step of the optimization strategy. SEP has a crucial impact on the

ion trajectories as it defines their radius. After defining the assessed experimental factors, it was necessary to define the desired response as a result of the optimization.

Table 1 : Coded composite face design (CCF) design matrix for experimental screening on NaTFA

Run N°	Run Order	Shi0	Shi90	Gat0	Gat90	SEP
1	10	-1	-1	-1	-1	1
2	28	1	-1	-1	-1	-1
3	23	-1	1	-1	-1	-1
4	9	1	1	-1	-1	1
5	1	-1	-1	1	-1	-1
6	24	1	-1	1	-1	1
7	20	-1	1	1	-1	1
8	12	1	1	1	-1	-1
9	29	-1	-1	-1	1	-1
10	8	1	-1	-1	1	1
11	6	-1	1	-1	1	1
12	5	1	1	-1	1	-1
13	25	-1	-1	1	1	1
14	16	1	-1	1	1	-1
15	26	-1	1	1	1	-1
16	17	1	1	1	1	1
17	2	-1	0	0	0	0
18	13	1	0	0	0	0
19	15	0	-1	0	0	0
20	19	0	1	0	0	0
21	4	0	0	-1	0	0
22	14	0	0	1	0	0
23	18	0	0	0	-1	0
24	22	0	0	0	1	0
25	3	0	0	0	0	-1
26	7	0	0	0	0	1
27	27	0	0	0	0	0
28	31	0	0	0	0	0
29	30	0	0	0	0	0
30	11	0	0	0	0	0
31	21	0	0	0	0	0

Following the classical optimization method, the intensity ratio between the ion of the NaTFA at m/z 702.86324 and its second harmonic at m/z 351.43164 was recovered. The optimization of the dynamically harmonized cell parameters was divided into two steps. The first screening step was performed with wide parameter intervals to evaluate the effects of each parameter on

the defined response. The second part, named optimization experimental design, focused on narrower intervals to reach the best set of parameters.

The applied chemometric approach for the two steps is based on a Composite Central Composite face design (CCF) which takes into account interactions between parameters. The coded CCF for the first step is given in **Table 1** formatted according to the required software input.

Basically, the table reflects the conducted experimental plan, where -1 means that the parameter was set at the respective low level (e.g., for Shi 0 at 1.465V) and +1 at the respective high level. Defined intervals for each parameter are given in **Table 2** for the screening and optimization step. These intervals have been defined quickly by varying each of the parameters until the intensity of the harmonic reaches a minimum level. This step takes about 5 minutes.

Table 2 : Predicted values for each factor for the screening and optimization step

Screening step			
Factor	Intervals	Predicted Value	Factor contribution (%)
Shim0	1.465 to 1.535 (V)	1.535V	15.4
Shim90	1.465 to 1.540 (V)	1.540V	33.3
Gat0	1.4 to 1.620 (V)	1.620V	12.6
Gat90	1.450 to 1.550 (V)	1.550V	10.7
Sweep	20 to 40 (%)	20%	28.0

Optimization step			
Factor	Intervals	Predicted Value	Factor contribution (%)
Shim0	1.530 to 1.550 (V)	1.542V	53.1
Shim90	1.530 to 1.550 (V)	1.550V	43.4
Gat0	1.600 to 1.640 (V)	1.600V	1.9
Gat90	1.540 to 1.570 (V)	1.551V	1.7

Multivariate modeling

Partial Least Squares regression (PLS) was used to model each CCF design and to find the best set of parameters regarding the response chosen using the optimizer tool implemented in MODDE 11. Thus, PLS coefficients are used to estimate factor effects.

A positive value of the coefficient indicates an increase of the response upon increasing the corresponding parameter, whereas a negative value indicates a decrease of the response upon increasing the corresponding parameter. The larger the absolute value of the PLS coefficient, the higher the effect of the parameter on the response. Analysis of variance (ANOVA) applied to the sum of squares of the PLS regression coefficient was used to assess the significance of the factor effect. For a statistically significant effect of a factor, the model p -value of ANOVA should not exceed 0.05([Montgomery 2008 \[29\]](#))([Montgomery 2008 \[30\]](#)).

3.1.3. Results and discussion

3.1.3.1. Screening experimental design using NaTFA

The design of experiments approach was performed using NaTFA to minimize the abundance of signals related to harmonics. Briefly, the influence of four pairs of factors and the SEP (Shi0, Shi90, Gat0, Gat90, and Sweep Excitation Power) was evaluated using an even harmonic peak intensity of the ion m/z 702.86324 at m/z 351.43164 as the response. A Central Composite Face design (CCF) was chosen as it is generally applicable for this type of optimization problems.

Figure 45a visualizes the effect of the different factors on the even harmonic abundance in the screening step. SEP, Shi0, and Shi90 revealed the most significant effects on the intensity of the second harmonic peak, with contribution factors of 28.0%, 15.4%, and 33.3% respectively. SEP played a positive effect with high values leading to high abundance of the harmonic.

In contrast, Shi0 and Shi90 showed a negative response, with high values resulting in low abundance of the harmonic. In addition, the interaction of Shi0 and Shi90 provided a significant positive effect, which is related to a high separation effect of these parameters. Hence these two couples of parameters have two very different effects on the abundance of the harmonic and, thus, on the ion cloud trajectories.

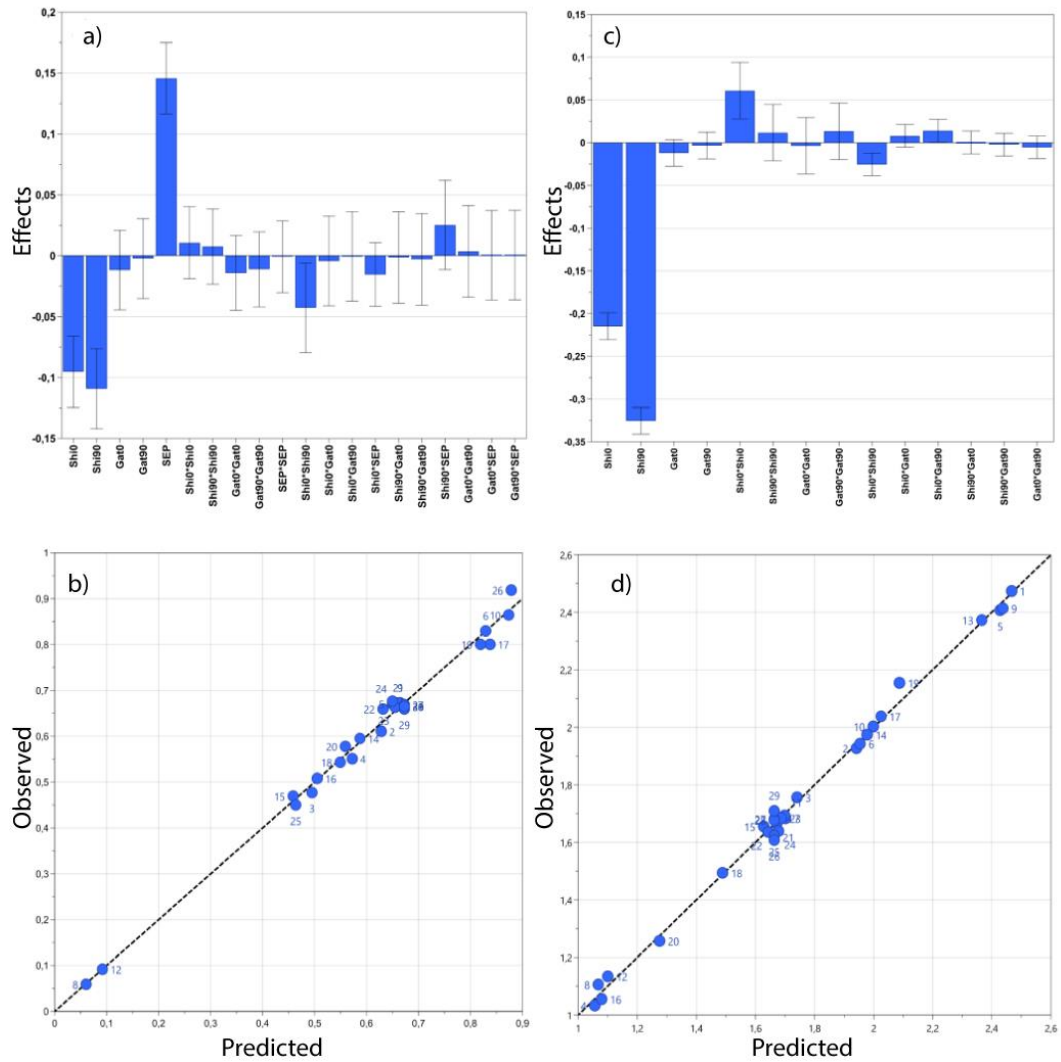


Figure 45 : **a)** Observation of the effects of the different factors on the even harmonic magnitude for the screening step, **b)** Prediction plot between the predicted and observed values of the second harmonic magnitude for the screening step, **c)** Observation of the effects of the different factors on the second harmonic magnitude for the optimization step, **d)** Prediction plot between the predicted and observed values of the second harmonic magnitude for the optimization step.

The prediction plot obtained based on the 27 experimental data points, between the predicted and observed values of the abundance of the second harmonic, is shown in **Figure 45b**. This representation allows to access the quality of prediction with this model based on the initial data set.

The cumulative modeled variation ($R^2X = 99.1\%$) and the cumulative predicted variation ($Q^2Y = 62.8\%$) reflect the explained variance and the predictability of the model. The near to one R^2X value proves the validity of the model. The Q^2Y value (which is above 50%) proves that the model can give predictable values of parameters for the defined response ([Eliasson, et al. 2012 \[18\]](#), [Eriksson 2008 \[19\]](#), [Montgomery 2008 \[30\]](#)). However, this value could be improved to obtain better optimization results, which will be the aim of the following part of the optimization experimental design. The values of the regression coefficients and their respective p -values are given in **Table S11** (Supplementary data). The fitted models' metrics, ANOVA and validation results of each model are presented in **Table S12** and **Table S13** (Supplementary data).

Using this built model, the instrumental parameters were optimized to reach the lowest harmonic intensity. The predicted optimized values are presented in **Table 2**. Nevertheless, the obtained set of predicted values reach the extremity of each defined interval. Therefore, an additional optimization step was performed focusing on tight intervals for Shi0, Shi90, Gat0, and Gat90. It should be noted that the SEP and shimming values are two decorrelated parameters. In the rest of this study, the SEP was fixed at a value of 30% in order to focus on fine tuning. This value is a percentage of the maximum excitation amplitude.

3.1.3.2. Optimization experimental design using NaTFA

As described above, for this step, the sweep excitation power (SEP) was removed from the studied factors to focus only on the cell parameters. It turned out, that Shi0 and Shi90 had a major negative response on the second harmonic abundance (**Figure 45c**), which is consistent with the results obtained in the previous screening step. Coded regression coefficients, ANOVA and model validation results are given in **Table S10**, **Table S13**, and **Table S14** (Supplementary data). The fitted model shows a cumulative modeled variation of $R^2X = 99.5\%$ and a cumulative predicted variation of $Q^2Y = 88.9\%$ as showed in **Figure 45d**.

These results improved compared to the screening step and proved the high predictability of the movement of ions in the dynamically harmonized cell. Using this built model, the optimized set of parameters provided an abundance of 1.3% of second harmonics compared to the base signal. In comparison, the non-optimized parameters (manufacturer default values) provided 8% and manually optimized parameters provided 4% of harmonic abundance, respectively.

This first study proved the possible optimization of the movement of ions in the ICR cell by lowering harmonic peaks. This DoE methodology was validated by the study of a NaTFA standard solution typically used for instrument calibration and optimization. However, the high interest of optimizing the ion trajectories in the ICR cell is related to the description of complex mixtures with unknown constituents. Consequently, the set of parameters yielded by the DoE methodology have been applied to study Titan Tholins. The results were compared to those obtained after the manual optimization.

3.1.3.3. Application for the analysis of complex mixtures: Titan Tholins

The largest moon of Saturn, Titan, is surrounded by a thick and nitrogen-rich fog with some fraction of methane([Niemann, et al. 2005 \[31\]](#) ,[Fulchignoni, et al. 2005 \[32\]](#) ,[Lebreton, et al. 2005 \[33\]](#) ,[Israel, et al. 2005 \[34\]](#)). Understanding how this haze is produced along with its molecular composition are crucial steps from a planetary and prebiotic chemistry perspective([Sagan 1974 \[34\]](#) ,[Sagan, et al. 2002 \[35\]](#)). Tholins are analogs of Titan's haze. They are laboratory-produced and used as a proxy on Earth as there is currently no sample return option. This material is an extremely complex mixture comparable to the complexity of petroleum and as such ideal for evaluating the tuning of the dynamically harmonized cell parameters([Maillard, et al. 2018 \[26\]](#)).. Understanding how this haze is produced along with its molecular composition are crucial steps from a planetary and prebiotic chemistry perspective([Sagan 1974 \[35\]](#) ,[Sagan, et al. 2002 \[36\]](#)). Tholins are analogs of Titan's haze. They are laboratory-produced and used as a proxy on Earth as there is currently no sample return option. This material is an extremely complex mixture comparable to the complexity of petroleum and as such ideal for evaluating the tuning of the dynamically harmonized cell parameters([Maillard, et al. 2018 \[27\]](#)).

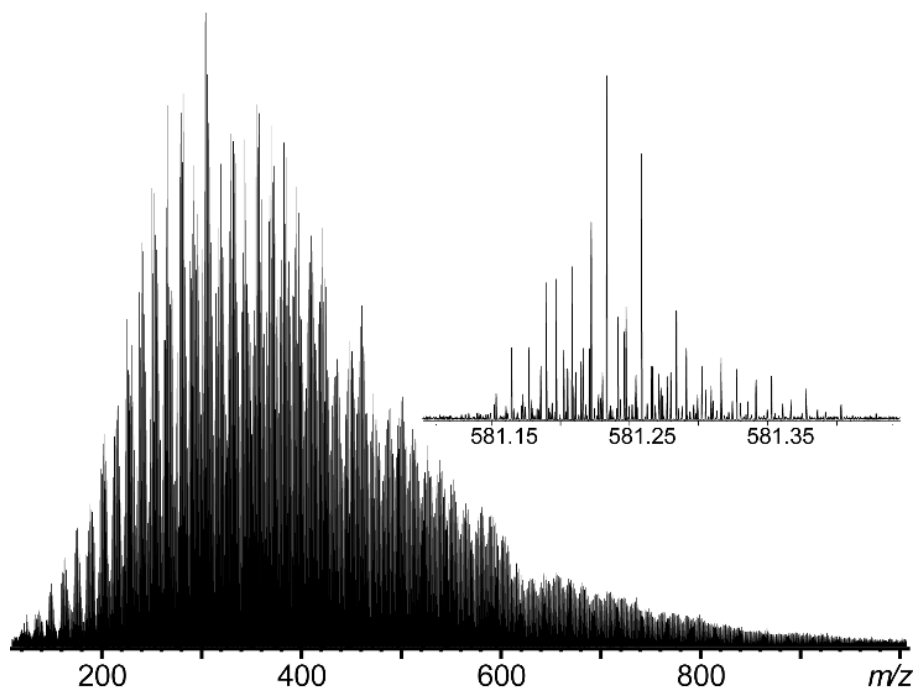


Figure 46 : LDI spectrum in positive mode of a Tholins sample obtained after deploying the cell parameter optimized with the DoE approach, revealing more than 50,000 peaks. The inset visualized a close zoom between m/z 581.15 and m/z 581.40 illustrating the high isobaric complexity.

Figure 46 shows an overview of a Tholins mass spectrum, containing more than 50,000 peaks. This mass spectrum was generated with LDI ionization together with the optimized cell tuning. In this study, we used this ultra-complex sample to compare the effects of the above-described optimization strategy with the conventional method relying on manual optimization.

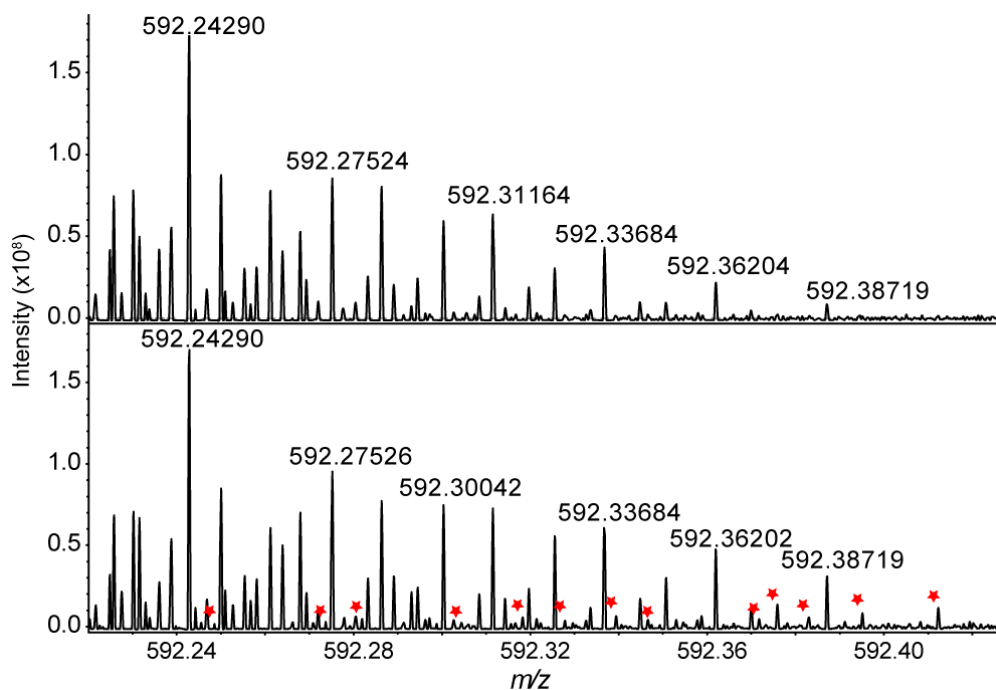


Figure 47 : (Top) Zoom on the Tholins spectrum obtained after a manual optimization (shimming) of the ICR cell parameters. (Bottom) Tholins spectrum obtained after applying the parameters given by the DoE optimization. Red dots indicate species found after DoE optimization and not detected after manual optimization.

The comparison between the detected species applying the parameters given by manual tuning and DoE optimization is illustrated in **Figure 47**. The mass spectrum at the top was recorded after the conventional manual optimization of the ICR cell. This manual optimization can take several hours easily to obtain high performance due to the manual iteration process. The spectrum at the bottom was recorded after deploying the optimized parameter set given by the newly developed DoE strategy, which takes approximately one hour. It should be mentioned that the shimming parameters are re-optimized once a year to compensate instrumental's drift. Except for the cell parameters, the two spectra were recorded with the exact same conditions. The number of detected species is significantly higher with the DoE optimization. **Figure 47**,

giving a small fraction of the mass spectrometric complexity revealed thirteen additional species (indicated with a red star) for the DoE optimized spectrum compared to the spectrum resulted after manual tuning. The attribution of these new peaks is given in **Table S15**.

This observation can be induced by two effects : 1) The ion packets is more centered in the cell thanks to the computational method, inducing a higher dynamic range or, 2) in the spectrum after manual adjustment, some peaks are in coalescence with their close neighbor. This effect is not present after the DoE tuning, which explain why more peaks are observed in this case. In order to reinforce this, through attributions, it can be justified that these peaks are not harmonics. Manual optimization is a difficult and limited process. Indeed, it is impossible for the human eye to take into account the interactions between the various parameters. Therefore, the location of the ions is not perfectly centered and could still be improved. Furthermore, this method is a time-consuming iterative process based on trial and error steps to reach acceptable values specified by the manufacturer (here below 6% for the second harmonic).

The above described DoE strategy, performed with NaTFA, allows a complete mapping of the different parameter effects along with their interactions, which allows modelling of the system. Thus, this strategy leads to a more fine-tuning and a greater reduction of harmonics compared to the conventional manual attempt.

Different metrics obtained for DoE originated and manually recorded spectra are listed in **Table 3**. These results were obtained after recording 5 replicates for each tuning. As illustrated in **Figure 47** applying the DoE generated parameters significantly increased the number of observed peaks. The comparison with the manually tuned cell revealed 13% more peaks attributed after the DoE tuning. The resolving power is also slightly increased after the experimental design optimization: approx. 1,440,000 with the DoE method and 1,350 000 with the manual method at m/z 400. A slight increase is also observed for the average mass accuracy of the elemental composition assignment. All these results are summarized in **Figure 48**.

The application of the new predicted dynamically harmonized cell parameters on Titan's Tholins showed a clear enhancement on almost every metric of the recorded spectrum in comparison

with the spectrum generated with the non-tuned cell but also with the manually tuned cell. Furthermore, this work will be useful for the implementation of quadrupolar ion detection, which commonly induces higher order harmonics with increased abundance. This newly developed optimization strategy is highly time efficient compared to the conventional method, taking only one hour including the recording of the spectra, data processing, and data analysis. It is a universal approach and can be applied to any complex mixture such as petroleum and biological sample materials. Those ultra-complex mixtures usually raise high analytical challenges regarding experimental optimization.

Table 3 : Comparison of the mass spectrometric response of the LDI Tholins spectra deploying manufacturer default values (before tuning), conventional manual tuning (manual), and parameters given by the DoE optimization method. p-values refers to the comparison between manual Tuning and the DoE strategy. (p-value > 0.05 is considered as significant)

	DoE (Mean)	Manual (Mean)	p-value	Fold Change
Attributed Peaks	1.76 10+04	1.55 10+04	9.69 10-05	1.13
Global Intensity	4.80 10+08	3.61 10+08	3.36 10-06	1.33
Resolution at m/z 400	1.44 10+06	1.35 10+06	1.24 10-07	1.07
RMS (ppm)	0.140	0.0654	2.18 10-06	2.14

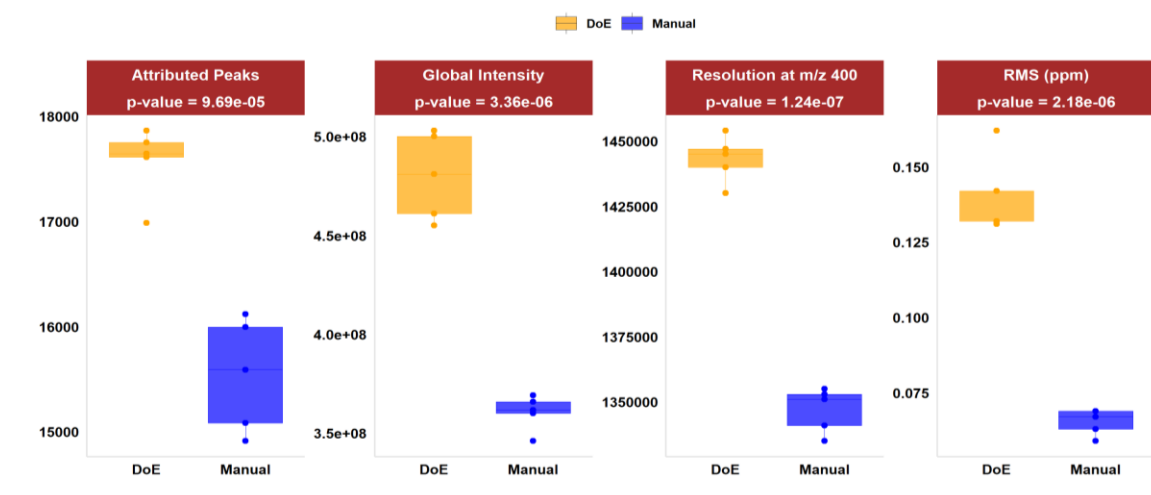


Figure 48 : Comparison of attributed peaks, global intensity, resolution and RMS between the manually optimized spectrum and the DoE spectrum of a Tholins sample.

3.1.4. Conclusions

The developed design of experiments (DoE) strategy has proven to be applicable for generating optimized parameter sets for a dynamically harmonized ICR cell. We could show that the approach results in settings leading to a reduction of harmonics to levels not reached by manual tuning and far below the manufacturer default values. Moreover, the optimization process takes significantly less time and can be performed by any instrument user. This substantial improvement allows for a better performance of the FTICR by significantly increasing all metrics of the recorded spectrum, i.e., mass accuracy, resolution and dynamic range, for both the standard as well as the ultra-complex mixture. Finally, this computational procedure based on a composite face design could be applied for any other mass spectrometric parameter optimization problem. The authors believe this will allow for a more transparent and more structured design when performing method development.

3.2. Vieillessement des analogues d'aérosols de Terre primitive

Dans cette deuxième partie, je présenterai l'étude de l'oxydation des Tholins suite à leur exposition à l'air. J'ai réalisé ces analyses au *National High Magnetic Fields Laboratory* (Tallahassee, Floride) sur un FTICR 21 Tesla. Les résultats sont reportés sous forme d'un article soumis au journal *Earth and Planetary Sciences Letters* (Maillard et al. 2020b).

Les Tholins sont produits en absence d'oxygène, cependant, un processus oxydatif apparaît lors que les Tholins sont exposés à l'oxygène de l'air ([Carrasco, et al. 2016 \[37\]](#)). J'ai décidé d'étudier ce phénomène et de l'inscrire dans l'étude des aérosols produit à l'époque de la Terre primitive. En effet, à cette époque, l'atmosphère régnant sur Terre est modérément oxydante, principalement composée d'azote et de dioxyde de carbone. Cependant, une atmosphère beaucoup plus réductrice existe aussi aux abords des cryovolcans, permettant la production d'aérosols organiques. Nous avons voulu étudier l'effet de la mise en contact des aérosols avec l'atmosphère oxydante afin de mieux comprendre leur réactivité et observer les nouvelles fonctions chimiques produites. Pour cela, plusieurs échantillons ont été laissés au contact de l'air et à l'abri de la lumière pendant des périodes allant de 1 mois à 3 ans. Ils ont ensuite été analysés en spectrométrie de masse, spectroscopie infrarouge et analyse élémentaire.

De nombreuses nouvelles espèces oxydées ont été détectées et la formation d'aldéhyde et d'acides carboxyliques a été observée. Ces fonctions chimiques sont de très bons précurseurs pour la formation de liaisons amide, même si ces dernières non pas été identifiées à ce jour. Un modèle de prédiction basé sur une étude PLS a aussi été proposé, permettant d'anticiper l'évolution de ces aérosols dans le temps.

Abstract

The study of the primitive earth remains a challenge for the scientific community because little information has been preserved over the passing of geologic time. It has been shown, through

the discovery of primitive rocks and ice cores, that the early Earth's atmosphere contained aerosols from sun-induced photo-chemistry. In volcanic regions, these aerosols were produced within reducing atmospheric conditions, and then exposed to the global oxidative atmosphere mainly made of CO₂ and N₂. With the oxidation event (2.4 billion years ago), these aerosols were then massively exposed to oxygen. Here, in order to investigate the bulk scale chemical changes in aerosols caused by the presence of life, analogues have been reproduced and then aged in the presence of oxygen. For this purpose, five samples were aged in a dark and water-free environment for periods ranging from 1 month to 3 years. The methanol-soluble fraction of each sample was then analyzed with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Thanks to the ultrahigh resolving power and dynamic range achievable with 21 Tesla FT-ICR mass spectrometry, isobaric overlaps less than the mass of an electron were resolved and subsequently assigned elemental compositions, and identified three times more species detected in the oldest sample compared to younger samples. Oxidized species become the major detected family and the addition of oxygen atoms in molecular formulas is observed with the increasing of the ageing. Then, thanks to the graphical treatment and a prediction model, more in-depth information is reported about the ageing process. Finally, an infrared and tandem mass spectrometry approach allows, in this work, to highlight the formation of aldehyde and carboxylic acid.

3.1.1. Introduction

Photo-chemical aerosols are suspected to be present in numerous atmospheres of exoplanets and other detected bodies ([Khare 1973 \[38\]](#) ,[Sagan and Salpeter 1976 \[39\]](#) ,[Sagan and Chyba 1997 \[40\]](#)). The presence of such hazes is also largely suspected during the Archean period due to geological evidence on the Earth ([Trainer, et al. 2004 \[41\]](#) ,[Kasting, et al. 1989 \[42\]](#) ,[Haqq-Misra, et al. 2008 \[43\]](#) ,[Trainer, et al. 2006 \[44\]](#) ,[Zerkle, et al. 2012 \[45\]](#) ,[Kurzweil, et al. 2013 \[46\]](#) ,[Izon, et al. 2015 \[47\]](#) ,[Arney, et al. 2016 \[48\]](#)). Today, the most accepted idea is that the original gas mixture producing these organic aerosols by photochemistry was composed of N₂, CO₂, H₂O, CO with a small amount of reducing species including CH₄ ([Trainer, et al. 2004 \[41\]](#) ,[Kasting 1993 \[49\]](#) ,[Cleaves, et al. 2008 \[50\]](#)). However, in particular locations such as volcanic plumes, the primitive soup could have been much more reducing, containing H₂ and CH₄ in addition to N₂ and other gases ([Tian, et al. 2005 \[51\]](#) ,[Parker, et al. 2011 \[52\]](#) ,[Johnson, et al. 2008 \[53\]](#)). In the case, interaction of haze produced in this location, with oxidative species in the rest of the primitive atmosphere is unknown. Furthermore, with the emergence of life, the explosion in the amount of oxygen caused a cascade of oxidative phenomenon. Studying these interactions would allow a better understanding of the prebiotic chemistry that occurred during that period. However it remains difficult to make progress because of the lack of real material to work on ([Haqq-Misra, et al. 2008 \[43\]](#) ,[Domagal-Goldman, et al. 2008 \[54\]](#)). One way to solve this issue is to simulate this primitive earth atmosphere with laboratory experiments ([DeWitt, et al. 2009 \[55\]](#) ,[Hasenkopf, et al. 2010 \[56\]](#)). These experiments are commonly used today because they are flexible and allow a reliable reproduction of the atmosphere of interest in term of composition and energy of photoionization. In addition, these analogues (called Tholins) are amenable to analysis via high end instrumentation ([Maillard, et al. 2018 \[27\]](#) ,[Ruger, et al. 2019 \[57\]](#)).

The goal of this work is therefore to study the ageing of such analogues of primitive earth's atmosphere in presence of oxygen. For this purpose, 5 samples were aged for periods ranging from 1 month to 3.5 years in the absence of light. Each sample was first, analyzed using a 21T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). This type of mass spectrometer is current state-of-the-art technology, and is the most suitable for retrieving information on highly complex organic mixtures ([McKenna, et al. 2010 \[4\]](#) ,[Marshall and Rodgers 2008 \[8\]](#) ,[Marshall and Chen 2015 \[58\]](#) ,[McKenna, et al. 2013 \[59\]](#) ,[McKenna, et al. 2013 \[60\]](#) ,[Podgorski, et al. 2013 \[61\]](#)). Then, in order to validate and push further our understanding of chemical functions present in these sample, infrared spectroscopy was performed. Here, we report the results obtained concerning the analysis of the oxidative process affecting primitive earth's aerosols.

3.2.2. Experimental methods

Tholins production

Analogues of primitive Earth's aerosols production. Aerosol analogues, also hereafter called *Tholins*, were produced with the PAMPRE experiment following the same procedure detailed in previous publications. The reactor is composed of a stainless steel cylindrical reactor in which a RF-Capacitively Coupled Plasma discharge is established thanks to an RF 13.56 MHz frequency generator. A gas mixture containing 95% of nitrogen and 5 % of methane was injected in the chamber as a continuous flow through polarized electrodes. It is then extracted by a primary vacuum pump to ensure that gases are homogeneously distributed. The plasma discharge was maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature. A brown powder (*Tholins*) was recovered after 1 day. The harvesting procedure was carried out under ambient air. *Tholins* are then stored at ambient atmosphere and protected from light.

The oxidation occurring is then only assimilated to the contact of samples with atmosphere's oxygen and the ambient humidity.

Sample preparation

High-performance liquid chromatography (HPLC)-grade methanol and water (J.T. Baker Chemicals, Phillipsburg, NJ, U.S.A.) were used as received. To recover the soluble part of the sample, 4 mg of laboratory Tholins were dissolved in 1 mL of methanol in a vial. The vial was vigorously stirred for 3 minutes to solubilize the maximum amount of species. The brown mixture was then filtered using a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter on a filter holder. Filtered solution was transferred in a vial, then dried with nitrogen flush and stored until analysis. All samples were dissolved in methanol to produce a stock solution of 500 $\mu\text{g/mL}$, and further diluted with 0.0125% (by volume) tetramethylammonium hydroxide (TMAH, 25% by weight in methanol) for negative-ion ESI (250 $\mu\text{g/mL}$).

FT-ICR Mass Spectrometry and Data Analysis

Ions were generated at atmospheric pressure via micro electrospray. ([Emmett, et al. 1998 \[60\]](#)) All samples were analyzed by 21 T FT-ICR MS. ([Hendrickson, et al. 2015 \[61\]](#) , [Smith, et al. 2018 \[62\]](#)) Ions were initially accumulated in an external multipole ion guide (21 T: 1-5 ms) and released m/z -dependently by decrease of an auxiliary radio frequency potential between the multipole rods and the end-cap electrode. ([Kaiser, et al. 2014 \[63\]](#)) Ions were excited to m/z -dependent radius to maximize the dynamic range and number of observed mass spectral peaks (m/z 150-1500; 21 T: 32-64%), ([Kaiser, et al. 2013 \[64\]](#)) and excitation and detection were performed on the same pair of electrodes. ([Chen, et al. 2014 \[65\]](#)) The dynamically harmonized ICR cell in the 21 T FT-ICR is operated with 6 V trapping potential. Ions were generated at atmospheric pressure via micro electrospray ([Emmett, et al. 1998 \[62\]](#)).

All samples were analyzed by 21 T FT-ICR MS. ([Hendrickson, et al. 2015 \[63\]](#) , [Smith, et al. 2018 \[64\]](#)) Ions were initially accumulated in an external multipole ion guide (21 T: 1-5 ms) and released m/z -dependently by decrease of an auxiliary radio frequency potential between the multipole rods and the end-cap electrode. ([Kaiser, et al. 2014 \[65\]](#)) Ions were excited to m/z -

dependent radius to maximize the dynamic range and number of observed mass spectral peaks (m/z 150-1500; 21 T: 32-64%),([Kaiser, et al. 2013 \[66\]](#)) and excitation and detection were performed on the same pair of electrodes.([Chen, et al. 2014 \[67\]](#)) The dynamically harmonized ICR cell in the 21 T FT-ICR is operated with 6 V trapping potential.([Boldin and Nikolaev 2011 \[68\]](#) ,[Kaiser, et al. 2011 \[69\]](#))

Time-domain transients of 3.1 seconds at 21 T were acquired with the Predator data station, with 100-500 time-domain acquisitions averaged for all experiments ([Blakney, et al. 2011 \[68\]](#)).(Blakney, et al. 2011 [70]). For 21 T FT-ICR MS, the Predator data station handled excitation and detection only, which were initiated by a TTL trigger from the commercial Thermo data station.([Smith, et al. 2018 \[62\]](#))([Smith, et al. 2018 \[64\]](#)) Mass spectra were internally calibrated with a high-abundance homologous series based on the “walking” calibration method.([Savory, et al. 2011 \[69\]](#))([Savory, et al. 2011 \[71\]](#)) Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale to the Kendrick mass scale([Kendrick 1963 \[70\]](#))([Kendrick 1963 \[72\]](#)) for rapid identification of homologous series for each heteroatom class (i.e., species with the same $C_cH_hN_nO_oS_s$ content, differing only by the degree of alkylation)([Hughey, et al. 2001 \[71\]](#)).(Hughey, et al. 2001 [73]).

For each elemental composition, $C_cH_hN_nO_oS_s$, the heteroatom class, type (double bond equivalents, $DBE = \text{number of rings plus double bonds to carbon}$, $DBE = C - h/2 + n/2 + 1$)([McLafferty and Turecek 1993 \[72\]](#))([McLafferty and Turecek 1993 \[74\]](#)) and carbon number, c , were tabulated for subsequent generation of heteroatom class relative abundance distributions and graphical relative-abundance weighted DBE versus carbon number images. Peaks with signal magnitude greater than 6 times the baseline root-mean-square (rms) noise at m/z 500 were exported to peak lists, and molecular formula assignments and data visualization were performed with PetroOrg © software.([Corilo 2014 \[73\]](#)) Molecular formula assignments with an error >0.5 parts-per-million were discarded, and only chemical classes with a combined relative abundance of $\geq 0.15\%$ of the total were considered.

1.4.3.2.3. Results and discussion

Aerosols dark-ageing

Production of analogues of primitive Earth aerosols, named here Tholins, is described in the material and method section. After their synthesis, the samples were stored in an enclosed, dark place. Therefore, all chemical aging is presumed the result of contact with air containing oxygen and a few percent water. Five ageing time-durations have been defined, 1 month, 4.5 months, 1.8 years, 2.2 years and 3.5 years after production.

Soluble fraction analysis

Mass Resolving Power. The experiments were recorded on a Fourier transform ion cyclotron resonance mass spectrometer equipped with a 21 Tesla superconducting magnet ([Smith, et al. 2018 \[5\]](#), [Hendrickson, et al. 2015 \[76\]](#)). All experiments were recorded in electrospray (ESI) positive ionization mode. Negative ionization mode was also performed and its results are reported in the supporting information section. **Figure 49** shows a mass spectrum in positive ion ESI for the methanol-soluble fraction of a sample of early earth analogues. Analytes are distributed across a wide m/z range (from $180 < m/z < 1200$) in a pseudo- Gaussian distribution with more than 30,000 peaks detected with signal magnitude greater than six times the baseline RMS noise level and the repetition patterns observed previously are recovered ([Somogyi, et al. 2005 \[24\]](#), [Pernot, et al. 2010 \[77\]](#)). A mass-scale zoom inset between m/z 558.24 and m/z 558.40, highlights the immense compositional complexity, with more than 100 peaks detected within a 0.16 Da mass window.

A second insert was included and displays the data over a 0.04 Da range with several fully-resolved mass spectral peaks corresponding to compounds that differ in mass by roughly the mass of an electron ($m_e = 548 \mu\text{Da}$) and further highlights the need for ultrahigh resolving power only achievable by FT-ICR MS.

For the 750 μDa split, the required mass resolution is about 800,000 at m/z 558, and is only achievable with ultra-high field FT-ICR MS, which justifies the use of this instrument in this study ([Hendrickson, et al. 2015 \[74\]](#)). For the 750 μDa split, the required mass resolution is about 800,000 at m/z 558, and is only achievable with ultra-high field FT-ICR MS, which justifies the use of this instrument in this study ([Hendrickson, et al. 2015 \[76\]](#)).

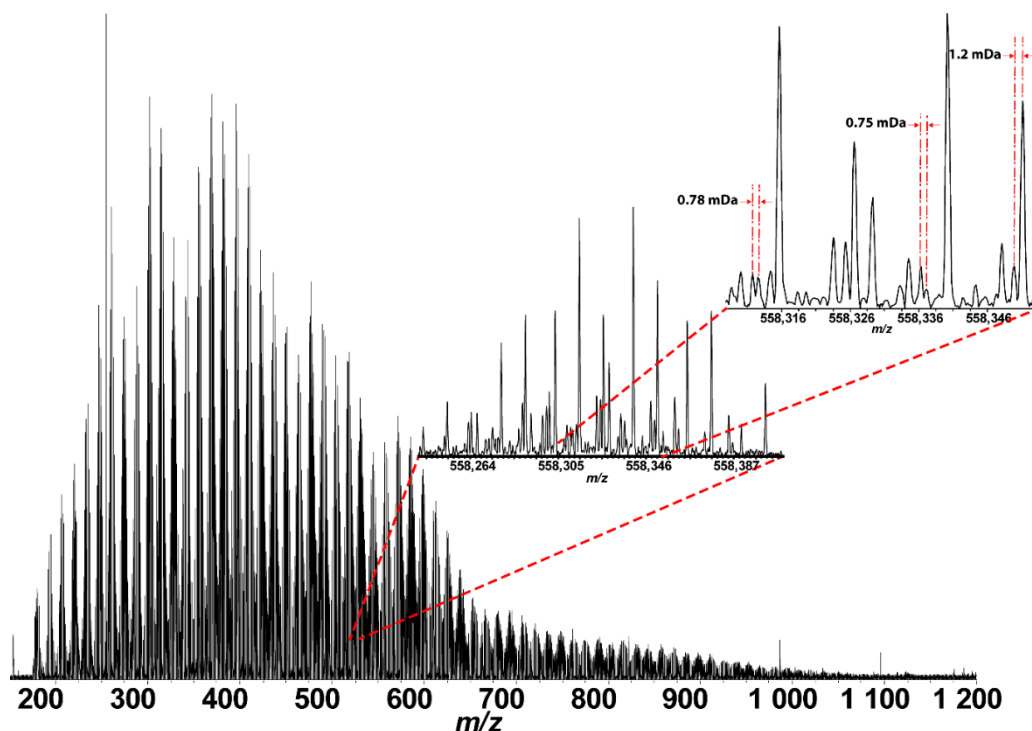


Figure 49 : Mass spectrum of soluble fraction of Tholins in methanol recorded with a 21 T FT-ICR MS.

Increased Compositional Complexity with Ageing Time. Comparison of the molecular composition between the five methanol-soluble fractions provides insight into the ageing of these fractions with time. **Figure 50** shows the comparison between 3 samples from ageing time of 1 month (**Figure 50a**), 1.8 years (**Figure 50b**) and 3.5 years (**Figure 50c**). For all three samples, the molecular weight distribution is centered at m/z 558.

An increase in compositional complexity corresponds to an increase in ageing, as shown by an increase in the number of detected peaks with time. From 1 month to 3.5 years, a more than two-fold increase in complexity occurs, which indicates the chemical transformation with exposure time.

Increased Oxidation with Ageing Time.

Figure 50 highlights mass spectral peaks that contain oxygen (red) and those without oxygen (black), with the number of oxygen-containing compounds annotated. The number of peaks that do not contain oxygen in their raw formula highlights the immense complexity of tholin precursors that lead to a widely variable chemical aging reaction rate. Due to practical limitations in the overall time period over which this study was conducted, it is reasonable and expected that minimal to no change may be observed for some species. However, the number of peaks that were assigned to oxygen-containing compounds classes varies from 16 in the youngest sample to 68 in the oldest sample. Thus, there is 4 times more oxygenated species observed after 3.5 years of ageing than after 1 month, and after 1.8 years (**Figure 50b**), the number of oxygenated species doubles. In addition, the most abundant compounds after 1 month correspond to non-oxygen containing compounds that decrease in relative abundance with time as oxygen incorporation occurs with time.

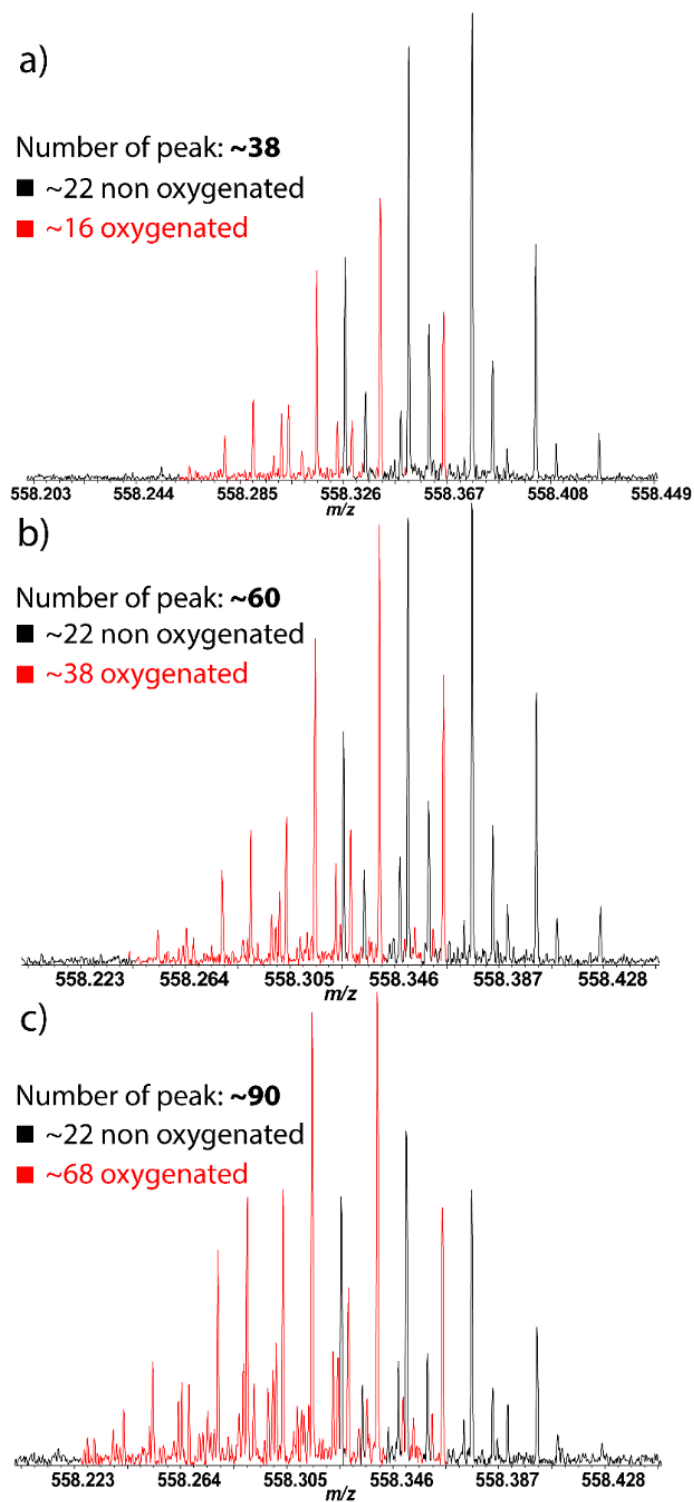


Figure 50 : Mass-scale zoom inset at $558.220 < m/z < 558.430$ of positive-ion ESI 21T FT-ICR mass spectra for methanol extracts after a) 1-month, b) 1.8 years and c) 3.5 years. An increase in the number of oxygen-containing compounds within the same mass range occurs from ~16 after 1 month to more than 68 after 3.5 years.

Amount of oxygen over time

In order to reveal more about the oxidative processes at work, we processed the mass spectral data and represent that data in several formats. First, the sum of the relative abundance or signal magnitude for oxygen-containing compounds were calculated and compared to the overall sum of the relative abundances for each sample and shown in **Figure 51a**. Plotted as number of weeks versus relative abundance, **Figure 51a** provides rapid visualization of global trends in the abundance of oxygen compounds detected with time, with all samples measured in triplicate. The relative abundance of oxygenated species increases logarithmically with time, and rapidly in the first year from ~30% after 1 month to ~60% and then plateaus at approximately 70% of the total relative abundance.

Then, in order to better understand the incorporation of oxygen into Tholins samples, a sorting between the detected elemental families was performed. Five compounds classes have been defined: compounds that do not contain oxygen and O1-O4 classes. The sum of the relative abundances of each compounds class was calculated and plotted in **Figure 51b**. As previously observed in **Figure 51a**, the N_xO_0 families are following a logarithmic decrease. However, the other families reveal different trends. The N_xO_1 family grows during the first year and then decreases while the N_xO_2 N_xO_3 and N_xO_4 families seem to increase as a function of time. This observation suggests that species that initially contain only one atom of oxygen oxidize over time to incorporate more oxygen into their structure. In addition, this illustrates that the chemical aging process is complex and often proceeds through multiple generations of oxidation reaction before the products reach greater stability limiting further oxidation.

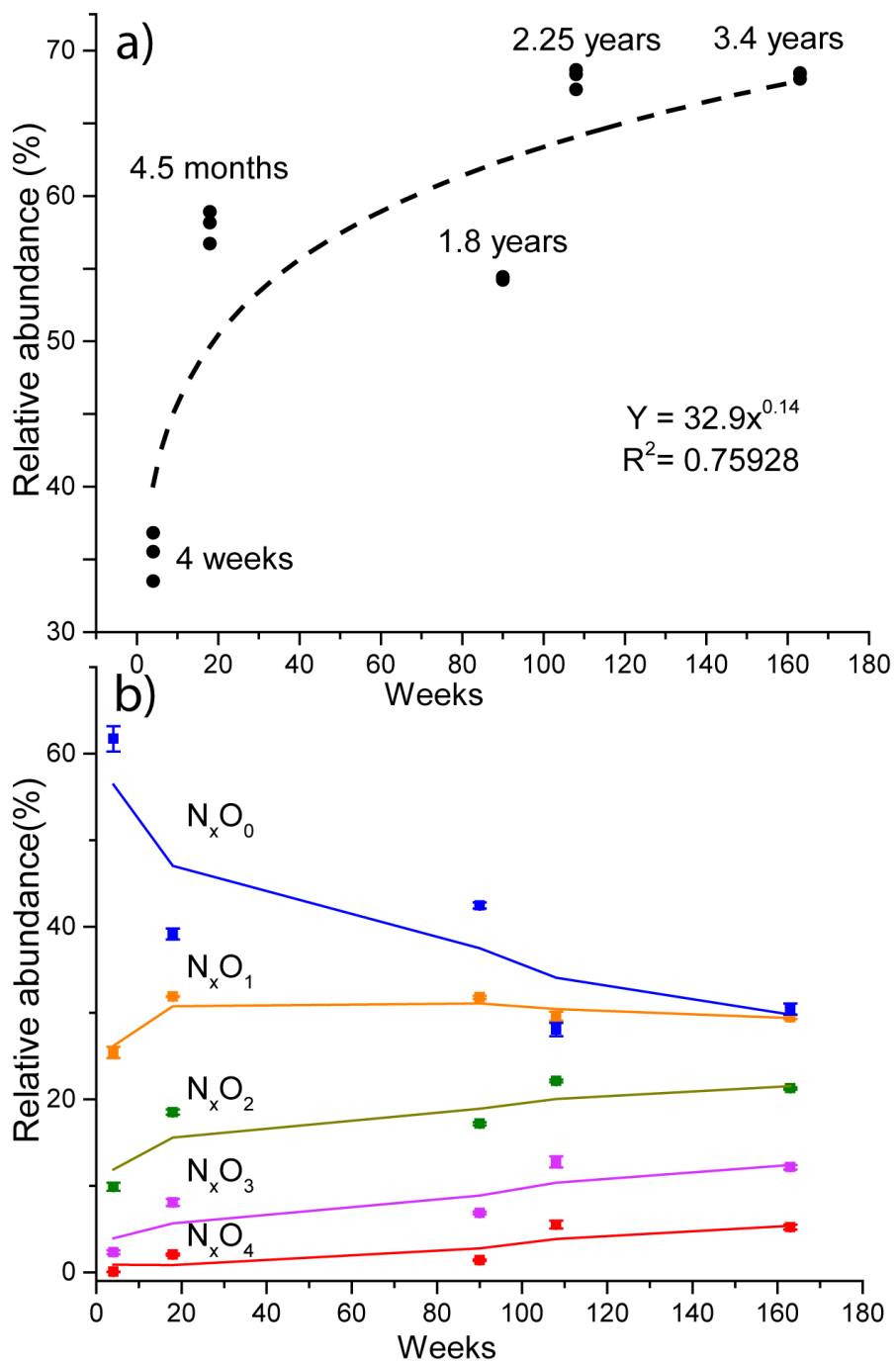


Figure 51 : Representation of the relative abundance of oxygenated species over time. A) Global observation of all oxygenated species. B) Relative abundance of the O_0, O_1, O_2, O_3, O_4 families over time.

Graphical analysis

In order to confirm the results obtained, and to facilitate the interpretation, graphical treatments were carried out. We opted for the use of Van Krevelen diagrams ([Van Krevelen 1950 \[76\]](#)). This type of graph was originally developed to assess the origin and maturity of kerogen and petroleum through the use of the H/C and O/C ratio and is now largely used to visualize high resolution mass spectrometry data ([Wu, et al. 2004 \[79\]](#), [Kim, et al. 2003 \[80\]](#)). It is very suitable for the study of the oxidative process. **Figure 52a, 9b,** and **9c** represent the Van Krevelen diagrams for the three samples aged of 1 month, 1.8 year and 3.5 years, respectively. Here each point is a single species present in the mass spectrum. The intensity of the points is given by the color code ranging from blue to red (Blue, lowest abundance and red, highest). All species are represented here. First of all, we can see that many species are close to the y-axis, these are the species that do not contain oxygen. The other species that spread in both dimensions are therefore oxygenated species. By comparing the three diagrams, we observe that the number of points is much higher in the third sample than in the first one. This confirms the observations made in **Figure 50**, with the increase in the number of oxygenated species over time. From these diagrams it is possible to see that oxygenated species are more intense as a function of time if we compare the three graphs, however, there is no significant change of the H/C ratio indicating that there is no change in the number of unsaturation of the molecules. This observation was also made in **Figure 51**. **Figure 52d, 9e** and **9f** represent the reconstructed mass spectra of the family of species containing 8 nitrogen atoms. This family was chosen as an example because it is the most abundant one in the sample. It has been divided into 5 sub-families according to the number of oxygen atoms present. Thus, we can see that the family not containing oxygen (Blue) is the major one in the first sample, but is no longer in the oldest one (3.5 years).

In addition, we observe an increase in oxygenated families following the trend observed in **Figure 51**. To reinforce previous results concerning the amount of oxygen incorporated in the sample over time, elemental analysis of two samples was carried out (FLASH 2000 from Thermo). Obtained percentages of each element composing one fresh sample and the 2.2 years old one are given in the Table 4. As observed, the amount of oxygen of the oldest sample is double that of the earliest sample.

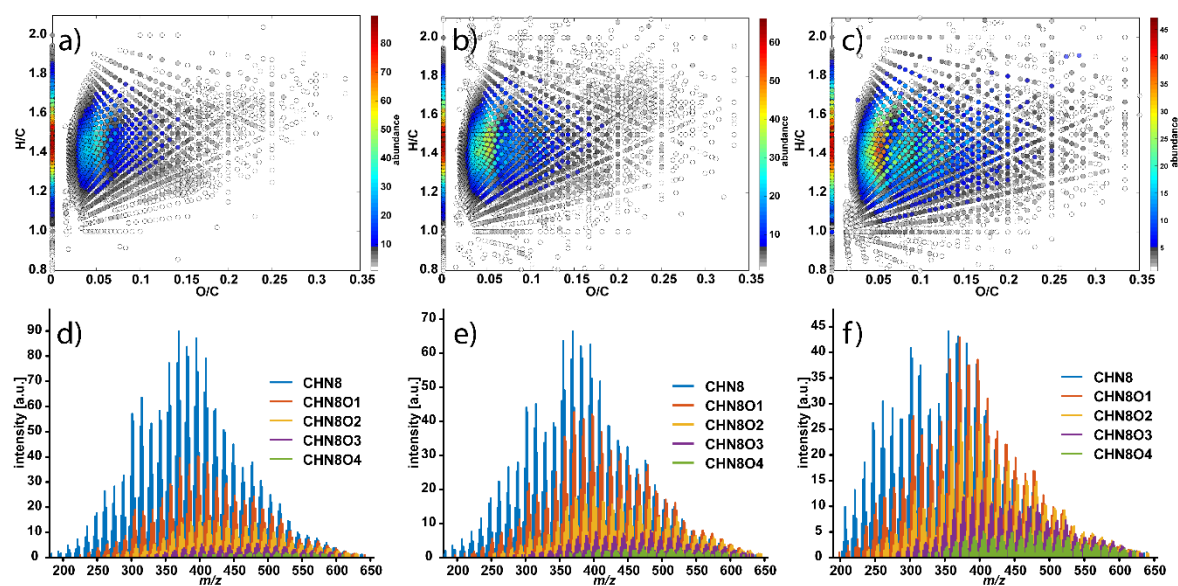


Figure 52 : Graphical analysis of mass spectra obtained in previous section. Van Krevelen diagrams of a) 2 months old sample, b) 1.8 years old sample, c) 3.5 years old sample. Reconstructed mass spectrum of five different families present in the sample for the d) 2 months old sample, e) 1.8 years old sample, f) 3.5 years old sample.

This validates what was observed with the mass spectrometry analyses. In addition to all these previous information, a simple linear regression model was designed in order to predict the amount of oxygen incorporated over time in these analogues. The result of the model based on a Partial Least Square algorithm is given in **Figure 53**.

This graphic shows that the amount of oxygen incorporated is predictable based on the molecular profile, which is useful for future experiments. After looking into details how the amount of oxygen is increasing in these analogues of primitive Earth’s haze, the next section will focus more on the chemistry behind this oxidation. The scope is to gain knowledge on the oxygen position and on the chemical functions formed.

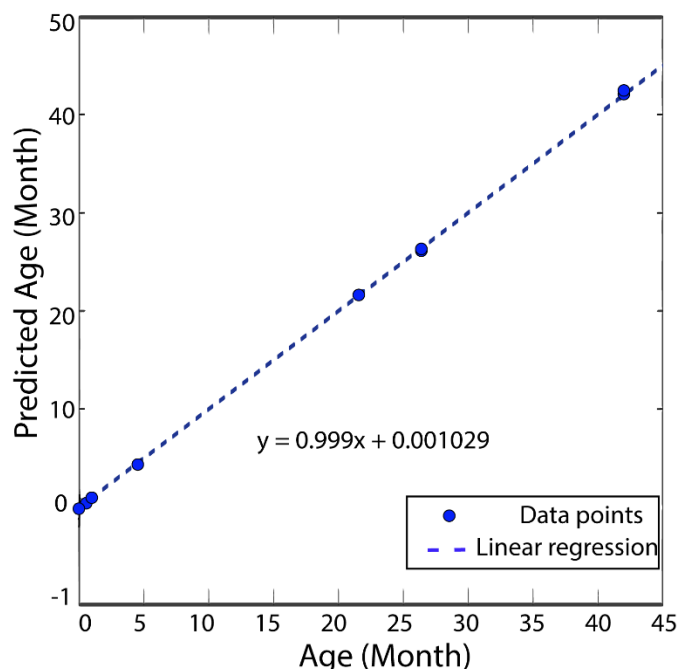


Figure 53 : Representation of results obtained for the PLS fitting on the broadband ultra-high resolution mass spectrometry data sets (elemental composition attribution lists). Predicted age (ordinate) and real/experimental (abscissa) age are well aligned, which proves the general predictability of the dark aging based on the molecular profile.

Table 4 : Elemental analysis of fresh and aged sample

Sample	%C	%H	%N	%O
1 month	36	40	20	4
2.2 years	27	41	24	8

Functional group bearing oxygen.

Transmission infrared spectroscopy (Nicolet 6700 FTIR from Thermo) was performed on different aged analogue samples. The aging process range from 1 month to 3.5 years in order to observe differences induced by the incorporation of oxygen. To focus on the evolution of shape of IR absorption bands, IR spectra were normalized according to the maximum peak in each wavenumber range studied. **Figure 54** focuses on a wave-number between 1000 cm^{-1} and 1900 cm^{-1} . The upper part (**Fig A and B**) of this figure shows the stacking of infrared spectra for the samples. The lower part (**Fig C and D**) highlights the evolution of spectra by subtracting the spectrum of the freshest sample. As observed, a band at 1700 cm^{-1} is increasing with the ageing. This band is attributed to the elongation of the double-bond between an oxygen and a carbon (carbonyl) ([Long 2004 \[79\]](#)), ([Long 2004 \[81\]](#)). Furthermore, the band of alcohol usually present between 1280 and 1420 cm, is absent in the reported IR spectrum ([Long 2004 \[79\]](#)), ([Long 2004 \[81\]](#)). Both information therefore involves a preferential fixation of oxygen in carbonyl form rather than alcohol or ether. **Figure C** shows the elongation of -CH and **Fig. D** the corresponding subtracted spectrum. As observed, there is a diminution of usual -CH bands at $2800 - 2900\text{ cm}^{-1}$ and an increase of the -CH bounded to aldehyde band at $2600 - 2800\text{ cm}^{-1}$. These observations highlight the presence of aldehyde in our samples. To go further, negative mode tandem mass spectrometry was performed and has brought to light the presence of carboxylic acid chemical function. These results are reported in supporting information. As a conclusion, both aldehyde and carboxylic acid functionalities were identified as the structure most likely present within early Earth analogues over the dark ageing time-period of this study.

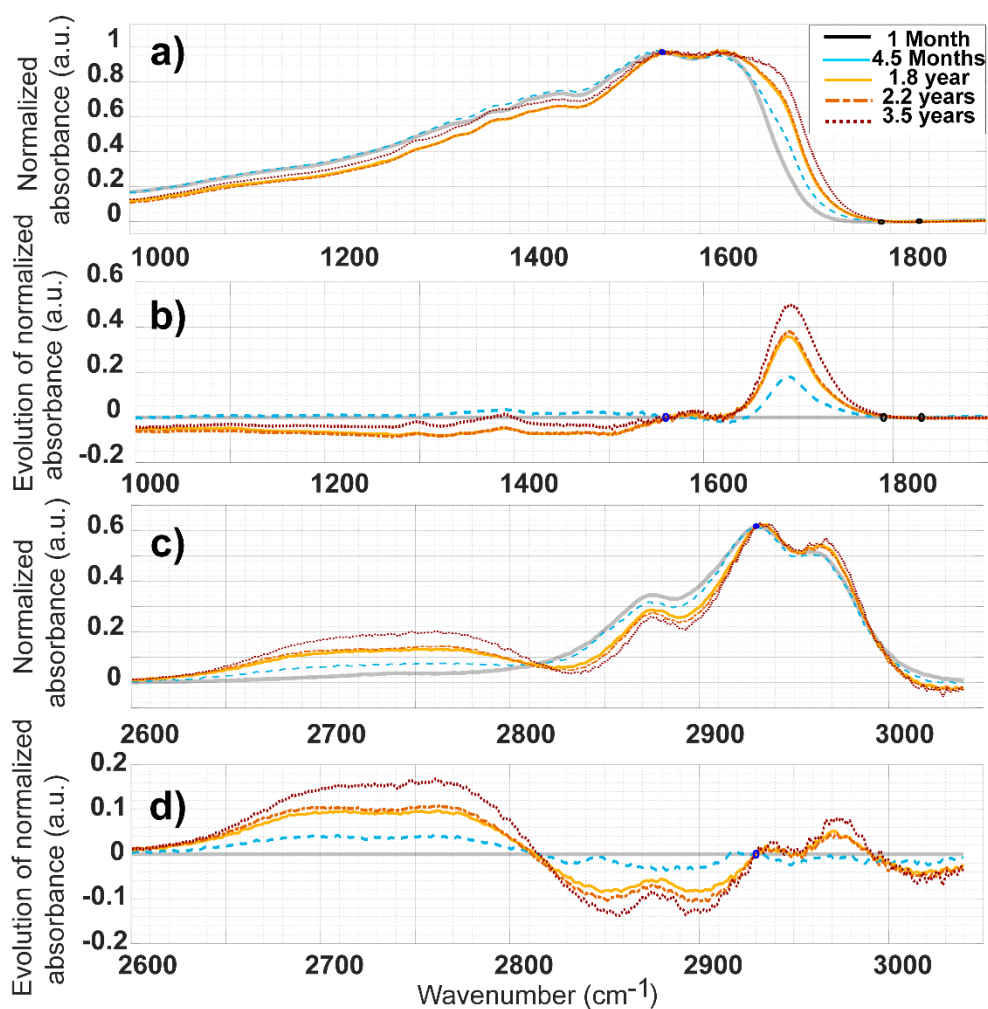


Figure 54 : Infrared spectra of ages analogues between 1000 cm^{-1} and 1900 cm^{-1} . A) and C) correspond to spectra in the 1000-1800 and 2600-3000 cm^{-1} range whereas B) and D) are residual spectra obtained by subtraction of the spectrum of the non-oxidized sample.

2.4. Conclusions

This work has allowed to have a better comprehension of the reactivity of analogues of primitive earth aerosols, produced in reducing atmosphere, with the carbon dioxide present in the atmosphere. The 21T FTICR deciphered the complexity of these aerosols and enabled to dig into the chemistry of every detected molecule. Incorporation of oxygen in their structure was observed over time and a prediction model based on Partial Least Square was proposed. A fast reactivity was observed during the first year of ageing meaning that these aerosols are very prone to oxidation. Concerning their structure, production of carboxylic acid and carbonyl function was revealed. This observation is an advance for prebiotic chemistry because such functions are precursor of amide bounds, which are the bases of peptidic structures.

3.3. Comparaison des fractions soluble et insoluble dans le méthanol

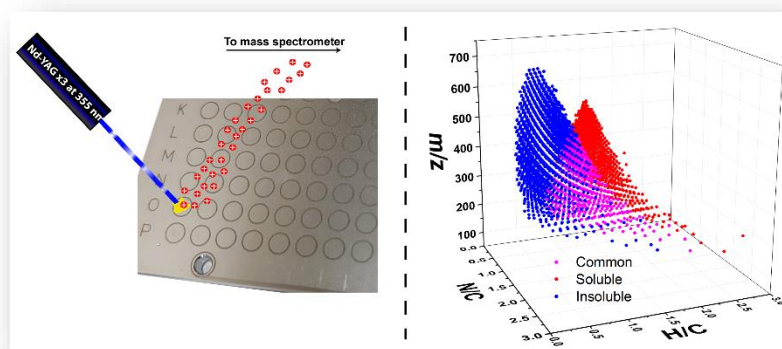
Cette troisième partie présente une étude de la composition chimique des fractions soluble et insoluble dans le méthanol d'un échantillon de Tholins. Ces résultats sont reportés sous forme d'article publié dans le journal *Earth and Planetary Sciences Letters* en 2018 (Maillard et al. 2018).

Comme présenté dans le **Chapitre 1**, les analyses précédemment réalisées sur les Tholins en spectrométrie de masse n'étaient portées que sur la fraction soluble dans le méthanol (40% en masse d'un échantillon de Tholins de PAMPRE). Le FTICR de Rouen étant équipé d'une source d'ionisation LDI permettant l'analyse d'échantillons sous forme solide, j'ai décidé de comparer les spectres de masse des deux fractions afin d'observer ce que nous manquions en analysant seulement la fraction soluble.

D'importantes différences ont été observés entre les deux fractions, plus particulièrement concernant leur rapport hydrogène sur carbone. Les espèces détectées dans la fraction insoluble ont révélé un rapport H/C moyen moitié moins important que les espèces solubles. Les molécules insolubles sont donc beaucoup en moyenne plus aromatiques que les solubles. De plus, j'ai mis en évidence une partie commune aux deux fractions à basse masse. La présence de ces espèces dans les deux fractions peut être lié à l'existence de différents isomères ou encore à la présence de molécules solubles dans des agrégats de molécules insolubles. Cependant, cela reste à confirmer au-delà de ces analyses.

Abstract

Titan, the biggest moon of Saturn, has a thick atmosphere which presents similarities with the one thought to be on Earth at its beginning. The study of Titan's photochemical haze is thus a precious tool in gaining knowledge of the primitive atmosphere of Earth.



The chemistry occurring in Titan's atmosphere and the exact processes that act in the formation of the hazes remain largely unknown. The production of analog samples on Earth has proved to be a useful tool to improve our knowledge of the aerosols formation on Titan. Such solid organic analog samples, named Tholins, were produced with the PAMPRE experiment (French acronym for Aerosols Microgravity Production by Reactive Plasma). PAMPRE Tholins were found to be mostly insoluble, with only one-third of the bulk sample that can be dissolved in methanol. This partial solubility limited the previous studies in mass spectrometry, which were done only on the soluble fraction. The goal of the present study is to compare the two fractions of PAMPRE's Tholins (insoluble and soluble) using a ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometer (FTICR) equipped with a laser desorption/ionization source. Using modified Van Krevelen diagrams, we compare the global distribution of the molecules within the samples according to their Hydrogen/Carbon ratio and Nitrogen/Carbon ratio. Major differences are observed in the molecular composition of the soluble and the insoluble fraction. The soluble fraction of Tholins was previously identified as a set of polymers of average formula $(C_2H_3N)_n$. In this work we observe that the insoluble fraction of Tholins is comprised of a significantly different set of polymers with an average composition of $(C_4H_3N_2)_n$.

3.3.1. Introduction

Titan, the largest satellite of Saturn, is surrounded by a thick atmosphere rich in nitrogen with a small percentage of methane. The chemistry in this atmosphere leads to the formation of a complex smog due to the radicalization and recombination of the two gases upon interaction with solar UV photons and charged particles from Saturn's magnetosphere. The observations of the Cassini mission and the information obtained during the landing of the Huygens probe provided partial information about the composition of the aerosols. Pyrolysis of the aerosols by the ACP experiment aboard Huygens released mainly NH₃ and HCN but was not sufficient to further elaborate on the structural composition of the aerosols because of the detection limit of the instrument ([Niemann, et al. 2005 \[31\]](#) ,[Fulchignoni, et al. 2005 \[32\]](#) ,[Israel, et al. 2005 \[34\]](#)).

Titan's aerosols also represent an interest for astrobiology ([Sagan, et al. 2002 \[35\]](#))([Sagan, et al. 2002 \[36\]](#)) due to their peculiar formation and their organic complexity. Some analogs, called Tholins, were produced on Earth to investigate the formation processes and the structure of Titan's aerosols ([Somogyi, et al. 2005 \[24\]](#) ,[Szopa, et al. 2006 \[25\]](#) ,[Imanaka and Smith 2010 \[26\]](#) ,[Cable, et al. 2012 \[82\]](#) ,[Toupance, et al. 1975 \[83\]](#)).

Infrared (IR) spectroscopy analyses have demonstrated that Tholins structure includes nitrile, amine, hydrocarbon and unsaturated functions ([Coll, et al. 1999 \[23\]](#) ,[Sciamma-O'Brien, et al. 2017 \[84\]](#) ,[Gautier, et al. 2012 \[85\]](#) ,[Imanaka, et al. 2004 \[86\]](#) ,[Cable, et al. 2014 \[87\]](#)). Primary amines were also identified in Tholins using laser-induced fluorescence with nonaqueous capillary electrophoresis ([Cable, et al. 2014 \[85\]](#)).(Cable, et al. 2014 [87]).

Additional analyses were performed on the soluble fraction of the Tholins by mass spectrometry showing a polymeric structure ([Gautier, et al. 2016 \[88\]](#) ,[Bonnet, et al. 2013 \[89\]](#) ,[Vuitton, et al. 2010 \[90\]](#)).

First analyses with ultra-high resolution mass spectrometry also brought to light repetition patterns (CH₂ and HCN) in the Tholins' soluble fraction ([Somogyi, et al. 2005 \[24\]](#) ,[He, et al. 2012 \[91\]](#) ,[Anicich, et al. 2006 \[92\]](#)). However, some Tholins are not completely soluble in solvents such as methanol ([Carrasco, et al. 2009 \[93\]](#)). This is the case of the Tholins synthesized with the PAMPRE experiment, described below ([Szopa, et al. 2006 \[24\]](#)).([Szopa, et al. 2006 \[25\]](#)). The soluble fraction of Tholins obtained from this experiment represents only one third of the total material. Only this soluble fraction was analyzed with mass spectrometry so far, using electrospray ionization and photoionization ([Carrasco, et al. 2009 \[93\]](#)). The soluble fraction provided numerous information about the general structure of these samples. Several studies compared various Tholins to a set of polymeric structures based on a CH₂-HCN repeating unit, spreading from m/z 100 to m/z 800, as emphasized by the Kendrick mass defect diagram using HCN or CH₂ normalization ([Hughey, et al. 2001 \[92\]](#) ,[Kendrick 1963 \[93\]](#)).([Hughey, et al. 2001 \[94\]](#) ,[Kendrick 1963 \[95\]](#)). This explained the periodic nature of the mass spectra of Tholins showing repeating ion clusters (Gautier et al. 2014, Pernot et al. 2010). A previous study emphasized that IR spectrum of the soluble and non-soluble fractions of Tholins were nearly identical ([Carrasco, et al. 2009 \[93\]](#)). As a consequence, it has been postulated that both fractions had a similar molecular content, and that the difference of solubility was only related to the difference of mass of the molecules composing Tholins, as larger molecules are expected to be less soluble. In the present study, we reexamine this insoluble fraction at the molecular level by using ultra-high resolution mass spectrometry.

3.3.2. Experimental

Sample production

Tholins were produced using the PAMPRE experiment and following the procedure detailed in previous publications ([Szopa, et al. 2006 \[24\]](#) ,[Gautier, et al. 2011 \[27\]](#)).([Szopa, et al. 2006 \[25\]](#) ,[Gautier, et al. 2011 \[28\]](#)). In this setup, the reactor is composed of a stainless steel cylindrical

reactor in which the Radio Frequency – Capacity Coupled Plasma (RF-CCP) discharge is established by an RF 13.56 MHz frequency generator. The gas mixture, containing nitrogen and 5% of methane, is injected in the chamber as a continuous flow through polarized electrodes and then extracted by a primary vacuum pump to ensure that gases are homogeneously distributed. The plasma discharge is maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature.

Sample treatment for mass spectrometry analysis

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

Mass spectrometry analyses

Different ionization methods can be used for the analysis of molecules in their solid state, especially Laser Desorption Ionization (LDI). LDI-MS has already been used for the analysis of Tholins ([Imanaka, et al. 2004 \[86\]](#) ,[Gautier, et al. 2017 \[96\]](#) ,[Mahjoub, et al. 2016 \[97\]](#) ,[Somogyi, et al. 2012 \[98\]](#) ,[Sagan, et al. 1993 \[99\]](#)). It has also been used for the analysis of asphaltenes, a fraction of petroleum non-soluble in pentane or heptane ([Sagan, et al. 1993 \[99\]](#)

[Pereira, et al. 2014 \[100\]](#) , [Tanaka, et al. 2004 \[101\]](#)). In this work a LDI source is coupled with a Fourier transform ion cyclotron resonance mass spectrometer (FTICR) to compare the soluble and non-soluble fractions of Tholins at the molecular level. A comparison between electrospray and LDI sources using the same analyzer parameters can be found in the supporting information (**Figure S90** and **Figure S91**). In addition, LDI-FTICR was compared to LDI-TOF analyses (**Figure S90**).

Previous works have demonstrated the significant differences in the molecules detected in Tholins depending on the ionization mode used ([Carrasco, et al. 2009 \[93\]](#) , [Somogyi, et al. 2012 \[98\]](#)). For this work we chose to investigate only positive ionization as this is the one most widely used in the community for Tholins analysis.

All analyses were performed on a FTICR Solarix XR from Bruker equipped with a 12 Tesla superconducting magnet and a laser desorption ionization source (laser NdYAg 355 nm). The mass spectrometer was externally calibrated with a solution of sodium trifluoroacetate. Mass spectra were afterwards internally calibrated with confidently assigned signals yielding a mass accuracy below 300 ppb in the considered mass range.

The soluble fraction was deposited on a LDI plate using the dry droplet method (5 x 1 μ L to ensure a good concentration of the sample). Insoluble fraction and the global sample were deposited using a solvent-free method, following a previously published procedure ([Barrere, et al. 2012 \[29\]](#)).

Mass spectra were recorded in positive mode at 8 million points with a sum of 500 scans, yielding a resolution of 1 500 000 at m/z 150 and 500 000 at m/z 500. The following instrumental parameters were implemented for the insoluble and total fractions: Plate offset 100 V, Deflector plate 210 V, Laser power 19 %, Laser shots 40, Frequency of laser shots 1000 Hz, Funnel 1 at 150 V, Skimmer 1 at 25 V. The same parameters were used for the soluble fraction, except the

laser power that was raised to 29% and the number of shots increased to 150. Fraction responses to the laser ionization were different for each fraction of the sample due to the molecular differences between each fraction, especially the hydrogen/carbon ratio. To obtain sufficient signal for the soluble fraction, it was necessary to increase the laser power compared to the parameter used for the non-soluble fraction spectra. A comparison between the global intensity of the spectra and the laser power used for the ionization is given in the supplementary material (**Figure S92**). We then defined a laser power value for each fraction in order to obtain similar signal intensities. We fixed the value at 5% of laser power above the determined threshold – 19 % and 29% for the insoluble and soluble fraction respectively. Peak picking was done with a signal/noise ratio of 5 and 0.01% of intensity. Molecular formulas were obtained using the SmartFormula tool from the Bruker Data Analysis 4.4 software with the following parameters: molecular formula $C_{0-x}H_{0-y}O_{0-2}$, even and odd electron configuration allowed, 0.5 ppm error tolerance.

It should be noted that while we try to limit oxygen contamination, Tholins are known to be prone to oxidation when exposed to oxygen. Numerous discussions can be found in the literature on this point (see for example ([Carrasco, et al. 2016 \[100\]](#))([Carrasco, et al. 2016 \[37\]](#)) for a discussion on PAMPRE Tholins). In our case, oxidation principally happens when Tholins are exposed to the air during their collection after production.

This exposition is intrinsic to the experimental setup used. Studies on plasma produced organic materials have shown that this oxidation happens within seconds of exposition of the sample to ambient air ([McKay 1996 \[102\]](#) ,[Swaraj, et al. 2007 \[103\]](#) ,[Tran, et al. 2003 \[104\]](#) ,[Hörst, et al. 2018 \[105\]](#)). In the case of the sample analysed here, the amount of oxygen contamination can be estimated through the detection of oxygenated molecules in the samples from the LDI-FTICR measurement. These measurements show the presence of ~20% of oxygenated signals (**Table 5**). This part is non negligible principally because the material is composed of thousands

of molecules in low abundance and so, thousands of molecules start to pick up oxygen after the exposition to ambient atmosphere. Nevertheless, especially because oxygenated species are produced from the bulk material and contain one or two oxygen atoms, no modification was observed between these oxygenated and non-oxygenated species concerning the number of unsaturation or the H/C ratio.

The formation of such oxygenated species was observed previously upon the addition of ammonium hydroxide and they were depicted as hydrolysis products ([Poch, et al. 2012 \[105\]](#), [Neish, et al. 2009 \[106\]](#)). ([Poch, et al. 2012 \[106\]](#), [Neish, et al. 2009 \[107\]](#)). We could expect some changes in composition due to exposure to water for our soluble fraction. However, the soluble fraction is prepared with water a few minutes before the analysis.

This is much shorter than the hydrolysis condition found in the literature for Tholins hydrolysis (10 weeks for Poch et al. 2012, 1 year for Neish et al. 2010). Neish et al. 2009 showed that Tholins hydrolysis rate in pure water was significantly slower than in the presence of ammonia, only yielding a total oxidation of ~2% of the Tholins after 53h exposure to water. To first order we can therefore consider hydrolysis negligible considering neutral pH of our solution and the hydrolysis kinetics. All molecular formulas were attributed thanks to the high precision m/z measures.

The oxygenated species were then excluded from the results presented here by filtering the CSV data file using Excel 2016 spreadsheet, keeping only formulas containing ^{12}C , ^1H and ^{14}N .

Table 5 summarizes all attributed formulas by the SmartFormula tool.

Table 5 : 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

3.3.3. Results and discussions

LDI-FTICR analyses

Figure 55 presents a general view of the recorded mass spectra for the two different fractions (soluble and insoluble) and for the global sample (soluble + insoluble). It can be seen that the base peaks are different in each fraction. For the soluble fraction, m/z 127.09784 is the base peak while it is m/z 200.09308 for the insoluble fraction and 214.10872 for the global sample. On the other hand, the ion distribution is relatively similar for all fractions but we observe that low m/z species are more abundant in the soluble fraction than in the other samples. The three mass spectra present a periodic profile with patterns consistent with the expected repeating unit of Tholins $\text{CH}_2 - \text{HCN}$ ([Pernot, et al. 2010 \[75\]](#))([Pernot, et al. 2010 \[77\]](#)).

The enlargement in the inset of **Figure 55** highlights that for the insoluble fraction and for the global sample, the formed repetition patterns are similar, while they appear shifted in the soluble fraction. This phenomenon is better illustrated on **Figure 56** by zooming in on several periods. We can observe that the soluble fraction has a period (two repetition patterns) of m/z 27.0109, which corresponds exactly to the HCN formula. In comparison, insoluble and the global sample have a period of m/z 26.0156, which corresponds exactly to the C_2H_2 formula. This explains why in some parts of the spectra, the waves of the soluble fraction present their lowest intensity and their highest intensity for the two different fractions.

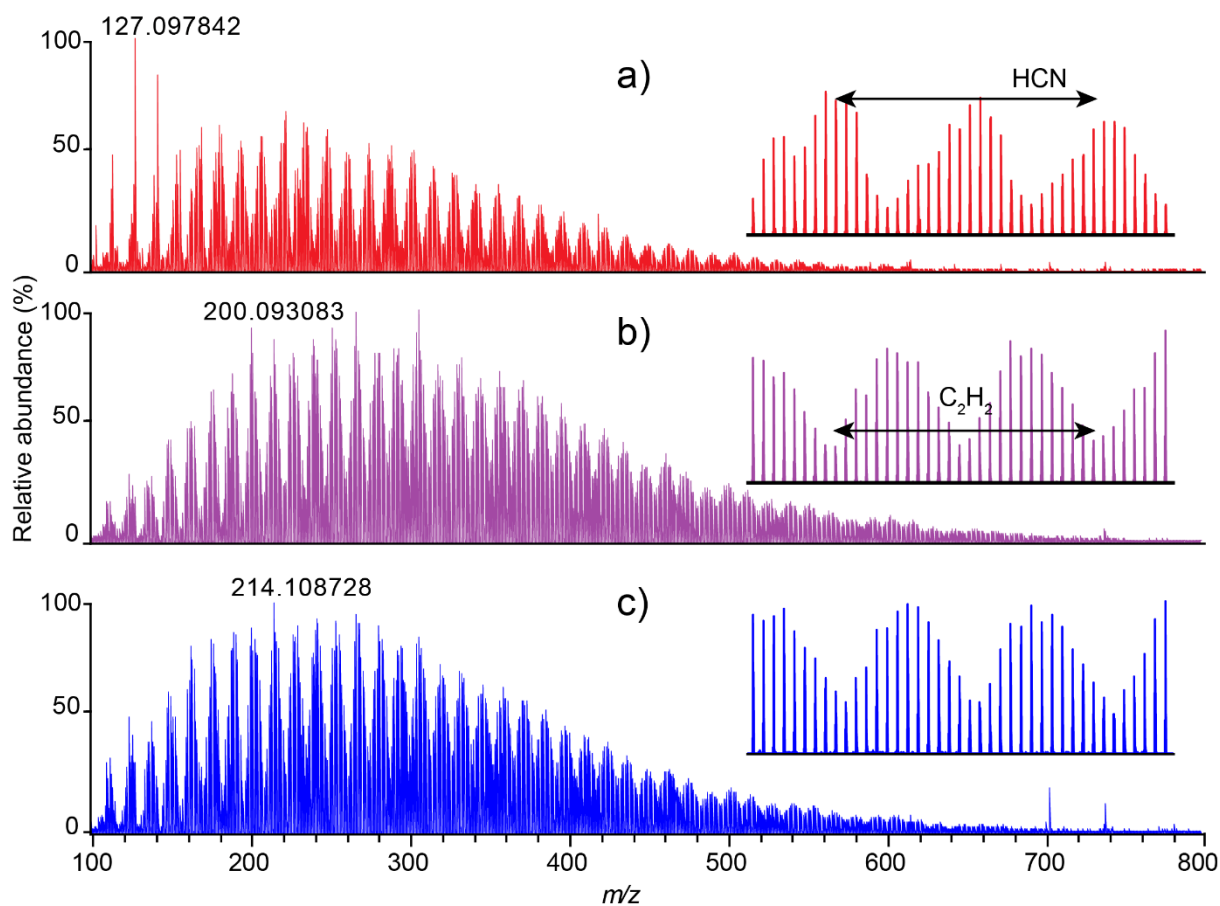


Figure 55 : Laser desorption/ionization FTICR MS spectra of a) soluble fraction, b) non-soluble fraction, c) global sample

These results highlight that significant differences exist between the soluble and non-soluble samples in the growth structure of the Tholins. However, these two specific periods are not the only one detected in each fraction. In addition to this C_2H_2 growth pattern, there are actually numerous ions series present in the samples. Previous work from Gautier et al. 2014 showed that one would need at least 8 different growth series to represent the samples, many of them bearing nitrogen thus explaining the presence of N-containing compounds at relatively high molecular masses.

We consider that presenting a full description for all ion series goes beyond the scope of the present paper and chose to focus solely the C_2H_2 as an illustration of the phenomenon.

The obtained results are also consistent with those recorded in previous studies on Tholins produced on another experimental setup according to the spectrum appearance ([Somogyi, et al. 2005 \[24\]](#) ,[He, et al. 2012 \[91\]](#)).

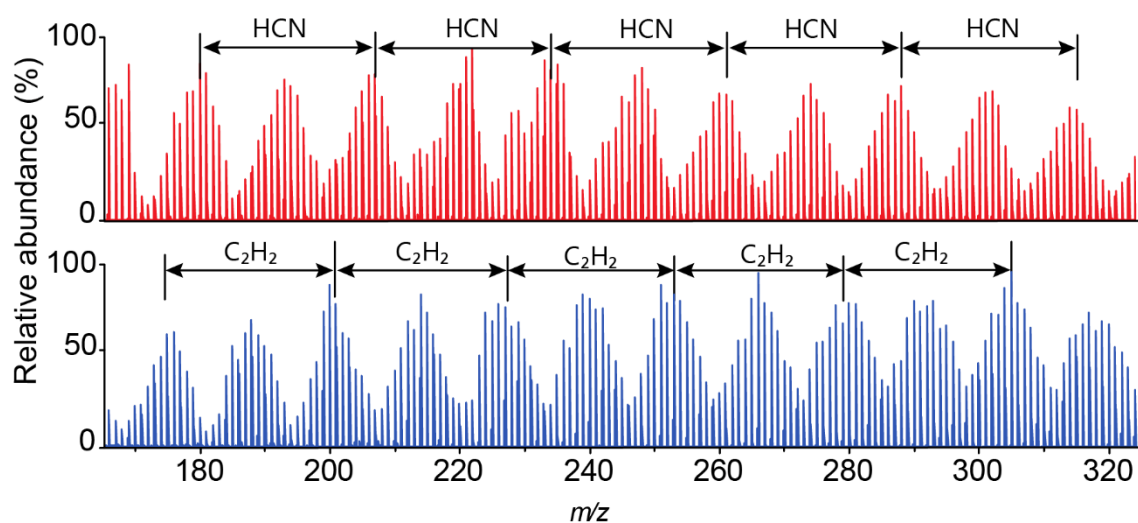


Figure 56 : Enlargement on the soluble (in red) and non-soluble fraction (in blue) to highlight the difference between the periodic phenomenon on each fraction

Figure 57 shows different sections of the mass spectra centered at m/z 148 and m/z 512, for the two different fraction and the global sample. These two parts of the spectrum were chosen to highlight the differences for the low and high m/z values. The ultra-high resolution of the FTICR instrument allows the separation of the isobaric ions even for highly complex ion distributions evidenced at the higher mass range. Interestingly, the three spectra are almost identical in the low mass range but differences can be observed for the higher mass range, with the detection of ions present in the soluble fraction but not in non-soluble fraction. As one can see on the enlargement of **Figure 57** on the m/z 512, the signal for the global sample seems to correspond to the sum of the signals present in the soluble and non-soluble fraction. The obtained ion relative abundance shows a higher amount of species specific to the non-soluble fraction in the total fraction. This is consistent with the amount of soluble species, which corresponds to only 35% of the total Tholins ([Carrasco, et al. 2009 \[93\]](#)).

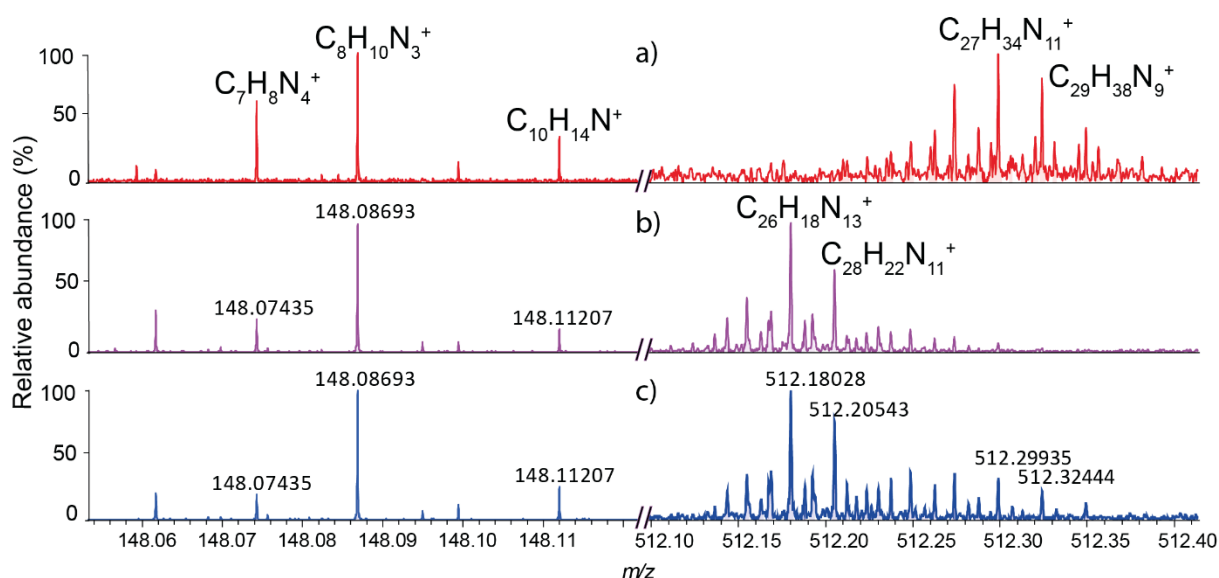


Figure 57 : Enlargement of the m/z 148 and m/z 512 ions for a) soluble fraction, b) non-soluble fraction, c) global sample

In order to obtain a broader picture on the detected species, molecular formulas were determined for each ion. This was done by considering only species presenting C, H and N

elements. Due to the mass accuracy of our measurement (<300 ppb), only one molecular formula could fit with each experimental mass. Looking into details at the molecular formula (see for example m/z 512 on **Figure 57**), the ions specifically detected in the global sample and non-soluble fractions present a low mass defect, consistent with less hydrogenated species. This comparison emphasizes once again important differences between the soluble and the non-soluble fractions: not on the mass of the molecules as initially thought, but rather in the type of molecules present in each sample.

Data treatment using modified Van Krevelen diagrams

Van Krevelen diagrams were originally proposed to assess the origin and maturity of kerogen and petroleum through the use of H/C and O/C ratio ([Van Krevelen 1950 \[76\]](#), [Kim, et al. 2003 \[107\]](#)), ([Van Krevelen 1950 \[78\]](#), [Kim, et al. 2003 \[108\]](#)). Such diagrams were used more recently for other research fields including global ‘omics’ analysis of metabolites but also Tholins ([Pernot, et al. 2010 \[75\]](#)), ([Pernot, et al. 2010 \[77\]](#)). In typical Van Krevelen diagrams O/C are plotted as a function of H/C ratio. These ratios are readily obtained from the molecular formulas. These diagrams particularly show evidence of unsaturation based on the H/C ratio and the number of hetero-elements with the O/C ratio. Previous studies in tholinomics demonstrated the interest of these diagrams by switching the x-axis that commonly represents the oxygen/carbon ratio with the nitrogen/carbon ratio ([Somogyi, et al. 2005 \[24\]](#), [Imanaka and Smith 2010 \[26\]](#), [Gautier, et al. 2014 \[109\]](#)). Van Krevelen diagrams are limited because they do not consider the m/z dimension and ions with very different m/z ratio can yield the same H/C or N/C ratio.

The m/z dimension can be added to the Van Krevelen plot to generate 3D plots ([Pernot, et al. 2010 \[77\]](#), [Gautier, et al. 2014 \[109\]](#)). Specific 2D plots H/C vs m/z or N/C vs m/z have also

been used ([Imanaka and Smith 2010 \[25\]](#)). . Specific 2D plots H/C vs m/z or N/C vs m/z have also been used ([Imanaka and Smith 2010 \[26\]](#)).

A comparison was done to choose the most appropriate axis to be used to represent our samples. Soluble and insoluble fractions were found quite equal in their nitrogen/carbon ratio as visible in the **Figure 58**-right. The nitrogen/carbon ratio bearing little information, the most interesting representation for our samples was determined to be the m/z value versus the ratio hydrogen/carbon (**Figure 58** - left). 3D Van Krevelen to highlight the interest for the first global view on the sample based on the data presented in the **Figure 58** can be found in supporting information (**Figure S93**).

Figure 58 shows the H/C versus m/z plot and the N/C versus m/z plots obtained from the soluble (in red) and the insoluble fractions (in blue). We focused our study on the major contributors to the sample. Thus only species with an intensity above 5% of the base peak (corresponding to 81% of all species detected in the samples according to the sum of all peaks intensities) are displayed. In this illustration, we observe for the soluble fraction (including the common part between the different samples in purple) a triangular distribution that converges toward a unique peak. This distribution has been observed in a previous work ([Pernot, et al. 2010 \[75\]](#)).(Pernot, et al. 2010 [77]). This structure starts at a low m/z value with a large base and then becomes thinner with the increase of the m/z value. This shape is in agreement with a set of copolymers involving the $-\text{CH}_2\text{-HCN-}$ repeating unit with an addition of some carbon in the structure that enlarges the distribution at low m/z values. This confirms the previous results on the soluble fraction described in Pernot et al., 2010, a previous study realized with an OrbitrapTM analyzer with a resolution of 200 000 at m/z 150 and 100 000 at m/z 450.

Figure 58 also presents the data from the non-soluble fraction. As evidenced above, both soluble and non-soluble fractions present very different molecular structures. For the lower

mass range numerous ions are present in both fractions but the difference between soluble and non-soluble fractions increases for m/z values larger than m/z 200, yielding to two distinct convergence points at high masses. The soluble fraction ions converge around H/C 1.5 and N/C 0.5 whereas the non-soluble species converge around H/C 0.75 and N/C 0.5. As discussed above, the two distributions have similar N/C ratio but they differ on the H/C ratio. The non-soluble fraction is composed of, relatively, the same amount of nitrogen as the soluble fraction, but is much less hydrogenated and so, presents a higher amount of unsaturation.

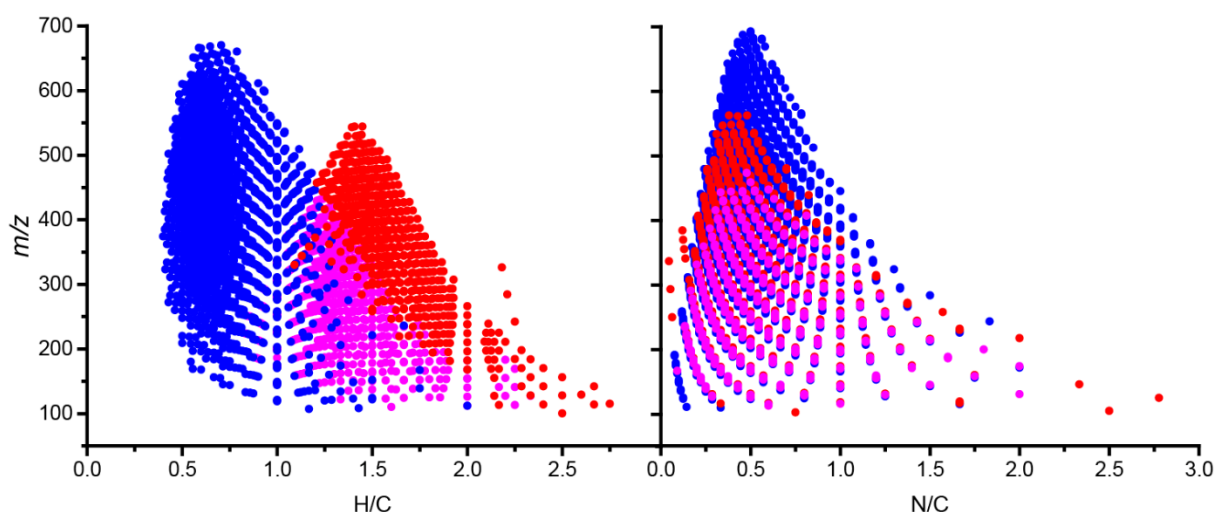


Figure 58 : Van Krevelen diagram of the comparison between soluble and insoluble fraction with a filter at 5% of intensity. Soluble is in red, insoluble in blue and common part in purple.

The distribution representative of the insoluble fraction tends to show that there is another set of polymeric structures in Tholins that has not been identified before from the analysis of the soluble fraction only.

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

Table 6 : 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

Table 6 summarizes the relative weight of each fraction in the global sample according to LDI source response and also the convergence point of each fraction for H/C ratio. The relative weight was calculated by doing the sum of all intensities of species for each fraction. We can see that the common part represents a third of all the species, the insoluble a half and the soluble less than a quarter. This emphasizes that the global sample is mainly composed of the insoluble part, and that the soluble part, the one mainly analyzed in previous mass spectrometry study of Tholins, is actually a minor component of Tholins.

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

Double Bond Equivalent (DBE) vs carbon number plots are generally used in petroleomic to observe the number of unsaturation in a family of species and to compare different oils ([McKenna, et al. 2010 \[3\]](#)).([McKenna, et al. 2010 \[4\]](#)). In **Figure 59** we adapted this graphic representation to highlight the differences between soluble species and non-soluble Tholins species.

A specific range of molecules was selected to create these graphics as the use of the all data set lead to the overlap of multiple species. The **Figure 59** presents the DBE vs Carbon atoms number plot for species containing between 6 and 9 nitrogen atoms for the soluble and non-soluble fractions. We added the Polycyclic Aromatic Hydrocarbon (PAH) line, which

represents the most unsaturated family of species containing only carbon and hydrogen as shown previously ([Cho, et al. 2011 \[109\]](#)). Soluble species are principally under the PAH line whereas non-soluble species are mainly above this reference line. This highlights the fact that the non-soluble species in the Tholins are much more unsaturated than the soluble species. This result is also consistent with what was observed on the Van Krevelen plot. It should be pointed out that this behavior presents several analogies with asphaltenes, which correspond to the fraction of crude oil non-soluble in pentane or heptane. The molecules present in the asphaltene fraction are mainly polyaromatic, which explain the specific properties of this fraction.

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

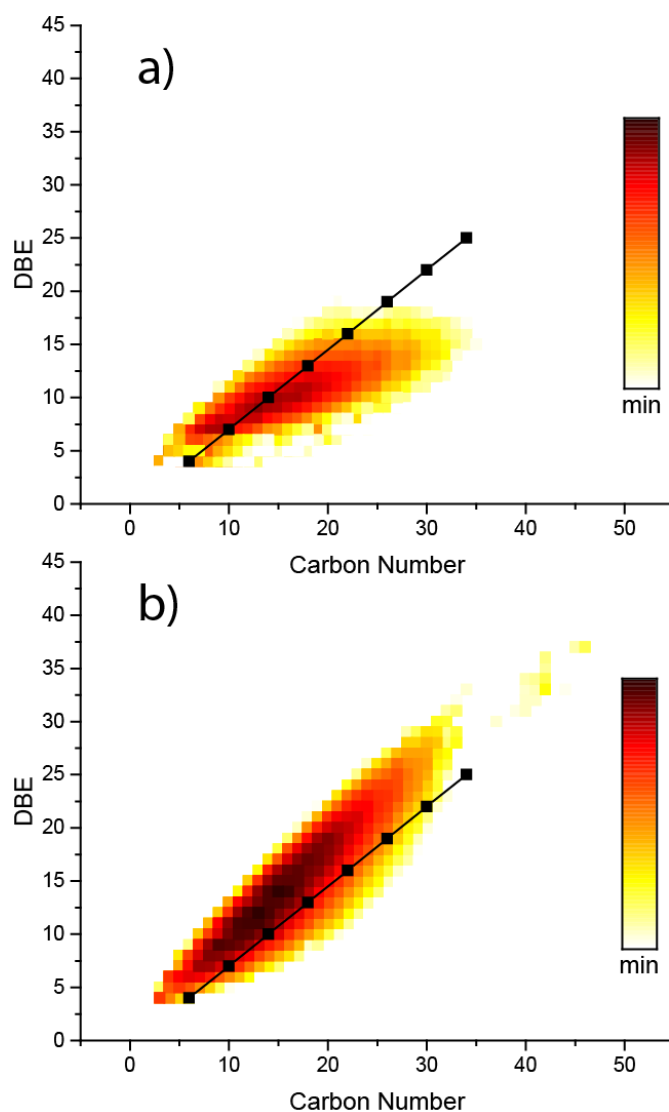


Figure 59 : a) Soluble species containing 6 to 9 Nitrogen, b) Non-soluble species containing 6 to 9 Nitrogen

3.3.4. Conclusions

With our FTICR mass spectrometer equipped with a laser desorption/ionization source it was possible to analyze all the fractions of a Tholins sample produced with the PAMPRE experiment (soluble in methanol, non-soluble and total) using solvent-free deposition method.

The high accuracy of mass measurement coupled with the ultra-high resolution of the FTICR instrument allowed us to assign molecular formula to all species contained in the Tholins. Modified Van Krevelen diagrams enabled us to easily compare the different fractions of the Tholins sample. Based on infrared absorption spectroscopy signatures, it was previously postulated that soluble fraction was representative of the global sample and the only difference between soluble and insoluble species would be the m/z values of the molecules composing the material. In the present work, we observed significant differences between the soluble and non-soluble fractions of this sample, and showed that soluble fraction was mainly not correlated to the insoluble fraction. We highlighted that these fractions are mostly different at the molecular level when m/z increases and that the non-soluble fraction is much less hydrogenated than the soluble fraction. We also confirmed that the soluble fraction does not represent the major part of the sample as expected before, but the ionization yield is similar for each fraction according to their respective proportions observed with the laser ionization. Furthermore, this work demonstrated the presence of a second set of copolymeric structures within the Tholins that had not been identified yet and which are less saturated than the one present in the soluble fraction.

This work highlights the importance to study of all the material and not only the soluble fraction. First, the non-soluble part represents 65% of the global mass sample and is then more representative of the bulk sample. Thus Titan's Tholins have been found less hydrogenated than previously thought, based on the analysis of the soluble fraction.

This high unsaturation level can be explained either by reticulation (interconnections of atoms to form a network rich in cycles and single bonds, see **Figure S94** for example), or by the presence of double and triple bonds. Yet the two structures involve different reactivities, which could impact our understanding of aerosols/gas interactions in Titan's atmosphere.

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

This work also confirmed the potential of the Laser Desorption Ionization source for the study of organic matter in planetary environment ([Gautier, et al. 2017 \[94\]](#))([Gautier, et al. 2017 \[96\]](#)), as being currently implemented for the analysis of Martian organic matter in the ESA/ExoMars mission ([Arevalo, et al. 2015 \[110\]](#)).([Arevalo, et al. 2015 \[111\]](#)). We also showed, on the case of analogs of Titan aerosol analogues, that this ionization method would be a compelling asset if coupled with high resolution mass spectrometry, as currently developed for future space missions ([Briois, et al. 2016 \[111\]](#)).([Briois, et al. 2016 \[112\]](#)). Finally, our results on Titan's Tholins can be compared to studies on the soluble (SOM) and insoluble (IOM) organic matter found in meteorites.

The insoluble matter is known to be constituted of highly branched aliphatics and substituted aromatics (Derenne and Robert 2010), whereas the soluble matter is comprised of more saturated molecules (Schmitt-Koplin, 2010). The origin of both IOM and SOM and their relation with each other is highly debated. They could originate from different reservoirs. One could be the result of the processing of the other, or they could be both produced by the same starting point, and then differ when they continue to grow as we showed in this study ([Derenne and Robert 2010 \[112\]](#)).([Derenne and Robert 2010 \[113\]](#)). Our work on a laboratory complex organic material shows that two fractions with different properties and structures can originate from the same formation processes.

3.4 Conclusions et perspectives

J'ai reporté dans ce chapitre les premières analyses réalisées durant ma thèse sur les Tholins de PAMPRE en spectrométrie de masse à ultra-haute résolution. Afin d'optimiser les performances de spectromètres de masse FTICR de nouvelle génération, ma première étude reportée dans ce chapitre s'est concentrée sur l'amélioration du *shimming* permettant l'ajustement de l'alignement du nuage d'ions avec le champ magnétique. Par la suite, les résultats obtenus en analysant des Tholins avant et après optimisation ont été comparés. Une augmentation de la résolution moyenne de 200 000 a été observée ainsi qu'une amélioration de 13% du nombre de pics attribués.

Dans un deuxième temps, afin d'observer les modifications apportées par l'oxydation induite par l'atmosphère sur les Tholins, j'ai réalisé des analyses sur plusieurs échantillons de Tholins de différents âges sur le FTICR 21 T du MagLab en Floride. L'analyse par ce spectromètre de masse aux performances inégalées a permis l'observation d'une très grande complexité moléculaire avec parfois plus de 120 espèces détectées à chaque masse nominale. Cela nous a permis de mettre en évidence de très nombreuses espèces qui n'étaient pas détectées lors d'une analyse en introduction directe sur des spectromètres de masse moins performants. Nous avons ensuite étudié les processus oxydatifs des Tholins. Cela nous a permis de réaliser un modèle de prédiction du phénomène d'oxydation ainsi qu'observer la formation progressive d'acide carboxyliques durant l'oxydation. Ces derniers sont des précurseurs pour la formation de liaisons peptidiques. Mon étude a des conséquences importantes pour la compréhension de la chimie prébiotique induite lors de l'oxydation des aérosols dans l'atmosphère de la Terre primitive.

Enfin, la présence d'une source LDI sur le FTICR de Rouen m'a permis de réaliser la première étude de la fraction insoluble des Tholins, ce qui n'avait encore jamais été effectué. De

grandes différences ont été observées entre chaque fraction et plus particulièrement sur leur rapport hydrogène sur carbone.

Ce premier chapitre a permis d'observer les différentes espèces chimiques retrouvées dans les Tholins et de faire une cartographie de la diversité moléculaire composant ce mélange. Afin d'aller plus loin dans la caractérisation, et notamment dans l'étude de la formation de ces Tholins, nous avons réalisé des analyses en spectrométrie de masse en tandem, reportées dans le **chapitre IV**.

Malgré la grande quantité d'information obtenu par ces approches en analyse directe par spectrométrie de masse nous avons accès seulement à la formule brute. Afin d'obtenir des informations sur les formules développées nous avons réalisé plusieurs couplages, et notamment un couplage mobilité ionique - spectrométrie de masse. Cette technique permet d'avoir un aperçu de la diversité isomérique présente dans un mélange ainsi que de la conformation structurale la plus souvent présente. Nous avons donc réalisé plusieurs études qui sont reportées dans le **Chapitre V**.

3.5. Références

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Chapitre 4 : Etude de la croissance chimique des Tholins

Sommaire

4.1: Spectrométrie de masse à haute résolution pour de futures missions spatiales : Etude comparative de matière organique complexe avec le LAb-CosmOrbitrap et un LDI-FTICR

4.2: Investigation des structures composants les Tholins grâce à la spectrométrie de masse en tandem.

4.3. Conclusions concernant les deux sections précédentes

4.4 : Spectrométrie de masse bidimensionnelle pour l'analyse des Tholins

Introduction

Les résultats présentés dans le **chapitre 3** ont permis de montrer la diversité chimique existante dans les Tholins grâce à la spectrométrie de masse à très haute résolution. Cependant, ces analyses directes en spectrométrie de masse ne fournissent pas d'information concernant la croissance ainsi que les structures chimiques présentes. Dans ce quatrième chapitre je présenterai les expériences de fragmentation des espèces chimiques détectées dans les Tholins que j'ai réalisées et qui nous renseignent sur des signatures structurales systématiques dans ce matériau.

Dans un premier temps, une étude a été réalisée en comparant un spectre de Tholins obtenu sur le FTICR de Rouen avec un spectre obtenu sur un prototype d'Orbitrap spatial. L'idée recherchée était de savoir si cet Orbitrap extrêmement simplifié était capable d'analyser de la matière organique complexe et d'en récupérer de précieuses informations. Les deux spectres ont été comparés et plusieurs observations ont été faites. Bien qu'une zone de gamme de masse commune aux deux spectres ait été observée, la plupart des ions détectés par CosmOrbitrap se situaient à une valeur de m/z bien inférieure à celle de ceux détectés par le FTICR. Nous nous sommes alors posés la question de l'origine de cette différence et avons attribué cela à de la fragmentation liée aux méthodes d'ionisation différente entre les deux instruments. Malgré ces différences, cette étude a validé les capacités analytiques du CosmOrbitrap pour l'analyse de matière organique complexe. Ces résultats sont reportés par **l'article 4**.

Dans un second temps, dans le but de mieux comprendre la structure de ces Tholins, une étude a été réalisée en spectrométrie de masse en tandem.

Ces expériences se sont portées sur la fragmentation par collisions induites des espèces détectés dans la fraction soluble et insoluble des Tholins. De grandes différences ont été observées entre les deux fractions. En effet, la fraction insoluble s'est révélée être beaucoup plus stable que la fraction soluble. Cette observation a été attribuée à la différence d'aromaticité entre les espèces détectées dans chaque fraction. J'ai ainsi, grâce aux résultats obtenus, proposé des structures pour chaque série de composés détectés. Ces résultats sont reportés dans **l'article 5**.

Bien que l'étude par fragmentation ait permis d'avancer dans la compréhension de la structure des Tholins, elle a été limitée par des problèmes instrumentaux. En effet, les étapes de fragmentation sont réalisées en isolant un certain rapport m/z dans un quadripôle puis en accélérant ce paquet dans un gaz tampon. Or, la résolution du quadripôle ne dépasse pas 3000, comme présenté dans le **chapitre 2**. Cette résolution n'est pas suffisante pour isoler une gamme de masse inférieure à un dalton. Dans un échantillon de Tholins, plusieurs dizaines voire centaines de pics sont présents dans une gamme de masse d'un dalton, ce qui signifie que les fragments obtenus sont difficilement attribuables à un pic spécifique. Dans le but de remédier à ce problème, une méthode d'isolation et de fragmentation directement dans la cellule ICR a été testée. Cette méthode permet de réaliser des cartographies bidimensionnelles d'un échantillon avec comme première dimension les ions précurseurs et comme second les fragments correspondants. Pour l'instant, peu de résultats ont été obtenus mais cette étude est reportée sous forme de perspectives dans la dernière partie de ce chapitre.

4.1: Spectrométrie de masse à haute résolution pour de futures missions spatiales : Etude comparative de matière organique complexe avec le LAb-CosmOrbitrap et un LDI-FTICR

Cette première partie présentera une étude comparative entre un prototype d'Orbitrap® spatial et un FTICR. Un échantillon de Tholins a été analysé par les deux appareils avec une méthode d'ionisation LDI. Le but est de confronter les capacités analytiques du prototype spatial lors de l'analyse de matrices complexes avec celle d'un FTICR, l'analyseur en masse aux meilleures performances de laboratoire à ce jour. Cette partie est présentée sous forme d'article publié en 2019 dans le journal *Rapid communication in mass spectrometry*.

Afin de résumer cet article, rappelons que le Laboratoire de Physique et Chimie de l'Environnement et de l'Espace (LPC2E) avec lequel j'ai collaboré pour cette étude fait partie d'un *consortium* ayant pour but la création d'une nouvelle génération de spectromètre de masse spatial afin d'améliorer significativement les capacités analytiques des instruments embarqués pour l'étude des matières organiques extraterrestres. Un prototype basé sur la technologie Orbitrap a vu le jour, équipé d'une source d'ionisation par laser. Un spectre de Tholins réalisé sur le FTICR de Rouen ainsi qu'un spectre du même échantillon enregistré sur l'Orbitrap spatial ont été comparés. Trois zones du spectre ont été définies. Une première zone à bas rapport m/z où les espèces détectées sont uniquement présentes en CosmOrbitrap; une seconde zone commune aux deux instruments, qui a permis la comparaison des espèces; et enfin, une troisième zone à haut rapport m/z uniquement présente en FTICR. La comparaison réalisée dans la seconde zone a permis de prouver la capacité de l'Orbitrap spatial à détecter les mêmes espèces qu'un instrument commercial. Cependant, l'apparition d'espèces à faible rapport m/z en CosmOrbitrap nous a amené à nous poser la question d'éventuelles fragmentations. Ces fragmentations seront étudiées dans la seconde partie de ce chapitre.

Abstract

Mass spectrometers are regularly boarded on spacecraft for the exploration of the Solar System. A better understanding of the origin, distribution and evolution of organic matter and its relationships with inorganic matter in different extra-terrestrial environments requires the development of innovative space tools, described as Ultra High Resolution Mass Spectrometry (UHRMS) instruments.

Analyses of a complex organic material simulating extraterrestrial matter (Titan's Tholins) are performed with a homemade space-designed Orbitrap™ equipped with a laser ablation ionization source at 266 nm: the LAb-CosmOrbitrap. Mass spectra are obtained using only one laser shot and transient duration of 838 ms. A comparison is made on the same sample with a laboratory benchmark mass spectrometer: a Fourier Transform Ion Cyclotron Resonance equipped with a laser desorption ionization at 355 nm (LDI-FTICR) allowing accumulation of 20,000 laser shots. Mass spectra and attributions of molecular formulae based on the peaks detected by both techniques show significant similarities. Detection and identification of the same species are validated. The formation of clusters ions with the LAb-CosmOrbitrap is also presented. This specific feature brings informative and unusual indirect detections about the chemical compounds constituting Titan's Tholins. In particular, the detection of HCN confirms previous results obtained with laboratory Electrospray Ionization (ESI)-UHRMS studies about the understanding of polymeric patterns for the formation of Tholins.

Capabilities of the LAb-CosmOrbitrap to decipher complex organic mixtures using single laser shot and a short transient are highlighted. In agreement with results provided by a commercial FTICR instrument in the laboratory, we demonstrate in this work the relevance of a space laser-CosmOrbitrap instrument for the future planetary exploration.

4.1.1. Introduction

Since the boarding of the first space mass spectrometers (MS) on a planetary exploration mission in the 1970s, these instruments have been used to decipher the chemical composition

of extraterrestrial bodies such as our closest neighbors Venus and Mars, the outer planets and their moons but also more Solar System primitive objects, as comets and asteroids. In some environments, such as the atmosphere of Titan, the organic matter detected was found to be extremely complex. The Cassini-Huygens mission explored the Saturnian environment from 2004 to 2017 using amongst other instruments two quadrupole MS: the Ion and Neutral Mass Spectrometer, INMS, aboard Cassini; and the Gas-Chromatograph Mass Spectrometer, GCMS, aboard the Huygens descent module. The interpretation of chemical analysis performed by these instruments was widely reduced due to their poor resolution and the high molecular complexity of the analyzed material resulting in extremely congested mass spectra with a low resolution. For instance, for INMS (Ion Neutral Mass Spectrometer), the identification of a part of the complex organic matter was not direct due to the mass resolution achieved by the instrument (~ 100 at m/z 100). Using modelling inversion, the team was able to propose possible attributions for a number of peaks in INMS data, up to $m/z < 100$. However, the mass resolution of the Cassini-Huygens instruments (INMS, Gas-Chromatograph-MS GCMS and Cassini Plasma Spectrometer CAPS) did not enable to directly decipher the composition of the complex organic matter. For the GCMS instrument, while tens of peaks are visible in the acquired mass spectra ([Niemann, et al. 2005 \[8\]](#)), only a handful of molecule have been identified so far in it. Deep analytical improvements of new space mass spectrometers are thus needed, in terms of mass resolving power and mass accuracy. Low mass resolving powers ($< 1,000$) from previous space missions have been increased up to 9,000 in more recent space mission (here the value is given for the Double Focusing Magnetic Mass Spectrometer-Rosetta Orbiter Spectrometer for Ion and Neutral Analysis instrument from the Rosetta mission, at m/z 28, as referenced in [Balsiger et al., 2007](#)). In the laboratory, they have been increased to ultra-high ones, higher than 100,000 ([Smith, et al. 2018 \[32\]](#), [Nikolaev, et al. 2011 \[35\]](#), [Denisov, et al. 2012 \[45\]](#)). These

enhancements, adapted to space MS, will allow a deeper understanding of the extraterrestrial material detected.

The Orbitrap™ technology ([Makarov 2000 \[46\]](#) ,[Perry, et al. 2008 \[47\]](#)) has been identified as a promising technique in the development of the new generation of space MS. A space-qualified version of the Orbitrap mass analyzer and its peripheral elements, called CosmOrbitrap, is currently under TRL (Technology Readiness Level, *i.e.* the advancement of the instrument development toward a flight able one) development ([Briois, et al. 2016 \[48\]](#)). The goal is to reach a mass resolution of *a minima* 50,000 (Full Width at Half Maximum) at m/z 56 under ultra-high vacuum conditions up to 10^{-9} mbar in the analyzer chamber. A simplified test-bench in the laboratory directly couples the CosmOrbitrap with a Nd-YAG laser at 266 nm (the LAb-CosmOrbitrap, standing for Laser Ablation CosmOrbitrap). As the instrumental configuration “laser-CosmOrbitrap” is highly considered for a space application ([Arevalo, et al. 2018 \[49\]](#)), the laboratory prototype has been designed as proof-of-concept, to challenge the performances of a future simple and compact spaceflight HRMS (High Resolution Mass Spectrometry)-CosmOrbitrap based instrument.

In the laboratory, highly complex organic matter, mimicking those of Titan’s atmosphere, has been synthesized by diverse experiments ([Szopa, et al. 2006 \[16\]](#) ,[Coll, et al. 1999 \[18\]](#) ,[Sciamma-O'Brien, et al. 2017 \[22\]](#) ,[Berry, et al. 2019 \[50\]](#) ,[Gautier, et al. 2017 \[51\]](#) ,[Hörst, et al. 2018 \[52\]](#) ,[Sciamma-O'Brien, et al. 2014 \[53\]](#)).

This material, named Titan’s Tholins, is an analogue of the photochemical smog surrounding Titan, well-known for its chemical complexity. A wide variety of organic compounds is thus produced and the whole forms a complex organic mixture. They are considered as primary

interest in planetary sciences and exobiology. They have been extensively studied using UHRMS laboratory techniques such as the ESI (Electrospray Ionization)-OrbitrapTM and ESI/LDI-FTICR (Laser Desorption Ionization – Fourier Transform Ion Cyclotron Resonance) commercial instruments. Results published in the Gautier et al., 2014; Pernot et al., 2010; and Somogyi et al., 2005 showed a polymeric material, with a highly complex chemical composition and a large number of chemical functionalities specific to nitrogen-rich species such as amino and nitrile compounds. As high analytical performances are now required for mass spectrometers selected on new space missions, Titan's Tholins are complex-enough organic standards for testing the capabilities, and thus demonstrating the relevance, of future space UHRMS instruments. Studying Titan's Tholins with the LAb-CosmOrbitrap enables to estimate (i) its capabilities to analyze a highly complex organic material, (ii) the chemical analysis allowed by these analytical performances and (iii) its relevance as a future space mass spectrometer dedicated to the study of Titan and other organic-rich environments in the Solar System.

In terms of analytical performances, one benchmark MS technique in laboratory is based on FTICR. Indeed, mass resolving powers higher than 20,000,000 obtained at nominal m/z of 608, *i.e.* on reserpine (Nikolaev et al., 2011; Smith et al., 2018) are, to this day, unmatched performances. A comparative study is presented here, between the LAb-CosmOrbitrap and a 12 Tesla FTICR. The FTICR is equipped with a LDI source. A laser ionization is thus performed, in both cases, on the same solid sample. Without any specific sample preparation, both techniques provide the analysis of the global material, and not only the soluble fraction as it is usual with the electrospray ionization technique. Using LDI-FTICR, Maillard et al., 2018 have demonstrated major differences in results obtained on the bulk Tholins sample and the soluble fraction, in terms of chemical composition. It is thus consistent to compare results between LAb-CosmOrbitrap and LDI-FTICR, both studying the global material of Titan's Tholins. Mass spectra obtained using these two MS techniques and molecular attributions inferred have been compared. This comparison aims at supporting the potential of the LAb-CosmOrbitrap as a potential future simple and compact space UHRMS instrument.

4.1.2. Material and methods

4.1.2.1. Synthesis of the complex organic compound studied

The samples chosen are solid spherical grains with a mean diameter of about 300 nm and made of nitrogen-rich organic molecules ([Sciamma-O'Brien, et al. 2010 \[15\]](#), [Hadamcik, et al. 2009 \[54\]](#)).

Tholins were synthesized in the radio-frequency plasma reactor called PAMPRE at LATMOS ([Szopa, et al. 2006 \[16\]](#)) at room temperature. A gas mixture containing 5% of methane and 95% of nitrogen flowed into the reactor with a 55 sccm (standard cubic centimeter per minute) total gas influx, leading to a total pressure of 0.9 mbar. A 30 W radio-frequency plasma discharge produced the reactive molecules leading to the formation of solid grains in the volume of the plasma box with limited wall effects. A few hundred mg of sample were collected in vials after several days of running experiment. We note that some oxidative processes occur on the surface of Tholins and probably during ionization. This process is minor and occurs only at the surface of the sample, affecting ~3% of the material ([Carrasco, et al. 2016 \[55\]](#), [Fleury, et al. 2014 \[56\]](#)). We were nonetheless careful not to over interpret this contamination, and as such we limited our study to molecules without oxygen atoms. These correspond to molecules which have not been altered by the oxidation and a representative of the pristine sample. This was possible thanks to the very good accuracy of measurements of each instrument, allowing the separation of oxygenated species that have lower mass defects than non-oxidized ones.

4.1.2.2. Simple sample preparation

Tholins were analyzed with LAb-CosmOrbitrap and LDI-FTICR. Both techniques allow a basic sample preparation and introduction in the ionization chamber. No chemical preparation of Tholins was done prior to the analysis. This simple deposit of the sample aims at mimicking an *in situ* study, as it would be done during a space mission. Collecting and bringing samples in front of the ionization system, with a space mass spectrometer, are key parameters for space exploration. In our study case, we assume the sample to be collected and a thin layer to be deposited onto one metallic sample-holder. Specifications of the two set-ups are classified in the Table 7. The first part of this table is related to the sample.

In the case of the LDI-FTICR set-up, the sample holder is a commercial stainless steel MALDI plate. The sample is deposited using a solvent-free method, following a previously published procedure([Barrere, et al. 2012 \[42\]](#)). For the LAb-CosmOrbitrap laboratory prototype, the holder is a homemade circular indium sample-holder (7 mm diameter) on which Tholins are pressed and embedded onto the surface (**Figure 60**). For both techniques, the sample thickness is not tightly controlled but is estimated to be less than 100 μm .



Figure 60 : Pictures of the Titan's Tholins powder pressed onto the indium sample-holder of the LAb-CosmOrbitrap before laser shot (left) and after laser shots (middle). On the middle picture, laser shots are visible on the brown Tholins powder, demonstrating the ablation of the sample. The scheme (right) illustrates dimensions of the sample-holder (8 mm height and 7 mm diameter).

4.1.2.3. Ultra-High Resolution Mass Spectrometry (UHRMS) techniques used for the study

The LAb-CosmOrbitrap laboratory prototype

The LAb-CosmOrbitrap is a simpler instrumental configuration than the one commercialized by Thermo Fisher Scientific (Bremen, Germany). No quadrupole, no linear trap nor C-trap are used in front of the Orbitrap cell. The Orbitrap cell is directly coupled with a laser ablation ionization source, with an Einzel lens to focalize the ions packet inside the analytical cell, as the C-trap would do in the commercial instrument. Specifications of the ionization source are summarized in the second part of the **Table 7**. The Nd-YAGx4 laser emitting at 266 nm (Brilliant model, Quantalet corporation) generates a 4 ns pulse. The energy reaching the sample can vary from 30 to 750 μJ , thanks to optics located between the laser output and the sample. Calculations give a fluence ranging from 2.9 to 12 J/cm^2 , indicating an ablation/desorption transition and pure ablation laser regimes. The CosmOrbitrap, as it has been used for these experiments, is currently at TRL 3, which means this is a laboratory prototype used as proof-of-concept, demonstrated analytically and experimentally. We note that the ionization source is not included in the TRL development. One mass spectrum was obtained at each laser shot. The signal transient was composed of 4,194,304 points recorded with a sampling frequency of 5 MHz (Alyxan software). Voltages were set to detect positive ions. A Hann window then a Fast Fourier Transform (FFT) were applied on the full 838 ms signal transient. Ultra-vacuum is needed with about 10^{-9} mbar and 10^{-8} mbar respectively measured in the mass analyzer and the ionization chambers. For the molecular formula attribution, the two most intense peaks of each nominal mass of the mass spectrum with a signal-to-noise ratio greater than 5 were selected. The molecular formula attributed to each peak selected was the $\text{C}_x\text{N}_y\text{H}_z$ formula with the closest mass.

The formula was considered acceptable if the resulting mass accuracy is lower than 2 ppm. Constraints applied for the molecular formula attribution were at most 30 hydrogen atoms, 20 carbon atoms and 20 nitrogen atoms. Even and odd electron configurations were considered.

With the same prototype configuration (TRL 3), analyses on pure organics and prebiotic compounds have demonstrated mass resolving power (FWHM) up to 70,000 at m/z 430 and mass accuracy better than 3 ppm ([Arevalo, et al. 2018 \[49\]](#) ,[Selliez, et al. 2019 \[57\]](#)). A full description of the set-up used in this work can also be found in these papers.

Table 7 : Specifications of both instrumental set-up: LAb-CosmOrbitrap and LDI-FTICR. The chart is separated in three main sections: sample, ionization and mass analysis.

	Instrumental set-up	LAB-CosmOrbitrap	LDI-FTICR
Sample	Sample	solid tholins powder (PAMPRE, 5% CH ₄)	
	Sample-holder	Homemade circular indium sample-holder	Commercial stainless steel MALDI plate
	Estimated thickness	< 100 μm	
Ionization	Laser type	Nd-YAG 4ω	Nd-YAG 3ω
	Wavelength	266 nm	355 nm
	Laser regime	Ablation/desorption	Desorption
	Laser pulse duration	4 ns	3 ns
	Laser power	42 kW	24 kW
	Used output	171 μJ	0.43 μJ
	Spot size (on sample)	18,558 μm ²	706 μm ²
	Fluence	2.60 J/cm ²	0.06 J/cm ²
	Number laser shot	1	40 * 500
	Repetition rate	N/A	1,000 Hz
Pressure inside mass analyzer chamber	10 ⁻⁹ mbar	3*10 ⁻¹⁰ mbar	
Mass analysis	Mass analyzer	Orbitrap™ cell (D30)	Paracell™ cell
	Transient duration	838 ms	2.2 s
	Apodization window	Hann	Half-sine
	Mass resolving power FWHM at m/z 430	Up to 70,000	600,000
	Mass range	m/z 1 to 2,000 (for the space configuration)	m/z 98 to 1,200

LDI-FTICR instrument

A FTICR (Solarix XR, Bruker Daltonics, Bremen, Germany) equipped with a 12 Tesla superconducting magnet and a laser desorption ionization source was used in this comparative study. The laser is a Nd-YAGx3 emitting at 355 nm with a shot frequency of 1,000 Hz. Experimental conditions were similar to those described in Maillard et al., 2018. Mass spectra were obtained using the broadband mode from m/z 98 to m/z 1,200 with the sum of 500 scans in positive ion mode. The analogue signal was amplified and digitalized with 8 million points resulting in the recording of transient of about 2.2 s which was transformed into the corresponding frequency domain by Fourier transform (one zero fill and half-sine apodization). Under these conditions a mass resolution of 600,000 at m/z 430 was obtained. Ionization parameters were set as follows: deflector plate at 210 V, plate offset at 100 V, laser power at 19% leading to an output energy of 0.43 μ J and a “small” laser focus . One scan represents 40 laser shots. Funnel 1 was set at 150 V and skimmer 1 at 25 V. Pressures of 10^{-3} mbar in the ionization chamber, 10^{-6} mbar through ions optics then $3 \cdot 10^{-10}$ mbar inside the ICR cell were measured. Molecular formulae were attributed after a peak picking with a signal to noise of 5 using Data Analysis 4.4. Following parameters were set to assign formula: $C_{0-x}H_{0-y}N_{0-z}$, with a maximum number of nitrogen atoms sets to 30, and even and odd electron configurations with a tolerance of 0.5 ppm.

Both instrumental configurations, LAb-CosmOrbitrap and LDI-FTICR, allow the analysis of solid Titan’s Tholins, leading to the study of the total fraction of the sample. Major differences are still observed in the two set-ups. On one hand, the LAb-CosmOrbitrap is a non-commercial instrumental configuration developed to be a space HRMS, with only one single laser shot, at 266 nm. Ionization is performed in a transitional regime between ablation and desorption, with a fluence about 2 J/cm².

In addition the source pressure is of 10^{-6} mbar in LAb-CosmOrbitrap and 10^{-3} mbar in the FTICR. Higher pressure yields to collisional cooling. A higher extent of fragmentation is thus expected as well as clustering and ion recombination with the LAb-CosmOrbitrap. A short transient duration of 838 ms leads to one mass spectrum of 4 million points. On the other hand, the LDI-FTICR is a commercial instrument accumulating 500 consecutives FID (Free Induction Decay), each one obtained with 40 laser shots. A pure laser desorption regime is observed. Heavy ions are suspected to be formed and detected, due to this softer ionization (in comparison to the LAb-CosmOrbitrap one). The transient duration is about 2.2 s.

4.1.3. Results

4.1.3.1. Global comparison between LAb-CosmOrbitrap and LDI-FTICR mass spectra

We compare mass spectra obtained with both techniques in the m/z range 8 to 600 (**Figure 61**). At the top, blue mass spectrum corresponds to LAb-CosmOrbitrap data while at the bottom red mass spectrum (in reverse scale) corresponds to LDI-FTICR data. This global comparison is separated in three distinct zones named A, B and C.

Zone A corresponds to the lowest masses, with $m/z < 100$. Zone B involves intermediate masses, $100 < m/z < 200$. Zone C corresponds to the highest masses, with $m/z > 200$.

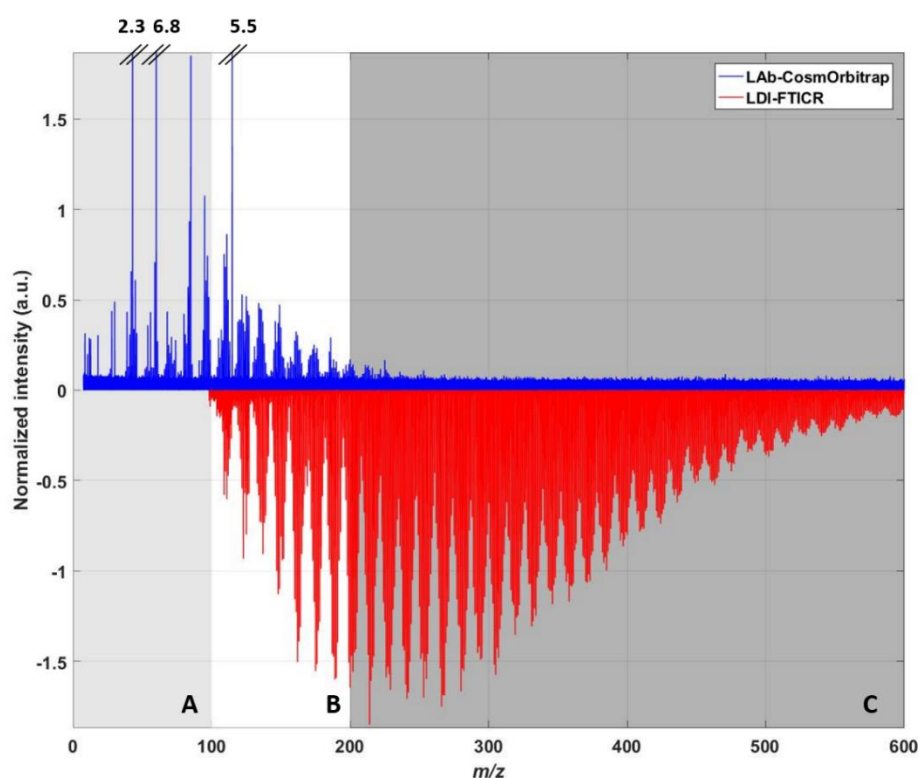


Figure 61 : Comparison of positive ions mass spectra (1) LAb-CosmOrbitrap, in blue, top panel and (2) LDI-FTICR, in red, lower panel in reverse scale. Intensities of three peaks (m/z 43.0290; 60.0556 and 114.9033) in the LAb-CosmOrbitrap mass spectrum are out of the window and thus reported at the top the window. Mass spectra are separated in three distinct zones named A, B and C. Each zone is detailed in a specific result section.

Zone A is only constituted by the contribution of the LAb-CosmOrbitrap data. The absence of peak detection in the m/z range 0 to 100, with LDI-FTICR technique, is due to an instrumental constraint of the set-up. Most of the commercial MS techniques used for the Titan's Tholins analyses allows data acquisition starting at a m/z rather high (*e.g.* $m/z > 50$). In the case of FTICR, starting detection at lower m/z requires a higher sampling frequency (due to the Nyquist Shannon criteria), a shorter transient and so, a lower resolution at higher mass. In this case, the choice of resolving higher masses was done. In addition, RF ion optics of the FTICR that transport ion from the high-pressure source region to the FTICR analyzer are not optimized for transmission of low m/z ions ([Le Vot, et al. 2011 \[58\]](#), [Page, et al. 2006 \[59\]](#)). The contrary is observed with the LAb-CosmOrbitrap, with low mass detections starting at m/z 8, with high mass resolution. These uncommon detections at low masses allow the study of a mass range unexplored so far using other commercial MS instruments but induce also some restrictions at higher masses. The observation of positive ions at $m/z < 100$ is also confirmed by the detection of cluster ions and will be detailed in the section 3.3.

In zone B, both mass spectra show peaks organized in a polymeric pattern, specific to the complex organics studied (*i.e.* Titan's Tholins). We observe (i) the end of the polymeric pattern detected with LAb-CosmOrbitrap and (ii) the beginning of the signal recorded by LDI-FTICR. It is the best zone for the comparison of results obtained in both techniques.

Unlike zone A, the contribution of LDI-FTICR data is dominant in zone C. The maximum of the polymeric pattern is observed around m/z 250, then it decreases down to m/z 600. As flat signal (noise level) is recorded in the LAb-CosmOrbitrap mass spectrum at $m/z > 200$, this range will not be further analyzed in this study but will be discussed in the perspectives.

Focusing on LAb-CosmOrbitrap data obtained with the instrumental parameters referenced in **Table 7**, peaks are mainly located on the m/z range 20 to 200. In this m/z range, three peaks are detected at higher intensities and outside of the window studied (their intensities are reported on the figure). One of them is identified as the indium positive ion ($^{115}\text{In}^+$) at m/z 114.903 (used as mass calibrant). Others are located at m/z 43.0290 and 60.0556 and will be studied in the following sections.

4.1.3.2 Comparison of LAb-CosmOrbitrap and LDI-FTICR spectra in zone B

This section will exclusively focus on the zone B identified on **Figure 61**. Three zoom on this mass window have been extracted (**Figure 62**). A first window (top) allows to visualize the whole zone B, on the m/z range 100 to 200, including detail of each polymeric pattern. Two other mass windows present m/z ranges more and more focused on one exclusive nominal mass: m/z 130 to 155 (middle) and m/z 138.0 to 138.2 (bottom).

Waves composing the polymeric pattern are observed, in both cases (LAb-CosmOrbitrap and LDI-FTICR), at the same location on the x-axis. This is particularly well-observed in the top mass window. The two other mass windows confirm, in addition to the same location of the waves, the same location of each peak inside of these waves. Visually, the LAb-CosmOrbitrap and LDI-FTICR mass spectra are consistent and seem to show the detection of the same species.

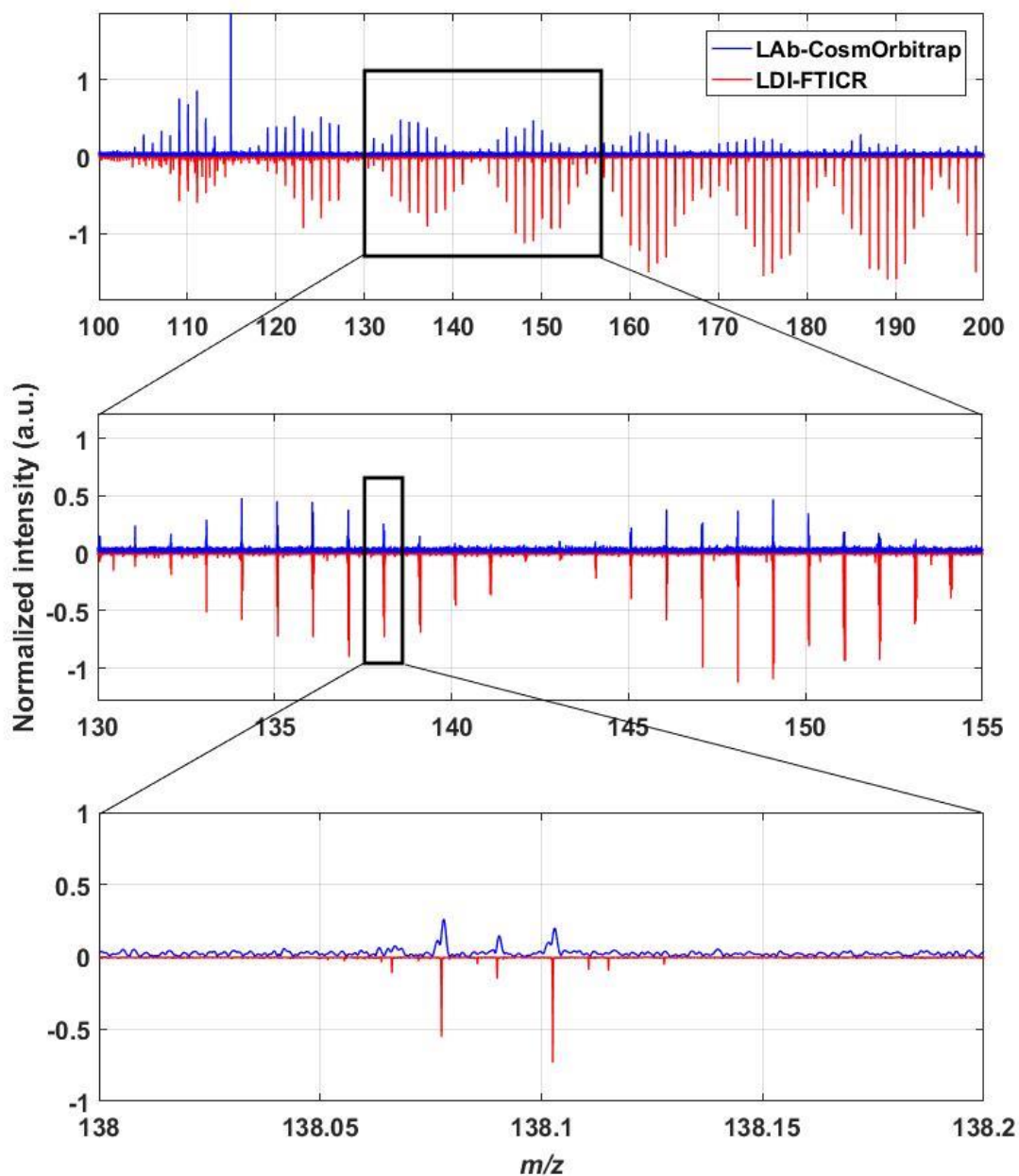


Figure 62 : Focus on three distinct zones in the LAB-CosmOrbitrap and LDI-FTICR mass spectra, as presented in **Figure 61**. The first window (top) shows m/z from 100 to 200. A black box indicates the next window (middle) from m/z 130 to 155. Again, another black box indicates the last window (bottom) from m/z 138 to 138.2. As a reminder from the **Figure 61**, the LAB-CosmOrbitrap mass spectrum is the blue top panel and the LDI-FTICR mass spectrum the red low panel in reverse scale.

Looking at a specific m/z value, the peak at m/z 138.077433 is detected with a mass resolving power of 1,551,160 in the LDI-FTICR mass spectrum. In the LAb-CosmOrbitrap mass spectrum, the same peak is detected at m/z 138.0771 with a mass resolving power of 118,778. We remind here that this study aims at showing results obtained with the LAb-CosmOrbitrap, a simple and compact instrumental configuration (with a D30 cell) developed for space. This is not a commercial Orbitrap, as no C-trap helps to the focalization and accumulation of ions and the transient duration is shorter than the LDI-FTICR instrumental configuration used for the comparison. It is thus expected to get mass resolving power lower with the LAb-CosmOrbitrap than with the LDI-FTICR, but the question is to know if the analytical performances of this homemade space-designed Orbitrap are high-enough to allow the peak attribution and species identification unambiguously.

To answer this question, the Table 8 sets out molecular formulae attributed to peaks similarly detected, for both techniques, in the m/z range 133 to 152. The first and second columns of the table report the theoretical m/z of positive ions detected in the mass range and the corresponding molecular formula. Then the table is separated in two main columns: the first one dedicated to the LAb-CosmOrbitrap results and the second one to LDI-FTICR. Each of them is composed of two columns: the experimental m/z value of positive ions detected within the precision of the technique considered (4 digits for the LAb-CosmOrbitrap values and 6 digits for the LDI-FTICR ones) and the mass accuracy (in ppm). In the 132-152 m/z range, 26 molecular formulae have been attributed based on the LAb-CosmOrbitrap mass spectrum and 38 based on the LDI-FTICR mass spectrum. The mass resolving power of each technique allows the detection of more than one ion for each nominal mass. For each ion, an attribution was done. For the m/z range studied in this section, 21 ions were detected in common in LAb-CosmOrbitrap and LDI-FTICR mass spectra (in bold in the Table 8).

Theoretical values		LAb-CosmOrbitrap		LDI-FTICR	
m/z (7 digits)	Molecular formulae	Measured m/z (4 digits)	Mass accuracy (ppm)	Measured m/z (6 digits)	Mass accuracy (ppm)
133.0760247	C ₈ H ₉ N ₂ ⁺	133.076	-0.19	133.076039	0.11
134.0712737	C ₇ H ₈ N ₃ ⁺	134.0713	0.20	134.071284	0.08
135.0665227	C ₆ H ₇ N ₄ ⁺	135.0665	-0.17	135.066535	0.09
135.0916748	C ₈ H ₁₁ N ₂ ⁺	135.0916	-0.55	135.091692	0.13
136.0617716	C ₅ H ₆ N ₅ ⁺	136.0617	-0.53	136.061784	0.09
136.0869237	C ₇ H ₁₀ N ₃ ⁺	136.0868	-0.91	136.086939	0.11
137.0821727	C ₆ H ₉ N ₄ ⁺	137.0821	-0.53	137.082189	0.12
138.0774217	C ₅ H ₈ N ₅ ⁺	138.0771	-2.33	138.077433	0.08
138.1025738	C ₇ H ₁₂ N ₃ ⁺	138.1024	-1.26	138.102588	0.10
145.0760247	C ₉ H ₉ N ₂ ⁺	145.076	-0.17	145.076038	0.09
146.0712737	C ₈ H ₈ N ₃ ⁺	146.0711	-1.19	146.071289	0.10
147.0665227	C ₇ H ₇ N ₄ ⁺	147.0663	-1.51	147.066536	0.09
147.0916748	C ₉ H ₁₁ N ₂ ⁺	147.0916	-0.51	147.091692	0.12
148.0869237	C ₈ H ₁₀ N ₃ ⁺	148.0868	-0.84	148.086939	0.10
149.0821727	C ₇ H ₉ N ₄ ⁺	149.082	-1.16	149.082187	0.10
150.0774217	C ₆ H ₈ N ₅ ⁺	150.0773	-0.81	150.077432	0.07
150.1025738	C ₈ H ₁₂ N ₃ ⁺	150.1023	-1.82	150.102583	0.06
151.0726707	C ₅ H ₇ N ₆ ⁺	151.0725	-1.13	151.072683	0.08
151.0978228	C ₇ H ₁₁ N ₄ ⁺	151.0976	-1.47	151.097833	0.07
152.0679196	C ₄ H ₆ N ₇ ⁺	152.0678	-0.79	152.067936	0.11
152.0930718	C ₆ H ₁₀ N ₅ ⁺	152.0928	-1.79	152.093087	0.10

Table 8 : Similar detections in the m/z range 133 to 152, between LAb-CosmOrbitrap and LDI-FTICR mass spectra. Theoretical m/z and molecular formula are given in the two first columns. Each molecular formula is correlated to the measured m/z of the corresponding peak, within the precision of the technique, and its mass accuracy (in ppm) for both techniques. The m/z value is given within the precision of the instrumental configuration: respectively 4 and 6 digits for the LAb-CosmOrbitrap and the LDI-FTICR set-ups. In this m/z range, 26 molecular formulae have been attributed from the LAb-CosmOrbitrap mass spectrum, 38 from the LDI-FTICR mass spectrum. Between both mass spectra, 21 ions are in common and referenced in the Table. Only common ions are indicated in this table. The global table starting at m/z 132, including similarities and discrepancies, is given in Supplementary Material.

These differences are based on (i) the non-detection of one given species by one of the two MS technique or (ii) the detection of two different species, at a same nominal m/z . In this case, the detection of two distinct species leads to two different attributions.

To illustrate this, we can focus on the nominal m/z 132 where two different species are detected: an ion at m/z 132.0679 is detected in the LAb-CosmOrbitrap mass spectrum, when the LDI-FTICR mass spectrum presents an ion at m/z 132.080789. These two m/z ratios are distinguishable thanks to the mass resolution of the two MS techniques considered. They lead to the attribution of two molecular formulae, respectively $C_8H_8N_2^+$ and $C_9H_{10}N^+$. The first case (non-detection of a species by one of the two techniques) is more complex as it reveals that some information are sometimes missed by one technique.

In the LAb-CosmOrbitrap mass spectrum, this is mostly caused by the low signal to noise ratio, having some peaks under the limit of detection. This is observable in the shorter m/z range 139 to 142, where peaks are too close to the noise level to be considered as peaks and are consequently not attributed. In the LDI-FTICR mass spectrum, the signal to noise ratio induced by the sum of 500 consecutives FID is very high, allowing the detection of low abundance ions. In addition, there is a strong difference about the energy applied on the sample, inducing two laser regimes different between both MS techniques: ablation for LAb-CosmOrbitrap and desorption for LDI-FTICR. Fragmentation is thus stronger in the LAb-CosmOrbitrap mass spectrum. Some fragment ions, detected in the LAb-CosmOrbitrap mass spectrum are possibly not formed with the LDI-FTICR technique and thus not observable in the mass spectrum.

4.2.3.3. Revealing low masses with the LAB-CosmOrbitrap

One of the most useful tool in order to visualize ions distribution in a complex organic mixture mass spectrum is the mass defect versus exact mass diagram (MDvEM). This diagram represents the mass defect (experimental mass – nominal mass) of the detected compounds as a function of their experimental m/z ([Kendrick 1963 \[28\]](#) ,[Murray, et al. 2013 \[60\]](#) ,[Sleno 2012 \[61\]](#)). Each dot in the diagram represents the most intense m/z value observed in the mass spectrum, at a given nominal mass.

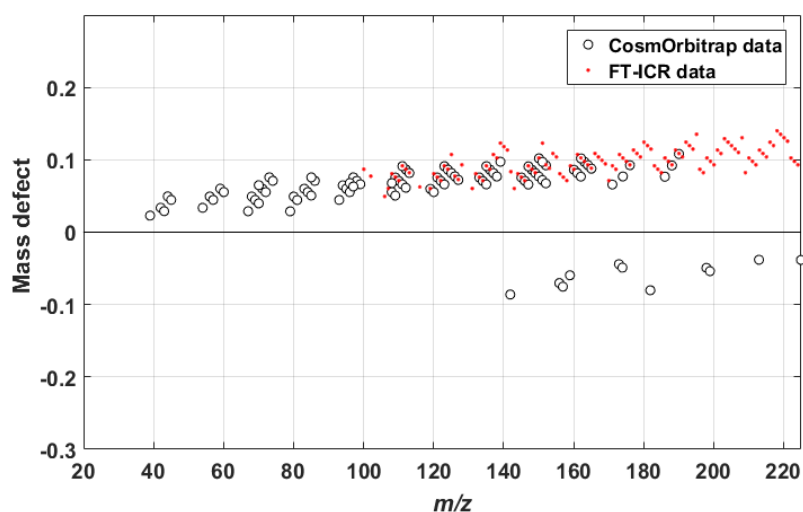


Figure 63 : Mass Defect versus Exact Mass diagram (MDvEM) derived from mass spectra presented in **Figure 61**. Red dots represent ions from the LDI-FTICR mass spectrum. Black open circles represent ions from LAB-CosmOrbitrap mass spectrum.

A first visual distinction is made between compounds presenting a positive mass defect against those presenting a negative mass defect. Dots with a positive mass defect are highly dominated by organic compounds. Indeed, elements mainly composing organic compounds such as hydrogen and nitrogen have positive mass defects (respectively +0.0078 and +0.0031 u, for H and N).

As Tholins are synthesized using a gas mixture of N_2 and CH_4 , their constituting molecules are composed of carbon, hydrogen and nitrogen, leading to positive mass defects regarding the formed compounds. They are observed on the **Figure 63**. On this diagram, red dots correspond to the LDI-FTICR ions and black circles to the LAb-CosmOrbitrap ones.

Thanks to the LAb-CosmOrbitrap instrumental configuration, including the sample-holder technique used, cluster ions between positive ions of the metallic sample-holder (here, $115In^+$) and the neutral organic sample are observed in the mass spectra ([Selliez, et al. 2019 \[57\]](#)).

Formation mechanisms of these cluster ions are similar to those of non-covalent bonds, as referenced in Bhardwaj and Hanley, 2014; Carrasco et al., 2016 and Le Roy et al., 2015. MDvEM diagrams derived from the LAb-CosmOrbitrap mass spectra show thus a contribution with a negative mass defect coming from the detection of the indium positive ion. This element is indeed characterized by a large negative mass defect of -0.0962 u. As a reminder, the indium peak is observed in the LAb-CosmOrbitrap mass spectrum because it composes the metallic surface of the sample-holder. As the indium peak in the mass spectrum allows a useful calibration point for the whole mass spectrum, the indium dot in the MDvEM diagram marks the location where cluster ions $[Organics + In]^+$ should be observed. Indeed, the main asset of this MDvEM diagram is to reveal some specific trend lines, dots alignments or dots grouping, giving information about the possible polymers constituting a polymeric pattern but also clusters formed with a common element such as indium.

In the **Figure 63**, we observe this specific contribution with a negative mass defect, only for the CosmOrbitrap data (black circles). Indeed, due to the ablation ionization source employed, elements coming from the sample and the sample-holder are produced and detected.

Dots composing this contribution are related to cluster ions including one positive ion of the main isotope of indium ($^{115}\text{In}^+$). In addition to the indium positive ion, neutral organic compounds are identified such as HCN. Indeed, we observe at m/z 141.9139 the cluster assumed to be $[\text{In}+\text{HCN}]^+$. As we know the positive ion of indium to be present, we can remove its m/z contribution: $141.9139 - 114.9033 = 27.0106$ leading to the neutral HCN value (i.e. 27.0109). Using this method, the following compounds have been identified: HCN, CH_2N_2 , CH_5N_3 , C_2HN_3 , $\text{C}_2\text{H}_4\text{N}_4$, $\text{C}_3\text{H}_6\text{N}_4$ and $\text{C}_4\text{H}_6\text{N}_4$. We note that such compounds are indirectly identified in their neutral form with indium clusters and also observed in their protonated and positive ion form, at lower masses.

Cluster ions are thus indirectly indicative of organic compounds composing Tholins. They allow the preservation of neutral organics. All these seven molecules provide a strong contribution of nitrogen elements in Tholins and draw a consistent picture of the molecular growth from the light HCN molecule to the first N-PAH structures. The detection of HCN is important: it confirms the polymeric pattern suspected in Tholins by Gautier et al., 2014 and Pernot et al., 2010 from a statistical analysis of the molecules formulae inferred from the high resolution mass spectra at higher m/z . CH_2N_2 has previously been observed in Tholins and identified as diazirine (cyclic isomer) after soft thermal desorption followed by VUV photoionization ([Cunha de Miranda, et al. 2016 \[62\]](#)). The CH_5N_3 species is identified as guanidine, already referenced in the study of Pernot et al., 2010. C_2HN_3 is consistent with dicyanamide (NC-NH-CN). This molecule has been previously observed as deprotonated (C_2N_3^- at m/z 66) in the context of Titan's laboratory experiments: it appeared as a main fragment ion after MS/MS analysis of Tholins ions in Carrasco et al., 2009 and as a major negative ion in the plasma discharge producing the Tholins ([Dubois, et al. 2019 \[63\]](#)).

$C_2H_4N_4$ has two major isomers: the linear cyanoguanidine $HN=C(NH_2)-NH-CN$, or the cyclic aminotriazole $NH_2-C_2N_3H_2$. The triazole cycle is aromatic and might be thermodynamically favored in the plasma discharge producing Tholins. It might be a first clue towards larger N-heterocyclic polyaromatic hydrocarbons (N-PAH). $C_3H_6N_4$ has also several isomers, among them the possible methyl-aminotriazole, corresponding to the aminotriazole with an addition of a methyl group CH_3 . The $C_4H_6N_4$ presence could be explained by the addition of C_2H_2 on $C_2H_4N_4$, possibly leading to the formation of a second adjacent cycle. Others species detected are informative on the chemical composition of Tholins material and still consistent with the presence of nitrogen-rich compounds.

Looking for the protonated positive ions related to these compounds at low masses, we note that three of them are among the most intense peaks observed: $[CH_2N_2+H]^+$ at m/z 43.0290; $[CH_5N_3+H]^+$ at m/z 60.0556 and $[C_2H_4N_4+H]^+$ at m/z 85.0507.

These direct and indirect observations of such organic compounds demonstrate analysis possibilities with the LAB-CosmOrbitrap instrumental configuration. Organic species detected at $m/z < 100$ are consistent with cluster ions observed at $m/z > 150$.

4.1.4. Conclusion and perspectives

Mass spectra of a complex mixture (*e.g.* Titan's Tholins) have been obtained for the first time with a homemade space-designed Orbitrap instrument (LAb-CosmOrbitrap). A comparison of results has been made with those obtained with a LDI-FTICR instrument, on the same sample. The two instrumental set-ups allow the study of the bulk Tholins sample, giving a relevant comparative study.

First of all, both set-ups were able to reveal the complexity of the sample. As expected, higher dynamic range and resolution were observed in the FTICR mass spectrum due to the use of a commercial instrument allowing ions accumulation and long transient duration. Nevertheless, the m/z range 100-200 allows, in both techniques, to recover the shape of a polymeric pattern. Looking at a same nominal mass, the main ions were detected and similarly attributed thanks to the high-enough mass resolving power. A few differences were observed, coming from the non-detection of a specific ion by one of the two techniques. This is well-explained by significant differences between the two set-ups, mostly about the transient accumulation and the laser regime.

In the LAb-CosmOrbitrap mass spectrum, cluster ions were also observed. They are composed by positive ion(s) coming from the metallic sample-holder and neutral organic compounds. Not detected in commercial MS techniques using softer ionization processes such as LDI-FTICR, cluster ions are indirect detections leading to the study of neutral organics composing the Titan's Tholins. Chemical compounds identified point out the presence of nitrogen-rich species. They confirm hypotheses suggested by previous studies, for instance the role played by the HCN polymer in the construction of the polymeric pattern specific to the Titan's Tholins.

With only one single laser shot and a short transient duration of less than one second, these results validate the capabilities of CosmOrbitrap to push forward the understanding of organic species in complex mixture encountered in space. The speed acquisition of a whole high resolution mass spectrum is a main asset of this instrumental configuration considering space missions with these specific limits. Other scenarios can be thought to improve the sensitivity of the data, such as using a higher number of laser shots and ions accumulation. A lower laser energy applied would allow a reduction of the Tholins fragmentation and thus a possible detection of higher m/z species. These assumptions have to be checked in the laboratory in order to determine how the best results can be obtained before being implemented on a space configuration.

In addition to the validation of our results by a benchtop laboratory technique, we can compare them with those obtained using space mass spectrometers and more specifically with the quadrupole mass spectrometers INMS and Huygens GC-MS, from the Cassini-Huygens mission, which studied *in situ* Titan's atmospheric composition.

These instruments were only able to study a reduced m/z range, respectively from m/z 1 to 99 (INMS) and m/z 2 to 141 (GC-MS) and at low resolution ($m/\Delta m$ of 100 at m/z 100 for both instruments). In our study, we were able to analyze Tholins over the m/z range 20 to about 200.

Our m/z range is thus more informative than these two instruments. Moreover, the LAB-CosmOrbitrap mass range is initially more extended. The laboratory prototype is able to cover a m/z range from 8 to few thousands. The space configuration in development and based on the LAB-CosmOrbitrap aims at covering a m/z range from 1 to 2,000 never reached neither by INMS nor the GC-MS.

And this would be done with a mass resolving power higher of several magnitude order than the INMS and GCMS, as it can reach few hundreds of thousands until m/z 136 and few tens of thousands at m/z 431 for the Lab-CosmOrbitrap ([Selliez, et al. 2019 \[57\]](#)).

To resume our study, we show in this work the relevance of a laser CosmOrbitrap instrument to provide a deep chemical analysis of a complex organic material. A laser CosmOrbitrap is the simplest instrumental configuration though for space. It provides an unprecedented mass-resolution allowing elemental identification of complex organic matter. A further issue to go deeper in the analysis will be to solve isomers and provide structural identification, as preliminary work by Gautier et al. 2016 demonstrated the need for with such complex samples using laboratory LC-Orbitrap. More complex configurations of the CosmOrbitrap could be proposed in that context, and the one that seems most suitable for this new challenge is the trapped ion mobility spectrometry (TIMS) ([Castellanos, et al. 2014 \[64\]](#), [Tose, et al. 2018 \[65\]](#)). This technique, located before the mass spectrometer, allows to estimate the number of isomers present in a mixture. It allows, thanks to the retention of ions in a small electric field and their diffusion according to their collision with the gas flowing through pumping, to separate the isomers according to their size and charge. Its utility has recently been proven for the analysis of laboratory Tholins and has allowed to show the isomeric diversity present in this complex environment ([Ruger, et al. 2019 \[30\]](#)).

4.2: Investigation des structures composants les Tholins grâce à la spectrométrie de masse en tandem.

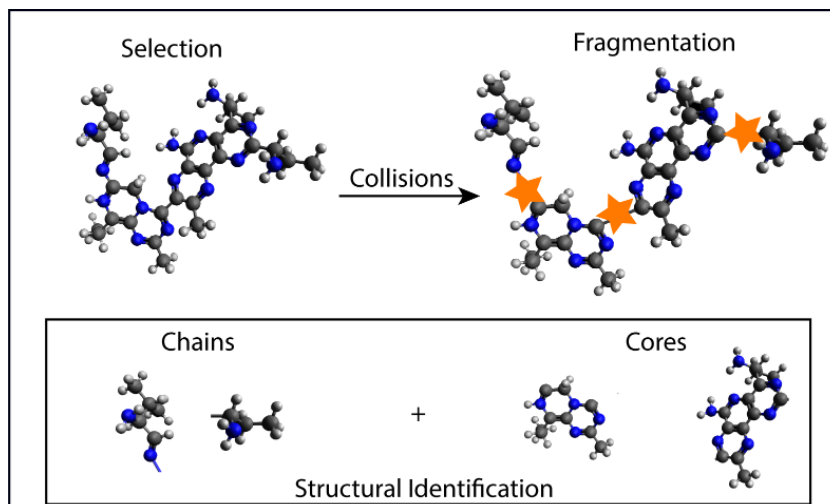
La deuxième partie présentée ici expose les résultats que j'ai obtenus en spectrométrie de masse en tandem lors de l'analyse des fractions soluble et insoluble des Tholins. Le but premier fut d'étudier les fragmentations caractéristiques présentes et d'observer la diversité structurale présente. Ces résultats sont présentés sous la forme d'une version d'article prête pour soumission.

Afin d'obtenir d'avantage d'informations concernant les structures composant les Tholins, nous avons décidé d'effectuer de la spectrométrie de masse en tandem. Cette technique permet de fragmenter des espèces sélectionnées grâce à leur collision avec un gaz tampon. Ces fragments sont caractéristiques de structures et permettent de récupérer de précieuses informations.

Ici, les deux fractions (soluble et insoluble) ont été étudiées individuellement. Trois séries principales de composés ont été mises en lumière : deux séries composant la fraction insoluble et une composant la fraction soluble. Les séries composant la fraction insoluble se sont révélées beaucoup plus résistantes à la fragmentation que celle de la fraction soluble, ce qui a permis d'en déduire des structures polyaromatiques peu ramifiées pour la première série, et des structures moins aromatiques pour la deuxième série. Celle composant la fraction soluble s'est révélée beaucoup plus sensible à la fragmentation, ce qui a permis de proposer des structures plus ramifiées pour cette fraction.

Abstract

Understanding the formation and composition of the organic photochemical haze produced in Titan's upper atmosphere is an important milestone in prebiotic chemistry, for the early Earth climate and for the origin of life.



However, retrieving knowledge about this organic fog is limited because of non-sample return possibilities. Chemical analyses from previous laboratory experiments allowed to obtain molecular information from analogues of this haze that were produced on Earth. In the current work, we push forward our comprehension of the structure composing this complex sample using tandem mass spectrometry. This type of analysis allows to recover structural information thanks to the observation of systematic fragmentation patterns. Strong differences are found between soluble and insoluble fractions of the aerosols and putative structures are proposed.

Introduction

Due to highly charged particles coming from Saturn's magnetosphere and UV photons from the sun, nitrogen and methane present in the upper atmosphere of Titan initiate an intense atmospheric chemistry chain (McKay et al., 1989; Sagan et al., 1993; Samuelson and A. Mayo, 1991). The very effective neutral and ion chemistry occurring induces the formation of large species than can be above 10 000 Da (Vuitton et al., 2009; Waite et al., 2007; Westlake et al., 2014) that end with the production of a complex organic haze lower in the atmosphere. This thick haze hides the surface of the largest satellite of Saturn (Israel et al., 2005). The Voyager and Cassini-Huygens space missions brought to light the importance of the carbon and nitrogen chemistry in the formation of this haze (Vuitton et al., 2006; Vuitton et al., 2019; Vuitton et al., 2007). However, due to the technical limitations of the onboard instruments, fully understanding the growing path of the haze particles is still out of range and many questions remain on their chemical composition. To address this issue, analogues of Titan's haze are produced on Earth (Coll et al., 1999; McKay et al., 1989; Poch et al., 2012; Sagan and Reid Thompson, 1984; Sciamma-O'Brien et al., 2010; Szopa et al., 2006; Trainer et al., 2006). These samples, called Tholins, are analyzed in the laboratory with different analytical techniques. Several analyses, including infrared spectroscopy (Cable et al., 2014; Gautier et al., 2012; Imanaka et al., 2004), capillary electrophoresis (Cable et al., 2014) and nuclear magnetic resonance (Derenne et al., 2012; He and Smith, 2014b, 2015) revealed the presence of molecular families such as nitriles, amines and hydrocarbons. Punctual structures, such as triazine, pyrazole and pyrazine were identified thanks to liquid and gas chromatography coupled to mass spectrometry (Gautier et al., 2014; Gautier et al., 2016).

Mass spectrometry analyses revealed repetitive wave shapes in the mass spectra (Somogyi et al., 2005; Somogyi et al., 2012). A polymeric structure was suggested by several research teams (Matthews and Minard, 2006; Vuitton et al., 2010). But even if a repetition unit of $(\text{CH}_2)_n$ - $(\text{HCN})_m$ was found statistically good to explain the structure of Tholins, the material is assumed as different from a pure polymer but rather a random one (Bonnet et al., 2013; Vuitton et al., 2010).

Recently, ultra-high resolution analyses through Fourier transform ion cyclotron resonance mass spectrometers have allowed to dig into the chemical complexity of these Tholins by generating global cartographies of the molecular diversity found in these samples (Maillard et al., 2018; Somogyi et al., 2012; Somogyi et al., 2016).

FTICR-MS is a state-of-the-art instrument, generally used to decipher the chemical composition of ultra-complex mixture such as petroleum (Hughey et al., 2002; Le Maître et al., 2019; Marshall and Rodgers, 2008; Smith et al., 2018). This instrument allows an unprecedented resolving power and dynamic range (Nikolaev et al., 2011), making it perfect for the analysis of Tholins samples which are an extremely complex material. These analyses allowed to get access to all the molecular formulae present in the sample. However, the isomeric and structural diversity remained unknown. In order to obtain a global overview of the global structure present in Tholins sample, ion mobility coupled with mass spectrometry was performed (Maillard et al., 2020; Ruger et al., 2019). These studies allowed to narrow the possibilities of structures by excluding several families of compounds. Hypothetical structures obtained by theoretical calculations were also proposed.

Several tandem mass spectrometry analyses were previously performed in order to push the chemical characterisation (Somogyi et al., 2005; Somogyi et al., 2012).

These studies have been performed on small species detected in the Tholins sample (maximum m/z 190). They found systematic losses such as HCN and CH_2N_2 as well as evidence of aromatic structures. However, higher species were not studied. In this work, in order to get further evidence about the growing path and the structure of these Tholins, tandem mass spectrometry (MS/MS) was performed at higher masses ($m/z > 400$).

Tholins samples studied here, are from the PAMPRE experiment. They are mainly insoluble in methanol (Maillard et al., 2018). Using the combinaison of *electrospray* (ESI) and laser desorption ionization (LDI) coupled to a 12 T FTICR, results concerning the fragmentation of both fractions are reported in this work.

2. Methods

2.1. Analogues of Titan's aerosols production

Titan's aerosol analogues were produced with the PAMPRE experiment following the same procedure detailed in previous publications (Gautier et al., 2011; Szopa et al., 2006). The reactor is composed of a stainless-steel cylindrical reactor in which a RF-CCP discharge is established thanks to an RF 13.56 MHz frequency generator. A N₂:CH₄ 95:5% gas mixture is injected in the chamber as a continuous flow through polarized electrodes and is then extracted by a primary vacuum pump to ensure that gases are homogeneously distributed. The plasma discharge is maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature. A brown powder is recovered after few hours. Tholins are then stored in an inert atmosphere and protected from light. Limited oxidation occurs during the harvest, thus the oxygenated species are sorted out and are not studied during the mass spectrometry data treatment. It should be noticed that the pressure and temperature are lower in Titan's ionosphere (respectively $\sim 10^{-5}$ - 10^{-8} mbar and 200 K) than in our experiment, but the ionization rate is the same (\sim ppm_v). As ion-molecule reaction rates are relatively insensitive to the temperature, the lower temperature in Titan's ionosphere (200K instead of 293K in the laboratory) is not an important issue in our case. The higher pressure ensures a faster kinetics in the experiment, and is low enough to limit termolecular reactions. The similar ionization rate enables a realistic contribution of ions into the whole ion-neutral coupled chemical network.

2.2. Sample preparation

To recover the soluble part of the sample, 4 mg of Tholins are dissolved in 1 mL of methanol in a vial. The vial is vigorously stirred for 3 minutes to solubilize the maximum of species.

The brown mixture is then filtered using a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter on a filter holder. The filtered solution is transferred into a vial and then analyzed after half dilution with a 50/50 water/methanol mixture. The insoluble part is as well recovered for analysis.

2.3. Tandem mass spectrometry analysis

All analyses are performed on a hybrid quadrupole - FTICR Solarix XR from Bruker equipped with a 12 Tesla superconducting magnet and a dynamically harmonized ICR cell. The mass spectrometer is also equipped with an electrospray ionization source and a laser desorption ionization source (laser NdYAG \times 3 355 nm). The dynamically harmonized cell of the instrument was optimized using a design of experiments in order to obtain spectral best performances (Maillard et al., 2019). The mass spectrometer is externally calibrated with a solution of sodium trifluoroacetate. Afterwards, mass spectra are internally calibrated with confidently assigned signals. This calibration procedure yields to a mass accuracy below 300 ppb in the considered mass range. The following ion transfer parameters are used for both electrospray (ESI) and laser desorption ionization (LDI) analyses in positive ion mode: capillary exit 150 V, deflector plate 200 V, funnel1 150 V, skimmer1 15 V, funnel RF amplitude 60 V_{pp} , octopole frequency 5 MHz, octopole RF amplitude 350 V_{pp} , lower cut-off of the quadrupole at m/z 93, time-of-flight 0.6 ms, frequency TOF 6 MHz, TOF RF amplitude 200 V_{pp} , side kick offset -1 V, front and back trapping plate 1.75 V. The soluble fraction was analyzed with *electrospray* ionization. Insoluble fraction is deposited using a solvent-free method, following a previously published procedure (Barrere et al., 2012) before being analyzed by laser desorption ionization.

Tandem mass spectrometry experiments are carried out using collision-induced dissociation (CID) in order to produce fragments (Chacon-Patino et al., 2017, 2018a, b; Ruger et al., 2017). This technique is based on the acceleration of mass selected ions species in a collision cell filled with a neutral gas such as argon or nitrogen. The extent of fragmentation generated depends on the nature of the target gas, the pressure and acceleration voltage applied (Shukla and Futrell, 2000). A scheme of the instrument is given in **Figure S95** in order to have a better comprehension of experiments carried out here. To sum up, the first selecting quadrupole allows to isolate a defined m/z range which is then fragmented in the collision cell. Resulting fragments are detected in the ICR cell. Tandem mass spectrometry is performed using collision energy values ranging from 0 eV to 60 eV. This variation of acceleration voltages allows to induce more fragmentation and so, the apparition of new fragments. This process allows a better understanding of the fragmentation pathway. For the nominal mass fragmentation, a window of one Dalton was set around the selected mass in order to isolate as less peaks as possible to simplify the comprehension of the analysis. Experiments were recorded in triplicates and only species recovered in the three triplicates were considered as real. Data treatment was carried out using DataAnalysis 5.0, OriginPro and Matlab. In addition, the collision energy in the laboratory frame of reference (E_{lab} in eV) is defined by $E_{\text{lab}}=z \times \Delta V$. Because the detected ions are singly-charged, the collision energy applied to the precursor ions is equal to the used acceleration voltage

3. Results

In order to obtain the maximum information about Tholins, several types of tandem experiments were conducted. Experiments without mass selection were evaluated for the insoluble and soluble species. In this experience, the selecting quadrupole is not set as a filter but only as an ion guide, meaning that all ions generated are fragmented in the collision cell. However, regarding the lack of information given by these experiments due to the extremely high complexity of this sample, results of this broadband CID are only reported in supporting information by the **Figure S96** and **Figure S98**. Nominal mass fragmentation was carried out on three nominal masses for the insoluble and soluble fraction. As the conclusions between each mass are the same, only the fragmentation of the nominal mass 504 will be discussed in the rest of this study. The two other masses (m/z 304 and m/z 404) are reported in supporting information by **Figure S99**, **Figure S100**, **Figure S101** and **Figure S102**.

Figure 64 displays representative tandem mass spectra obtained from mass selected ions (m/z 504 nominal mass) for the insoluble and soluble fraction. The inset above each spectrum shows the same isolated species without acceleration voltages. Many species are co-isolated by the selecting quadrupole due to its low mass resolution (< 3000). Therefore, it is impossible to study particular/individual species, and all following results are reported using global views and molecular maps.

The first observation that is recovered from **Figure 64** is the strong difference in the fragment ion distribution between both fractions. In order to have a better understanding of what is happening, breakdown curves are given in **Figure 65** for each fraction showing the evolution of precursor ions relative intensity depending on the collision energy.

As observed, when the fragmentation starts for the insoluble fraction around 20 eV, soluble precursors are already fragmented at 66%.

This highlights the fact that species present in the insoluble fraction are harder to break than in the soluble fraction. In our previous work, we have shown that the difference at the molecular level between each fraction is related to the number of unsaturations (Maillard et al., 2018). The insoluble fraction species present in average a higher number of unsaturations compared to the soluble fraction. With the insoluble fraction, at 40 eV collision energy, major fragment peaks are close to the precursor peak at m/z 504 indicating that they are produced through small neutral molecules losses. In contrast, major peaks in the soluble fraction are located under m/z 200 indicating that mainly relatively high mass neutral losses are produced. The insoluble fraction presents a relatively low H/C ratio which is consistent to more aromatic species that are more stable and are therefore harder to fragment. In addition, the loss between major peaks is different in both fractions. Several HCN are lost in the insoluble part whereas several NH_3 are lost in the soluble one. In order to go further in the description of both fractions, each one will be discussed separately in the rest of the manuscript.

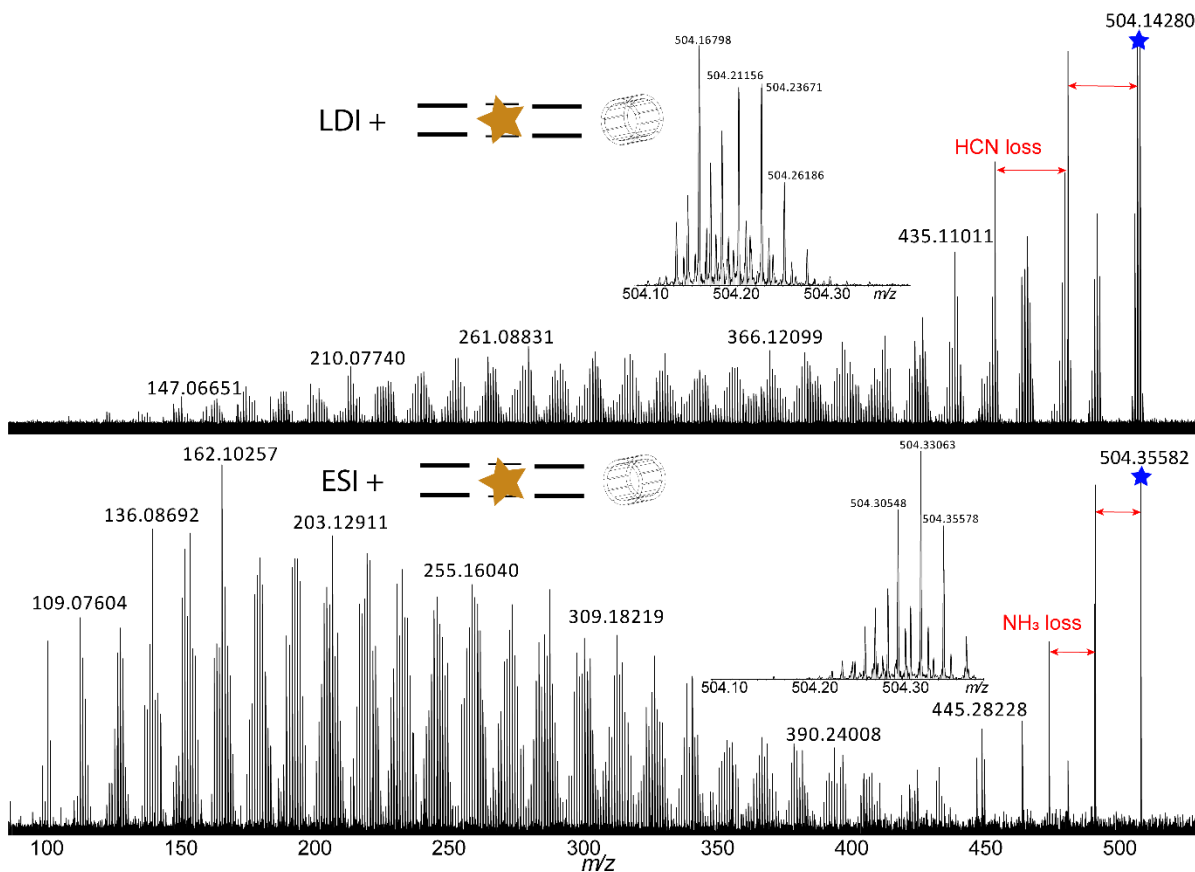


Figure 64 : Fragmentation of the nominal mass 504 Da at 40 eV. (Top) Insoluble fraction, (Bottom) Soluble fraction.

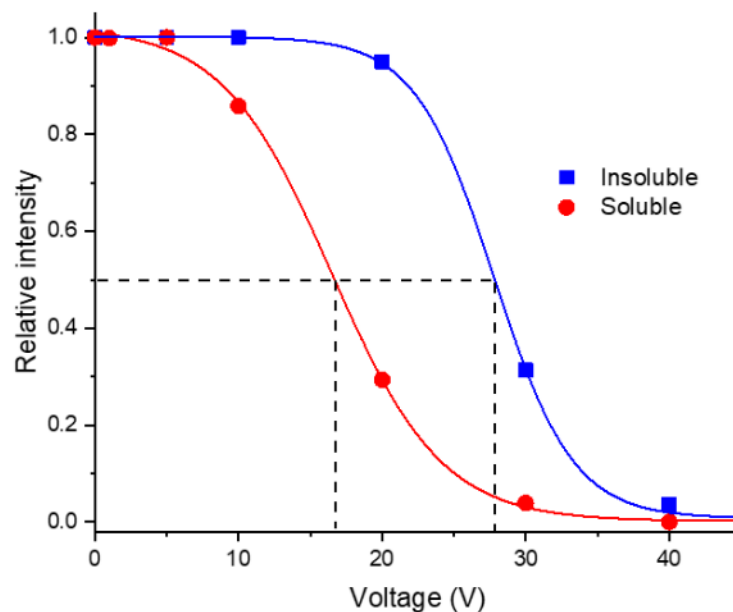


Figure 65: Breakdown curves of the insoluble (blue) and soluble (red) precursor species of the nominal mass 504.

3.1. Fragmentation of the species detected in the insoluble fraction

In this section, the fragmentation of species presents in the methanol insoluble fraction is studied. **Figure 66** displays the results obtained for one nominal mass (m/z 504) which was isolated and fragmented at different voltages. The Double Bond Equivalent (DBE) vs carbon number map is commonly used to visualize the diversity of compounds present in a complex sample or to observe particular fragmentation patterns (Chacon-Patino et al., 2017, 2018a, b; Le Maître et al., 2019). In addition, to highlight the degree of aromaticity of the fragment ions, polyaromatic hydrocarbon (planar limit) family has been added and is displayed by the red line (Cho et al., 2011).

In **Figure 66**, each ion intensity is represented by the size of the circle and the amount of nitrogen contained in each species by a color code. **Figure 66a** plots the detected species present at m/z 504 with no collision energy. As mentioned before, many different ions at nominal masses m/z 504 are co-isolated due to the low resolution delivered by the quadrupole. Two main ions series appear at higher and lower DBE values. These series seem to have a similar number of carbons but a strong difference on the aromaticity degree. The more aromatic one will be called **series 1** and the other **series 2**. To illustrate this observation, putative structural formulas of compounds representative of the two major series were added in the Figure. These putative structures are based on hypotheses made in previous works (Gautier et al., 2017; Maillard et al., 2020; Ruger et al., 2019).

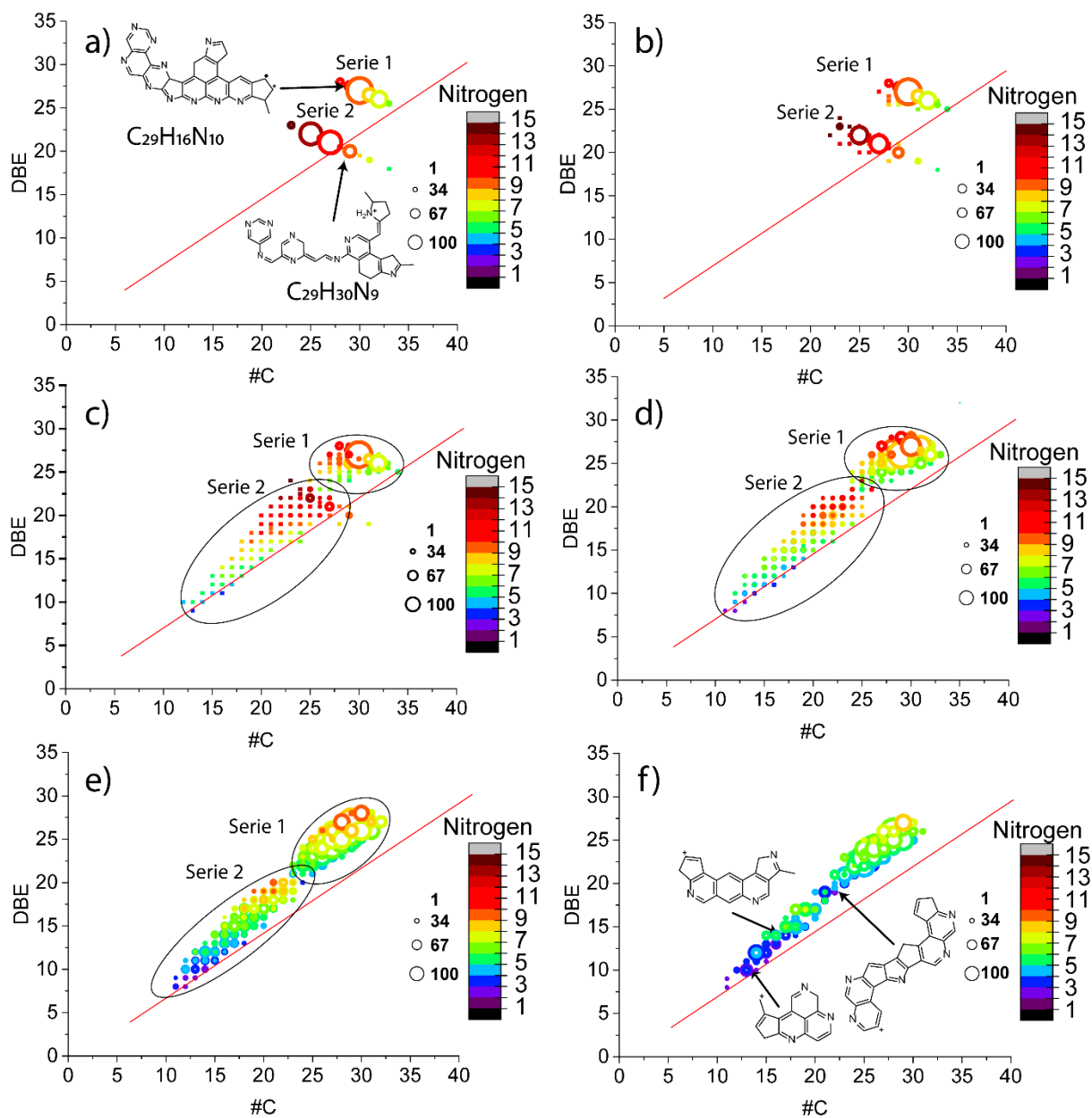


Figure 66: Nominal mass fragmentation of the m/z 504 insoluble displayed by DBE vs #C maps at a) 0 eV, b) 20 eV, c) 30 eV, d) 40e V, e) 50 eV and f) 60 eV collision energy, including some putative representative structures. The ions intensity is represented by the size of the circle and the amount of nitrogen contained in each species by the color code.

Two types of aromatic materials are observed by the **series 1** and **series 2**. The first series is much more aromatic than the other one and thus these two series will have a different fragmentation pattern. **Figure 66b** reports the results for m/z 504 fragmented at 20 eV. Fragments for the two series are observed.

Less fragments are produced in the **series 1** in agreement with the fact that this series corresponds to more aromatic compounds and so, much more resistant to fragmentation. Due to the low resolution of the quadrupole used for mass selection, attributing fragments to one precursor is difficult regarding the number of isolated isobaric precursor ions. However, it can be observed that at 20 eV, the amount of DBE remains nearly constant for each series and mainly the amount of carbon is slightly decreasing (DBE value around 25 for the **series 1** and 20 for the **series 2**). This is consistent with a dealkylation process, meaning that peripheral alkyl branches are broken first.

Results at 30 eV are reported in **Figure 66c**. For the **series 2**, fragment ions presenting low DBE values compared to the precursor ions are observed. The N/C ratio which was at 0.330 for precursor species is measured for the fragments at a value of 0.325 which is roughly the same. In the case of Tholins unlike petroleum molecules, nitrogen atoms should also be present in the neutral losses that are not visualised in these DBE vs C# diagrams (*vide infra*)(Le Maître et al., 2019). The **Figure 66c** color code reveals a decrease of the amount of nitrogen with the apparition of fragments at lower DBE. This observation highlights the loss of nitrogen atoms during the fragmentation. In addition, the **Figure S103** presents the corresponding van Krevelen diagrams showing the H/C vs N/C ratio in order to highlight the losses of nitrogen as well as the losses of DBE (through the increase of H/C ratio).

Figure 66d, 7e and 7f present the results obtained at 40 eV, 50 eV and 60 eV. The first observation is that no new fragments are observed in comparison with **Figure 66c** but there is mainly an increase of the intensity of previously detected species. This means that most easily breakable bounds were fragmented first.

Two types of aromatic materials can be considered from a previous study on asphaltene fractions of petroleum (Chacon-Patino et al., 2018a): (i) small aromatic cores linked together by chains named *archipelago* or (ii) a large aromatic core with side chains named *island*. The rest of the section focuses on the obtained fragments in order to gain clues on the consistency the proposed models for the insoluble fraction of Tholins.

For **series 1**, it can be observed that fragments remain around the precursor's region even at 40 eV and 50 eV. There is also no observation of nitrogen loss. At 60 eV, DBE, carbons and nitrogen losses starts to be observable. This means that the fragmentation of this series can be explained by three steps: In the first one, at low energy, peripheral bounds are broken, revealing in **Figure 66a** dealkylation pattern with some losses of DBE and nitrogen. Then, the fragmentation remains stable with an increasing energy until a certain level (between 50 eV and 60 eV). Finally, as observed in the **Figure 66f**, at high energies, large losses of DBE, carbons and nitrogen are observed, meaning that stronger bounds, such as aromatics rings are broken. This fragmentation process goes in favour of the island model for the **series 1**.

For **series 2**, the fragmentation process is slightly different from the one and can be explained as follows. The first step of the fragmentation, at low energy, involves the rupture of weak bounds. As mentioned, during this step, large amount of DBE, carbons and nitrogen are lost. Then, no new fragments are observed during the increase of the collision energy.

Finally, such as for the **series 1**, between 50 eV and 60 eV, the fragmentation occurs starts again with losses of DBE, carbon and nitrogen. Large neutral losses during the first step go into favour of an *archipelago*-like model. Links between cores are broken and then at higher energy, ring opening occurs.

In conclusion, **Figure 67** presents two putative structures with the fragmentation scheme in agreement with all our observations for **series 1** and **series 2**.

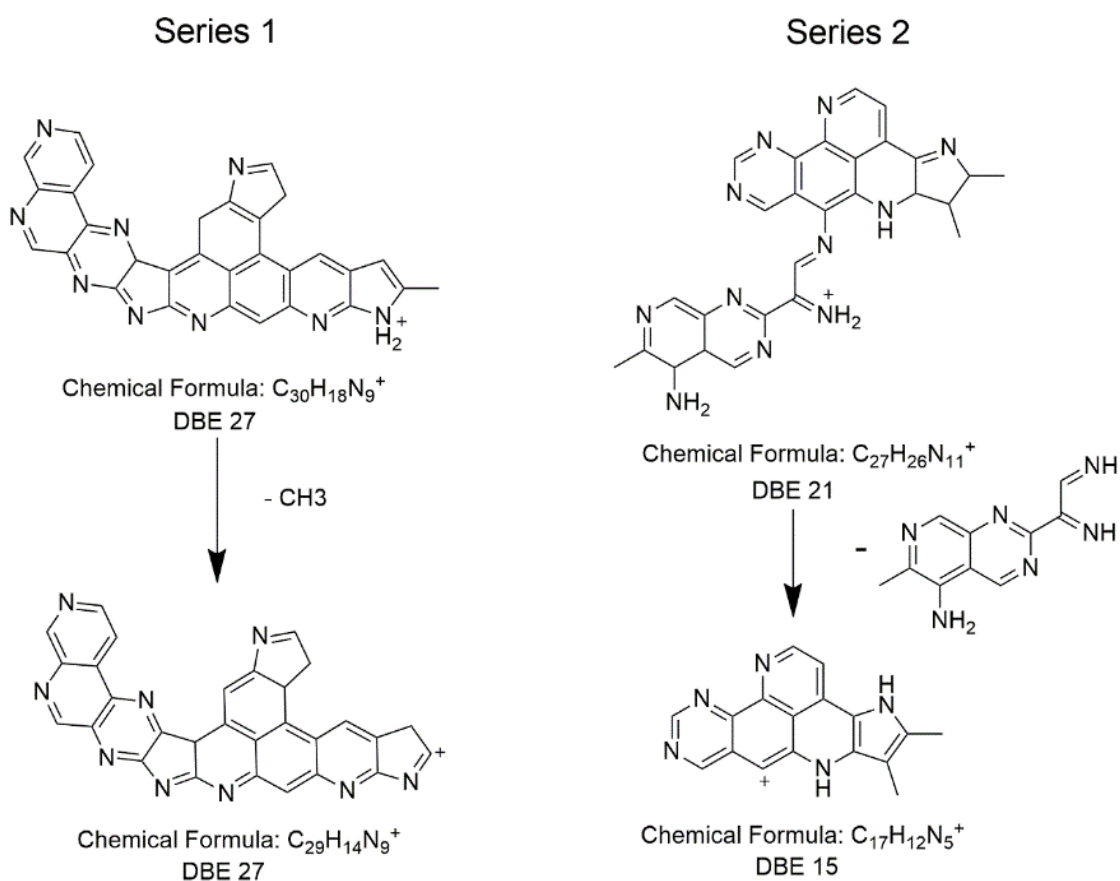


Figure 67: Hypothetical structures of one detected species at m/z 504 with (a) Archipelago type, (b) island type

3.2. Fragmentation of the species detected in the soluble

In this part, as in the **section 3.1.**, the ions at m/z 504 were isolated and fragmented at different acceleration voltages in order to retrieve additional information about structures composing the soluble fraction. Results are illustrated by the **Figure 68**. In opposition with the insoluble fraction, only one series of compound is detected (**Figure 68a**); this series will be called **series 3**. As reported in previous work, the molecules of this fraction contain in average more hydrogen atoms than the insoluble fraction and present therefore a lower amount of unsaturations (Maillard et al., 2018). A hypothetical formula of one detected compound is described in **Figure 68a**.

At 5 eV (**Figure 68b**), many fragments are already observed. Detected species are located lower and more on the left on the map, meaning that large losses of DBE and #C are observed. Such fragmentation at this low energy means that weak bounds are broken. Furthermore, the large losses of DBE reveal that the broken part of the precursor species contain DBE and aromatic rings because of the nonlinear intensities of the detected fragments (the most intense are highlighted by a red star).

At other acceleration voltages, we observe no more losses of DBE. However, a dealkylation and denitrogenation is present as fragments are moving closer to the PAH line with the increase of the collision energy. **Figure S104** presents the corresponding van Krevelen diagrams showing the H/C vs N/C ratio in order to highlight the losses of nitrogen as well as the losses of DBE (through the increase of H/C ratio).

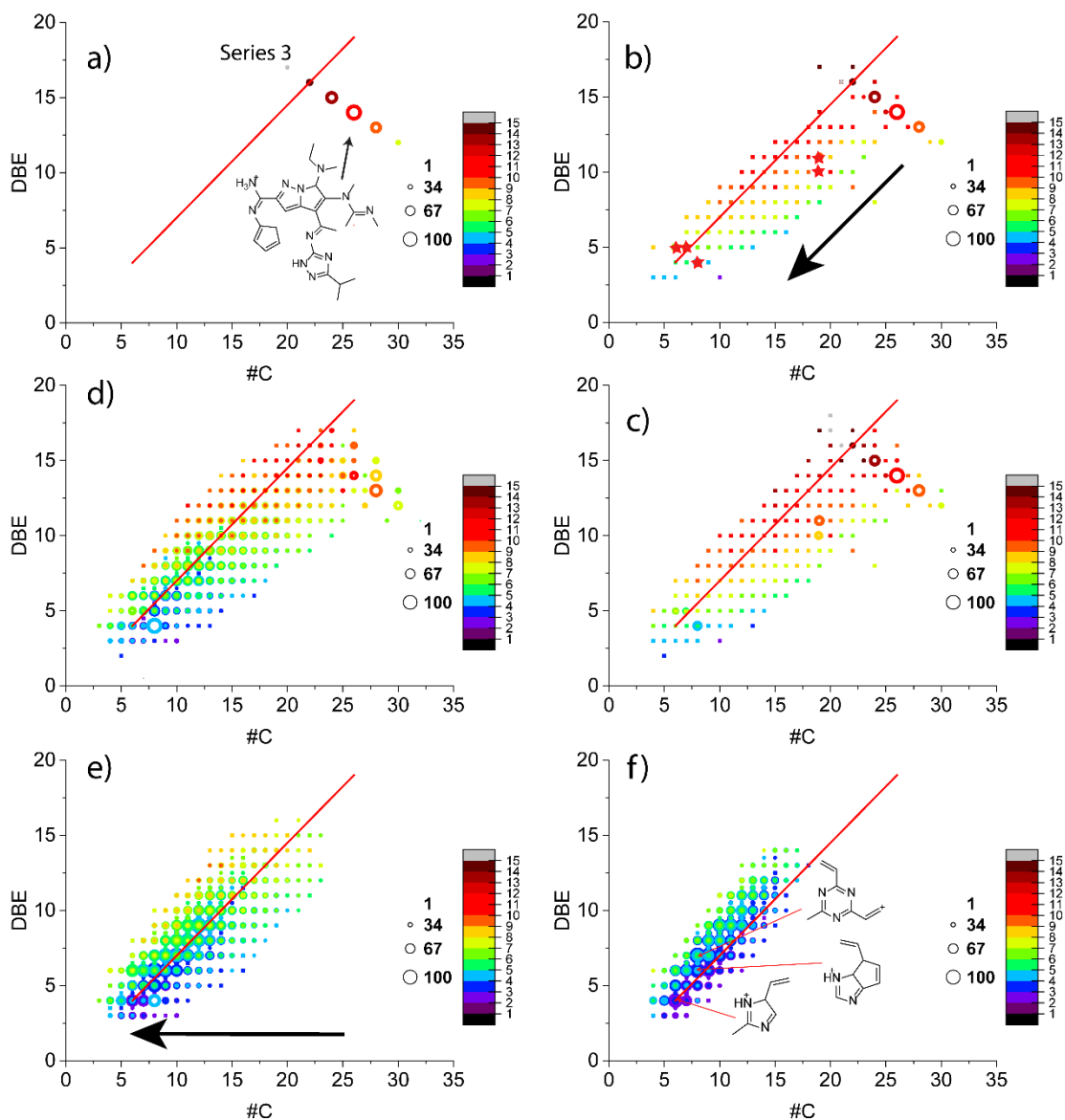


Figure 68 : Nominal mass fragmentation of the m/z 504 soluble displayed by DBE vs #C maps at (a) 0 eV, (b) 5 eV, (c) 10 eV, (d) 30 eV, (e) 40 eV, (f) 60 eV collision energy, including some putative representative structures. The ions intensity is represented by the size of the circle and the amount of nitrogen contained in each species by the color code.

As for the insoluble species, this fragmentation can be explained in two distinct steps. First, at low energy, weak bonds between aromatic moieties are broken, showing large losses of DBE, carbon and nitrogen. Then, at higher energies, these moieties are dealkylated and denitrogenated. This fragmentation pattern is characteristic of an archipelago.

Regarding binding energies, the lower possible energy corresponds to the energy of a N – N binding, at 1.7 eV. The next one is the C – N binding with an energy of 3.192 eV. Imine bounds (C = N) have an energy of 7.856 eV: these kinds of bonds are not broken at this acceleration voltage. Thus, preferential encountered links between cores should be C – N bounds or N – N bounds in the soluble fraction. Several structures of the most intense detected species are given in the **Figure 68f**.

Finally, both models, islands and archipelagos, seem to be present in the sample, with islands more located in the insoluble fraction and archipelagos located in both fractions.

4. Conclusions and discussions

In this study, tandem mass spectrometry was performed on analogues of Titan's haze. Due to the complexity of the analyzed sample, cartographies were used to facilitate the comprehension of the fragmentation patterns. Both insoluble and soluble fractions were studied. Fragmentation patterns were compared to island and archipelago models proposed in the literature. Island fragmentation was observed for the most aromatic family of the insoluble fraction. The second family was attributed to archipelagos because of the large losses of DBE observed at low collisional energies. Soluble structures were also attributed to archipelagos. As a summary, **Figure 69** shows three hypotheses of structures present in the Tholins sample.

This work confirms structural hypotheses proposed in previous works performed by ion mobility concerning the presence of bulky structures in the soluble molecules (Maillard et al., 2020; Ruger et al., 2019). Such part of these structure were identified in several NMR studies (Derenne et al., 2012; He and Smith, 2014a, b) and Chromatography-mass spectrometry analyses (Gautier et al., 2016). It has also allowed to go further into the chemical characterisation of the Tholins by studying the major part, the insoluble fraction.

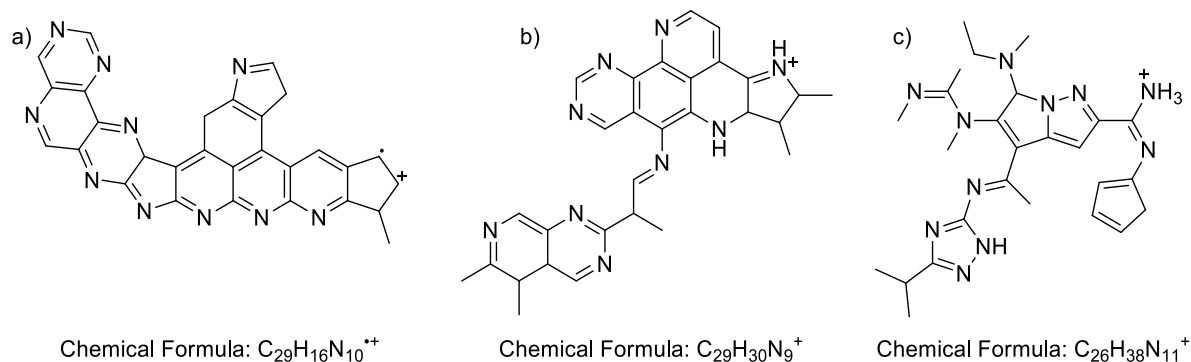


Figure 69 : Hypotheses of structures for (a) Insoluble fraction **series 1**, (b) Insoluble fraction **series 2** and (c) Soluble fraction **series 3**

Retrieving such diversity of families for the compounds contained in Titan's Tholins is an important information about Titan's haze. Firstly, such Nitrogenated-PAH structures are consistent with *in situ* measurements performed by the Cassini Huygens mission (Dinelli et al., 2013; López-Puertas et al., 2013; Yoon et al., 2014). Then, regarding the recovered structures, soluble species are more fragile and much more reactive so they will react with their environment as a contrary to the insoluble ones which will deposit on the surface of the satellite. Such insoluble species can aggregate and be at the reason of the formation of dune on the surface (Pirim et al., 2015).

Furthermore, looking at **Figure 69**, having such compounds in a non polar solvent (for example Titan's lakes) could lead to the formation of azotosomes, as suspected by Stevenson et al in 2015 (Stevenson et al., 2015). An azotosome is the formation of a membrane equivalent to the liposomes in our biology. The formation of such structures presents a huge interest for the origin of life because this allows the formation of micelles and therefore different environments within a solvent.

Finally, in a previous work, Tholins sample were analysed comparatively with FTICR and the LAb-CosmOrbitrap, an Orbitrap that is intended to be boarded in a space probe(Selliez et al., 2019). Large differences were observed between each analysis: higher masses were detected by FTICR compared to LAB-Cosmorbitrap, but the ions detected in the 100-200 u mass range were found the same. Fragmentation was very likely present with the cosmorbitrap analysis, induced by the difference of power delivered by the ionization laser. It is interesting to see that the fragmentation of such a complex mixture leads to new fragmented molecules consistent with the smaller non-fragmented molecules contained in the sample. And thus, studying these species is representative of larger masses. Laser with moduable energy could be a promising equipment to perform fragmentation experiments in space.

4.3: Conclusions concernant les deux parties précédentes

J'ai reporté dans les deux précédentes parties, les résultats obtenus durant cette thèse concernant l'étude de la fragmentation des Tholins.

Dans un premier temps, j'ai présenté une comparaison spectrale entre un prototype d'Orbitrap spatial et un FTICR de Rouen. Dans cette étude, le prototype spatial a été validé pour l'analyse de mélange complexe, et en particulier des Tholins. Une zone identique aux deux spectres (Orbitrap et FTICR) a été observée. Cependant, une zone unique à chaque instrument a aussi été révélée. L'Orbitrap, équipé d'un laser beaucoup plus puissant que la source LDI du FTICR a révélé des espèces de faible rapport m/z non détectées en ICR. En opposition, le spectre ICR a montré des espèces à des masses beaucoup plus élevées. Nous nous sommes posé alors la question d'une éventuelle fragmentation des Tholins, et cela nous a amené à construire une nouvelle étude uniquement pour étudier ce phénomène.

J'ai reporté les résultats de cette étude dans la deuxième partie de ce chapitre. Les deux fractions, soluble et insoluble, des Tholins ont été fragmentées. Deux séries de composés distincts ont été révélés dans la fraction insoluble et une série pour la fraction soluble. La fraction insoluble s'est révélée beaucoup plus stable que les espèces solubles dans le méthanol. J'ai pu, en faisant varier l'énergie de fragmentation, accéder à de précieuses informations structurales concernant les deux fractions. Trois schémas de fragmentations ont été proposés pour chaque série de composés identifiée. Enfin, en corrélant la littérature à nos résultats, j'ai pu proposer des structures putatives pour chaque série.

Cependant, vu la complexité de cet échantillon, les informations récupérées restent limitées et seules des hypothèses concernant les structures majeures ont pu être proposées grâce aux modèles de fragmentations trouvés dans la littérature. J'ai ainsi observé que les structures solubles présentes étaient plutôt de type archipels (des molécules aromatiques liées entre elles par des ligands). Dans la fraction insoluble, la diversité structurale semble plus importante, des îles et des archipels plus aromatiques que celles de la fraction soluble ont été proposés.

Cependant, les limitations techniques ne permettent pas aujourd'hui, d'approfondir l'étude structurale par la spectrométrie de masse en tandem. En effet, malgré les résultats obtenus, cette étude a été limitée par la résolution de quadripôle, limitant la sélection des espèces fragmentées à 1 Da maximum. C'est pourquoi une nouvelle méthode d'analyse a été étudiée dans la dernière partie de ce chapitre. Cette méthode appelée spectrométrie de masse bidimensionnelle permet de réaliser la sélection et la fragmentation des espèces directement dans la cellule ICR qui est beaucoup plus résolutive que le quadripôle.

4.4: Spectrométrie de masse bidimensionnelle pour l'analyse des Tholins

En 1987, l'équipe de Gäumann essaya de générer des spectres FTICR bidimensionnels en se basant sur les méthodes appliquées en résonance magnétique nucléaire et sur la désexcitation des ions discutée par Marshall ([Bensimon, et al. 1989 \[66\]](#), [Pfändler, et al. 1987 \[67\]](#), [Kumar, et al. 1980 \[68\]](#), [Marshall, et al. 1984 \[69\]](#)). Le but de cette équipe était de pouvoir visualiser les transferts de masse et donc les réactions ions-molécules existantes en phase gaz. Pour cela, ils ont adapté une séquence bidimensionnelle RMN (la NOESY) afin de l'appliquer à une cellule ICR. Cette séquence est définie par l'Équation 21 et illustrée par la Figure 70.

Équation 21 : $P1 - t1 - P2 - tm - P3$

Dans un premier temps, un *pulse* d'excitation est imposé au nuage d'ions qui va voir son rayon cyclotronique augmenter. Un temps $t1$ de relaxation est laissé au nuage d'ions qui est ensuite suivi d'un second *pulse* d'excitation identique au premier. Cette seconde excitation aura plusieurs effets : (1) les ions dont la phase sera identique à celle du *pulse* seront excités et (2) les ions ayant une phase opposée seront désexcités ([Marshall, et al. 1984 \[69\]](#)). Il est ensuite possible, pendant le tm (temps de mixage) suivant ces deux excitations de réaliser de la fragmentation par laser sur les ions désexcités ou encore des réactions ions-molécules en ajoutant du gaz dans la cellule. Enfin, un troisième *pulse* de détection classique différent des deux premiers permet d'amener tous les ions à un rayon cyclotronique suffisant pour pouvoir enregistrer un FID et récupérer le spectre de masse.

La particularité de cette séquence est que la phase des ions est directement proportionnelle de façon linéaire au temps $t1$ entre les deux *pulses* P1 et P2 ([Pfändler, et al. 1987 \[67\]](#)).

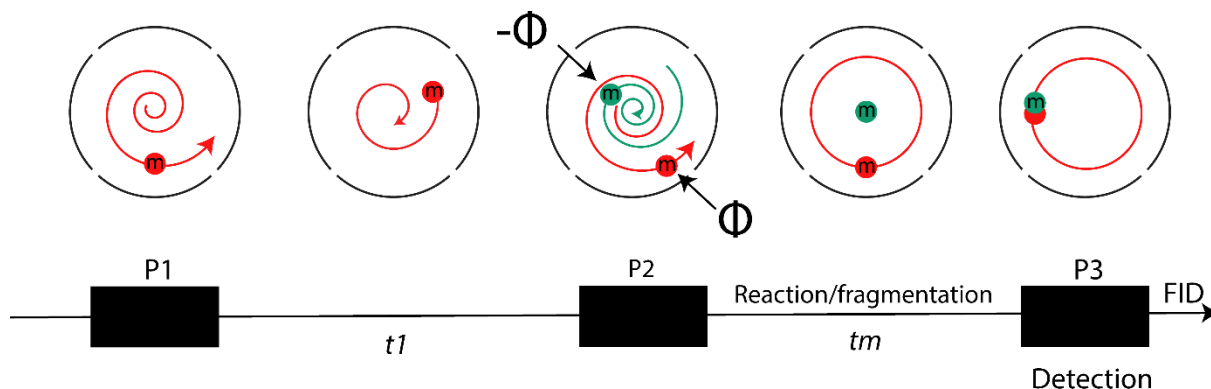


Figure 71 : Représentation d'une séquence d'excitation bidimensionnelle et du mouvement résultant du nuage d'ion de masse m dans la cellule ICR.

Cette proportionnalité permet d'enregistrer un spectre FTICR avec un incrément du temps $t1$ entre chaque scan et ainsi, par reconstruction graphique, de réaliser une carte bidimensionnelle où chaque ligne représentera un ion et ses fragments ou ses produits de réaction correspondants. Gäumann et al., étudièrent la réaction de formation de $\text{CH}_3^+(\text{OH})\text{CH}_3$ à partir d'un ion CH_3CO^+ et de gaz réactif CH_3COCH_3 ajouté durant le temps de mixage. Ils obtinrent le spectre reporté par la **Figure 72**.

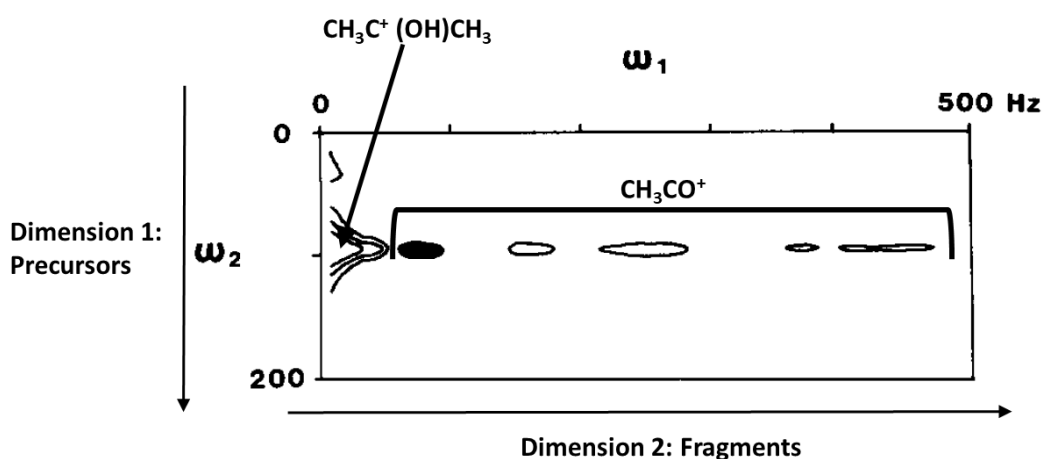


Figure 73 : Premier spectre FTICR bidimensionnel obtenu par Gäumann et al ([Pfändler, et al. 1987 \[67\]](#)).

Durant les années qui ont suivi, plusieurs améliorations ont été apportées à la 2D-FTICR notamment grâce à l'apparition des excitations SWIM (Stored Waveform Ion radius Modulation) ([Ross, et al. 2002 \[70\]](#) ,[Ross, et al. 1993 \[71\]](#)) et de la transformation d'Hadamard ([McLafferty, et al. 2002 \[72\]](#) ,[Williams, et al. 1990 \[73\]](#) ,[Haebel and Gaumann 1995 \[74\]](#)). Aujourd'hui, la FTICR bidimensionnelle généralement réalisée en fragmentation grâce à un laser IRMPD tiré durant le temps de mixage tm ([van Agthoven, et al. 2011 \[75\]](#)) et est appliquée à l'analyse de macromolécules comme les protéines ([van Agthoven, et al. 2016 \[76\]](#)).

Durant la thèse, nous avons installé un IRMPD sur le FTICR de Rouen et avons mis en place des premières analyses en 2D dans le but d'adapter cette technique à l'analyse de matrices complexes tel que les Tholins. La première expérience a été réalisé récemment sur un composé standard, la leucine enképhaline qui est un peptide bien connu pour l'étalonnage. La cartographie obtenue est reportée par la **Figure 74**. Sur cette cartographie, on retrouve l'ion précurseur de la LeucEnph à m/z 556 ainsi que ses fragments sur la ligne horizontale. Beaucoup de bruit se trouve sur la cartographie qui sera éliminé avec un traitement ultérieur. Cette première expérience a surtout permis de vérifier le bon fonctionnement de la méthode d'analyse et d'adapter les paramètres pour passer à des analyses plus complexes. C'est ce qui a été réalisé durant les derniers mois de cette thèse sur les Tholins.

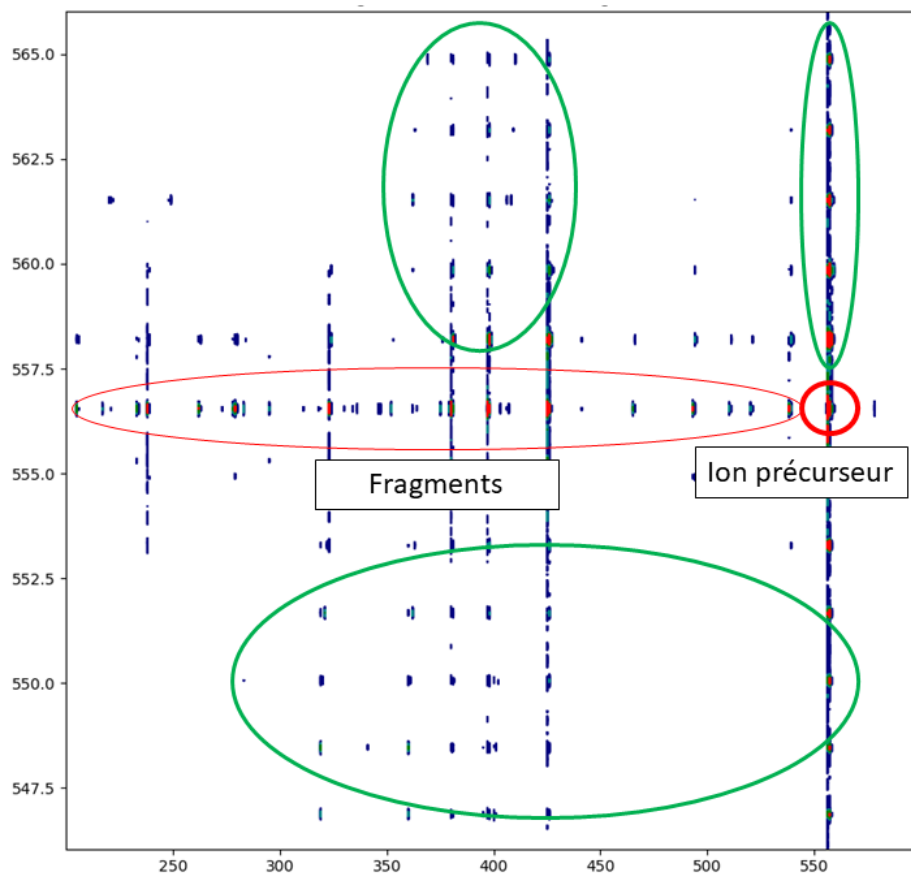


Figure 74 : Première cartographie bi-dimensionnelle de la Leucine Enképhaline réalisée à Rouen. En rouge on retrouve la zone d'intérêt avec l'ion précurseur et ses fragments, en vert sont les zones de bruits qui disparaîtront avec le traitement de donnée.

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Chapitre 5 : Etude structurale

Sommaire

5.1: Première élucidation structurale par mobilité ionique de la matière organique soluble composant les analogues d'aérosols de Titan

5.2: Etude structurale d'analogues d'aérosols de Titan par TIMS-FTICR

5.3 : Clichés de structures composant les analogues d'aérosols de Titan par microscope à force atomique

Introduction

Les résultats reportés dans les **chapitres III** et **IV** ont permis d'étudier la composition chimique globale de l'échantillon de Tholins ainsi que d'obtenir de précieuses informations quant à leur formation. Des premières informations structurales ont aussi été récupérées grâce à la spectrométrie de masse en tandem. Dans ce cinquième et dernier chapitre, je présenterai les résultats obtenus par le couplage de la mobilité ionique avec la spectrométrie de masse. Les études que je présenterai ici ont été réalisées dans un but complémentaire aux résultats présentés dans le **chapitre IV**. En effet, j'ai voulu aller plus loin dans la caractérisation structurale de l'échantillon de Tholins en utilisant la technique de mobilité ionique décrite dans le **chapitre II**.

La première étude a été réalisée sur un spectromètre de masse de type TOF (Résolution < 40 000 à m/z 200) possédant une cellule de mobilité TWIMS. Seule la fraction soluble des Tholins a pu être analysée car l'appareil n'est pas équipé de source LDI. De plus, la gamme de masse d'étude a été limitée à une zone allant de m/z 50 à m/z 250 afin d'être sûr de résoudre toutes les espèces détectées. Cependant, malgré ces inconvénients, de précieuses informations ont pu être récupérées grâce à la dimension de mobilité ionique. J'ai pu, dans un premier temps grâce à l'analyse de composés standard et à la comparaison aux valeurs de la littérature, exclure certaines familles chimiques suspectées présentes dans les Tholins grâce à leur section efficace de collision. J'ai ensuite utilisé le fait que la CCS est une valeur intrinsèque et prédictible pour réaliser des calculs théoriques sur des molécules choisies afin d'en récupérer leur mobilité. Cela m'a permis de proposer des structures de molécules compatibles au regard des valeurs de m/z et de CCS obtenus. Ces résultats sont reportés dans l'**article 6**.

Dans un deuxième temps, nous avons eu l'occasion de réaliser des mesures sur un FTICR équipé d'une cellule de mobilité TIMS. La fraction soluble des Tholins a ainsi pu être étudiée. L'utilisation d'un FTICR en guise d'analyseur a permis d'étendre l'analyse en termes de gamme de masse, ce qui a permis de récupérer la CCS d'espèces allant jusqu'à m/z 550. J'ai ainsi comparé directement les résultats de l'étude précédente (article 6) avec ceux obtenus sur cet appareil. Un repliement de la structure de Tholins apparaît vers m/z 350,. Cela m'a permis d'optimiser mes calculs théoriques afin de proposer une structure plus appropriée. Ces résultats sont reportés dans l'**article 7**.

Enfin, j'ai pris contact avec IBM *research* (Zurich, Suisse) qui réalise des images en microscope à force atomique à l'échelle de l'atome (AFM). Ce groupe a accepté de mesurer notre échantillon de Tholins avec leur instrument. J'ai ainsi obtenu directement des clichés de molécules composants les Tholins (Echelle \approx 30 nm x 30 nm). Ces clichés m'ont permis de comparer les structures observées en AFM avec nos précédentes informations obtenues dans les **chapitres III, IV** et **V**. Nous avons été surpris d'observer des espèces majoritairement catacondensées contenant des cycles à 5 et à 6 carbones. Ces résultats sont reportés dans l'**article 8**.

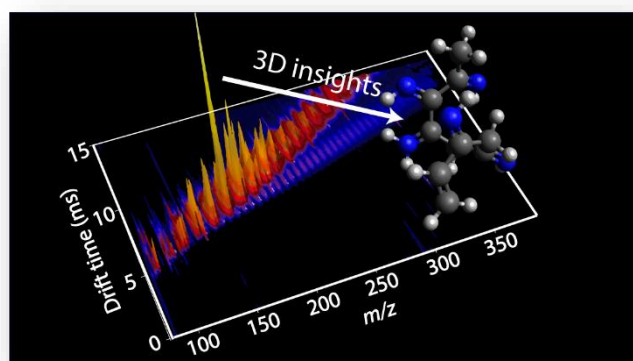
5.1: Première élucidation structurale par mobilité ionique de la matière organique soluble composant les analogues d'aérosols de Titan

La première partie présentée ici expose les résultats obtenus lors de l'analyse de la fraction soluble des Tholins par mobilité ionique couplée à la spectrométrie de masse haute résolution. Le but premier fut d'observer la conformation globale adoptée par les molécules composant ce mélange. Ces résultats sont présentés sous la forme d'un article accepté lors de la troisième année de thèse dans le journal *Icarus*.

Comme présenté dans le **chapitre II**, la mobilité ionique est une technique de séparation d'ions en fonction de leur conformation. Plus un ion possède une section efficace de collision grande, plus il sera retenu longtemps. En opposition un ion possédant une petite section efficace ne sera que peu retenu. Ce principe permet, couplé à la spectrométrie de masse de récupérer deux informations intrinsèques à chaque molécule, leur m/z et leur CCS. Nous avons donc réalisé des analyses de la fraction soluble des Tholins sur un spectromètre de masse équipé d'une cellule de mobilité ionique. Dans un premier temps, j'ai comparé les résultats obtenus avec différentes familles chimiques afin de les comparer à notre mélange. J'ai ainsi observé que les HAP purs, le poly-HCN ainsi que les sels de tetraalkylammonium étaient très éloignés de la conformation principale adoptée par les Tholins. Comme il est possible de prédire une CCS à partir d'une formule développée, j'ai réalisé des calculs théoriques sur plusieurs molécules afin de trouver les familles chimiques les plus adaptées aux Tholins. Une famille de triazines et une famille de pyrazoles se sont révélées les plus proches de la conformation globale des Tholins. J'ai ainsi proposé une structure correspondant à un mélange de ces deux familles.

Abstract

The origin and evolution of organic matter in the solar system intertwines astrobiology and planetary geochemistry issues. To observe the contribution of atmospheric processes in the formation of complex organic matter, one of the most intensively studied objects in the outer



solar system is Titan, the largest moon of Saturn. Its reducing atmosphere of methane and nitrogen hosts a thick, permanent, nitrogen-rich, organic haze, whose complex composition remains largely unknown.

Due to the measurement of species at large mass-to-charge ratio and infrared information from the Cassini-Huygens mission, polyaromatic hydrocarbons (PAHs) based structure for this haze has been suggested. Here, we propose a snapshot of the global chemical structure based on the analysis of laboratory analogue of Titan's haze with ion mobility spectrometry coupled with mass spectrometry. This robust analysis, validated with other geochemical complex mixtures, such as petroleum, allows for the observation of the size and three-dimensional structure of detected species. By comparison, with standards molecules, we exclude several structures such as pure PolyHCN and pure polycyclic aromatic hydrocarbons to be present in the principal trend of the laboratory Tholins. Using theoretical calculations, we propose a plausible structure consistent with our results, which is a branched triazine-pyrazole. We observe that the larger the aerosols molecules are, the more they tend towards a structure containing small aromatics cores linked together by short chains. We suggest that the use of such an analytical approach could help advance our understanding of other complex organic compounds in the Solar System such as soluble organic matter in meteorites.

5.1.1. Introduction

Organic matter is present all over the solar system. Understanding its formation and history remains a major challenge for the fields of astrobiology and planetary geochemistry. A key approach to do so is to study the elemental composition and chemical structure of this organic material. In this work, we propose to tackle the question of the chemical structure of complex, soluble organic matters of interest for Earth field samples, meteorites, interplanetary dust collected in the Earth's upper atmosphere, sample return from space missions and in situ future space developments oriented towards complex organic worlds such as Titan and the ocean worlds. The case study chosen here is the challenging analysis of Titan's aerosols analogues. These are reduced nitrogen-rich $C_xH_yN_z$ organic samples synthesized in the laboratory from processes simulating the formation of the atmospheric organic haze surrounding Titan. The high degree of complexity of this material, with only carbon, nitrogen and hydrogen atoms, the absence of oxygen, and its formation in cold conditions leads us to consider this model organic material of interest not only for Titan, but for understanding the formation of primitive organic material in the outer reduced solar system, for example comets ([Jost, et al. 2017 \[1\]](#)) and the early Earth ([Trainer, et al. 2006 \[2\]](#)). In addition, the hydrolysis of these analogues has been shown to lead to a variety of amino acids very close to the result of the Miller-Urey experiment, making it a key component for astrobiology ([Neish, et al. 2010 \[3\]](#), [Neish, et al. 2009 \[4\]](#)).

These proxies have been extensively analyzed with state-of-the-art instruments, e.g., ultrahigh resolution mass spectrometers, such as FT-ICR and Orbitrap to push our understanding of this organic matter ([Toupance, et al. 1975 \[5\]](#), [Szopa, et al. 2006 \[6\]](#), [Somogyi, et al. 2005 \[7\]](#), [Cable, et al. 2012 \[8\]](#), [Danger, et al. 2016 \[9\]](#), [Danger, et al. 2013 \[10\]](#)). Unfortunately, even with Earth best instruments, revealing the structure of these analogues remains a challenge.

Several analyses were performed on Titan's aerosol analogues, including infrared spectroscopy ([Cable, et al. 2014 \[11\]](#) ,[Gautier, et al. 2012 \[12\]](#) ,[Imanaka, et al. 2004 \[13\]](#)), capillary electrophoresis ([Cable, et al. 2014 \[11\]](#)) and nuclear magnetic resonance ([He and Smith 2015 \[14\]](#)). These previous analyses revealed the presence of molecular families such as nitriles, amines and hydrocarbons. Mass spectrometry analysis revealed a great deal of structural information on laboratory Tholins, including a polymeric trend with CH₂ and HCN as repetition pattern ([Somogyi, et al. 2005 \[7\]](#) ,[Bonnet, et al. 2013 \[15\]](#) ,[Vuitton, et al. 2010 \[16\]](#) ,[Gautier, et al. 2016 \[17\]](#) ,[Anicich, et al. 2006 \[18\]](#) ,[Somogyi, et al. 2016 \[19\]](#)). Recently, mass spectrometry analyses were performed with a laser ionization desorption source ([Somogyi, et al. 2012 \[20\]](#) ,[Maillard, et al. 2018 \[21\]](#) ,[Gautier, et al. 2017 \[22\]](#)). This source allowed for the comparison of both liquid and solid state of non-totally soluble Tholins and showed the chemical difference between these two fractions with the presence of half the amount of hydrogen in the insoluble fraction ([Maillard, et al. 2018 \[21\]](#)).

The main limitation of mass spectrometry analyses has been the absence of conformational and isomeric information about the analyzed sample. Tandem mass spectrometry remains a solution to recover structural information but is difficult to apply here due to the extreme complexity of such organic samples. One way to get around this issue is to use chromatographic separation (in gaseous or liquid state) upstream of the mass spectrometer to recover isomeric information. The retrieved retention time is dependent of the chemical properties of the molecule and allows, using standard molecules, identification of species. This technique revealed a strong potential for the elucidation of several compounds such as triazole, triazine and cyanoguanidine ([Gautier, et al. 2016 \[17\]](#)).

However, chromatography does not allow obtaining structural information without comparison to a standard molecule and therefore remains unable to solve the structure of the thousands of molecules comprising laboratory Tholins.

Ion mobility spectrometry (IMS) is a gas phase separation method that can be coupled to mass spectrometry (IMS-MS) ([Mason and Schamp 1958 \[23\]](#) ,[Revercomb and Mason 1975 \[24\]](#)). IMS-MS has been used recently for the analysis of complex mixtures such as petroleum ([Fernandez-Lima, et al. 2009 \[25\]](#) ,[Castellanos, et al. 2014 \[26\]](#) ,[Maleki, et al. 2016 \[27\]](#)). IMS is based on the separation of ions in a neutral gas, according to their charge state, size and shape. Three main ion mobility techniques allow the determination of collision cross section (CCS): drift tube ([May, et al. 2014 \[28\]](#)), Trapped Ions Mobility Spectrometry (TIMS) ([Tose, et al. 2018 \[29\]](#)) and Travelling Waves Ion Mobility Spectrometry (TWIMS) ([Hines, et al. 2016 \[30\]](#) ,[Fasciotti, et al. 2013 \[31\]](#)) technology. Direct measurement of the ion collision cross section (CCS) can be done with a drift tube. For TIMS or TWIMS, it is possible to determine the CCS ([Campuzano, et al. 2012 \[32\]](#)) from the experimentally determined drift time (t_D) after calibration. The CCS is an intrinsic property of the ions (for a particular buffer gas) that can be compared to theoretical CCS values obtained from putative tridimensional structures.

The following study presents the advantage of IMS-MS for the study of complex organic matter, in our case analogous of the Titan's hazes, to provide a snapshot of their global structure.

5.1.2. Methods

Tholins production

Titan's aerosol analogues, also hereafter called *Tholins*, were produced with the PAMPRE experiment (French acronym for *Aerosols Production in microgravity with reactive plasma*) following the same procedure detailed in previous publications ([Szopa, et al. 2006 \[6\]](#), [Gautier, et al. 2011 \[33\]](#)). The reactor is composed of a stainless steel cylindrical reactor in which a RF-Capacitively Coupled Plasma discharge is established thanks to an RF 13.56 MHz frequency generator. A gas mixture containing 95% of nitrogen and 5 % of methane was injected in the chamber as a continuous flow through polarized electrodes. It is then extracted by a primary vacuum pump to ensure that gases are homogeneously distributed. The plasma discharge was maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature. A brown powder, called *Tholins*, was recovered after 1 day. The harvesting procedure was carried out under ambient air. *Tholins* are then stored in an inert atmosphere and protected from light. Some oxidation occurs during the harvest but these new oxygenated species can be sorted and are not studied during the mass spectrometry analysis. It should be noticed that the pressure and temperature are lower in Titan's ionosphere (respectively $\sim 10^{-5}$ - 10^{-8} mbar and 200K) than in our experiment, but the ionization rate is the same ($\sim \text{ppm}_v$). As ion-molecule reaction rates are relatively insensitive to the temperature, the lower temperature in Titan's ionosphere (200K instead of 293K in the laboratory) is not an important issue in our case. The higher pressure ensures a faster kinetics in the experiment without being high enough to enable molecular reactions, whereas the similar ionization rate enables a realistic contribution of ions into the whole ion-neutral coupled chemical network.

1.5.

Sample preparation

To recover the soluble part of the sample, 4 mg of laboratory Tholins were dissolved in 1 mL of methanol in a vial. The vial was vigorously stirred for 3 minutes to solubilize the maximum amount of species. The brown mixture was then filtered using a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter on a filter holder. Filtered solution was transferred in a vial and then analyzed after half dilution with a 50/50 water/methanol mixture to be analyzed under the same conditions than previous studies by electrospray ionization. Based on electrospray mass spectrometry and elemental analysis, we have evidenced the presence of less than 10% of oxygen containing species. Oxidation occurred most likely during sample harvesting and storage but we cannot rule out that some oxidation might occur also during ionization. The oxygen containing species have been discarded for data treatment. It must be notified that around 40% of the initial quantity of laboratory Tholins is soluble in methanol, so the following study will only focus on a part of the sample and will not be fully representative of the entire sample ([Carrasco, et al. 2009 \[34\]](#)).

Chemicals

Methanol (LCMS grade) was purchased from Fisher scientific. All other chemical products (tetra-alkylammonium, polyglycine and drug-like compounds) were purchased from Sigma Aldrich.

Ion mobility - mass spectrometry experiments

Laboratory Tholins were analyzed in positive ion mode on a hybrid quadrupole-time of flight mass spectrometer equipped with a Travelling Wave Ion Mobility cell (TWIMS) and electrospray ion source (Synapt G2 HDMS, Waters, Manchester, UK). Although it has been shown that different species ionize in negative ionization mode ([Somogyi, et al. 2012 \[20\]](#)), it was decided, in this work, to focus on the positive mode in order to present an introduction to ion mobility analysis. Following ionization parameters were applied: For the source, the capillary was set at 3 kV, the temperature at 100 $^{\circ}\text{C}$, the Sampling cone at 25 V. The extraction cone was also at 5 V and the desolvation temperature at 250 $^{\circ}\text{C}$.

Nitrogen cone gas flow was set at 10 L/Hour and nitrogen desolvation gas flow at 400 L/Hour. For Ion mobility parameters, helium cell gas flow was set at 180 mL/min; Nitrogen gas flow was set at 90 mL/min, the IMS wave velocity at 400 m/s. and the IMS wave height at 13 V. Trap wave velocity was set at 311 m/s and Trap wave height at 6 V. Finally, transfer wave velocity was set at 191 m/s and transfer wave height at 4 V. The m/z values in recorded spectra were first externally calibrated using sodium formate and then internally calibrated using several well-known ions (Maillard et al., 2018)[21]. Drift times were extracted for each species using DriftScope 2.8 and MassLynx 4.1. Mobility dimension was calibrated using tetraalkylammonium ions (TAA) ([Campuzano, et al. 2012 \[32\]](#)) with reference values in helium. Even if the instrument is working under nitrogen, it is commonly used to calibrate the CCS values in helium. Indeed, it was proven that for small molecules, there is no bias in the calibration ([Bleiholder, et al. 2015 \[35\]](#)). Therefore, every recovered laboratory Tholins CCS is calculated in the same gas. Calibration peaks were fitted with a Gaussian shape using Origin 2016. The resulting uncertainty after calibration for cross sections was estimated at $\pm 0.8 \text{ \AA}^2$ by taking into account the error of the fitting (**Figure S107**). Consequently, CCS for laboratory Tholins will be stated without decimal. **Table S16** shows the comparison between experimental measurements and database CCS values in order to validate our calibration. All reported CCS values are given following the recommendation of the ion mobility community in terms of rating, calibration and results reporting ([Gabelica, et al. 2018 \[36\]](#)).

Due to the resolution of the time of flight mass analyzer ($m/\Delta m$ 40 000), we choose to focus on species below m/z 250 to prevent isobaric interferences and misassignments (See **Figure S105-Figure S106**). We have shown that at this mass range, detected species are similar in the soluble and the insoluble fractions ([Maillard, et al. 2018 \[21\]](#)).

Collision Cross Section calculation using theoretical model

From two-dimensional structures of chosen references ions, three-dimensional structures were geometrically optimized (including partial charges) with Avogadro 1.1.1 using MMFF94 force field, 10,000 steps, a steepest descent algorithm and a convergence of $10e^{-7}$. Conformation search was performed when molecules were containing chiral center.

Theoretical calculation was achieved with MOBCAL ([Shvartsburg and Jarrold 1996 \[37\]](#), [Mesleh, et al. 1996 \[38\]](#)) with the trajectory method following Lennard-Jones parameters ([Campuzano, et al. 2012 \[32\]](#)): hydrogen with atomic energy at 0.6175 meV and van der Waals distance at 2.2610 Å. carbon with atomic energy at 1.3266 meV and van der Waals distance at 3.0126 Å and finally nitrogen with atomic energy at 1.4740 meV and van der Waals distance at 3.3473 Å. Each calculated species were obtained thanks to 400,000 points (10 cycles. 1000 points in Monte Carlo integrations of impact and 40 points in velocity integration.). All calculations were performed considering helium as buffer gas. Helium was chosen because it is a much easier gas to model than nitrogen.

5.1.3. Results

IMS-MS experiments

Two pieces of observable information are recovered from the analysis of complex organic matter by IMS-MS: first, the usual mass-to-charge (m/z) dimension, and then the drift time dimension. The second dimension corresponds to the time taken by the ions to get across the mobility cell (in practice, it includes a short period of time spent by the ions in the ion optics to reach the detector). This time is characteristic of the ion size, shape and charge in the gas phase. The resulting spectrum is given in **Figure 75**. This three-dimensional map allows a first overview of the analyzed sample.

The major sequence (blue area) corresponds to singly charged species. In the case of laboratory Tholins, a typical wave-formed structure is recovered ([Bonnet, et al. 2013 \[15\]](#), [Gautier, et al. 2016 \[17\]](#), [Pernot, et al. 2010 \[39\]](#)) with a succession of high intensity clusters characteristic of known repetition patterns such as CH₂ and HCN ([Somogyi, et al. 2016 \[19\]](#)). The minor sequence below the major one on the map (green area) is composed of doubly charged species naturally separated from singly charged species due to the ion mobility. These doubly charged species are centered around m/z 275, which represents molecules of mass 550 u. Their presence is not surprising because laboratory Tholins contains a large amount of nitrogenous compounds ([Sciamma-O'Brien, et al. 2010 \[40\]](#)), which easily allow double protonation. These double charged species will not be discussed in this study.

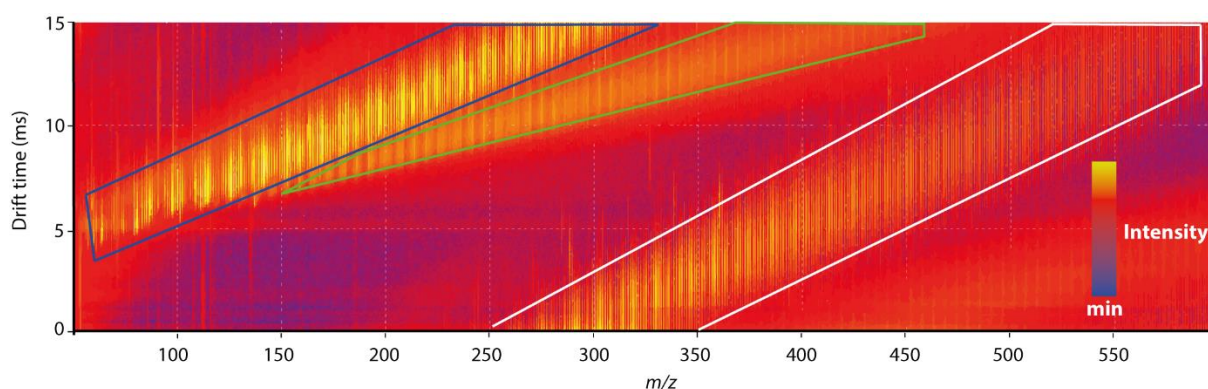


Figure 75 : CCS vs m/z plots obtained from laboratory Tholins recorded on Synapt G2 (intensity in logarithm scale). Three areas are surrounded: (blue) main trend corresponding to singly charged species, (green) secondary trend corresponding to doubly charged species, (white) wrap around phenomenon corresponding to low mobility singly charged species coming from the precedent scan.

A third sequence (white area) is observable on **Figure 75** far below the doubly charged one. This represents the larger singly charged species collected from the previous scan. Indeed, these species would need a longer time to be expelled from the mobility cell than the time stated for the next ion packet to be released in the cell.

This phenomenon is often called “wrap-around”. It could have been avoided by changing parameters, such as wave velocity and wave height, but the used experimental conditions were chosen to optimize the ion mobility separation in the desired drift time range. In this study, we focused on the separation of ions between m/z 50 and m/z 250, as discussed in the methods section. Therefore, we optimized the drift time separation for this m/z range. The following results will focus on the extracted peaks in the major sequence. Their collision cross section (CCS) are recovered using tetra-alkylammonium salt (TAA) ions ([Campuzano, et al. 2012 \[32\]](#)) as reference for CCS calibration in helium.

All recovered peaks are presented in **Figure 76**, which provides CCS in helium vs mass-to-charge ratio for each detected peak. With this overview, we obtain the general trend for our sample. To have a snapshot of the global structure of laboratory Tholins sample, we added two well-known families from our experimental measurements: tetra-alkylammonium ions (TAA), which were used as reference CCS, and polyglycine. In addition, polycyclic aromatic hydrocarbon was added from precedent measurements (PAH) ([Campuzano, et al. 2012 \[32\]](#), [Lim, et al. 2018 \[41\]](#)). All these families are listed in the **Table S17** and a comparison is given between experimental measurements and database CCS in order to validate our results.

Furthermore, two other families are described using theoretical calculation: linear and branched polyHCN (See **Figure S108** for detailed formulas of plotted species).

PAHs represent a family of planar compounds containing only carbon and hydrogen. This family is generally used in DBE vs. carbon number plot because it is the most unsaturated known family ([Cho, et al. 2011 \[42\]](#)) and was suspected to be present in Titan’s aerosols and in meteoritic organic matter ([Waite, et al. 2007 \[43\]](#)).

Due to the planar conformation of PAH molecules, it is interesting to observe the trend of the analyzed sample in comparison to this family. It can be noticed that, even if the amount of heteroatoms is not the same between laboratory Tholins and PAHs, the PAH line remains under all detected species of laboratory Tholins. This means that the planar conformation, and so PAHs, does not seem to be the preferred structure of our sample at this m/z range.

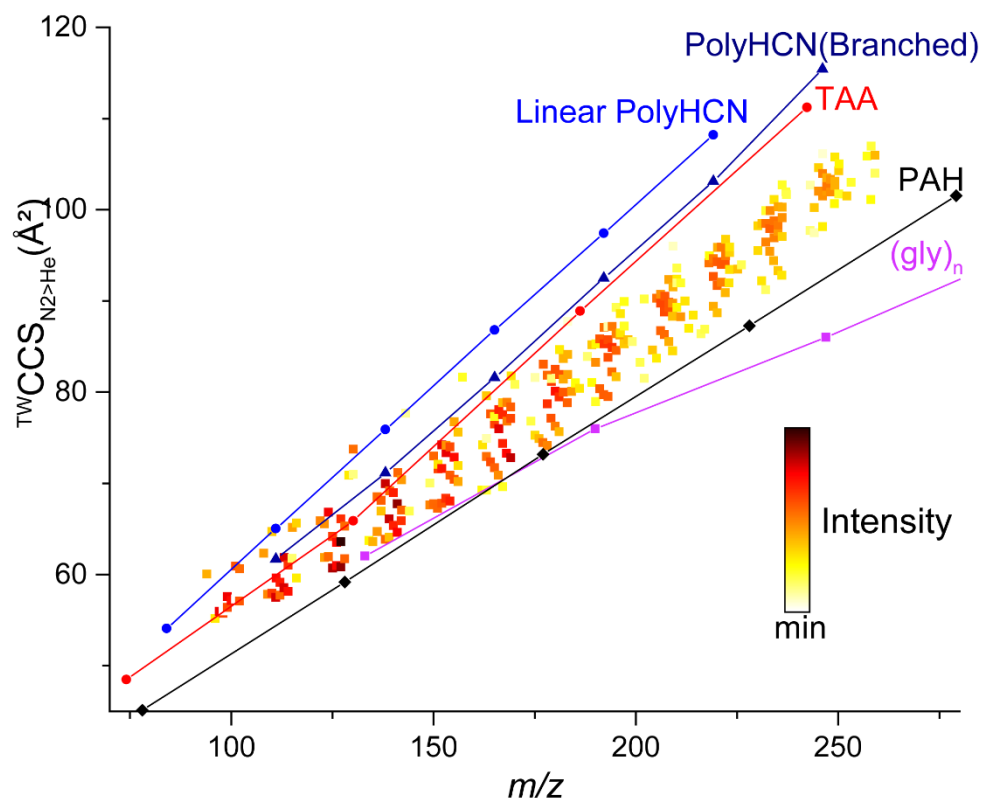


Figure 76 : Comparison of collision cross section (\AA^2) vs m/z of ions of laboratory Tholins sample with experimental measurements (*), polyglycin (purple) and tetraalkylammonium salts (red). Taken from database (+), polyaromatics hydrocarbons (black) and calculated (o) polyHCN (dashed lines)

TAA family is composed of molecules containing an ammonium core with four carbon chain arms. This group, in opposition to PAHs, tends to form spherical conformations with the folding of the carbon chains. This line remains above the laboratory Tholins one, leading to the conclusion that this specific spherical conformation is not the one preferred in our sample. Finally, polyglycine was also considered as possible laboratory Tholins structures.

Polyglycine is a peptide, which tend to form a helical structure according to our 3D optimization (See **Figure S109a** for a structure). The shorter Gly2 peptide presents a CCS close to that of Tholins but larger peptides present a more compact structure. Small polyglycines are not long enough to form a helical structure. This explain why they fit with the Tholins sample. As a short summary of the comparison of these tree families with our sample, the preferential conformation present in these laboratory Tholins seems to be seems to be none of the three observed conformations. Two ideal PolyHCN polymers were added thanks to calculated CCS as presented in the next section. The linear PolyHCN can be assimilated to a straight stick shape (3D structure in **Figure S109b**). The branched one form a non-perfectly flat network (3D structure in **Figure S109c**). These 3D structures are also given in **Figure S109b** and **Figure S109c** . This family has been proposed to be the one composing Titan's aerosols. In agreement with Vuitton *et al.*, 2010 ([Vuitton, et al. 2010 \[16\]](#)), laboratory Tholins are clearly not pure ideal polyHCN because representative lines of this family are far above the detected sample. We can then exclude this structure from our measurement. They are potentially present in small quantities in the sample but not detected in these measurements.

In this first overview, we have recovered important information concerning the global structure of laboratory Tholins sample. Spherical and pure planar shapes were excluded as well as helical structures.

A middle range zoom, described in **Figure 77a**, allows for the observation of four clusters between m/z 120 and m/z 180. Ion mobility spectrometry naturally acts as a Kendrick mass defect diagram ([Kendrick 1963 \[44\]](#) ,[Hughey, et al. 2001 \[45\]](#)). In our case, two main repetition patterns are recovered: CH₂ and HCN. This result is consistent with previous studies ([Somogyi, et al. 2005 \[7\]](#) ,[Anicich, et al. 2006 \[18\]](#)).

A short-range zoom is given in **Figure 77b**. This sub-figure focuses on a single cluster located between m/z 134 and m/z 144. All molecular formulas are determined for the detected species, allowing the direct observation of the collision cross section evolution according to the compounds formula. Two main areas are shown according to the number of nitrogen atoms in each species. The first region (in blue) is located at the top of the cluster. It contains ions with a lower amount of nitrogen atoms (between N2 and N3). The other region (in red), which contains ions with a higher nitrogen content (between N4 and N7) is located under the first one. Therefore, the number of nitrogen atoms plays a major role in the conformation of our organic matter samples and also allows the production of different isomers that are not separated with the current instrumental resolution. The collision cross section decreases with an increasing number of nitrogen atoms. Such evolution of polymeric species as a function of the number of polar heteroelements has been observed previously ([Farenc, et al. 2017 \[46\]](#), [Woods, et al. 2004 \[47\]](#)). It suggests that laboratory Tholins molecules have some flexibility that can yield to partial folding as the number of heteroelements increase by the formation of intramolecular hydrogen bonding.

This overview allowed the study of the global molecular structure of the laboratory Tholins sample. Several typical structures (such as polyHCN, PAH, TAA and polyglycine) are excluded without doubt from our sample due to the difference in the CCS values at this m/z range. After working by exclusion, the next step is to use the CCS calculation tool to propose possible structures of laboratory Tholins.

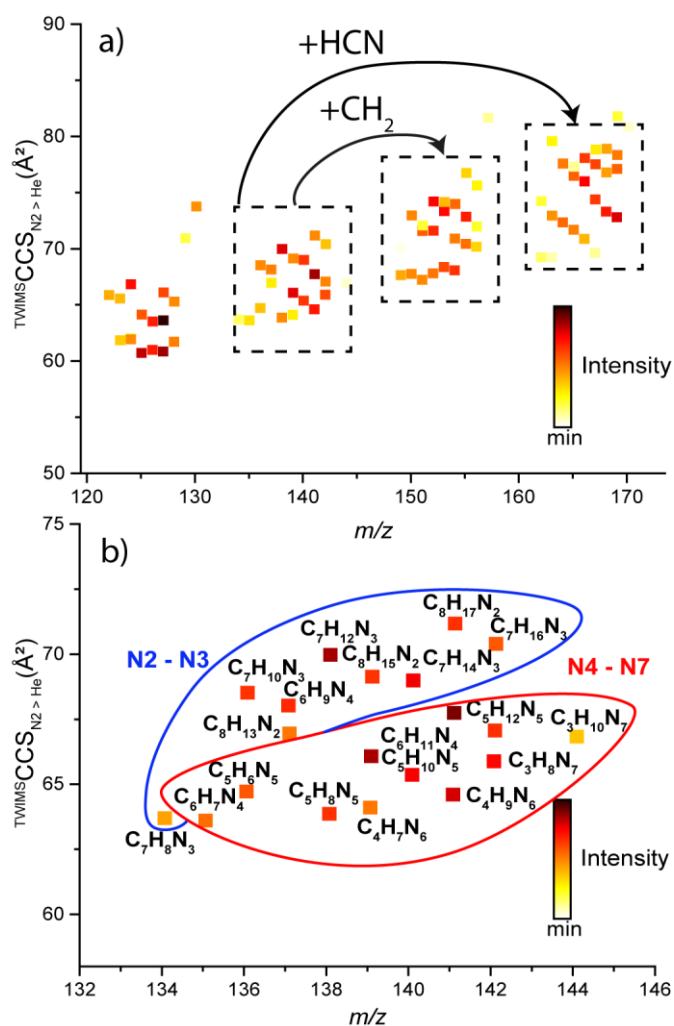
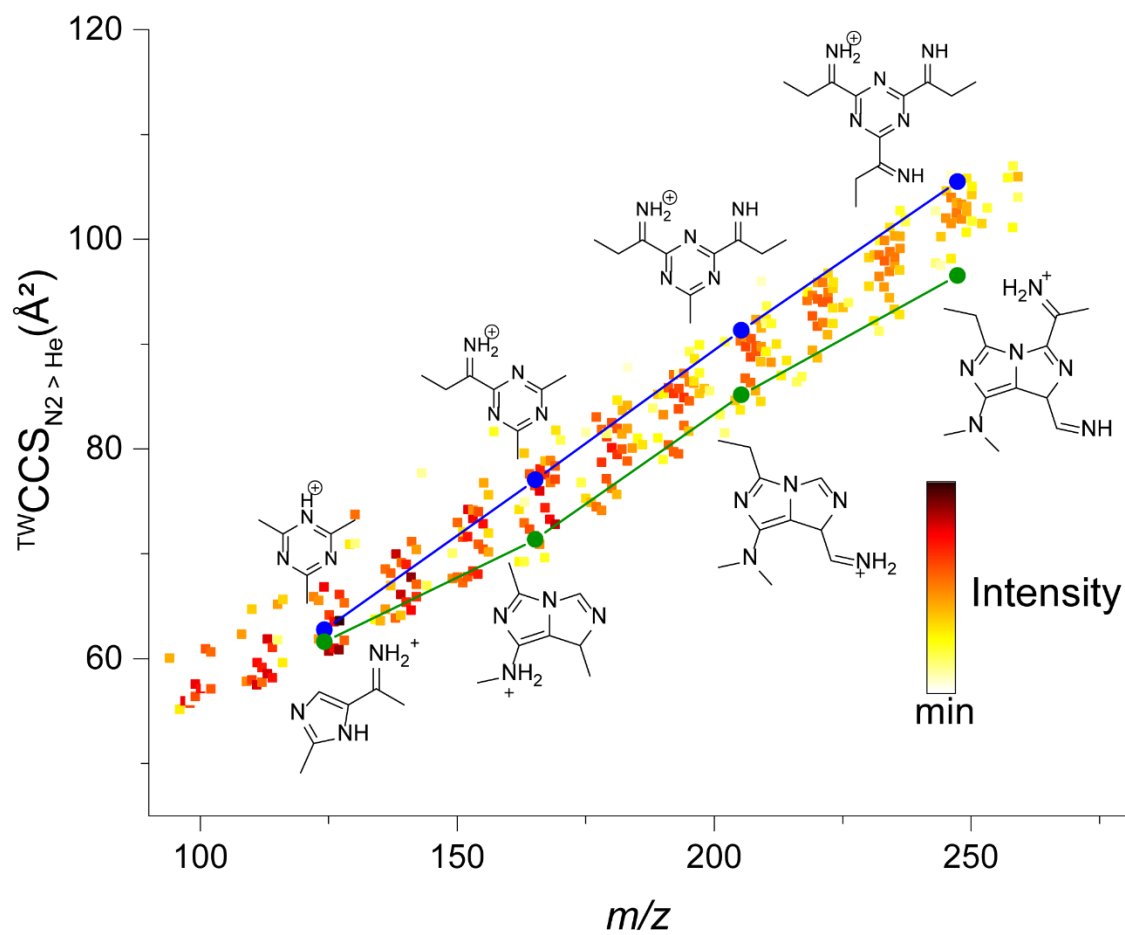


Figure 77 : Middle range zoom of the **Figure 76** for detailed comparison of collision cross section (\AA^2) vs m/z of laboratory Tholins ions. (a) Close zoom of **Figure 76** on clusters between m/z 120 and m/z 170, b) Zoom on one cluster between m/z 134 and m/z 144 with molecular formula.

CCS calculation of potential structures

Due to the actual limited knowledge of such organic material molecular structures, finding a family of standard compounds that would fit the entire range of collision cross sections can be difficult. To solve this problem, CCS calculation is the easiest way to probe possible structures.

Theoretical CCS in helium are obtained using MOBCAL software ([Shvartsburg and Jarrold 1996 \[37\]](#), [Mesleh, et al. 1996 \[38\]](#)) modified with new Lennard-Jones parameters ([Campuzano, et al. 2012 \[32\]](#)). The calculation method is first trained on standards to ensure its robustness (See **Table S17** for further details). CCS values of two series of compounds are represented on **Figure 78**. The blue curve presents a family of compounds based on a triazine core substituted with different numbers of branches. These branches are composed of an imine connected to a carbon chain. The green curve shows a family based on pyrazole cores connected with imine groups. Triazine and pyrazole cores have been chosen because their presence has been previously identified in laboratory Tholins ([Gautier, et al. 2016 \[17\]](#), [Quirico, et al. 2008 \[48\]](#)). As observed, the CCS of these two set of compounds corresponds to the CCS of Tholins compounds. Thus, these two families could be consistent with laboratory Tholins structure, in opposition with compounds presented in the previous section. It should be pointed out that several structures can match a particular CCS value, the structures presented here are likely hypothesis in agreement with laboratory Tholins.



1.1

Figure 78 : Comparison of CCS vs m/z with (blue) calculated CCS of triazine family (green) calculated CCS of pyrazole family.

5.1.4. Discussion

Triazine and pyrazole were identified in laboratory Tholins by NMR studies ([He and Smith 2014 \[49\]](#)) and liquid chromatography coupled to high resolution mass spectrometry ([Gautier, et al. 2016 \[17\]](#)). The first described triazine family in this study was based on these precedent works ([Gautier, et al. 2016 \[17\]](#), [He and Smith 2014 \[49\]](#)) and seems to fit well with the global trend of laboratory Tholins samples between PAHs and TAA families. However, the CCS values of this family correspond to the highest CCS values of laboratory Tholins typical ions. In opposition, the second family based on a pyrazole core described in precedent works have CCS values in agreement with the lowest CCS values for the laboratory Tholins species. We propose, regarding these results, a structure based on a Nitrogenated-PAH cores (Triazine and pyrazole) linked together with short chains. The core of this set is completely conjugated. This would induce a strong absorption in the UV/visible, consistent with Titan's aerosols color. A growing path perspective of this family is given in **Figure 79**. This hypothetical structure is consistent with recent works that highlighted the wide diversity of production pathways based on aromatic starting cores ([Gautier, et al. 2017 \[22\]](#), [Mahjoub, et al. 2016 \[50\]](#)).

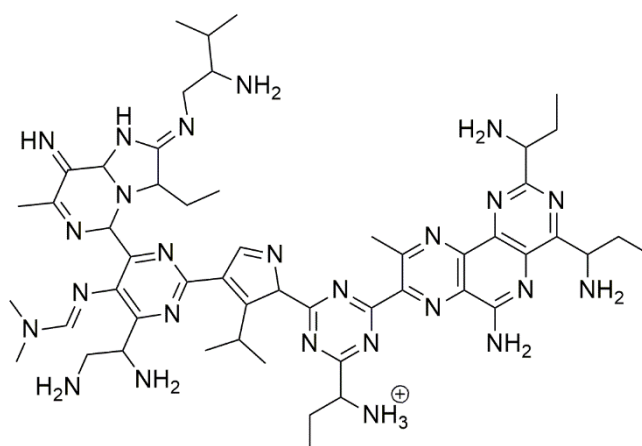


Figure 79 : Growing path possibility of families containing triazine and pyrazole cores. As example here, a compound with raw formula $C_{52}H_{78}N_{25}$ Note: This structure by itself was not detected directly in the laboratory Tholins but represents a general idea of molecules that could be embedded in them.

Regarding Titan, it was proposed that the aerosols should have a PAHs structure based on several observations obtained thanks to the Visual and Infrared Mapping Spectrometer (VIMS) boarded on the Cassini probe ([Dinelli, et al. 2013 \[51\]](#) ,[López-Puertas, et al. 2013 \[52\]](#)). This scenario was largely approved because the aromatisation of small compounds is usually favoured due to their strong stability. While our results agree with the presence of aromatic rings in the aerosol structure, we observe that a pure PAH structure cannot fit the aerosol CCS. However, an aromatic structure based on N-PAH cores could still be in agreement with VIMS observation and would fit the structure of studied laboratory Tholins sample much better.

5.1.5. Conclusions

In summary, this work introduces ion mobility spectrometry coupled with mass spectrometry analysis for the structural study of soluble organic matter by presenting a case study on analogues of the Titan's haze. This new analysis allows for an isomeric separation of the sample. In addition, the collision cross section, additional structural information, is determined for each ion. The collision cross sections give an overview of the main structural shape of the organic matter present in the sample. Moreover, as this information is intrinsic (for a certain gas) and predictable, the calculation of theoretical CCS is possible for several series of compounds. Our method allows the exclusion of several structures, in the case of laboratory Tholins such as pure polyHCN, PAH, polyglycine and tetra-alkylammonium salts at this range of mass-to-charge ratio. These are definitely not present in the major structure of laboratory Tholins. To conclude, we proposed two families composed of small aromatic cores linked together with short chains that could be potentially be present in laboratory Tholins sample.

We suggest using the methodology detailed here to study other material containing complex organic matter, such as meteoritic soluble organic matter or Earth's kerogens. We would like to point out that this first introduction provides an opportunity for further studies to complete this research, including the study of negative ions as well as the insoluble fraction of Tholins, both proved to be an important part of the material ([Somogyi, et al. 2012 \[20\]](#) ,[Maillard, et al. 2018 \[21\]](#)).

5.2: Etude structurale d'analogues d'aérosols de Titan par « Trapped Ion mobility » couplé avec un « Fourier Transform Ion Cyclotron Resonance »

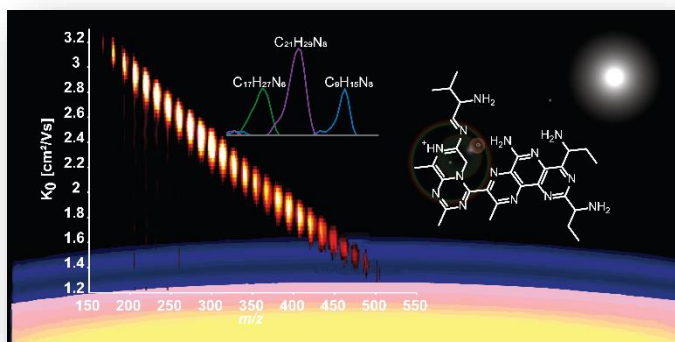
Cette seconde étude s'insère dans la continuité de la partie précédente. En effet, les mêmes expériences de mobilité ionique ont été réalisées mais cette fois-ci couplée à un spectromètre de masse à ultra-haute résolution. Les limitations observées dans la première partie ont pu être améliorées et la gamme de masse analysée élargie. Cette partie est présentée sous la forme d'une communication courte publiée lors de la deuxième année de thèse dans *Journal of The American Society for Mass Spectrometry*.

Dans le but de développer une technologie de mobilité ionique à bas champ couplable aux restrictions d'un analyseur FTICR (qui est un analyseur très lent), Bruker a mis au point un guide d'ions permettant de retenir des paquets d'ions et de les relâcher par tranche de voltage. Ces tranches sont caractéristiques de la section efficace de collision des ions piégés. Cette technologie TIMS est présentée en détail dans le **chapitre II**. Ce prototype de couplage a été réalisé à Billerica (Etats-Unis) dans le laboratoire de recherche de Bruker. Nous avons eu l'occasion de réaliser une semaine d'expériences sur cet appareil afin d'en observer les performances. Sur les Tholins, les résultats obtenus ont été comparés dans cet article aux précédents résultats obtenus sur l'IMS-QTOF de Rouen. Une plus large gamme de masse a pu être analysée ce qui m'a permis d'observer l'apparition d'un repliement de la structure globale vers la masse 350 qui n'avait pas encore été vu. Ce repliement montre qu'à partir d'un certain point, les structures commencent à devenir de moins en moins flexibles et de plus en plus proches de structures HAP. Cela a confirmé l'hypothèse de structures azotées polyaromatiques cycliques. De plus, la structure théorique calculée dans l'article précédent a été affinée afin d'être en adéquation avec ces nouveaux résultats.

Abstract

Non-earth planetology relies on artificial laboratory-made materials. The aerosol constituents of the Saturn's moon Titan are of particular interest as several spacecraft missions already allowed to gather spectroscopic data. Titan haze's

analogs, so-called Tholins, were produced on earth to push forward the comprehension of this phenomenon.



In this study, this incredibly complex mixture was firstly described by trapped ion mobility spectrometry coupled to ultra-high resolution mass spectrometry (FT-ICR MS). Electrospray ionization revealed the characteristic CHN_x -class components, with CHN_{5-6} and DBE 6-7 most abundant. Deploying specialized visualization, enabled by the exact mass measurement and elemental composition assignment, the adapted Kendrick mass defect analysis highlights the $\text{C}_2\text{H}_3\text{N}$ homolog series, whereas the nitrogen-modified Van Krevelen diagram is exhibiting a clear trend towards H/C 1.5 and N/C 0.5. More interestingly, the representation of m/z versus collision cross section (CCS) allowed hypothesizing a ramified N-PAH structural motive. IMS will not be able to resolve the isomeric continuum of ultra-complex mixtures; thus peak parameter different as CCS are explored. As such, analyzing the peak width versus m/z allows suggesting a lowering increase of isomeric complexity with increasing size of the N-PAH-like structure. Due to the tremendous complexity, these structural insights are only to be revealed by TIMS FTMS.

5.2.1. Introduction

Titan, the second largest moon in our solar system possess a thick atmosphere mainly composed of nitrogen with few percentages of methane. In this gaseous environment, nitrogen radicals are created. Their reaction with methane leads to the formation of a thick haze ([Israel, et al. 2005 \[53\]](#)). Because of this particular formation, the study of this fog represents a substantial interest for prebiotic chemistry and planetary sciences. Space missions (Voyager and Cassini-Huygens) provided the first information on the chemical composition of this haze. They reveal the presence of mainly NH_3 and HCN chemical motives. However, due to technical limitations of the on-boarded instruments, the exact composition of this dust remains mostly unknown ([Vinatier, et al. 2010 \[54\]](#)). These limitations can be overcome, and further knowledge on the Titan atmosphere can be gained by producing analogs of the haze on earth, so-called Tholins ([Szopa, et al. 2006 \[6\]](#)).

Tholins were investigated by multiple mass spectrometric approaches, such as by liquid chromatography and various, direct infusion experiments ([Somogyi, et al. 2005 \[7\]](#), [Vuitton, et al. 2010 \[16\]](#), [Gautier, et al. 2016 \[17\]](#), [Maillard, et al. 2018 \[21\]](#), [Carrasco, et al. 2009 \[34\]](#), [Gautier, et al. 2014 \[55\]](#)). Very recently, ion-mobility coupled with a time of flight mass spectrometer (IM-MS) was performed in order to obtain a snapshot of the global structure. By comparison with common structural elements several structural motives could be excluded, and an overview of potential shapes was allowed. However, the conclusions of this work and other previous investigations revealed limitations due to the restricted resolution of the mass spectrometer and the extreme complexity of Tholins samples. This previous investigation was restricted to a relatively narrow mass range focusing on small constituents.

Commonly, far heavier species are detected; thus, a significant proportion of the structural information might be lost.

The presented work was motivated to overcome the limited view on the molecular structure of Tholins by hyphenation of a powerful separation approach, through trapped ion mobility spectrometry (TIMS), to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). FT-ICR MS with its unbeaten mass accuracy and resolving power serves as the ideal analyzer for the ultra-complex Tholins samples. TIMS FT-ICR MS was recently deployed for the description of petroleum and other complex natural mixtures ([Fernandez-Lima, et al. 2011 \[56\]](#), [Benigni, et al. 2015 \[57\]](#)). To the best of the author's knowledge, this is the first proof of principle study addressing a planetary science subject. Exemplary, the soluble fraction of a Tholins sample is investigated by ESI. The unique parameter derived by ion mobility separation, such as peak position, width, and shape, serves as a measure for a more detailed structural insight.

5.2.2. Experimental

Tholins were produced according to the PAMPRE experiment ([Szopa, et al. 2006 \[6\]](#)). Briefly, after a few hours of plasma discharge of a nitrogen/methane mixture, a brown powder, the Tholins sample material, is recovered. For electrospray analysis, the sample was dissolved in methanol, stirred and filtered giving the soluble fraction. Directly prior analysis, a water/methanol (50/50 v-%) mixture was prepared. A standard mixture containing phosphazene derivatives (Agilent Tune Mix) was used for collision-cross section calibration.

Molecular level analysis of the Tholins soluble fraction was performed by a trapped ion mobility spectrometry (TIMS) 12 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS).

The prototype was equipped with an electrospray ionization source, and spectra are recorded in positive ion mode (5 kV, sample flow rate 120 $\mu\text{L}/\text{h}$). The trapped ion mobility cell replaced the inlet funnel. Briefly, the TIMS ramp voltage is stepwise increased from -110 V with 1 V per frame and 220 pulses per IMS scan were accumulated. Absorption mode spectra are recorded with a ParaCell® and a 2 mega-word transient length (full-sine apodization, one zero-fill). Consequently, a resolving power of over 500,000 at m/z 400, is achieved.

After mass calibration utilizing characteristic CHN homolog rows, each scan of the time-resolved data was peak picked (S/N 9) and exported. The exported mass spectra were processed by self-written MATLAB routines. The extracted mobilograms are fitted to an asymmetric Gauss shape and peak parameter are exported for each individual signal. Finally, elemental compositions were assigned with the following common boundaries for Tholins: $\text{C}_{1-50}\text{H}_{1-120}\text{N}_{1-15}\text{O}_{0-3}\text{S}_0$. A mass error of 1 ppm and only protonated species were considered. The mean assignment error was found to be below 100 ppb. More details can be found in the supplemental material (S1).

5.2.3. Results and Discussion

The results give evidence of the high complexity of this artificial planetology material, and roughly 1,300 elemental compositions could be attributed. The complexity of Tholins was previously pointed out but the direct infusion experiments missing an added dimension for separation and, thus, missing further structural information ([Benigni, et al. 2015 \[57\]](#)). The mass spectral inset in **Figure 80** emphasizes the mass spectral complexity. The ultra-high resolving power of the FTICR mass analyzer could fully resolve those close signals. Most critical for the Tholins sample was a 2.6 mDa split caused by N_6 versus $\text{C}_4\text{H}_4\text{O}_2$ and a 4 mDa split resulted by N_2O_2 versus C_5 , requiring at least a resolving power of 150k and 100k at m/z 400, respectively.

More importantly, **Figure 80** reveals the global view of the ion mobility dimension in combination with the fully resolved mass spectral pattern. Previous work performed with IMS TOF-MS was not able to resolve the increasing spectral complexity at higher m/z -values, and, thus, was focusing on the lower m/z -proportion of the tholin chemical space (**Figure 80**, red square). The TIMS FT-ICR MS coupling was able to significantly enlarge the accessible chemical space revealing CCS-values ranging from 70-170 Å² with an intensity-weighted CCS-value of 120 Å² almost in the center of the distribution.

In an initial attempt for insights into Tholins structural motives, the global trend of CCS versus m/z can be compared to CCS-values of different characteristic homolog series.

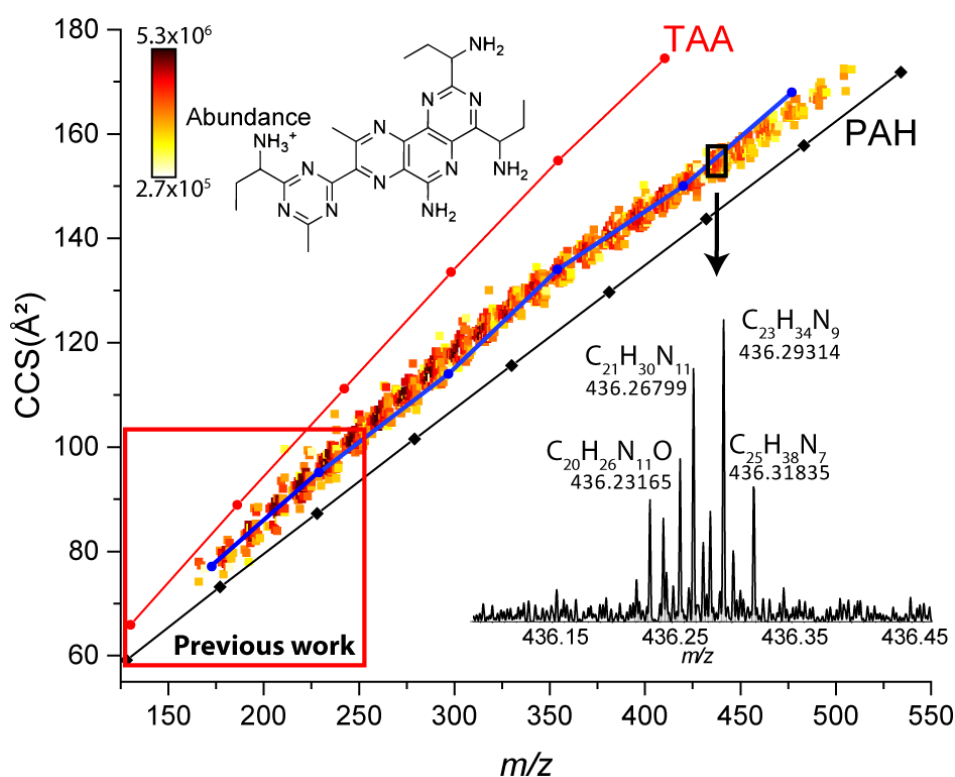


Figure 80. Apex collision-cross-section (CCS, in N₂ at room temperature) versus m/z color-coded by abundance. The behavior of the theoretical cross-sections of different structural motives is provided for Tetraalkylammonium salt species (TAA, red) and polycyclic aromatic hydrocarbons (PAH - black). A proposed structural motive for the Tholins homologue series is presented in blue (archipelago ramified N-PAH). Detailed information on the homologue rows can be found in **Table S18**.

The global trend of CCS versus m/z can be compared to CCS-values of different characteristic homolog series. This approach is visualized in **Figure 80** for the Tetraalkylammonium species (red line) and the polycyclic aromatic hydrocarbons (PAHs, black line). Noticeably, both series agree not very well with the observed pattern and moreover an overall slightly flattening of the pattern is revealed. Based on previous molecular level studies on Tholins, we hypothesize ramified archipelago-type nitrogen-containing PAHs (N-PAHs) as the primary structural element. Theoretical calculations of the cross-sections for those species, given as blue line in **Figure 80**, revealed an excellent agreement to the experimentally observed data. This first comparison already allows for structural information only to be retrieved by TIMS FTICR MS.

The elemental compositions assignments reveal CHN_x -class constituents to be prevailing, and compositions with up to 13 nitrogen could be found ($\text{O}\#\text{N}$ 6.8). This observation is in good agreement to the literature, which is pointing out the dominant presence of pure CHN_x -class constituents ([Maillard, et al. 2018 \[21\]](#), [Gautier, et al. 2014 \[55\]](#)). Oxidized components (artifact from the rapid oxidation) were found to be below 15 % of the overall abundance and as the focus of this study is to proof TIMS coupled to FT-ICR MS as a preferable servant for the investigation of planetology relevant materials, on an acceptable level.

In contrast to the classical Van-Krevelen diagram visualizing H/C versus O/C, the modified representation utilizing the N/C can be ideally deployed for Tholins ([Gautier, et al. 2014 \[55\]](#)). In **Figure 81** the respective N/C-Van-Krevelen diagram is given for all CHN_x -class constituents.

The pattern turns out to pursue to a center close to the respective intensity weighted values of 1.56 and 0.44 for the H/C and N/C, respectively. Species with up to a nitrogen-number equal to the carbon number were found.

Noticeably, the peak apex CCS-value as a measure of the mean size of the specific molecular formula is low at the edges of the pattern and increasing towards the central point of the pattern. This behavior is caused by the stepwise addition of C_2H_3N -building blocks with N/C and H/C similar to the central point. Thus, the start of an isomeric tholin series was found to be rather non-characteristic covering an ample space in the Van-Krevelen diagram, whereas with increasing m/z caused by the addition of C_2H_3N -fragments to the molecular formula the size increase and consequently the CCS-value rise towards H/C 1.5 and N/C 0.5. An interpolated N-PAH (5-ring structure) with the elemental composition of $C_{30}H_{43}N_{14}$ would almost meet this condition.

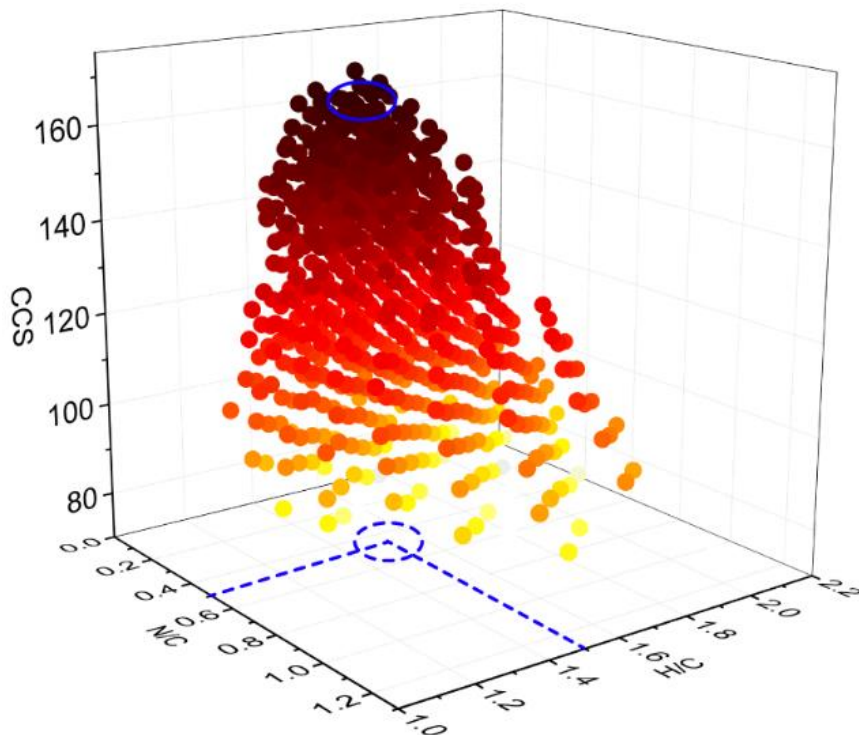


Figure 81 : H/C versus N/C Van-Krevelen representation of all $C_xH_yN_z$ -class constituents color-coded according to peak apex CCS-value. A clear center of the pattern can be spotted at H/C 1.5 and N/C 0.5 (marked in blue).

As C_2H_3N was identified as an essential building block, the respective Kendrick mass defect diagram can be drawn with the C_2H_3N -homolog rows at the horizontal lines (see **Figure S110**). The correlation pointed out in Figure 1 between m/z and CCS can be seen. Moreover, a less prevailing effect of increasing CCS with increasing KMD- C_2H_3N is observed.

Thus, different homolog rows might exhibit the same nominal mass but different size (reflected by CCS), which correlates with KMD- C_2H_3N . Nonetheless, this effect is weak, and we can conclude, that the size-span of the homolog rows is rather low compared to the increase with raising m/z .

Aside from the CCS, allowing to hypothesize structural aspects, the full-width-at-half-maximum (FWHM) serves as a measure for the isomeric complexity ([Farenc, et al. 2017 \[58\]](#)). Due to time-of-flight mass spectrometric instrumentation, this approach was previously limited to simple mixtures or a low m/z interval. TIMS FTICR MS allows to push these limitations drastically, and **Figure 82** visualizes the FWHM versus m/z of the Tholins signals. It can be observed that the standard tune mix FWHM (containing a specific isomer) increases linearly, whereas the Tholins signals reveal an apparent flattening of the pattern. In the lower m/z range from 150-350 a linear trend can be found, whereas for the heavier constituents ($> 350 m/z$) a lower increase or eventually at above m/z 400 a plateau effect is observed. Thus, the isomeric complexity is increasing linearly for the low m/z interval. Thus, the addition of a building block leads to multiple isomers by far exceeding the isomeric complexity of the precursor.

At a certain point, despite the increasing number of theoretical attachment positions, this increase in isomeric complexity is drastically lowered. We hypothesize that this is due to sterically aspects of the ramified aromatic structure.

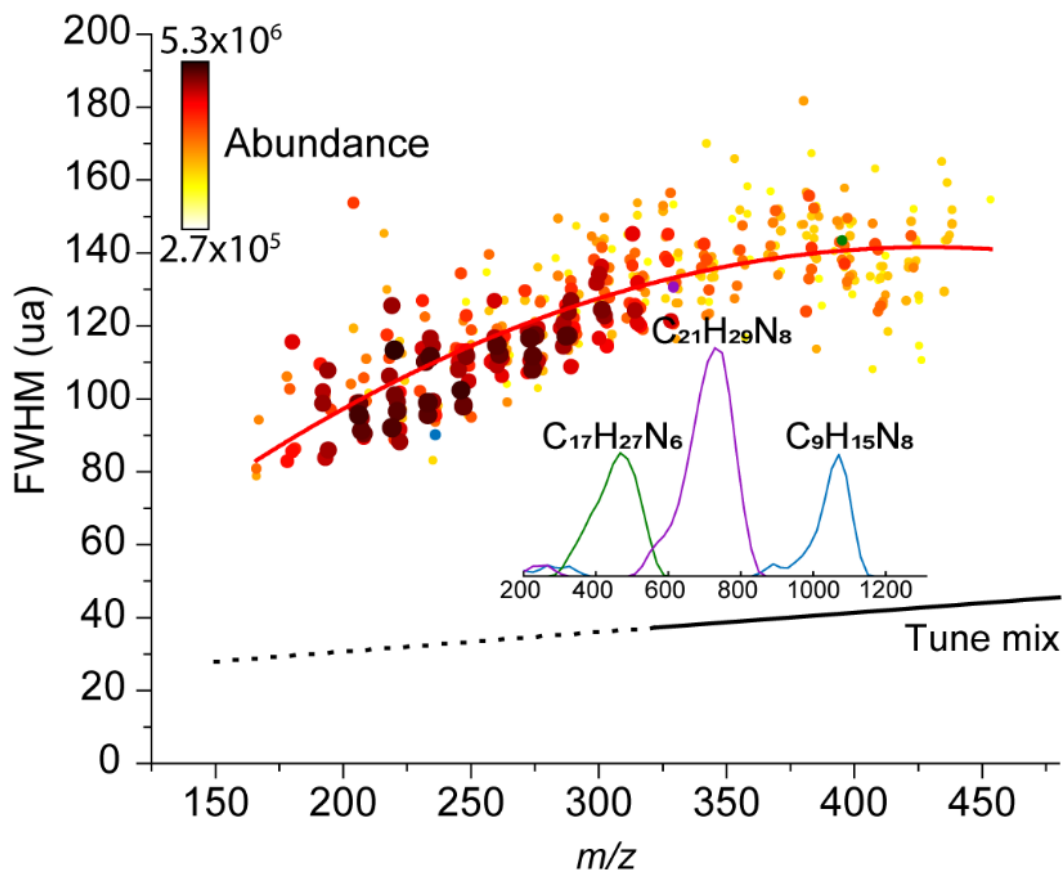


Figure 82 : Full width at half maximum (FWHM) versus m/z color- and size-coded to the summed abundance. Interestingly, the pattern of the FWHM is flattening towards higher m/z allowing for implication of the isomeric complexity growth. As comparison, the standard mix values are displayed and interpolated for lower m/z -values (dotted black line).

5.3 : Clichés de structures composant les analogues d'aérosols de Titan par microscope à force atomique

La dernière partie présentée ici expose les résultats que nous avons obtenu en réalisant de la microscopie à force atomique sur les échantillons de Tholins. Le but étant d'observer directement les structures présentes dans le mélange. Ces résultats sont présentés sous la forme d'une version d'article prêt pour soumission.

Cette étude a été réalisée en collaboration avec IBM *research* (Zürich, Suisse) qui possède une technologie de microscope AFM à haute résolution. Grâce à ceux-ci, ils sont capables d'observer la structure atomique de molécules (photo généralement de taille 30 nm x 30 nm). Ces instruments leur servent à développer des micropuces et autres composants informatiques.

Je suis entré en contact avec eux afin de pouvoir réaliser des mesures sur un échantillon de Tholins. Ils ont ainsi imagé plus de 110 molécules composant l'échantillon. Nous avons été surpris par la qualité des images obtenues, notamment pour une douzaine de molécules. Des structures polyaromatiques ont été observées contenant des cycles à 5 et à 6 membres, ainsi que des structures plus ramifiées. La mesure de composés standard comme d'adénine et la benzacridine a permis d'identifier les atomes d'azote dans nos structures mesurées. Nous avons ainsi mis en évidence la présence d'azote dans les structures polycycliques. Nous avons aussi observé que la plupart des molécules observées étaient catacondensées, ce qui nous a permis de discuter leur mode de formation. De plus, les petites molécules se sont montrées moins insaturées que les plus grande, ce qui nous a amené à nous poser la question de leur croissance et leur aromatisation.

Abstract

The atmosphere of Saturn's largest moon, Titan, is filled by a photochemical organic haze composed of nitrogen, carbon and hydrogen. This haze is one of the most complex organic material in the Solar System. The comprehension of its chemical composition presents a huge interest for prebiotic chemistry. However, the actual chemical structure of this haze remains unknown so far. Here, we report the first pictures of molecules composing Titan haze laboratory analogues using high-resolution atomic force microscopy (HR-AFM). Through direct imaging, we confirm the hypothesis that nitrogenous polycyclic aromatic hydrocarbons (N-PAH) constitute the major fraction of the aerosols. Surprisingly, elongated N-PAH containing five members aromatic rings are found to be the predominant structures. These observations represent a major step forward our understanding of processes at act on Titan. Furthermore, having actual pictures of molecules using HR-AFM is a milestone for all scientists working on structural elucidation in planetary sciences.

5.3.1. Introduction

Thanks to the observational constraints given by the successful Cassini-Huygens space mission, reproductions of Titan photochemical haze are performed on Earth in order to gain additional knowledge using state-of-the-art instruments. Reproductions of the Titan's haze on Earth are currently the best method to obtain information about the chemical processes occurring in the atmosphere of the icy moon ([Cable, et al. 2012 \[8\]](#)). Understanding the formation of such aerosols and their chemical structure is a key for the comprehension of the origin of life ([Sagan and Salpeter 1976 \[59\]](#), [Sagan 1974 \[60\]](#)). Titan's atmospheric chemistry is also often used as a proxy to understand the chemistry present during the primitive Earth age ([Cleaves, et al. 2008 \[61\]](#), [Kasting 1993 \[62\]](#)). State-of-the-art analyzes were realized on these analogues in order to decipher their structure ([Cable, et al. 2014 \[11\]](#), [Gautier, et al. 2012 \[12\]](#), [Vuitton, et al. 2010 \[16\]](#), [Gautier, et al. 2016 \[17\]](#), [Somogyi, et al. 2016 \[19\]](#)). Recently, new analytical tools were used, in particular ion mobility coupled to mass spectrometry, and investigations were made concerning their tridimensional structures ([Maillard, et al. 2020 \[63\]](#), [Ruger, et al. 2019 \[64\]](#)). A N-PAH structure has been defined as the most likely scenario. However, this structure remained a hypothesis.

In recent years, high-resolution Atomic Force Microscopy (AFM) has emerged as a new tool to study complex molecular mixtures. Functionalizing the apex of an AFM tip with a carbon monoxide (CO) molecule allows for molecules to be imaged with atomic resolution ([Gross, et al. 2009 \[65\]](#), [Moll, et al. 2010 \[66\]](#)) and *a priori* unknown molecules to be identified ([Gross, et al. 2010 \[67\]](#), [de Oteyza, et al. 2013 \[68\]](#)). Taking advantage of the AFM's single-molecule sensitivity, this approach was used recently to unravel the chemical structures of asphaltenes ([Schuler, et al. 2015 \[69\]](#)), image the molecular constituents of soot ([Schulz, et al. 2019 \[70\]](#), [Commodo, et al. 2019 \[71\]](#)), identify fuel pyrolysis products ([Fatayer, et al. 2018 \[72\]](#)) and study marine dissolved organic carbon ([Fatayer, et al. 2018 \[73\]](#)).

Here, we employ AFM with CO-functionalized tips to study Titan haze analogues based on individually resolved molecules. We examine the samples produced using the PAMPRE experiment ([Szopa, et al. 2006 \[6\]](#)) (see Methods), assign their chemical structures and compare our results to recent high resolution mass spectrometry (HR-MS) study ([Maillard, et al. 2018 \[21\]](#)).

This allows us to identify key signatures in the molecular structure of this complex N-rich organic matter, thus shining light into their structure and their formation precursors.

5.3.2. AFM results on Titan haze analogues

For AFM analysis, the sample is sublimed at low temperatures onto a Cu(111) single crystal partially covered by bilayer, (100)-oriented NaCl islands [denoted as NaCl(2ML)/Cu(111)] (see Methods, and Supplementary **Figure S111** for typical overview image). Based on its solubility in methanol, the sample can be separated into a soluble and insoluble fraction. We first study the common fraction containing all molecules (in the following denoted by ‘A’) but find only very few molecules for which atomic resolution can be achieved by AFM. In a second set of measurements we study the insoluble fraction (in the following denoted by ‘B’), which contains a much larger share of molecules that can be resolved by AFM. We ascribe this difference to a larger amount of chain-like linker structures in the common fraction compared to the insoluble one. In total, around 110 individual molecules are analyzed by AFM. The majority of those are either too bulky, too small or too mobile to achieve sufficient atomic resolution to assign their chemical structure. But for ~12% of the analyzed molecules, we are able to propose either their entire chemical structure or at least significant parts of it.

AFM images for all molecules for which a structure can be assigned are presented in **Figure 83**. The AFM images show a map of the frequency shift of the oscillating cantilever as the tip is scanned at constant height over the molecule (see Methods). The molecular structure reveals

itself as a bright modulation (more repulsive forces) on top of a dark background (more attractive). Our AFM data show several cyclic or polycyclic molecules. We find molecules containing only one or two cyclic units, e.g., **B6** and **B32**, as well as larger ones, with up to at least seven cyclic units, e.g., **A4** and **B23**. The intensity variations of the AFM contrast along the molecular backbones suggests non-planar adsorption geometries as well as the incorporation of heteroatoms and aliphatic moieties.

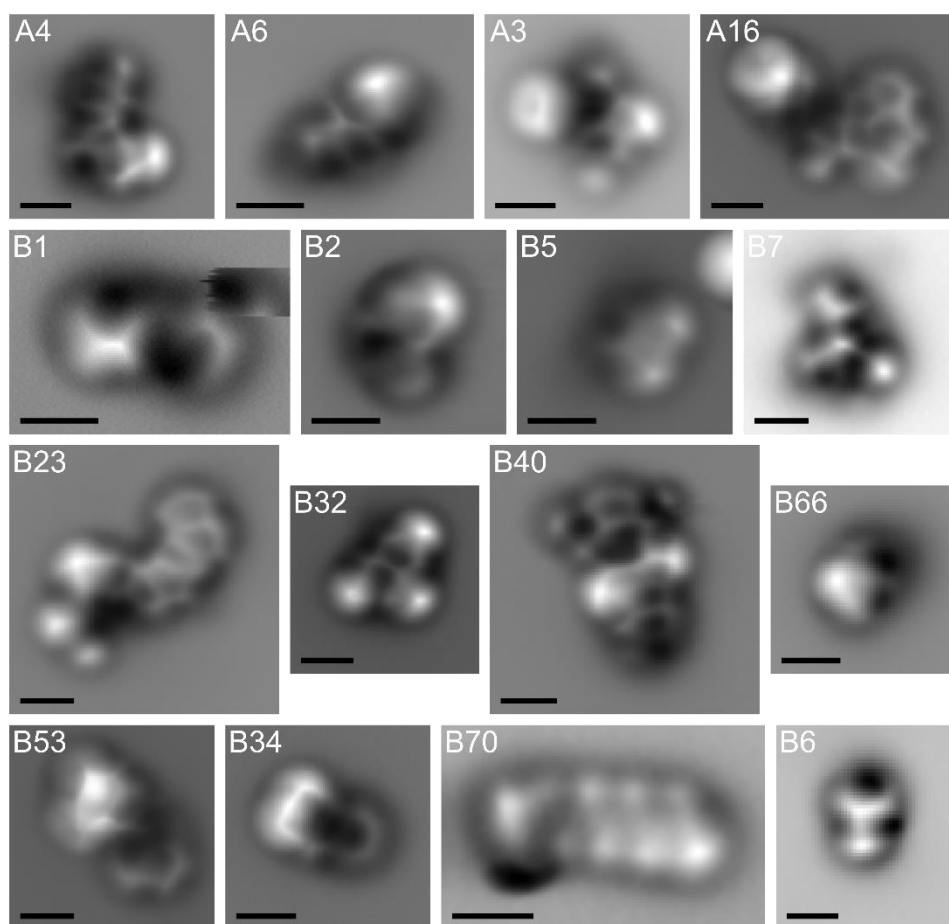


Figure 83 : High-resolution AFM images of Tholins. ‘A’ refers to molecules from the common fraction, while ‘B’ denotes the insoluble fraction. All scale bars are 5 Ångström.

Molecules containing aliphatic chains or linker structures were also observed, see **B1** and **B70**.

In addition, **A16** and **B7** are examples of molecules for which the AFM images indicate the presence of cyclic units but strong distortions in the image contrast or strongly repulsive features do not permit inferring the molecule’s structure.

We assign these distortions to significant non-planarity of the molecule and how it affects the tilting of the CO at the tip apex ([Pavlicek, et al. 2012 \[74\]](#), [Hapala, et al. 2014 \[75\]](#), [Hamalainen, et al. 2014 \[76\]](#)).

AFM signatures of N-rich standard molecules

Detailed structure assignment of unknown molecules on the basis of high-resolution AFM images can benefit from studies of model compounds.

These are used to characterize the AFM contrast of specific chemical moieties under different imaging conditions (*e.g.*, different substrates, adsorption sites, tip heights, tip functionalizations) and subsequently identify them in *a priori* unknown molecules. Model compounds containing different linear alkyl chains and cycloaliphatic moieties were studied in detail previously ([Schuler, et al. 2017 \[77\]](#)). There are also some studies regarding the AFM image contrast of different heteroatoms in polycyclic hydrocarbons ([van der Heijden, et al. 2016 \[78\]](#), [van der Heijden, et al. 2016 \[79\]](#), [Ellner, et al. 2017 \[80\]](#), [Kawai, et al. 2018 \[81\]](#))

In contrast to the previous molecular mixtures that were studied by high-resolution ([Schuler, et al. 2015 \[69\]](#), [Fatayer, et al. 2018 \[72\]](#)), the organic sample contains a large amount of nitrogen heteroatoms. Moreover, based on the sample synthesis, nitrogen should be the only heteroatom present. However, the number and relative amount of nitrogen atoms can vary between individual molecules, and the nitrogen atoms can have different chemical environments. Therefore, we studied a set of model compounds with different N-containing moieties and different numbers of nitrogen atoms in various chemical environments to facilitate the structure assignment for our measurements. The different model compounds and their AFM images are shown in **Figure 84**.

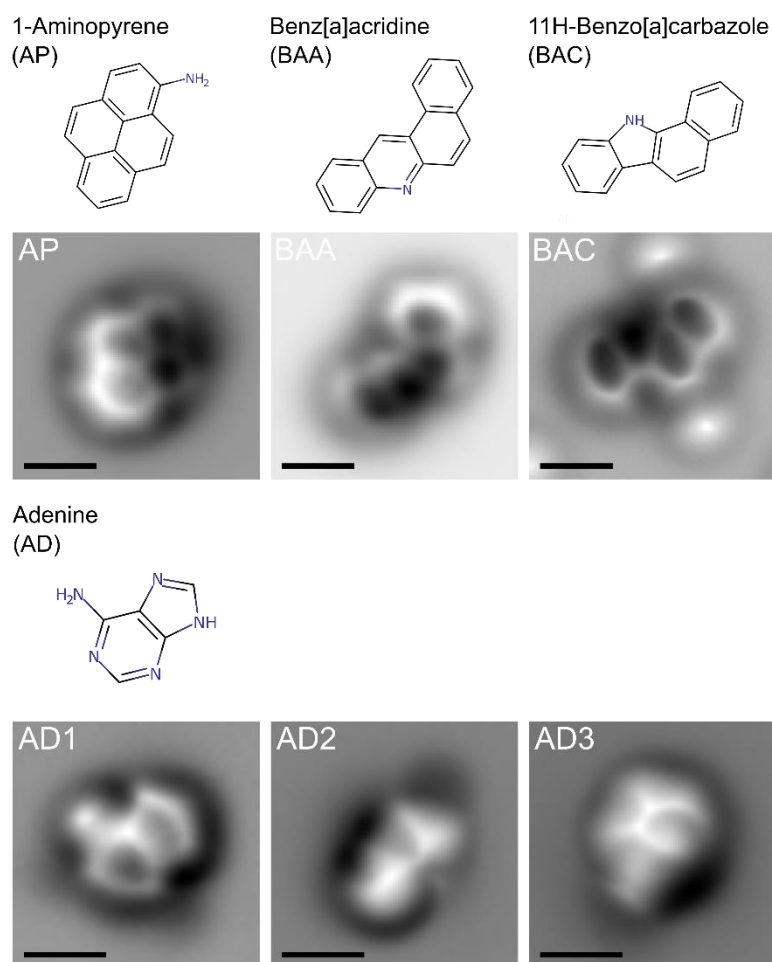


Figure 84 : Molecular structure models and AFM images of nitrogen-containing model compounds. Images of AP, BAA and BAC are acquired on NaCl(2ML)/Cu(111). Image AD1 is acquired on Cu(111) and AD2, AD3 on NaCl(2ML)/Cu(111). The two bright features adjacent to the molecule in the image of BAC are two CO molecules adsorbed on the NaCl film. All scale bars are 5 Ångström

First, we studied three model compounds containing each only one nitrogen but in different chemical environments, either as amine (1-Aminopyrene, AP), pyridine (Benz[a]acridine, BAA) or pyrrole (11H-Benzo[a]carbazole, BAC). The AFM images show that for each molecule, the part containing the nitrogen appears darker, indicating less repulsive interaction compared to the carbon backbone of the molecule.

The dark contrast of the nitrogen sites is explained by a locally reduced adsorption height because nitrogen interacts stronger than carbon with the surface, resulting in a tilted adsorption geometry with N being closer to the surface compared to the remaining carbon backbone. In addition, the larger electronegativity of nitrogen (with respect to carbon) results in a different electron density, which is relevant for AFM imaging. However, it is not trivial to conclude if this will result in more attractive or more repulsive contrast compared to, *e.g.*, carbon ([Moll, et al. 2012 \[82\]](#)). The images for AP, BAA and BAC in **Figure 84** show molecules adsorbed on bilayer NaCl islands, but we find the same qualitative adsorption behavior for these molecules on Cu(111).

Finally, we study a model compound with a larger N/C ratio to qualitatively assess how the presence of several nitrogen atoms affects the adsorption behavior. In AP, BAA and BAC the adsorption is governed by the single nitrogen forming a chemical bond with the surface resulting a tilted adsorption geometry. In a molecule containing several N atoms these will compete to form a bond with the surface. Adenine (AD) contains five nitrogen atoms in different chemical environments and it was previously identified in the sample ([Horst, et al. 2012 \[83\]](#)). From our AFM study, we find several different adsorption geometries of AD, both on Cu(111) and NaCl(2ML)/Cu(111), three of which are shown in **Figure 84**. For some molecules, such as depicted in AD1, the uniform AFM contrast on the entire molecule indicates a planar adsorption geometry. Other adsorption geometries in which the molecule is adsorbed in a tilted geometry indicate that only one or two nitrogen atoms on one site of the molecule form a chemical bond to the surface (see AD2 and AD3). In the planar adsorption geometry (AD1), the backbone of AD can be readily identified. Two important observations can be made. First, the presence of several N atoms within a heterocycle can lead to significant distortions in the AFM images, highlighted by the six-member ring of AD (lower part of the molecule in AD1) appearing almost triangular-shaped.

A similar contrast was predicted in a theoretical study of the AFM contrast ([Ellner, et al. 2017 \[80\]](#)) and can be ascribed to the redistribution of electron density due to the nitrogen heteroatoms and its effect on the CO tilting. Second, in this planar adsorption geometry the nitrogen atoms usually appear slightly brighter than the neighboring carbon atoms.

In the AFM images of AD, BAA and BAC, the N and NH groups within the heterocycle have a very similar contrast, demonstrating that these two groups are difficult to differentiate in the images of unknown molecules. This is similar for C and CH groups at the periphery of a PAH, where the absence of a hydrogen in the plane of the PAH is very challenging to be resolved ([Schuler, et al. 2016 \[84\]](#)). In the proposed structures, N and NH groups are tentatively assigned such that the number of unpaired pi-electrons is minimal. However, even under these constrictions the assignment of N and NH is not always unique (see Supplementary **Figure S112** and Supplementary Discussion).

5.3.3. Structural identification in Titan haze analogues

Figure 85 shows the proposed chemical structures for the molecules depicted in **Figure 84**. The assigned structures are based on the AFM measurements of the model compounds in **Figure 85**, previously published AFM studies ([Schuler, et al. 2017 \[77\]](#) ,[van der Heijden, et al. 2016 \[78\]](#) ,[van der Heijden, et al. 2016 \[79\]](#) ,[Ellner, et al. 2017 \[80\]](#) ,[Kawai, et al. 2018 \[81\]](#) ,[Zahl and Zhang 2019 \[85\]](#)) as well as insights from HR-MS ([Maillard, et al. 2018 \[21\]](#)). Interestingly, the structures indicate the frequent occurrence of five-membered rings, either with (e.g., **B23**, **B53**) or without N (e.g., **A4**, **B23**) as well as partially aliphatic rings (e.g., **B34**, **B32**). Another noteworthy observation from the proposed structures is the significant partial *cata*-condensed structure of some of the larger molecules (e.g., **A4**, **B23**). We also detected a polycyclic molecule containing no nitrogen (**B34**). The finding of the molecule **B70** was unexpected and is assumed as a washing contaminant (Heptane)

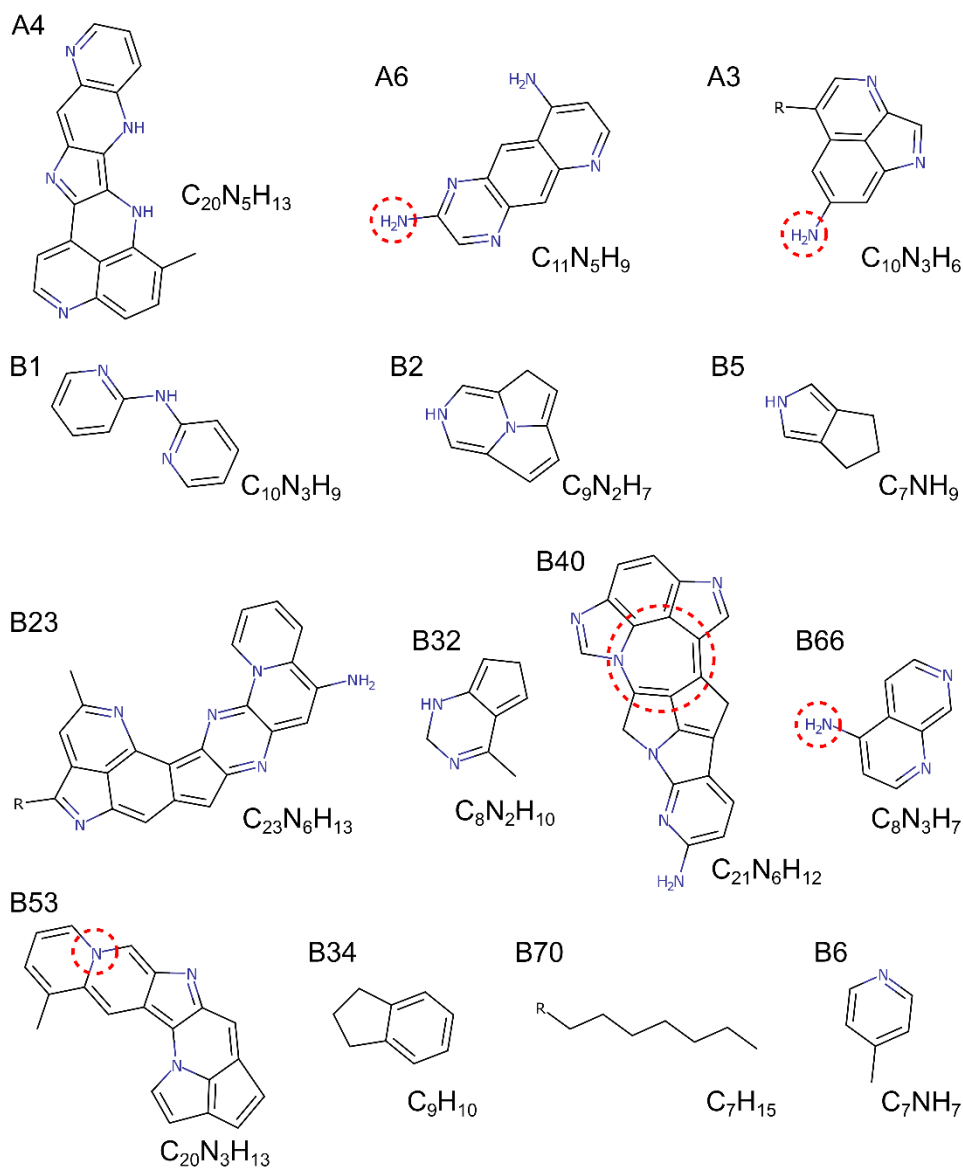


Figure 85 : Proposed structures for the molecules shown in **Figure 83**. Red dashed circles indicate uncertain moieties. ‘R’ denotes unidentified parts of a molecule, which are neglected in the molecular formulas. No structures were assigned to A16 and B7 (see main text).

5.3.4. Discussion

A quantitative analysis of the proposed structures is presented in **Figure 86**, where we plot H/C over N/C (**Figure 86a**) as well as double-bond equivalent (DBE) over number of carbon atoms (**Figure 86b**) for each molecular formula. The range of H/C values from our AFM study agrees well with a recent HR-MS study carried out on the same sample, with the majority of molecules having values between 0.5 and 1.5. Even though our AFM-derived structures indicate that *cata*-condensation is common in the PAH structures of the sample, plots of H/C over number of carbon atoms do not allow for identification of a dominating condensation pathway (see Supplementary **Figure S113** and Supplementary Discussion).

Surprisingly, the largest N/C within the molecules identified by AFM is just under 0.5, while the HR-MS study found that a large portion of Tholins have N/C values between 0.5 and 1.0. As could be seen from our AFM model compound Adenine (**Figure 84**), an increased number of nitrogen atoms can result in a complex adsorption behavior with significant non-planarity and strong distortions in the AFM contrast. This makes it challenging to achieve atomic resolution on molecules with higher N content and assign their structure. A possible example for such molecules could be **B7** in **Figure 84**. This suggests an inherent measurement bias in our AFM study towards molecules with lower N/C ratios. In addition, previous AFM studies [AFM14, AFM15] indicate that N atoms embedded in the polycyclic structure and bonded to three carbon atoms are particularly difficult to identify based purely on AFM images, thus our proposed chemical structures are more likely to underestimate rather than overestimate the amount of nitrogen atoms.

The double bond equivalent *versus* carbon number plotted in **Figure 86b** is a measure of the degree of unsaturation for a given molecular formula. For molecules containing only carbon and hydrogen, PAHs represent the family of species containing only carbon and hydrogen with the highest degree of unsaturation.

Most of the proposed structures for the insoluble fraction are above the PAH line, indicating their higher degree of unsaturation, in agreement with previous HR-MS results.

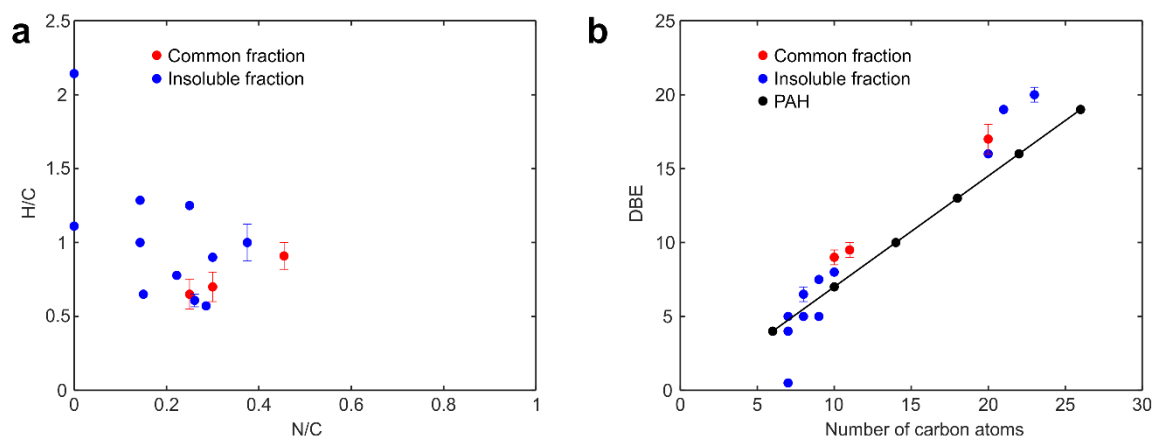


Figure 86 Quantitative analysis of assigned molecular formulas. (a) H/C ratio over N/C ratio. (b) Double-bond equivalent (DBE) over number of carbon atoms. In (b), the corresponding DBE values for linearly fused (cata-condensed) benzenoid PAH are shown as well for comparison. Unassigned parts of molecules denoted as ‘R’ in the proposed structures are neglected. Error bars correspond to the different possibilities of assigning N and NH for some of the structures (see Supplementary **Figure S112** and Supplementary Discussion).

PAH signatures were detected in Titan’s haze based on the onboarded Visual and Infrared Mapping Spectrometer (VIMS) ([Gautier, et al. 2017 \[22\]](#) ,[Dinelli, et al. 2013 \[51\]](#) ,[López-Puertas, et al. 2013 \[52\]](#) ,[Yoon, et al. 2014 \[86\]](#)) But as shown in these previous works, this signature is also compatible with N-PAH molecules and do not allow to distinguish between PAH or N-PAH scenarii. N-rich material have a higher interest in prebiotic chemistry, and their radiative impact is also expected to be higher, N-rich aerosols being more efficient UV absorbers (Mahjoub 2012). It is therefore important to clearly identify the structures composing Titan’s aerosols, using their laboratory analogues. Concerning Titan’s haze analogues synthesized in the present work, previous analysis already suggested the presence of at least small aromatic signatures in the material: Two-Step Laser Desorption Ionization Time-of-

Flight Mass Spectrometry highlighted the presence of small aromatics with one to 3 aromatic rings, with or without nitrogen (Mahjoub et al.).

Then global analytical techniques gave an overview of the overall structure of the material. Solid-phase NMR showed that Tholins structure is mainly based on unsaturated C=N bonds such as imines or triazine units (Derenne et al. 2012); and ion mobility highlighted a global structure with a slightly higher DBE than PAH, more in agreement with a N-PAH global structure. But the intermediate scale was missing: how does the material grow chemically from one or two aromatic rings towards a large PAH and/or a N-PAH structure; and what are the key-structures composing this complex material? The present results, imaging directly intermediate key structures composing the material gave invaluable insights on these open questions.

First we highlight mostly nitrogen-rich structures, orienting the scenario towards NPAH structures, also in agreement with VIMS measurements on Titan. Preciser un peu : pas n'importe quels N PAH : info sur leur organisation, plutôt linéaire/catacondensed. In fact, several reports have shown evidence about such structures([Imanaka, et al. 2004 \[13\]](#) ,[Gautier, et al. 2016 \[17\]](#) ,[Gautier, et al. 2017 \[22\]](#) ,[Derenne, et al. 2012 \[87\]](#)). Structures B5, B6 and B66 are very close to structures identified in previous solid-state NMR analyses([He and Smith 2014 \[49\]](#) ,[He and Smith 2014 \[88\]](#) ,[McGuigan, et al. 2006 \[89\]](#)). Such structures as A4, B23 and B53 were hypothesized in recent works([Maillard, et al. 2020 \[63\]](#) ,[Ruger, et al. 2019 \[64\]](#)). Furthermore, as stated before, recovered structures could contain even more nitrogen atoms that are not revealed now because it induces non-planar structures. On the other hand, these results are in opposition with previous findings in thermal analysis were aromatic structure and PAH were excluded from the composition of such Tholins([Nna-Mvondo, et al. 2013 \[90\]](#)).

Regarding the impact of such molecules present in the atmosphere of Titan, several effects can be discussed. First of all, finding such N-PAH could have important meanings. These molecules are good UV absorbers and can thus, modulate the photochemical process occurring in the atmosphere ([McKay, et al. 1989 \[91\]](#)). In addition, it can therefore be assumed that they could also modulate the aggregation process of the particles composing the haze due to their charging properties ([Bakes, et al. 2002 \[92\]](#)). Such molecules were also discussed as the reason of the formation of dunes over the Titan's surface ([Pirim, et al. 2015 \[93\]](#)).

The other information suggested by these results concerns the formation of this particle haze. Indeed, it can be observed that small detected molecules such as **B5**, **B32** and **B34** contain non-aromatic moieties. In opposition, large ones such as **A4**, **B23** and **B53** are fully conjugated. The production of this haze is initialized by the formation of small molecules followed by their growth by ion-molecule chemistry ([Vuitton, et al. 2019 \[94\]](#), [Vuitton, et al. 2006 \[95\]](#), [Lavvas, et al. 2013 \[96\]](#)). Thus, detected small molecules can be attributed to precursors for larger ones. And so, a catacondensation and an aromatisation is occurring during the growth of the Tholins. The molecule **B1** is interesting because its structure suggests that a cyclisation will possibly occur afterward. Having this diversity of molecules tends to suggest that a frozen image of the reacting mixture was taken at a certain moment.

However, as stated before, AFM measurements are particularly adapted to reveal catacondensed molecules. In fact, over that 110 molecules imaged, only a small portion (12%) was assigned because most of the structures were not enough planar. We can therefore assume that molecules imaged are definitively in the laboratory Tholins sample, but their overall quantity remains inaccessible. Even if most of the molecules are not as the ones reported in this study, it won't change the general conclusion regarding Titan's effect.

5.3.5. Methods

Tholin production:

Atomic force microscopy: AFM experiments were carried out in a home-built combined atomic force microscope/scanning tunneling microscope, operated in ultrahigh vacuum (UHV, base pressure $\sim 1 \times 10^{-10}$ mbar) and at low temperature (~ 5 K). The Cu(111) single crystal was cleaned by repeated Ne⁺ sputtering and annealing cycles. Bilayer NaCl islands were grown by evaporating NaCl onto the clean Cu(111) held at 273 K. Tholins were sublimed *in situ* from a silicon wafer onto the substrate held at ~ 10 K. CO for tip functionalization was dosed onto the substrate held at ~ 10 K by admitting CO into the UHV system through a leak valve.

AFM measurements were performed with a qPlus quartz force sensor ([Giessibl 1998 \[97\]](#)) operated in the frequency-modulation mode ([Albrecht, et al. 1991 \[98\]](#)). The qPlus sensor had a resonance frequency of ~ 28.8 kHz, quality factor of $\sim 100,000$, and was operated at an oscillation amplitude $A = 50$ pm. All AFM images were acquired in the constant height mode at 0 V bias voltage with a CO-functionalized tip. To facilitate structure assignment, several AFM images at different tip-sample distances were acquired for a given molecule.

AFM model compounds Adenine, 1-Aminopyrene, Benz[a]acridine and 11H-Benzo[a]carbazole were purchased from Sigma-Aldrich.

5.4. Conclusions

J'ai reporté dans ce chapitre les résultats obtenus durant cette thèse concernant la caractérisation structurale de l'échantillon de Tholins.

Dans la première étude, j'ai réalisé des mesures sur un spectromètre de masse temps de vol équipé d'une cellule de mobilité ionique. Cela m'a permis d'observer l'apport de la mesure des sections efficaces de collision pour l'étude des Tholins. Cette technique m'a permis d'obtenir des informations sur la conformation globale de l'échantillon pour les espèces allant de m/z 50 à m/z 250. J'ai pu ainsi, grâce à la mesure de familles de composés standards, exclure différentes conformations, notamment celles planaire et linéaire. Par la suite, grâce aux calculs théoriques, j'ai proposé une conformation cohérente avec les résultats obtenus. Cette conformation est plutôt planaire mais avec un peu de flexibilité apportée par la présence d'azote dans les molécules composant les Tholins.

Dans une deuxième étude, nous avons réalisé des mesures sur un spectromètre de masse de plus haute résolution (FTICR) équipé d'une cellule de mobilité ionique afin d'améliorer nos résultats. J'ai observé une forte amélioration de la gamme de masse étudiée, avec la détection d'espèces allant jusqu'à m/z 550. Cela m'a permis d'observer la présence d'un repliement dans la conformation des Tholins, vers m/z 350. Ce repliement n'avait pas encore été observé et m'a permis d'adapter la conformation proposée précédemment afin de coller au mieux avec ces nouveaux résultats. Cette étude nous a aussi permis d'étudier l'évolution de la largeur à mi-hauteur des pics de mobilité des Tholins. Cette valeur est caractéristique du nombre d'isomères différents présents dans un pic de mobilité. J'ai ainsi observé que le nombre d'isomères augmentait fortement dans les bas rapport m/z pour atteindre un pallier à plus haute masse. Cela a montré une forte diversité isomérique dans les premières espèces formées qui s'atténue avec la croissance de ces Tholins.

Enfin, dans la dernière étude, nous avons eu la chance de réaliser des mesures sur un microscope à force atomique à haute résolution. Cela nous a permis d'obtenir directement des images des molécules présentes dans l'échantillon de Tholins. Ces résultats ont confirmé nos précédentes hypothèses en montrant la présence d'hydrocarbures polycycliques azotés ainsi que de petites molécules moins insaturées. Nous avons ainsi discuté du mode de formation de ces Tholins, qui semble tendre vers une catacondensation et aussi étudié leur croissance et leur aromatisation.

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Conclusions Générales

Le but de cette thèse fut l'étude de la composition chimique d'analogues du brouillard régnant sur Titan, le plus gros satellite de Saturne. La caractérisation détaillée de ce brouillard permet une meilleure compréhension des phénomènes se produisant sur la lune ainsi que d'avancer sur l'étude de la chimie prébiotique, ou chimie organique complexe antérieure à l'apparition de la vie. De plus, par ses similitudes avec l'atmosphère de la terre primitive, la compréhension de ce brouillard permet aussi d'étudier les phénomènes de grande oxydation liée à l'apparition de la vie sur Terre.

Dans un premier temps, il a été rapporté dans la première partie de résultats de cette thèse les études réalisées en introduction directe sur FTICR-MS. Ces premières analyses ont mis en évidence la diversité chimique présente au sein de ce matériau en révélant une très grande complexité. L'impact du contact des Tholins avec l'oxygène de l'atmosphère a été étudié afin de mettre en évidence les processus oxydatifs et de mieux comprendre la formation de fonctions chimiques prébiotiques comme les liaisons amides et aldéhydes. Dans cette même partie, la comparaison entre les fractions soluble et insoluble dans le méthanol des Tholins a été réalisée pour la première fois, ce qui a mis en lumière les grandes différences au niveau du nombre d'hydrogène entre ces deux fractions. Cela a permis notamment de réétudier les hypothèses concernant la formation des dunes sur Titan.

Dans le second chapitre de résultats de cette thèse a été reporté les études réalisées sur la formation et les briques élémentaires composant la structure des Tholins. Pour cela, des expériences de fragmentation en FTICR ont été réalisées. La fragmentation permet, grâce à l'observation de motifs spécifiques lors de la rupture de liaisons chimiques, d'obtenir des informations structurales sur les espèces fragmentées. En réalisant une étude sur chaque fraction composant les Tholins, il a été possible de mieux comprendre les différences structurales entre les deux fractions. De plus, grâce à la comparaison avec des modèles détaillés dans la littérature, des structures putatives d'espèces composant chaque fraction ont été proposées.

Enfin, dans la dernière partie des résultats, la technique de mobilité ionique couplée à la spectrométrie de masse ainsi que de la microscopie à force atomique ont été utilisés afin d'obtenir de plus amples informations sur les structures composant les Tholins. J'ai, grâce à la mobilité ionique, obtenu les indices concernant la structures globales composant la fraction soluble des Tholins en excluant des familles de composés standard mesurés au préalable ainsi qu'en réalisant des calculs de CCS théoriques. La microscopie à force atomique m'a permis, ensuite d'obtenir directement des clichés de molécules composant les Tholins et ainsi de pouvoir les comparer aux résultats précédemment obtenus.

Cette thèse a permis d'avancer sur la compréhension de la formation du brouillard de Titan, dans sa composition ainsi que des ses interactions avec la surface et les lacs du satellite. Nous avons, grâce à la littérature, comparés nos résultats aux suppositions de la communauté et ainsi valider ou invalider certaines hypothèses notamment concernant la composition de chaque fractions (soluble et insoluble) ainsi que sur la présence ou non de N-PAH.

Plusieurs perspectives s'ouvrent aujourd'hui pour la continuité de ce projet. En ayant balayer une large gamme de techniques analytiques durant cette thèse, il a été possible de juger de la pertinence de chacune et ainsi mieux orienter les prochains projets portant sur l'analyse de matériaux atypiques comme les Tholins. Certaines analyses restent encore à développer, comme la spectrométrie de masse bidimensionnelle présenté à la fin du chapitre 4. La comparaison d'échantillon provenant de différentes expériences pourra être effectuer afin d'observer les similitudes et les différences au niveau chimique entre les échantillons. Cela permettrait d'obtenir un schéma global de composition général afin d'être plus représentatif du brouillard de Titan.

Concernant la spatialisation de futurs instruments, la comparaison entre le FTICR de Rouen et le CosmOrbitrap a permis de confirmer d'importance de l'ultra-haute résolution en spectrométrie de masse dans les sondes spatiales destinées à l'étude de la matière organique extraterrestre. En effet, ces matériaux ne ressemblent en rien à ce que l'on trouve sur Terre et nécessitent donc des performances instrumentales très élevées afin d'obtenir des informations sûres. La mobilité ionique s'est aussi avérée extrêmement pertinente pour obtenir directement des informations structurales sur un mélange complexe. Spatialiser ce genre d'instrument permettrait d'obtenir de précieuses informations *in situ* lors de prochaines missions.

Annexes 1

- **Productions scientifiques**

- Publications liées à la thèse
- Publications non liées à la thèse
- Communications orales
- Communications liées à la thèse
- Rédaction de projets durant la thèse

Production scientifique

Publications liées à la thèse

1. **J. Maillard**, N. Carrasco, I. Schmitz-Afonso, T. Gautier and C. Afonso (2018). "Comparison of soluble and insoluble organic matter in analogues of Titan's aerosols." *Earth and Planetary Science Letters* 495: 185-191.
2. **J. Maillard**, J. Ferey, C. P. Ruger, I. Schmitz-Afonso, S. Bekri, T. Gautier, N. Carrasco, C. Afonso and A. Tebani (2019). "Optimization of ion trajectories in a dynamically harmonized Fourier-Transform Ion Cyclotron Resonance cell using a Design of Experiments strategy." *Rapid Commun Mass Spectrom.*
3. C. Ruger, **J. Maillard**, J. Le Maitre, M. Ridgeway, C. J. Thompson, I. Schmitz-Afonso, T. Gautier, N. Carrasco, M. A. Park, P. Giusti and C. Afonso (2019). "Structural Study of Analogues of Titan's Haze by Trapped Ion Mobility Coupled with a Fourier Transform Ion Cyclotron Mass Spectrometer." *J Am Soc Mass Spectrom* 30(7): 1169-1173.
4. Selliez, L., **J. Maillard**, B. Cherville, T. Gautier, L. Thirkell, B. Gaubicher, I. Schmitz-Afonso, C. Afonso, C. Briois and N. Carrasco (2019). "High resolution mass spectrometry for future space missions: comparative analysis of complex organic matter with LAb-CosmOrbitrap and LDI-FTICR." *Rapid Commun Mass Spectrom.*
5. **J. Maillard**, S. Hupin, N. Carrasco, I. Schmitz-Afonso, T. Gautier and C. Afonso (2020). "Structural elucidation of soluble organic matter: Application to Titan's haze." *Icarus*: 113627.
6. **J. Maillard**, Christopher P. Rüger, Audrey Chatain, Isabelle Schmitz-Afonso, Chad R. Weisbrod, Laetitia Bailly, Emilie Petit, Thomas Gautier, Amy M. McKenna, Nathalie Carrasco and Carlos Afonso (2020). "Dark-ageing of analogues of aerosols in the atmosphere of the early Earth." Submitted to *EPLS*.
7. **J. Maillard**, Isabelle Schmitz-Afonso, Thomas Gautier, Carlos Afonso and Nathalie Carrasco (2020). "Deciphering chemical structures in Titan Tholins using tandem mass spectrometry." Ready for submission.
8. Fabian Schulz, **J. Maillard**, Katharina Kaiser, Isabelle Schmitz-Afonso, Thomas Gautier, Carlos Afonso, Nathalie Carrasco and Leo Gross (2020). "Imaging the molecular structures of Titan haze with atomic force microscopy." Ready for submission.

Publications non liées à la thèse

9. Caroline Barrère-Mangote, Johann Le Maître, **Julien Maillard**, Julien Roussely, Anne Roubaud, Marie Hubert-Roux, Carole Garnier, Carlos Afonso, Pierre Giusti, (2020). "Chemical characterization of 15 biocrudexes obtained from hydrothermal liquefaction of industrially cultivated wild micro algae." Ready for submission.
10. Grégoire Danger, Alexandre Ruf, **Julien Maillard**, Jasmine Hertzog, Vassilissa Vinogradoff, Philippe Schmitt-Kopplin, Carlos Afonso, Laurent Remusat, Nathalie Carrasco, Isabelle Schmitz-Afonso, Louis Le Sergeant d'Hendecourt (2020). "Unprecedented molecular diversity revealed in meteoritic insoluble organic matter: the Paris meteorite's case." Submitted to PNAS.

Communications orales

1. 13^{ème} congrès EFTMS du 24 au 27 Avril 2018 (European Fourier transform mass spectrometry) à Freising (Allemagne) - Comparison of soluble and insoluble organic matter in Titan's aerosols – Julien Maillard
2. Advances in Space Mass Spectrometry for the Search of Extraterrestrial Signs of Life du 16 au 18 Mai 2018 à Orléans - Comparison of soluble and insoluble organic matter in Titan's aerosols – Julien Maillard
3. European Planetary Science Congress du 16 au 21 Septembre 2018 à Berlin (Allemagne) - Comparison of soluble and insoluble organic matter in Titan's aerosols – Julien Maillard
4. 3^{ème} Ecole thématique de spectrométrie de masse à transformée de Fourier du 3 au 6 Avril à Cabourg - Comparison of soluble and insoluble organic matter in Titan's aerosols – Julien Maillard
5. European Planetary Science Congress + DPS du 16 au 21 Septembre 2019 à Genève – Introduction to ion mobility for the study of extra-terrestrial materials. – Julien Maillard
6. Rencontres du club jeune de spectrométrie de masse 2019 à Quiberon – Introduction to ion mobility for the study of extra-terrestrial materials. – Julien Maillard

Communications liées à la thèse

- Article dans le magasin Fields (MagLab) :
<https://nationalmaglab.org/fieldsmagazine/archives/amazing-haze>
- Poster 13^{ème} congrès EFTMS
- Poster ASMS 2019

Rédaction de projets durant la thèse

1. Projet Très Grands Equipement (plateforme française de FTICRs) - Analyse d'analogues du brouillard de Titan par LDI-FTICR (2017)
2. Projet Très Grands Equipements (plateforme française de FTICRs) – Caractérisation de la fraction insoluble des Tholins par spectrométrie de masse en tandem avec ionisation LDI (2019)
3. Projet European_FTICR_MS (Plateforme Européen de FTICRs) – AeroSimTitan – Caractérisation par GC-FTICR des aérosols de Titan (submitted to Rostock laboratory)
4. Projet National High Magnetic Fields Laboratory, (Tallahassee, Floride) – Etude de l'oxydation des Tholins -

Annexes 2

- **Chapitre 3, informations complémentaires pour :**
 - **Article 1 :** Optimisation de la cellule ICR
 - **Article 2 :** Vieillissement des analogues d'aérosols de Terre primitive
 - **Article 3 :** Comparaison des fractions soluble et insoluble dans le méthanol

- **Chapitre 4, informations complémentaires pour :**
 - **Article 5 :** Investigation des structures composants les Tholins grâce à la spectrométrie de masse en tandem

- **Chapitre 5, informations complémentaires pour :**
 - **Article 6 :** Première élucidation structurale par mobilité ionique de la matière organique soluble composant les analogues d'aérosols de Titan
 - **Article 7 :** Etude structurale d'analogues d'aérosols de Titan par « Trapped Ion mobility » couplé avec un « Fourier Transform Ion Cyclotron Resonance »
 - **Article 8 :** Clichés de structures composant les analogues d'aérosols de Titan par microscope à force atomique

Chapitre 3, Article 1 : Optimisation de la cellule ICR

This part contains table S1 to S7.

Table S9 : Fractional Factorial design matrix with response values the screening step.

Run N°	Run Order	Shim0 (V)	Shim90 (V)	Gat0 (V)	Gat90 (V)	SEP (%)	Second harmonic (%)
1	10	1.465	1.465	1.400	1.450	40	
2	28	1.535	1.465	1.400	1.450	20	4.083
3	23	1.465	1.540	1.400	1.450	20	2.996
4	9	1.535	1.540	1.400	1.450	40	3.558
5	1	1.465	1.465	1.620	1.450	20	4.694
6	24	1.535	1.465	1.620	1.450	40	6.77
7	20	1.465	1.540	1.620	1.450	40	
8	12	1.535	1.540	1.620	1.450	20	1.143
9	29	1.465	1.465	1.400	1.550	20	4.714
10	8	1.535	1.465	1.400	1.550	40	7.312
11	6	1.465	1.540	1.400	1.550	40	
12	5	1.535	1.540	1.400	1.550	20	1.237
13	25	1.465	1.465	1.620	1.550	40	
14	16	1.535	1.465	1.620	1.550	20	3.937
15	26	1.465	1.540	1.620	1.550	20	2.946
16	17	1.535	1.540	1.620	1.550	40	3.228
17	2	1.465	1.503	1.510	1.500	30	6.332
18	13	1.535	1.503	1.510	1.500	30	3.494
19	15	1.500	1.465	1.510	1.500	30	6.305
20	19	1.500	1.540	1.510	1.500	30	3.788
21	4	1.500	1.503	1.400	1.500	30	4.716
22	14	1.500	1.503	1.620	1.500	30	4.56
23	18	1.500	1.503	1.510	1.450	30	4.599
24	22	1.500	1.503	1.510	1.550	30	4.747
25	3	1.500	1.503	1.510	1.500	20	2.819
26	7	1.500	1.503	1.510	1.500	40	8.281
27	27	1.500	1.503	1.510	1.500	30	4.659
28	31	1.500	1.503	1.510	1.500	30	4.627
29	30	1.500	1.503	1.510	1.500	30	4.567
30	11	1.500	1.503	1.510	1.500	30	4.629
31	21	1.500	1.503	1.510	1.500	30	4.624

Table S10 : Fractional Factorial design matrix with response values for the optimization step.

Run N°	Run Order	Shim0 (V)	Shim90 (V)	Gat0 (V)	Gat90 (V)	Second harmonic (%)
1	27	1.530	1.530	1.600	1.540	2.475
2	2	1.550	1.530	1.600	1.540	1.928
3	20	1.530	1.550	1.600	1.540	1.757
4	4	1.550	1.550	1.600	1.540	1.033
5	5	1.530	1.530	1.640	1.540	2.407
6	23	1.550	1.530	1.640	1.540	1.942
7	12	1.530	1.550	1.640	1.540	1.684
8	24	1.550	1.550	1.640	1.540	1.107
9	19	1.530	1.530	1.600	1.570	2.414
10	6	1.550	1.530	1.600	1.570	2.003
11	26	1.530	1.550	1.600	1.570	1.693
12	3	1.550	1.550	1.600	1.570	1.135
13	1	1.530	1.530	1.640	1.570	2.373
14	11	1.550	1.530	1.640	1.570	1.975
15	28	1.530	1.550	1.640	1.570	1.654
16	29	1.550	1.550	1.640	1.570	1.054
17	17	1.530	1.540	1.620	1.555	2.039
18	18	1.550	1.540	1.620	1.555	1.493
19	8	1.540	1.530	1.620	1.555	2.156
20	10	1.540	1.550	1.620	1.555	1.256
21	21	1.540	1.540	1.600	1.555	1.665
22	22	1.540	1.540	1.640	1.555	1.637
23	16	1.540	1.540	1.620	1.540	1.684
24	15	1.540	1.540	1.620	1.570	1.639
25	25	1.540	1.540	1.620	1.555	1.623
26	14	1.540	1.540	1.620	1.555	1.608
27	13	1.540	1.540	1.620	1.555	1.682
28	7	1.540	1.540	1.620	1.555	1.676
29	9	1.540	1.540	1.620	1.555	1.708

Table S11 : PLS model regression coefficients generated by the screening step.

Second harmonic

	Coeff. SC	Std. Err.	P
Shim0	-0.08870	0.01303	0.00049
Shim90	-0.11241	0.01455	0.00025
Gat0	-0.00704	0.01455	0.64563
Gat90	0.00193	0.01455	0.89872
SEP	0.14123	0.01303	0.00004
Shi0*Shi0	-0.00072	0.01310	0.95773
Shi90*Shi90	0.00442	0.01369	0.75791
Gat0*Gat0	-0.01040	0.01368	0.47577
Gat90*Gat90	-0.00789	0.01368	0.58525
SEP*SEP	0.00569	0.01310	0.67912
Shi0*Shi90	-0.04575	0.01633	0.03106
Shi0*Gat0	-0.00388	0.01633	0.81987
Shi0*Gat90	-0.00052	0.01633	0.97560
Shi0*SEP	-0.01725	0.01162	0.18826
Shi90*Gat0	-0.00212	0.01669	0.90321
Shi90*Gat90	-0.00107	0.01669	0.95099
Shi90*SEP	0.03057	0.01633	0.11029
Gat0*Gat90	0.00019	0.01668	0.99123
Gat0*SEP	-0.00032	0.01633	0.98493
Gat90*SEP	0.00031	0.01633	0.98555

N = 27
DF = 6
Comp. = 4

Q2 = 0.628
R2 = 0.991
R2 adj. = 0.959

RSD = 0.03919
Confidence = 0.95

Table S12 : ANOVA test results of the PLS model generated by the screening step.

Second harmonic

	DF	SS	MS (variance)	F	p
Total	27	11.0417	0.40895		
Total corrected	26	0.972344	0.0373979		
Regression	20	0.96313	0.0481565	31.3577	0
Residual	6	0.00921428	0.00153571		

N = 27
DF = 6
Comp. = 4

Q2 = 0.628
R2 = 0.991
R2 adj. = 0.959

Cond. no. = 12.05
RSD = 0.03919

Table S13 : PLS model regression coefficients generated by the optimization step.

Second harmonic	Coeff. SC	Std. Err.	P
Constant	1,66208	0,0126069	4,58E-23
Shim0	-0,214759	0,0072904	5,37E-14
Shim90	-0,325479	0,00729039	1,69E-16
Gat0	-0,0120755	0,0072904	0,119882
Gat90	-0,00339587	0,0072904	0,648523
Shi0*Shi0	0,0608795	0,0154223	0,00145892
Shi90*Shi90	0,0117455	0,0154223	0,458943
Gat0*Gat0	-0,00371134	0,0154223	0,813317
Gat90*Gat90	0,0132228	0,0154223	0,405672
Shi0*Shi90	-0,0255319	0,00619991	0,00104456
Shi0*Gat0	0,00798444	0,0061999	0,218685
Shi0*Gat90	0,0138399	0,0061999	0,0424476
Shi90*Gat0	0,000118387	0,0061999	0,985034
Shi90*Gat90	-0,0022854	0,0061999	0,717926
Gat0*Gat90	-0,00542158	0,0061999	0,39662

N = 29	Q2 = 0,889		
DF = 14	R2 = 0,995	RSD =	0,03858
Comp. = 5	R2 adj. = 0,991	Confidence =	0,95

Table S14 : ANOVA test results of the PLS model generated by the optimization step.

Second harmonic	DF	SS	MS (variance)	F	p
Total	29	92,3573	3,18473		
Total corrected	28	4,41765	0,157773		
Regression	14	4,39681	0,314058	211,033	0
Residual	14	0,0208347	0,00148819		

N = 29	Q2 = 0,889	Cond. no. =	6,106
DF = 14	R2 = 0,995	RSD =	0,03858
Comp. = 5	R2 adj. = 0,991		

Table S15 : Attribution of new peaks detected after the DoE optimization

Measured <i>m/z</i>	Molecular formula	Err. (ppm)
592.24866	C25H30N13O5	0.13
592.27374	C26H32N13O3 ¹⁸ O	-0.04
592.28203	C27H35N10O5 ¹³ C	-0.05
592.31836	C38H38N7	-0.06
592.32791	C19H41N14O7 ¹⁵ N	-0.14
592.33949	C25H43N10O6 ¹³ C	0.03
592.34673	C19H43N14O7 ¹³ C	-0.01
592.37013	C33H43N10 ¹³ C	-0.11
592.37597	C26H47N10O5 ¹³ C	-0.12
592.38314	C30H46N11O2	-0.17
592.39528	C35H47N8 ¹³ C	-0.17
592.41238	C37H50N7	-0.11

Chapitre 3, Article 2 : Vieillissement des analogues d'aérosols de Terre primitive

This part contains the supplementary text for the dark ageing process study as well as supplementary figures S1 to S3.

Negative mode analysis

In the same approach as for the positive mode, negative ionization mode results are reported here in this section. **Figure S87** shows a zoom between m/z 558.12 and 558.46 for three different ageing samples, ranging from 2 months to 3.5 years. As observed in the manuscript, the same effect is present when we look at these zooms. The number of species containing oxygen (in red in the figure) increases as a function of ageing time.

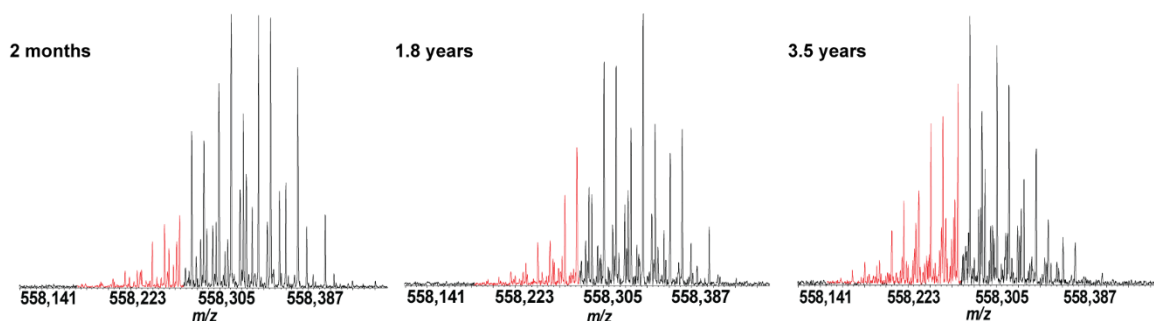


Figure S87 : Zoom on the mass spectra between m/z 558.12 and 558.46 for the 2 months old sample, the 1.8 years and the 3.5 years

Figure S88 shows the data treatment for negative ionization mode. On the first line are presented the distributions of the families present in the analyzed samples with an intensity greater than 1%. We can observe that in the 1.8-year-old sample, major families are those that do not contain oxygen. This is not the case in the other two samples where major families are those containing between 1 and 2 oxygen. This same effect can be observed on the second line, where the distributions of the number of oxygen atoms present in the samples is presented. We can see that there is a shift between the three samples, with the increase in the number of oxygen present. This same conclusion was observed and discussed in the manuscript.

Finally, to confirm all these observation, the third line shows us the distribution of oxygenated species in a Van Krevelen type diagram. We can see that there are much more species spreading along the x-axis for the 2.25 and 3.5-year-old samples than for the 1.8-year-old sample.

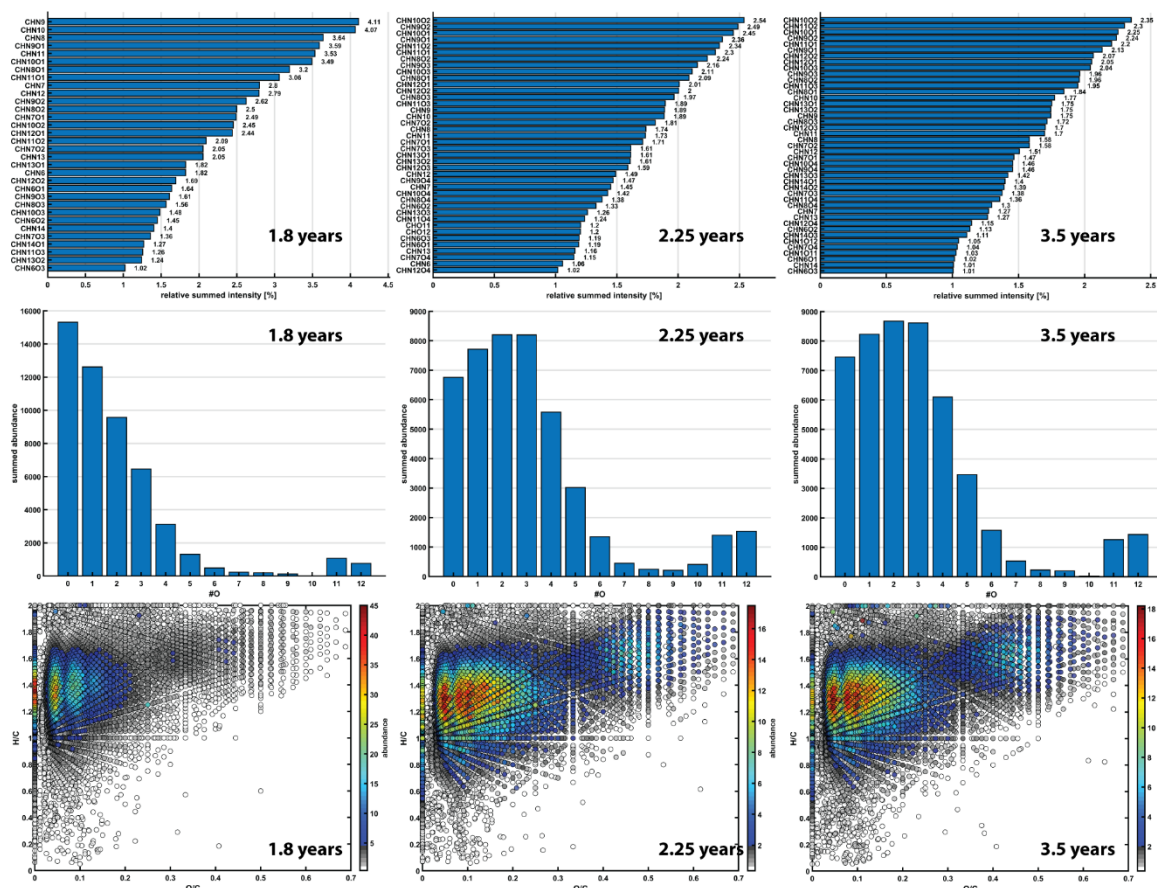


Figure S88 : Data treatment of assigned species for three samples with an ageing time of 1.8, 2.25 and 3.5 years. **First line:** Amount of each family present in the sample above 1% of intensity. **Second line:** Distribution of the amount of oxygen in the detected species. **Third line:** Van Krevelen representation for each sample.

Tandem Mass spectrometry

In order to improve our understanding of the incorporation of oxygen into primitive earth analogues, fragmentation analysis was performed. The objective was to highlight the oxygenated chemical functions present in the aged sample. Experiments were carried out on a FTICR (Solarix XR, Bruker) equipped with a 12T superconducting magnet. The spectrometer is, among other things, composed of a quadrupole for isolation of nominal mass followed by a collision cell.

The collision induced dissociation (CID) is performed with Argon as a buffer gas. 20 V of acceleration voltage were set between the entrance of the collision cell and the exit. Electrospray in negative mode was performed in order to focus on oxygenated species. Aldehyde compound were already identified in the manuscript. Therefore, the goal of this section is to find out if acid compounds are also present. For this purpose, the 3.5 years old sample was used.

Single nominal mass isolation was done at m/z 270. Because of the low resolution of the quadrupole, more than 25 peaks were isolated in the same time. All the peaks were assigned thanks to the high accuracy and resolution delivered by the FTICR. In the **Figure S89**, three specific species are presented. Two ions containing 2 oxygen atoms and 1 containing 3 oxygen atoms were chosen. If we look at the fragmentation of these species, we can observe that each ion has a loss of precisely 43.98986 Da. This loss is exactly the one of a CO_2 molecule. This fragmentation pattern is well known to be assigned to the fragmentation of an acid chemical function. This observation highlights the presence of such functions in early earth analogues.

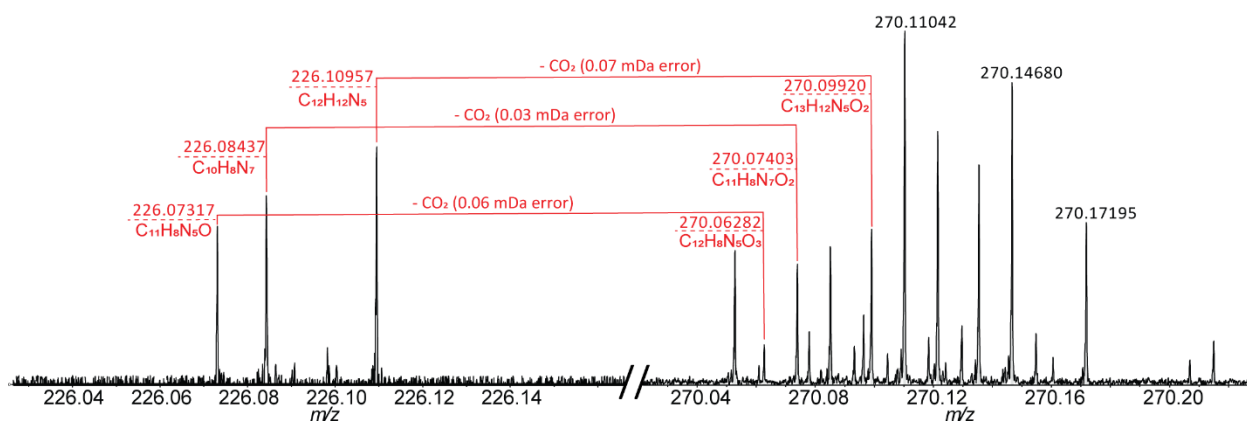


Figure S89 : Mass spectrum of the fragmentation of the nominal mass 270 by collision induced dissociation at 20V.

Partial least-squares regression

Partial least-squares (PLS) regression based on the SIMPLS algorithm was performed on the positive-mode broadband ultra-high resolution mass spectrometric data using Matlab 2018b. In brief, the elemental composition attribution lists for all data files were compared. Formulae not found were assigned with zero abundance. Data were centered, and column means were subtracted. Figure 1 displays the regression result given as experimental age versus predicted age based on the broadband molecular profile. Despite the fact that PLS is only able to depict linear behaviors, it was deployed in the presented study. The individual aging properties might be more complex and of higher-order functionality. Nonetheless, for simplification, a linear response was assumed for overall dark aging. The limited number of samples and replicates and the tremendous molecular complexity (over 18,000 different elemental composition attributions in positive-mode) restrict the applicable model types. Figure 5 displays the experimental age at the abscissa versus predicted age based on the PLS model at the ordinate. Samples with comparable low aging times (< 5 months) revealed a higher relative error between prediction and theoretical value. This finding is in good agreement with the aging trend of the individual compound classes (Figure 3), for which a fast non-linear functionality could be found. Aging above 5 months could be well described, and relative deviations are below 2-3 %.

Chapitre 3, Article 3 : Comparaison des fractions soluble et insoluble dans le méthanol

Comparison between different ionization sources and instruments on Tholins 5%

As we can see on **Figure S90**, a study was made to validate our results obtained with laser desorption ionization method.

First, we compared spectra recorded on a LDI-TOF instrument and LDI-FTICR instrument to observe if different analyzer equipped with the same ionization source gave the same results. Except for the resolution, no differences were founded on spectra. **Figure S90a** and **Figure S90b**: the general pattern of the mass spectrum is similar except for higher intensities for lower m/z values. This is due to transmission parameters of the SolariX mass spectrometer which tends to be more discriminating than the TOF analyzer. Nevertheless, we confirm that we detect the same species with the same repetition patterns with both analyzers and therefore we validate the species detected by FTICR.

We, then, compared two different ionization sources on the same analyzer (**Figure S90b** , **Figure S90c**). We recovered quite the same distribution, with a base peak located at a low m/z value.

Figure S91 shows a zoom on m/z 155 and m/z 504. Same species were recovered for each ionization method and this allowed us to validate our results.

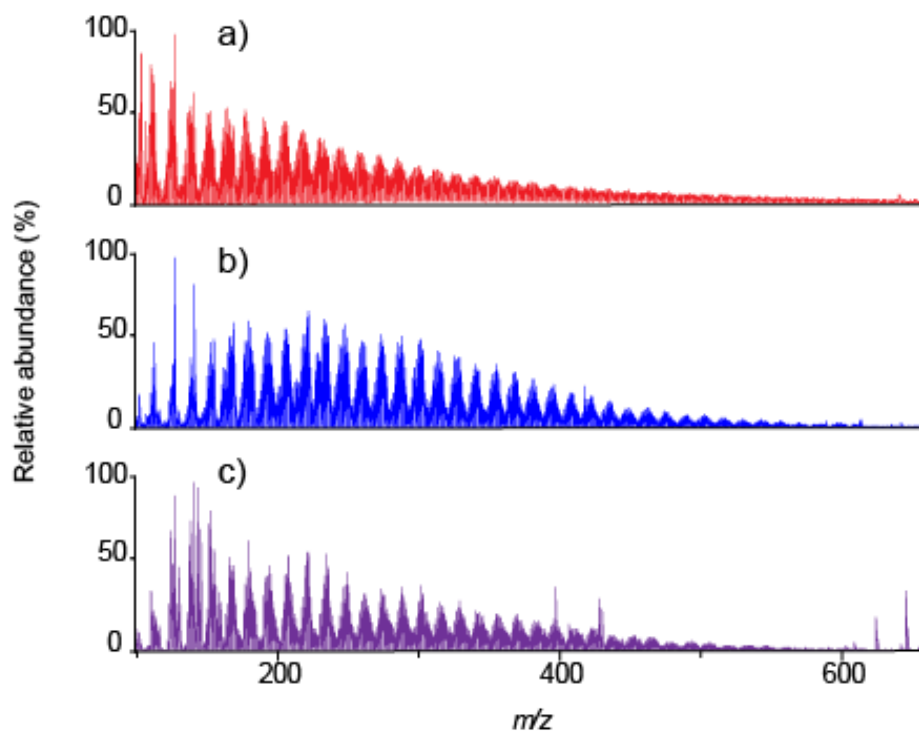


Figure S90 : Comparison between a) LDI-TOF b) LDI-FTICR and c) ESI-FTICR of soluble fraction of Tholins 5%

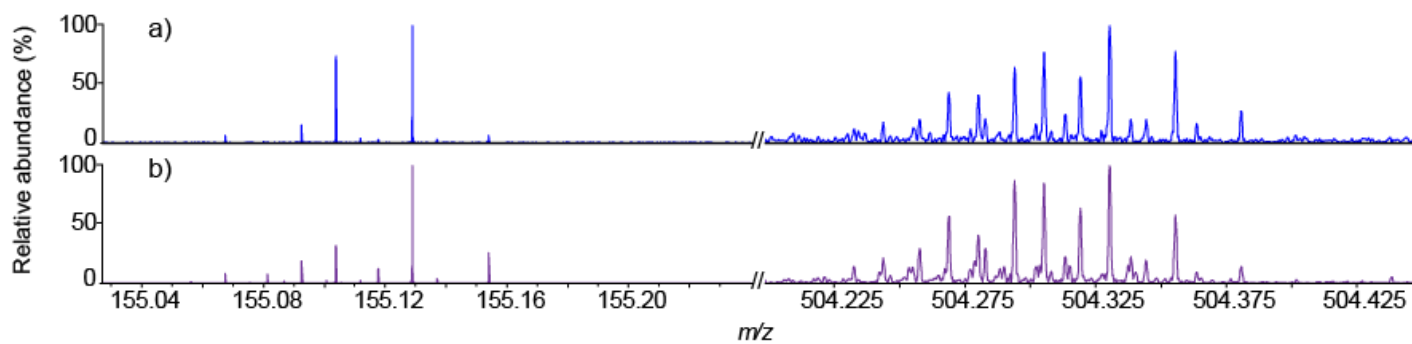


Figure S91 : Comparison at m/z 155 and m/z 504 for the a) LDI-FTICR and b) ESI-FTICR soluble fraction of Tholins 5%

Threshold

Because fractions responses to the laser ionization are different due to their composition (one is quite more saturated than this other), it was impossible to compare them with the same laser power. In fact, with the same ionization energy, spectra were not usable. Insoluble fraction was overexpressed and the peak shape was not good and soluble fraction got a global intensity which was very low. We decided, to avoid this problem, to observe the variation of the global intensity with the rise of the laser power for each fraction. **Figure S92** shows the result of this comparison after the normalization of each sum of intensities. We observe that each fraction got quite the same curve shape.

To accommodate the different behaviour of soluble and non-soluble fractions, and the global sample, we have to define the best laser power to be used for each sample. The laser delivers a maximum power of 166.7 kJ per shot. We first determined the minimum power to be applied to detect the first ions, 14%Pmax for the soluble and 24%Pmax for the insoluble. The best compromise between a good signal and avoiding ion coalescence, was found by settling the laser power at 5% above the threshold power, i.e. 19%Pmax and 29%Pmax for respectively the insoluble and soluble fractions. The global intensity was for all samples $1.10e7$ for 10 scans.

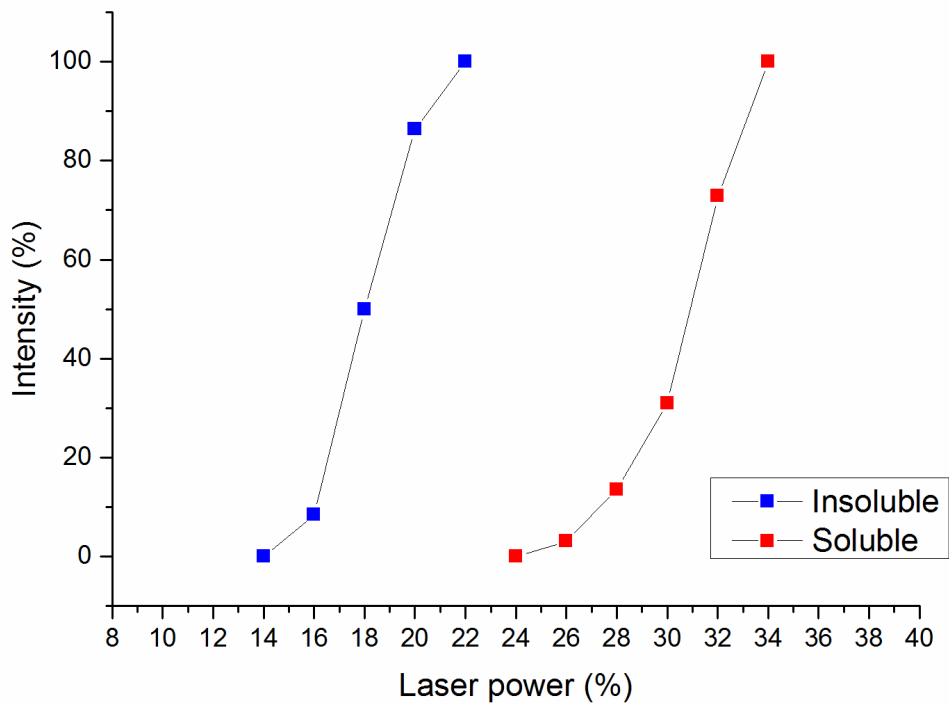


Figure S92 : Evolution of the signal intensity in LDI as a function of the laser power for the non-soluble in blue and soluble in red Tholins fractions.

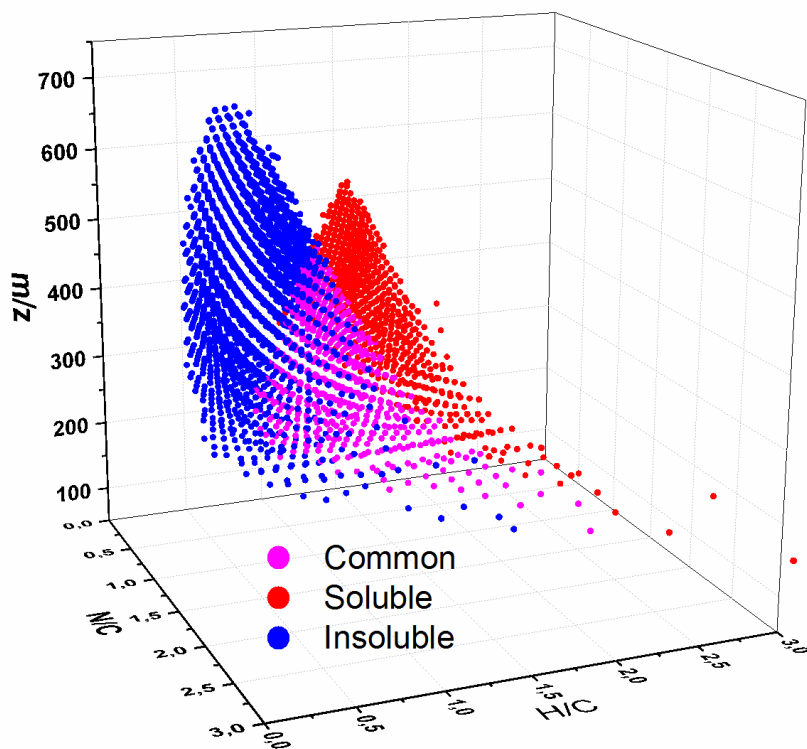


Figure S93 : 3D Van Krevelen for the comparison of Tholins fractions with a filter at 5% of intensity. Soluble is in red, insoluble in blue and common part in purple.

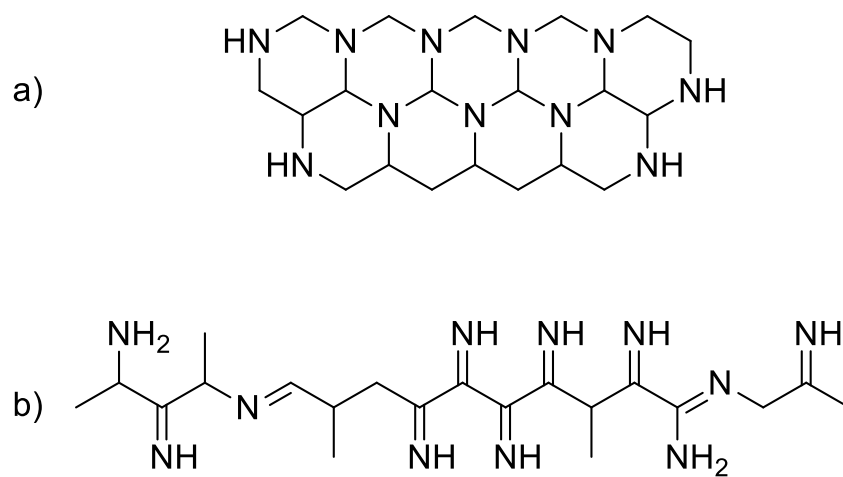


Figure S94 : Example of two different structures corresponding to $C_{20}H_{35}N_{11}$ a) Reticulated,
b) Unsaturated

Chapitre 4, Article 5 : Investigation des structures composants les Tholins grâce à la spectrométrie de masse en tandem

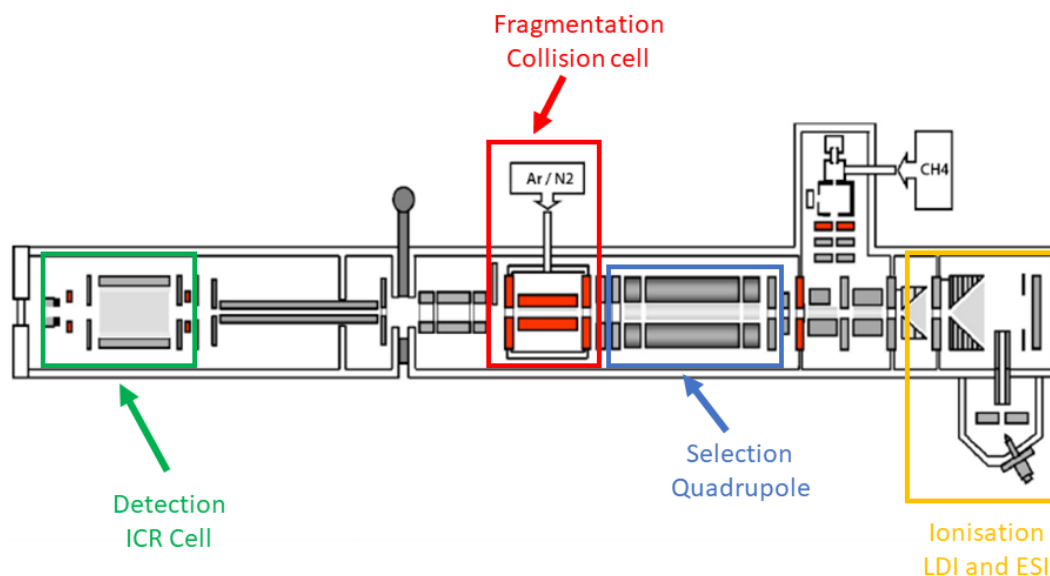


Figure S95 : Scheme of the instrument used for the tandem experiments

Figure S96a, Figure S96b and Figure S96c present the fragmentation obtained at respectively 0 eV, 10 eV and 20 eV using such visualization. At these collision energies, the cartographies look roughly similar except a shift in the DBE and number of carbons of the most intense species, those being represented with a red square on **Figure S96**. At 0 eV, the square is located between DBE 6 - 15 and #C 7 - 18. At 20 eV, the square is now located between DBE 5- 11, and #C 6 - 14. The area given by the square also becomes smaller as the energy increases. A loss of DBE and carbon is observed meaning that functional groups containing double bond are broken for voltages lower than 20 eV.

Figure S96d, Figure S96e and Figure S96f present the fragmentation of the insoluble fraction at 40 eV, 60 eV and 80 eV. At 40 eV, the global shape is thinner than at 20 eV.

The species with DBE smaller than the aromatic red line are no more observable. The area of the red square continues to decrease, passing from DBE 5 – 12 and #C 6 – 14 at 30 eV to DBE 7 – 11 and #C 8 – 11 at 60 eV. At 80 eV, the same area as 60 eV is observed. By comparing the 0 eV and the 80 eV, a loss of DBE and #C is observed, passing from DBE 6 – 15 to DBE 7 – 11 and #C 7 – 18 to #C 8 – 11. We conclude that some fragmentation occurs, definitely with a loss of functions containing DBE and carbons. However, regarding these results, it is not excluded at this point that the loss of species is induced by the scattering in the collision cell. This phenomenon occurs at high acceleration energy when an ion collides with an atom of neutral gas and is deviated without being fragmented. It results in a loss of the ion without the formation of any fragment. In order to retrieve more information, nominal mass fragmentation is performed in the next section.

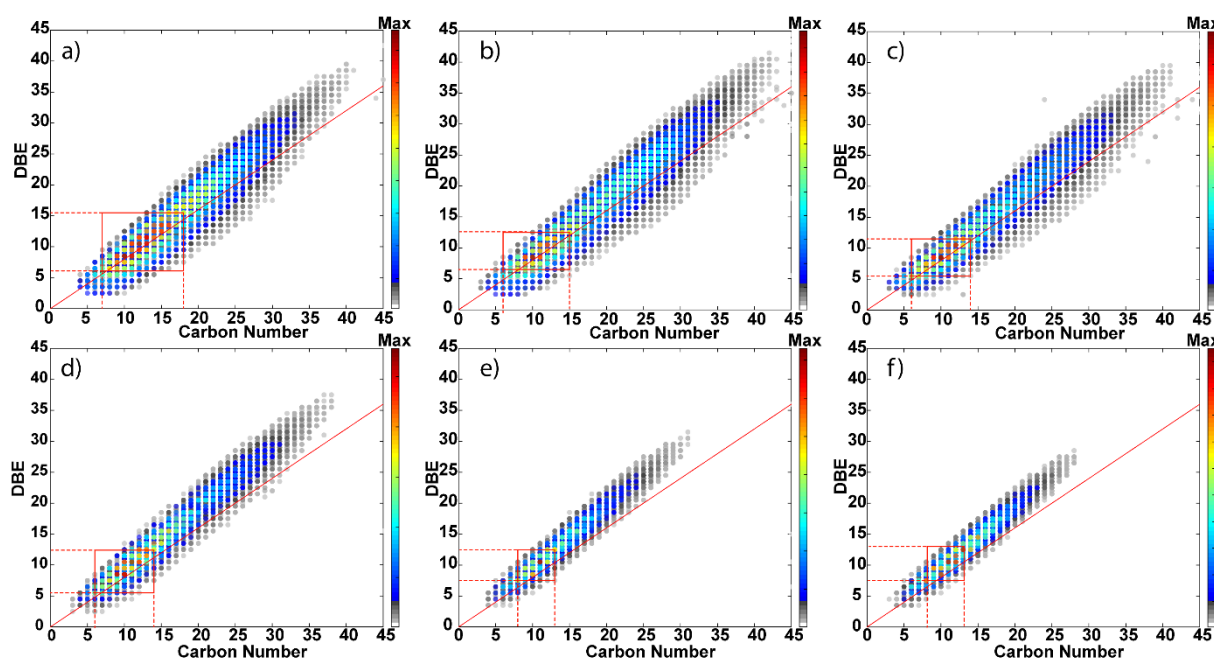


Figure S96 :Broadband fragmentation of the Insoluble fraction displayed by DBE vs #C maps at a) 0 eV, b) 20 eV, c) 30 eV, d) 40 eV, e) 50 eV and f) 60 eV.

This section targets the fragmentation of the second fraction recovered from the bulk Tholins sample: the soluble fraction in methanol. At first, broadband CID has been performed on the sample and results are reported on **Figure S97**. By comparing the 0 eV maps and the results obtained for the 10 eV, we can see major peaks moving from DBE 3 – 11 to DBE 3 – 8 and the carbon number from 5 – 20 to 5 – 17. A loss of DBE and carbon is already observed even at this low energy. Between the 20 eV and the 60 eV energy, the value of DBE stays the same with a value between DBE 3 – 8. However, the #C decreases with the increase of the collision energy. Here, a large loss of DBE is observed at low energy in the first step. Then, the amount of carbons decreases with the increase of the collision energy.

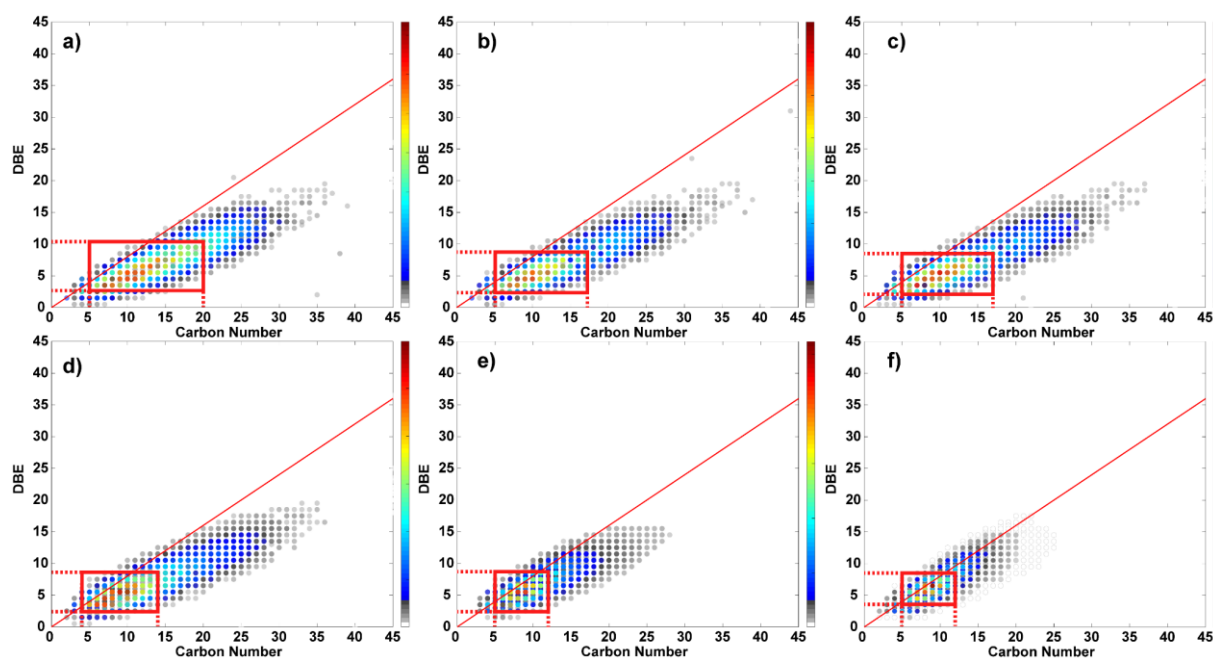


Figure S98 : Broadband fragmentation of the soluble fraction displayed by DBE vs #C maps at a) 0 eV, b) 10 eV, c) 20 eV, d) 30 eV, e) 40 eV and f) 60 eV.

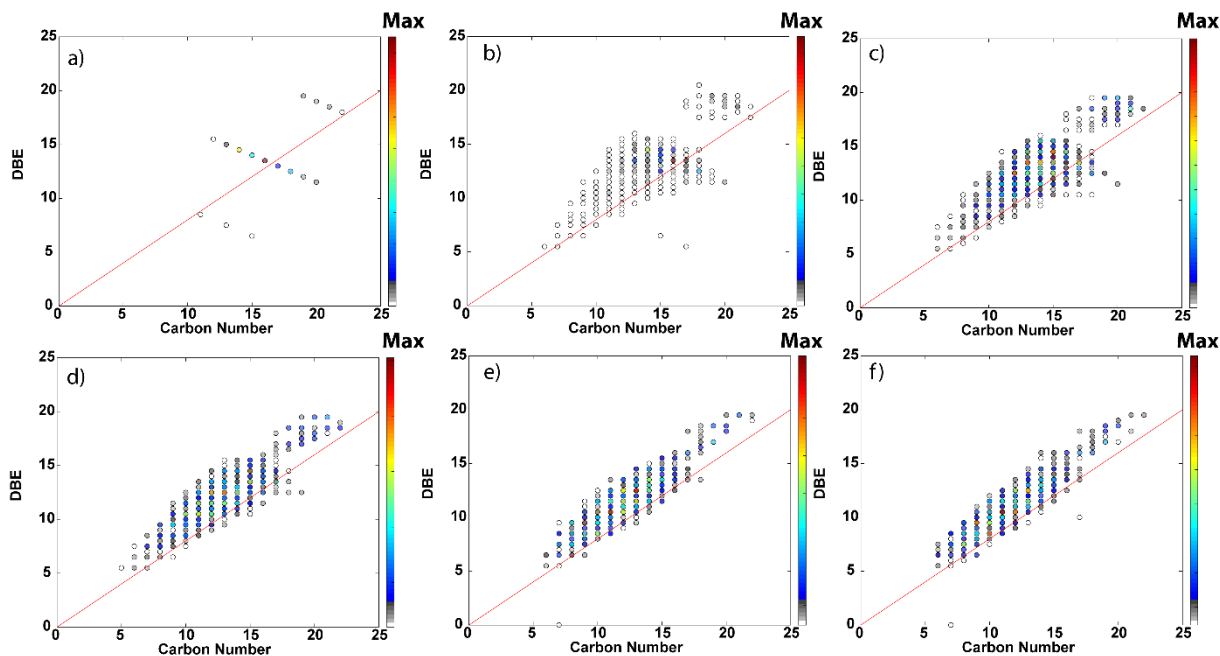


Figure S99 : Nominal mass fragmentation of the m/z 404 insoluble displayed by DBE vs #C maps at a) 0 eV, b) 20 eV, c) 30 eV, d) 40 eV, e) 50 eV and f) 60 eV.

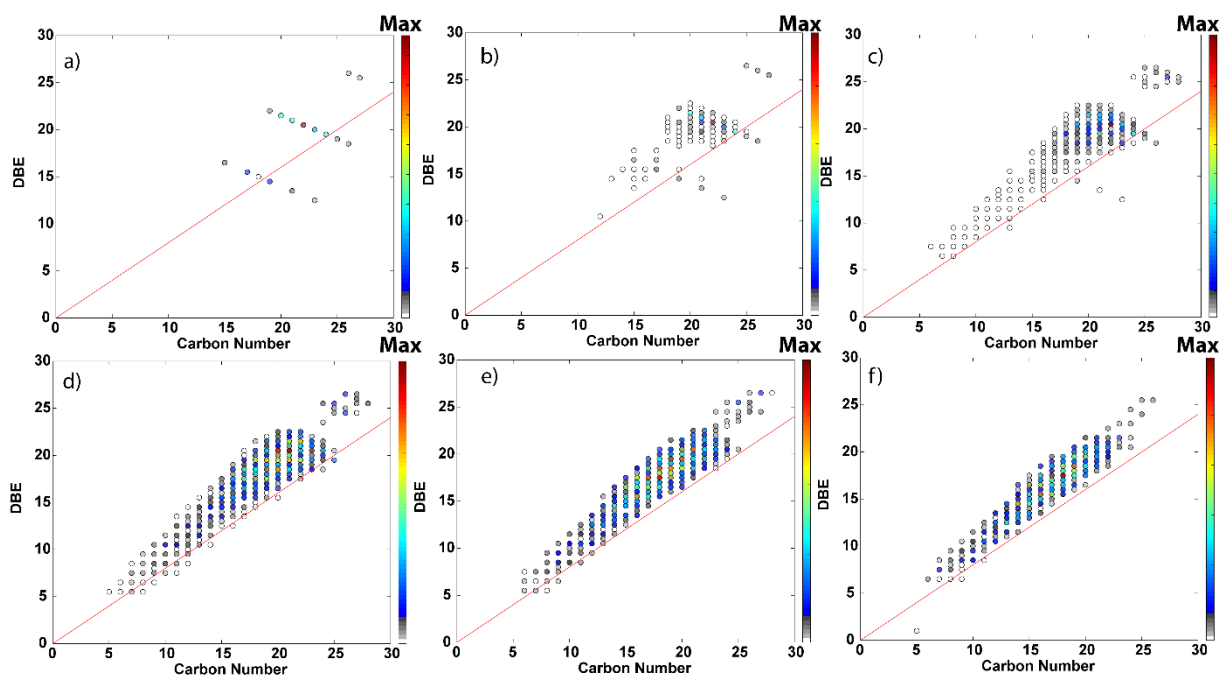


Figure S100 : Nominal mass fragmentation of the m/z 404 soluble displayed by DBE vs #C maps at a) 0 eV, b) 20 eV, c) 30 eV, d) 40 eV, e) 50 eV and f) 60 eV.

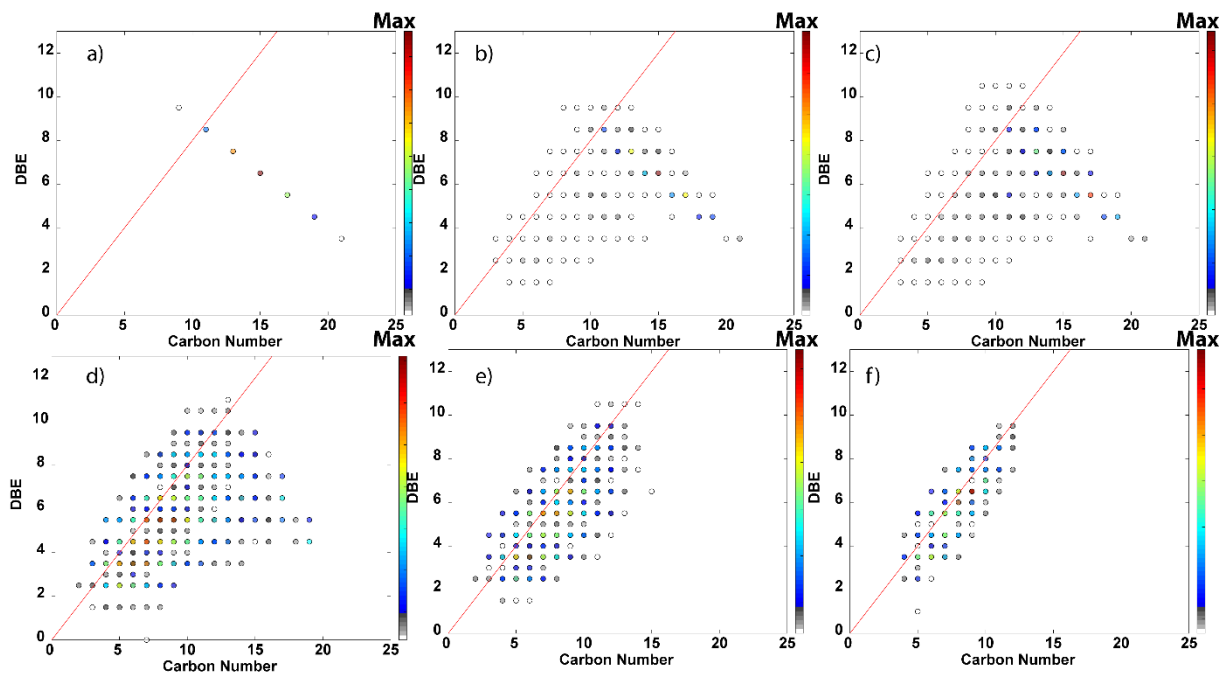


Figure S101 : Nominal mass fragmentation of the m/z 304 Soluble displayed by DBE vs #C maps at (a) 0 eV, (b) 5 eV, (c) 10 eV, (d) 30 eV, (e) 40 eV, (f) 60 eV.

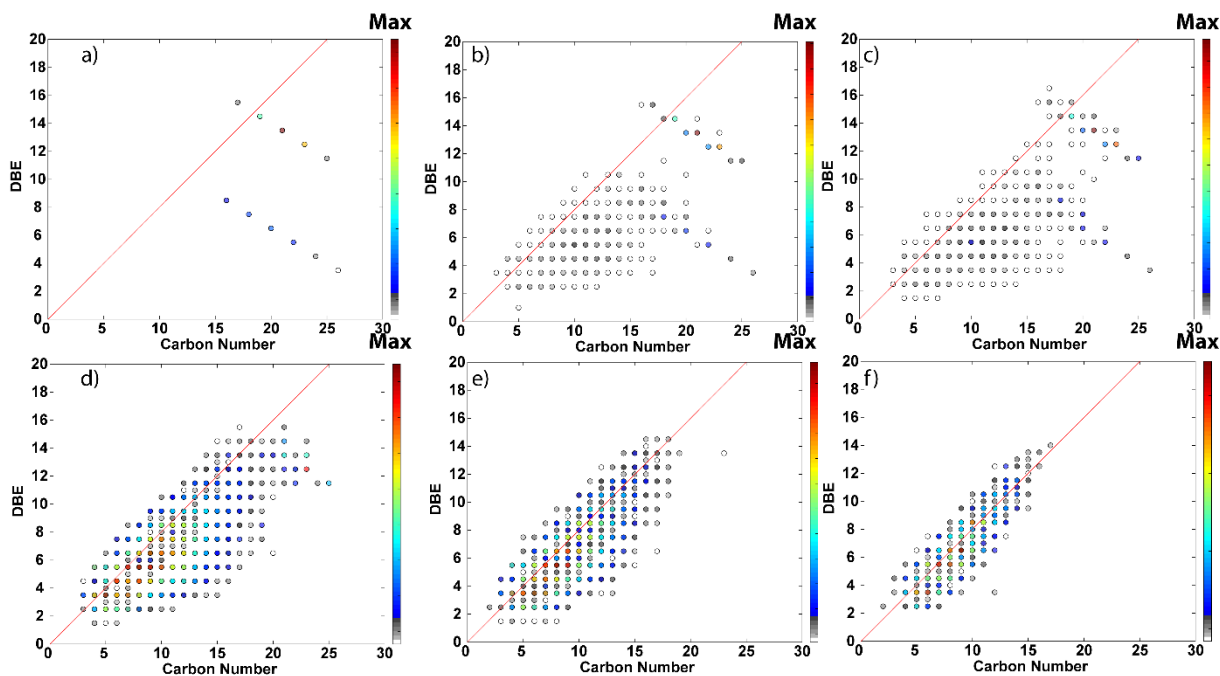


Figure S102 : Nominal mass fragmentation of the m/z 304 insoluble displayed by DBE vs #C maps at (a) 0 eV, (b) 5 eV, (c) 10 eV, (d) 30 eV, (e) 40 eV, (f) 60 eV.

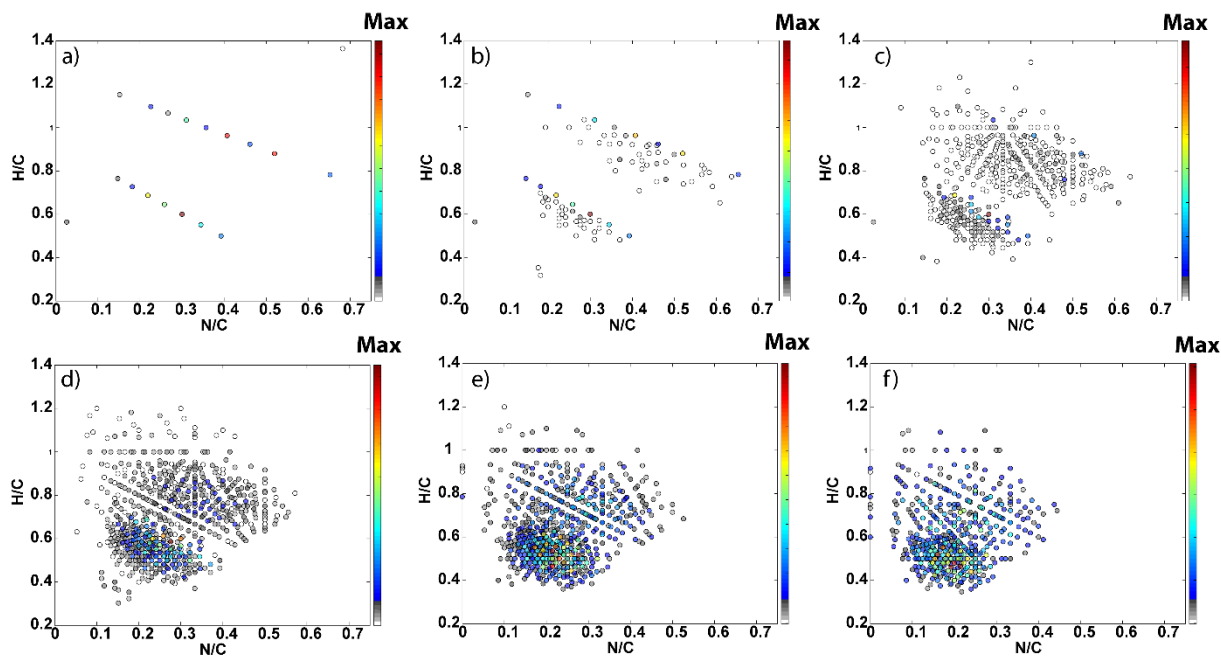


Figure S103 : Van Krevelen diagrams obtained from the MS/MS spectra of m/z 504 recorded at a collision energy of a) 0 eV, b) 10 eV, c) 20 eV, d) 30 eV e) 40 eV, f) 60 eV.

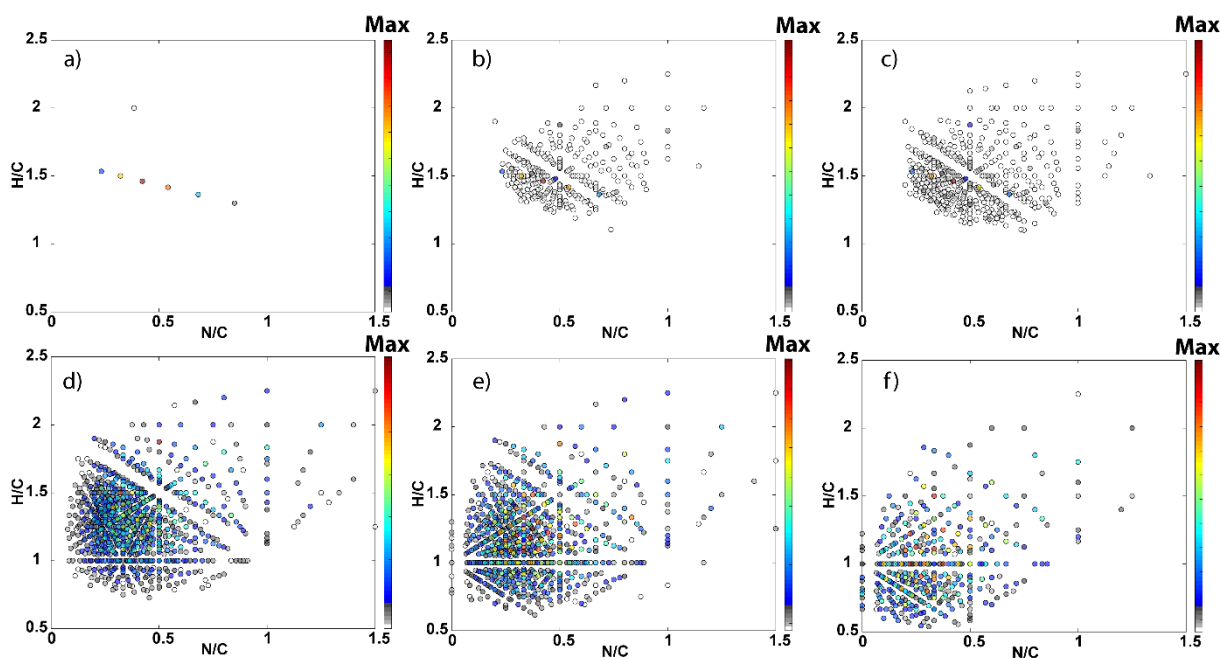


Figure S104 : Van Krevelen diagrams obtained from the MS/MS spectra of m/z 504 recorded at a collision energy of a) 0 eV, b) 5 eV, c) 10 eV, d) 30 eV e) 40 eV, f) 60 eV.

Chapitre 5, Article 6 : Première élucidation structurale par mobilité ionique de la matière organique soluble composant les analogues d'aérosols de Titan

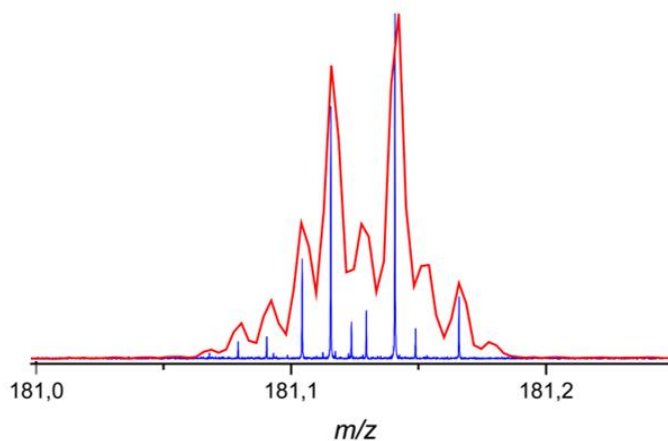


Figure S105 : Comparison between Synapt G2 resolving power and FTICR at m/z 181. All species are resolved with both analyser.

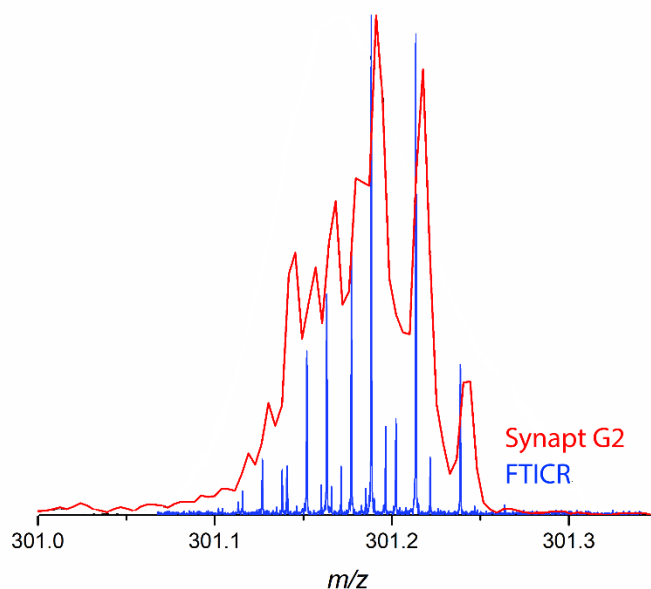


Figure S106 : Comparison between Synapt G2 resolving power and FTICR at m/z 301. Above m/z 250, Tholins species are not resolved with the Synapt G2 analyser.

Table S16 : TAA calibrant CCS values. Drift time was measured for each ion and added in the table.

	CCS _{He} (Å ²)	M	charge	t _D (ms)	Ω'
Methyl	48.5	74.0970	1	5.18	94.51
Ethyl	65.9	130.1596	1	7.43	129.86
Propyl	88.9	186.2222	1	10.65	175.98
Butyl	111.2	242.2848	1	14.4	220.66

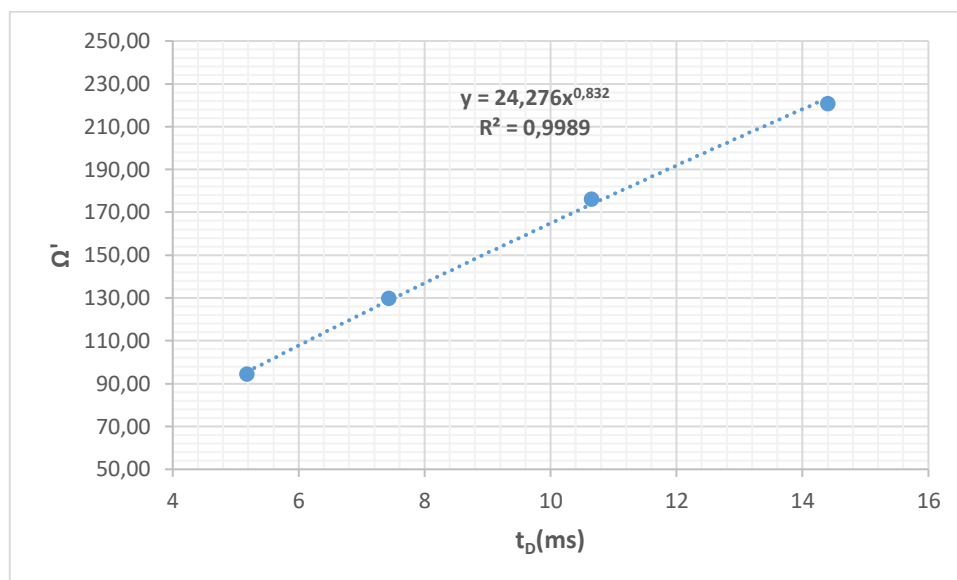


Figure S107 : Calibration curve for the tetraalkylammonium salts in helium with a power fitting.

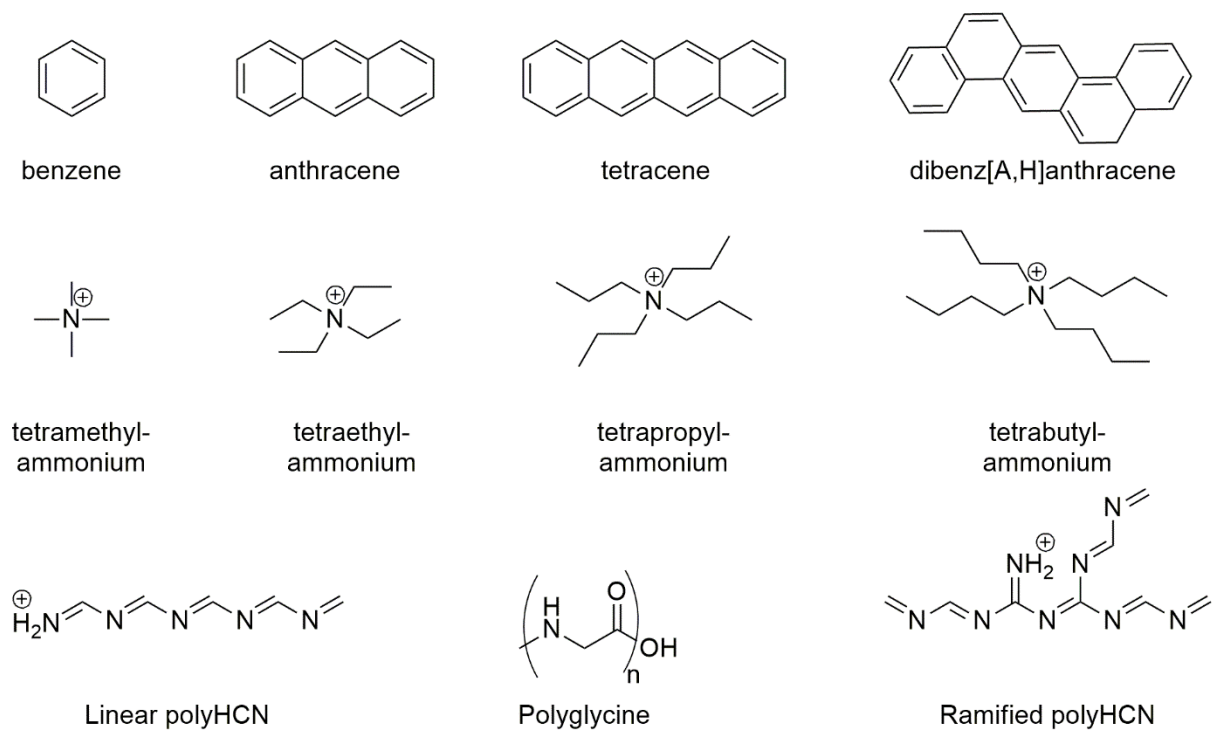


Figure S108 : 2D structures of compounds used for the comparison with Tholins

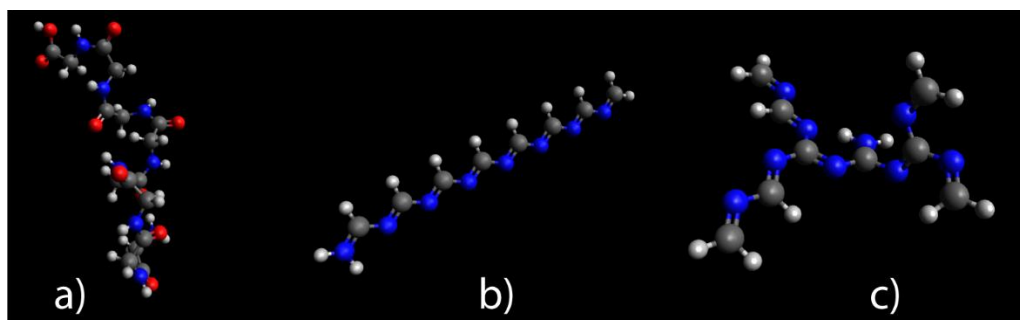


Figure S109 : 3D structures of a) polyglycine, b) Linear polyHCN, c) branched polyHCN

Table S17 : Validation of calibration parameters

Tetraalkylammonium salts (CCS calibrant)			
	Experimental CCS (Å²)		Calculated CCS (Å²)
	Literature	This work	
Methyl	49 ^a	49	48
Ethyl	66 ^a	66	66
Propyl	89 ^a	89	90
Butyl	111 ^a	111	114
Polycyclic Aromatic Hydrocarbons			
Benzene	/	/	45
Naphtalene	59 ^a	/	59
Antracene	72 ^a	/	73
dibenz[A,H]anthracene	98 ^c	/	100
Polyglycines			
Glc₂	62 ^b	64	60
Glc₃	76 ^b	76	78
Glc₄	86 ^b	87	88
Glc₅	97 ^b	/	101
Drug-like compounds			
N-Ethylaniline	63 ^a	63	62
Acetaminophen	67 ^a	69	64
Alprenolol	97 ^a	98	98
Ondansetron	106 ^a	109	108

a : Campuzano et al. Anal. Chem. 2012,

b: Wyttenbach et al. J. Am. Chem. Soc. 1998

c: Dongwan et al. Bull. Kor. Soc. 2018

Chapitre 5, Article 7 : Etude structurale d'analogues d'aérosols de Titan par « Trapped Ion mobility » couplé avec un « Fourier Transform Ion Cyclotron Resonance »

Experimental details

A) Sample generation

Tholins were produced according to the PAMPRE experiment following the same procedure, in detail given in previous publications [1, 2]. Briefly, in a stainless steel cylindrical reactor, a capacity coupled plasma discharge is generated by an RF 13.56 MHz frequency generator. A nitrogen-based gas mixture containing 5 v-% of methane is injected in the chamber as a continuous flow through the polarized electrodes. A primary vacuum pump ensures a homogeneous distribution of the gases. The plasma discharge is maintained at a pressure of 0.9 ± 0.1 mbar and at room temperature (20 °C). Finally, a brown powder, the tholin sample material, is recovered after a few hours of treatment.

B) Sample preparation

For electrospray analysis in positive ion mode, 4 mg of the tholin sample was dissolved in 1mL of methanol. The mixture was vigorously stirred for three minutes to dissolve the maximum proportion of the solid sample material. Subsequently, the brown dispersion was filtered using a 0.2 μ PTFE membrane filter giving the soluble fraction as the eluate. Other studies on the same tholin material have shown, that the soluble fraction accounts for approximately 35 w-% of the global sample. Directly prior electrospray ionization mass spectrometric analysis, a water/methanol (50/50 v-%) mixture was prepared. A standard mixture consisting of 10 phosphazene derivatives (Agilent Tune Mix) spanning a broad mass range of 118-2712 m/z was used for collision-cross section calibration

C) Ion mobility and mass spectrometry

Molecular level analysis of the prepared tholin soluble fraction was performed by a trapped ion mobility spectrometry (TIMS) 12 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). Briefly, the prototype instrument (a modified SolariXR) was equipped with a commercial Bruker electrospray ionization source and the following settings were applied for the positive mode ionization: capillary voltage 5kV, drying gas temperature 180 °C, drying gas flow rate 4 L/min, nebulizer gas flow rate 1.4 bar, spray shield voltage 500 V, sample flow rate 120 µL/h. The trapped ion mobility cell replaced the inlet funnel and is basically of similar design to the commercial Bruker TIMS TOF system. Briefly, the TIMS ramp voltage is stepwise increased from -110 V with 1 V per frame (dwell 2 frames). 220 pulses per IMS scan were accumulated. Mass spectrometric detection of the mobility separated ion cloud was performed in the ParaCell[®]. Absorption mode spectra are recorded with a transient length of corresponding to 2 mega-word data size (full-sine apodization, one zero-fill) from m/z 123-3,000. Consequently, a resolving power of over 500,000 at m/z 400, and an overall analysis time of approximately 25 min is resulting.

D) Data analysis

External calibration of the mass spectra was performed using the exact masses of the phosphazene-containing standard mixture. Internal calibration was performed on an average spectrum generated over the complete analysis using different homolog series of CHN-class compounds. After calibration, each individual scan of the time-resolved data was peak picked (S/N 9) and exported with Bruker Data Analysis (5.0) visual basic scripts. The exported mass spectra were processed by self-written MATLAB routines. The extracted mobilograms are fitted to an asymmetric Gauss shape and peak parameter, e.g., abundance, position (CCS), peak width and skewness, are exported for each individually signal. Finally, elemental compositions were assigned with the following common boundaries for Tholins: $C_{1-50}H_{1-120}N_{1-15}O_{0-3}S_0$. A mass error of 1 ppm and only protonated (even electronic configuration) were considered. The mean assignment error was found to be below 100 ppb. Visualization of the data was done OriginPro (2016).

Table S18 : Detailed information on the constituents of the three different homologue series represented in Figure 1. CCS-values are taken from Mccllemmer database, Young et al. [3] and Borsdorf et al. [4] (*) or calculated by modified MOBCAL with new Lennart Jones parameter set taken from Campuzano et al. [5] (#).

Name	Formula	Series type	<i>m/z</i>	CCS [Å ²]
Benzene	C ₆ H ₆	PAH	128.05	59.2*
Naphthalene	C ₁₀ H ₈	PAH	177.08	73.2*
Anthracene	C ₁₄ H ₁₀	PAH	228.09	87.3*
Tetracene	C ₁₈ H ₁₂	PAH	279.10	101.6*
Pentacene	C ₂₂ H ₁₄	PAH	330.12	115.6*
Hexacene	C ₂₆ H ₁₆	PAH	381.14	129.7 [#]
Heptacene	C ₃₀ H ₁₈	PAH	432.15	143.7 [#]
Octacene	C ₃₄ H ₂₀	PAH	483.17	157.8 [#]
Nonacene	C ₃₈ H ₂₂	PAH	534.18	171.9 [#]
Tetramethylammonium	C ₄ H ₁₂ N	TAA	130.16	65.9*
Tetraethylammonium	C ₈ H ₂₀ N	TAA	186.22	88.9*
Tetrapropylammonium	C ₁₂ H ₂₈ N	TAA	242.28	111.2*
Tetrabutylammonium	C ₁₆ H ₃₆ N	TAA	298.35	133.5*
Tetrapentylammonium	C ₂₀ H ₄₄ N	TAA	354.41	154.9*
Tetrahexylammonium	C ₂₄ H ₅₂ N	TAA	410.47	174.5*
2 cycles	C ₈ H ₈ N ₅	Archipelago N-PAH	174.08	76.7 [#]
2 cycles ramified	C ₁₁ H ₁₃ N ₆	Archipelago N-PAH	229.12	95.1 [#]
3 cycles	C ₁₄ H ₁₇ N ₈	Archipelago N-PAH	297.15	114 [#]
3 cycles ramified	C ₁₇ H ₂₄ N ₉	Archipelago N-PAH	354.21	134 [#]
4 cycles	C ₂₀ H ₂₆ N ₁₁	Archipelago N-PAH	420.23	150 [#]
4 cycles ramified	C ₂₃ H ₃₃ N ₁₂	Archipelago N-PAH	477.29	168 [#]

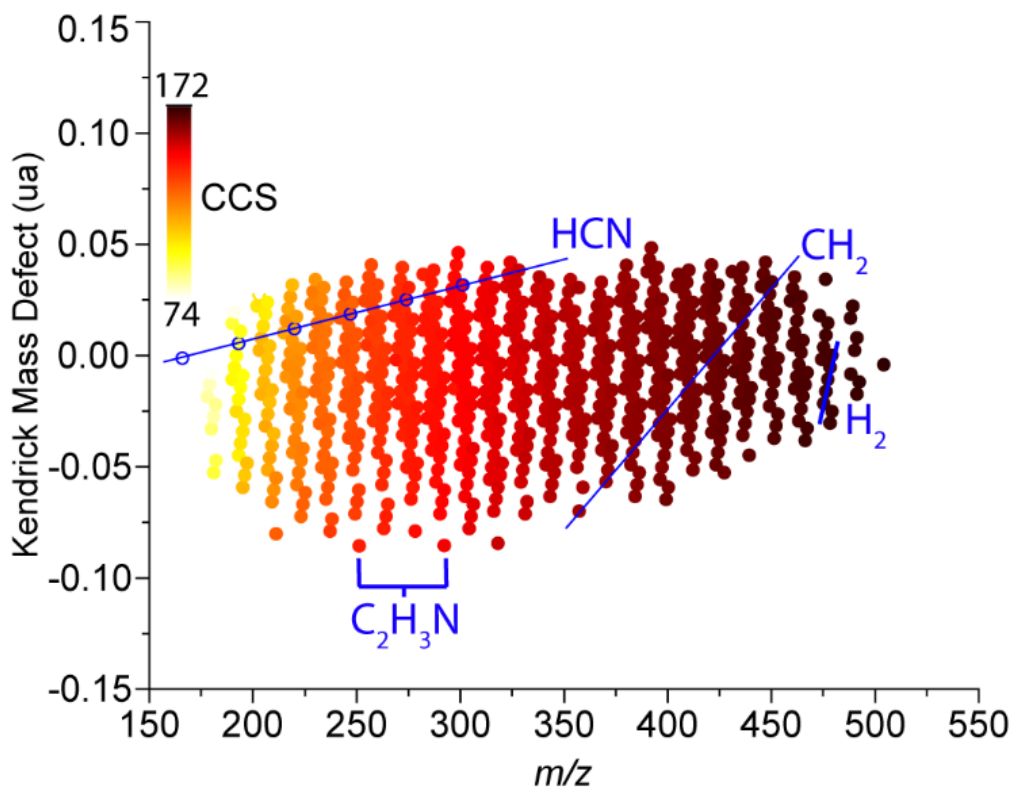


Figure S110 : Adapted Kendrick mass defect visualization normalized on C_2H_3N increment exposing these homologue rows on the horizontal lines. Further homologue rows, such as H_2 , CH_2 -, and HCN-series can be easily observed on diagonal lines.

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1. Szopa, C., Cernogora, G., Boufendi, L., Correia, J.J., Coll, P.: PAMPRE: A dusty plasma experiment for Titan's Tholins production and study. *Planetary and Space Science* (2006). doi: 10.1016/j.pss.2005.12.012
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3. Young, D., Douglas, K.M., Eiceman, G.A., Lake, D.A., Johnston, M.V.: Laser desorption-ionization of polycyclic aromatic hydrocarbons from glass surfaces with ion mobility spectrometry analysis. *Analytica chimica acta* (2002). doi: 10.1016/S0003-2670(01)01402-7
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Chapitre 5, Article 8 : Clichés de structures composant les analogues d'aérosols de Titan par microscope à force atomique

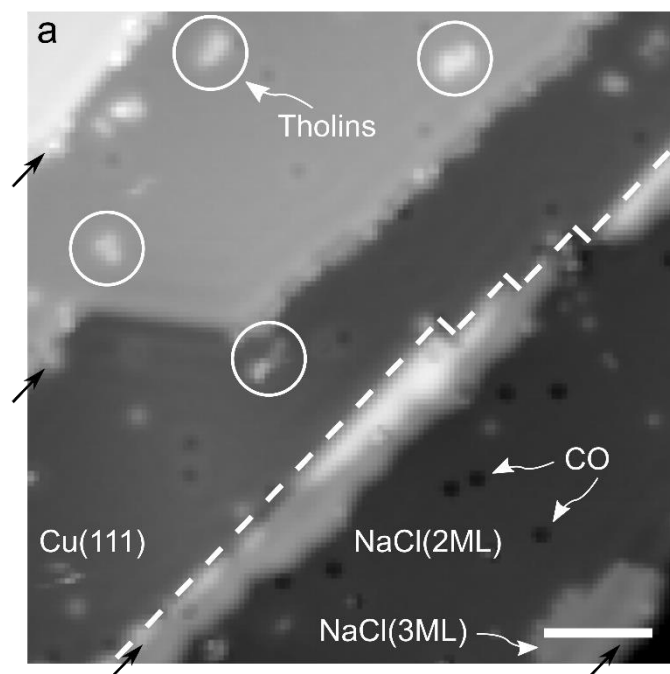


Figure S111 : Typical STM overview image of the NaCl(2ML)/Cu(111) substrate after deposition of Tholins via sublimation at low temperature (0.2 V, 0.5 pA). The white dashed line indicates the edge of a bilayer NaCl island on the right-hand side. A small island of trilayer NaCl, CO molecules and some Tholins (white circles) are marked as well. The black arrows indicate monatomic steps of the underlying Cu(111) surface. Scale bar is 5 nm.

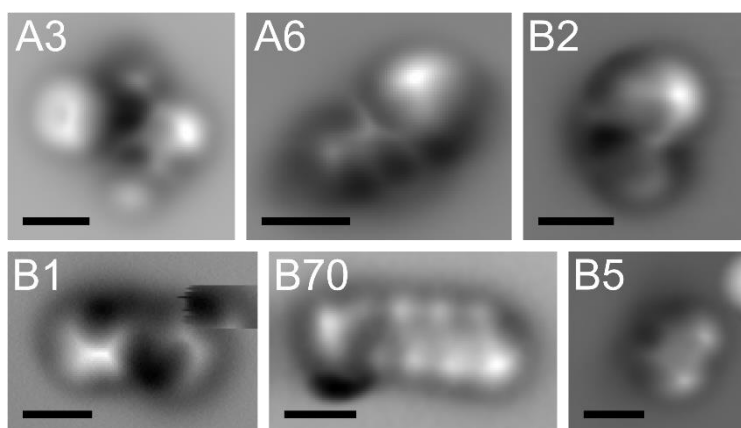


Figure S112 : High-resolution AFM images of additional molecules for which a chemical structure could be proposed. ‘A’ refers to molecules from the common fraction, while ‘B’ denotes the insoluble fraction. All scale bars are 5 Angstroem.

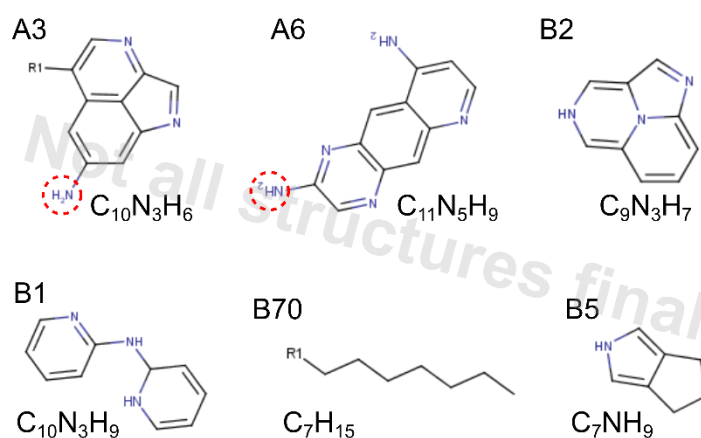


Figure S113 : Proposed structures for the molecules shown in Supporting Figure S2. Red dashed circles indicate moieties with high uncertainty in the assignment. ‘R’ denotes unidentified parts of a molecule and are neglected in the molecular formulas.

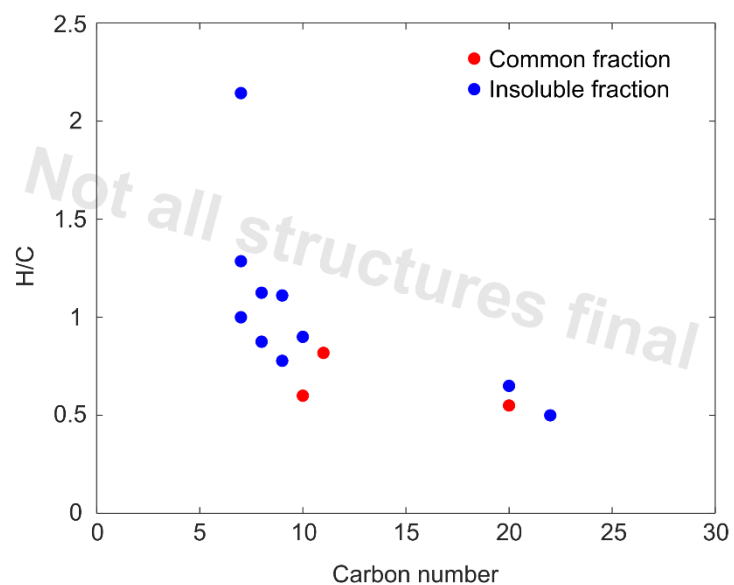


Figure S114 : Plot of H/C ratio over the number of carbon atoms for each molecular formula shown in Figure 3 and Supporting Figure S3. Unassigned parts of molecules denoted as 'R' in the proposed structures are neglected for the purpose of the plot.

Titre : Composition chimique du brouillard de Titan

Mots clés : Titan, aérosols, spectrométrie de masse, mobilité ionique, FTICR

Résumé : Bien que l'enjeu prébiotique soit important, l'étude des aérosols présents dans l'atmosphère de Titan, la plus grosse lune de Saturne, reste un challenge pour la communauté scientifique. En effet, les données récoltées par les sondes Voyager et Cassini-Huygens ont permis de récupérer les premières informations mais pas de percer les secrets de ce brouillard de particules. Le but de cette thèse est d'étudier la composition chimique de ces aérosols grâce à leur reproduction sur Terre. La première approche présentée est la caractérisation moléculaire par le biais de la spectrométrie de masse à ultra haute résolution (FTICR). Dans cette partie sera présenté la comparaison entre les fraction soluble et insoluble de ces échantillons et leur vieillissement en présence d'oxygène.

Ensuite, l'investigation de la croissance de ces aérosols est présentée par l'utilisation de spectrométrie de masse en tandem. La dernière partie de cette thèse présente l'étude de la structure tridimensionnelle. Pour cela, la mobilité ionique couplée à la spectrométrie de masse a été utilisée et sera reporté en deux partie, l'une en TWIMS-Synapt et l'autre en TIMS-FTICR. Enfin une étude en *Atomic Force Microscopie* sera présentée et permettra de montrer de vraies structures de molécules présentes dans les aérosols grâce au grossissement fournit par ce type de microscope.

Title : Chemical composition of the Titan's haze

Keywords : Titan, aerosols, mass spectrometry, ion mobility, FTICR

Abstract: Although the prebiotic challenge is important, the study of aerosols in the atmosphere of Titan, Saturn's largest moon, remains a challenge for the scientific community. Indeed, the data collected by the Voyager and Cassini-Huygens probes made it possible to retrieve the first information but not to unlock the secrets of this particle fog. The purpose of this thesis is to study the chemical composition of these aerosols through their reproduction on Earth. The first approach presented is molecular characterization using high resolution mass spectrometry. In this section, the comparison between the soluble and insoluble fraction of these samples and their ageing will be presented.

Then, the investigation of the growth of these aerosols is presented through the use of tandem mass spectrometry.

The last part of this thesis presents the study of the three-dimensional structure. For this purpose, ion mobility coupled with mass spectrometry has been used and will be reported in two parts, one in TWIMS-Synapt and the other in TIMS-FTICR. Finally, a study in Atomic Force Microscopy will be presented and will make it possible to show real structures of molecules present in aerosols thanks to the magnification provided by this type of microscope.