NOVEL APPLICATIONS OF MASS SPECTROMETRY ON SYNTHETIC POLYMERIC MATERIALS

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ABSTRACT

This dissertation focuses on the application of new mass spectrometry approaches for the characterization of different types of synthetic materials. Combination of the classical and innovative methods, such as electron transfer dissociation (ETD) and ion mobility mass spectrometry (IM-MS) enabled the conclusive and unambiguous determination of macromolecular structures, end groups and architectures, as well as stoichiometry of high molecular weight complexes whose detection is often obscured by charge overlapping, viz. [M+Na]$^+$ and [2M+2Na]$^{2+}$.

Chapter III concerns the investigation of phosphazenes, a broad class of important inorganic compounds. For this goal, different mass spectrometry techniques were employed to better understand the reaction products but also their particular chemistry under mass spectrometry conditions. The tadpole architecture was detected for the first time among the products of the reaction between NH$_4$Cl and PCl$_5$ by utilizing IM-MS and tandem mass spectrometry (MS$^2$). The reaction [PCl$_2$N]$_3$ with MX$_n$ to form [PCl$_2$N]$_3$HMX$_{n+1}$ superacids was confirmed by detecting both the protonated weak base [PCl$_2$N]$_3$ and the corresponding labile anion species [MX$_{n+1}$].

Chapter IV evaluates of ETD, a new MS$^2$ technique, for the structural analysis of polymers, specifically polyester homo- and copolymers, and also a comparison between this new method and the classical collisionally activated dissociation (CAD). Advantages
of ETD over CAD, include less congested MS\(^2\) spectra due to site specific dissociations, fragment ions in a lower charge state than the precursor ion and absence the of consecutive dissociations of the first generation of fragment ions, which lead to more specific end group information and more readily interpretable spectra.

The last chapter covers an investigation, by electrospray ionization mass spectrometry (ESI-MS), of the noncovalent interactions between differently substitued POSS molecules and sorbitol-type nucleating agents for developing nanocomposite materials with isotactic polypropylene (iPP). The complexes detected and their stoichiometries were confirmed not only by mass measurements but also by their dissociation (MS\(^2\)) and by examination of their charge states and size by IM-MS. These studies confirmed the formation of high order heterocomplexes between POSS particles carrying both silanol and phenyl groups and sorbitol molecules substituted by phenyl groups, underscoring that both hydrogen bonding and \(\pi-\pi\) interactions are necessary to form POSS-sorbitol self-assemblies. Such self-assembled structures can be evenly blended with iPP to yield hybrid materials with superior physical and mechanical properties.
Results from this dissertation have been reported in the following publications:


DEDICATION

To my parents Maria and Salvatore, my sisters Francesca and Antonella and my grandmother Francesca. Your love and encouragement made this possible.
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CHAPTER I

INTRODUCTION

The application of mass spectrometry (MS) to the characterization not only of biological samples but also of synthetic materials has been revolutionized in the last decades by the introduction and improvement of new soft ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). The capability of ionizing intact high molecular weight molecules with less or no fragmentation can provide the exact molecular weight of each component in mixtures. The dispersion of the resulting ions according to their $m/z$ values (mass-to-charge ratios) after ionization allows the detection of slight differences from the expected products, such as mutation or post-translational modifications in the case of the analysis of biomolecules or undesired by-products in the case of characterization of synthetic materials. As the need for producing new and innovative materials with enhanced properties and low cost production increased, different analytical techniques had to move forward and more precise and fast approaches were developed in order to provide synthetic researchers with information concerning the quality of final products, purity, degradation or presence of unwanted by-products.
In this dissertation, innovative approaches for the investigation of synthetic materials have been explored and improved to thereby extrapolate as much information as possible from materials analyses.

Chapter III focuses on the characterization of different phosphazene compounds by different mass spectrometry techniques. The term phosphazene identifies a broad class of inorganic compounds. These materials are characterized by an alternating phosphorus-nitrogen bond sequence, while at the phosphorus atom two other side groups are attached. Along with another important class of inorganic compounds, “siloxanes”, phosphazenes have occupied a central role in the inorganic synthetic field during the last decades. The first time that these compounds came to the public eye was in 1834 with the work of Liebig, Wohler\(^1\) and Rose,\(^2\) who discovered that phosphorus pentachloride reacts with ammonia to give a crystalline product, which can be distilled without decomposition. However, it took a couple of decades with the study of Gerhardt and Laurent\(^3\) first, and Gladstone and Holmes\(^4\) and Wichelhaus\(^5\) later, before both the empirical formula and the structure of this compound were assigned. It was just in the late nineteenth century that Stokes not only proposed a cyclic structure for the compound (PCl\(_2\)N)\(_3\), assuming the presence of additional, higher cyclic homologues up to the seven membered ring, but he also realized that when heated at high temperature these compounds give rise to elastomeric materials.\(^6\)

The substitution of the chlorine atoms on poly(dichlorophosphazene) can provide an array of different polyphosphazene compounds. Different nucleophiles have been considered and tested for this purpose, such as halogen, alkoxy or aryloxy groups, primary and secondary amino units, metallocenes, borazines, carboranes, transition metal
carbonyls, amino acids, steroids, and carbohydrates. The physical and chemical properties of phophazenes depend on and can be fine-tuned by their side chain groups. Just by changing these groups, phosphazens can be used as elastomers, films, coatings, fire retardants, fibers for optical, electro-optical and biomedical materials, solid battery electrolytes, fuel cell components and a variety of different membranes.

The inorganic backbone by itself has also particular properties that are difficult or sometimes impossible to find in any traditional organic polymer. Properties like fire-resistance, high flexibility, near-ultraviolet transparency, gamma-radiation stability and biomedical compatibility. For biomedical applications, phosphazenes can be designed to hydrolyze under physiological conditions and in aqueous environment to liberate biocompatible side groups and to convert the backbone to a pH-buffered mixture of phosphate and ammonia, where the resulting phosphate can be metabolized, and the ammonia, which is innocuous at low concentrations, can be easily excreted.

Although these compounds appear to have extraordinary properties, their industrial production is relatively expensive due to the low yield of the synthesis of the starting polymer material, polydichlorophosphazene, and its considerable degradation upon storage.

Very few investigations by mass spectrometry have been reported up to date for the characterization of these compounds, either the small cyclic species or the long chain polymers, in particular for the chloro-substituted ones, which are the starting materials for the different classes of functionalized phosphazenes. The major reason has been their intrinsic physical and chemical properties, which make chlorophoshazenes very reactive and unstable at the working condition of most mass spectrometers. Their high tendency to
hydrolyze in presence of water molecules and their fast degradation when exposed to air pose challenges to their analysis by the new soft mass spectrometry developed during the last decades.

One of the first phosphazene studies by mass spectrometry was the analysis of the phosphonitrilic diphenoxide trimer, carried out by Allcock and Best. The instrument used at that time was an electron impact (EI) mass spectrometer. It was during the same period that Coxon, Palmer and Sowerby investigated bromocyclophosphazene, by EI mass spectrometry with the aim to broaden the MS database of heterocyclic systems that do not contain carbon atoms. Following these studies, additional electron impact analyses on other homologous halogenated compounds were carried out by Brion and Paddock on phosphonitrilic fluorides, and by Schmulbach, Cook and Miller on the hexachlorocyclotriphosphazene and octachlorocyclotetraphosphazene.

In chapter IV, a different tandem mass spectrometry techniques, electron transfer dissociation (ETD), is considered and tested for the analysis of synthetic materials and the results are compared with those obtained by the traditional approach, viz. collisionally activated dissociation (CAD). The development of instruments with the capability of performing tandem mass spectrometry experiments has allowed to obtain more detailed information about the polymer architectures and insight on how a polymer’s constituents are connected to each other. ETD employs ion-ion reactions as ion excitation method, while the most common MS technique used to perform tandem MS analysis of polymers is CAD, where ions of a specific m/z value are isolated first and then fragmented by collisions with an inert gas (e.g., Ar or He). ETD was initially developed for the characterization of proteins and peptides. It was observed that this alternative
fragmentation technique could generate fragments from the cleavage of the N-C\textsubscript{a} bond of the amide functionality, giving rise to the c and z fragment ion series\textsuperscript{15,16} while the classical CAD on the same analytes provides mostly the y and b fragment ion series, arising from the CO-N bond cleavage of the amide group.

The synthetic materials that have been considered for ETD investigation belong to the family of polyesters. This class of polymers is widely used in industry because of their different applications. They are employed in the production of plastics, composite materials, coatings and fibers; because of their biodegradable properties, recently they have also been used for biomedical applications\textsuperscript{17}. Their synthesis is straightforward; they are synthesized by condensation reactions between diols and dicarboxylic acids via step growth polymerization. The common acids and diols involved in the generation of this class of materials are ethylene glycol, neopentyl glycol and trimethylol propane for the diols, and phtalic acid isomers, adipic acid, sebacic acid and lactic acid for the dicarboxylic acids. Diverse architectures and end groups arise from these polymerization reactions\textsuperscript{17}. Their characterization can be performed by gel permeation chromatography (GPC), which is able to provide both the average molecular weights and polydispersities, and by NMR. The problem related to the analysis by NMR is that this technique can reveal information about the average microstructure of the materials but cannot provide information on each single component in molecular distributions\textsuperscript{18}. Because of its dispersive character, mass spectrometry can separate based on $m/z$ value and detect each oligomer resulting from different architectures and end groups by single-stage mass spectrometry experiments (MS). Structural composition or oligomeric sequence can then
be derived by performing tandem mass spectrometry (MS/MS) experiments as will be illustrated in this dissertation.

Ultimately, chapter V focuses on the investigation of non-covalent interactions between polyhedral oligomeric silsesquioxane (POSS) and sorbitol molecules by ESI mass spectrometry and ion mobility mass spectrometry. These compounds are used in engineering applications aiming at the development of polymer composite materials. Non-covalent interactions are very common and they occur in our everyday life constantly. Weak intermolecular forces organize cell membranes, help in the precise information read-out during DNA replication, control the secondary and tertiary structures of proteins, and mediate the formation of multiprotein complexes in biochemistry.\textsuperscript{19} Displays based on liquid crystalline materials or the semicelles formed by detergents used to solubilize greasy dirt in water and help to wash it away are also examples of non-covalent interaction that happen around us.\textsuperscript{20} Because these interactions are relatively weak, they provide reversibility and capability to adapt to changes in the environment, providing highly dynamic systems compared to purely covalent bonds where, under the same conditions, the system is more static.\textsuperscript{20}

In recent years POSS compounds have received considerable attention as hybrid organic-inorganic filler materials for the development of polymer nanocomposites.\textsuperscript{21,22,23} In comparison with more conventional inorganic fillers, such as talc, layered silicate clay, and silica, POSS molecules are more promising due to the advantages of monodispersed size ranging between 1 and 3 nm, isotropic molecular shape, low density, high thermal stability, and an array of functionalities. POSS molecules can be functionalized to aid
incorporation in several polymer systems via copolymerization and grafting reactions and to render them as integral parts of polymer chains.

Melt blending of nonreactive POSS molecules with the host polymers presents a much simpler alternative to grafting and copolymerization reactions for the preparation of POSS-polymer nanocomposites. Nonreactive POSS molecules are easier to synthesize and can be mixed with the polymer using an array of extruders and internal mixers. However, good dispersion and the desired improvements in mechanical properties strongly depend on POSS-polymer compatibility. Thus, the polar nature of hydroxyl-functionalized POSS molecules (silanol-POSS) deters their dispersion in isotactic polypropylene (iPP) melt.\textsuperscript{24,25}

Previous work reported that dispersion of nonreactive trisilanol phenyl-POSS (tri-POSS) in iPP can be improved in the presence of dibenzylidene sorbitol (DBS).\textsuperscript{26,27,28} The improvement was attributed to the development of non-covalent interactions between tri-POSS and DBS, which facilitated the dispersion of the former component in nonpolar iPP, because the phenyl groups in DBS can interact favorably with iPP. Indirect evidence for the occurrence interactions between tri-POSS and DBS molecules was gleaned from rheological data, and such interactions were attributed to hydrogen bonding. However, it has remained unclear whether the number of Si-OH groups or the nature of the organic substituents in silanol-POSS molecules has any impact on such interactions. It is also not known if POSS-sorbitol interactions lead to molecular complex formation. These issues are addressed here in detail.
CHAPTER II

MASS SPECTROMETRY ON THE ANALYSIS OF SYNTHETIC POLYMER

2.1 Introduction

The development of soft ionization techniques, in particular electrospray ionization (ESI)\textsuperscript{29} and matrix-assisted laser desorption ionization (MALDI),\textsuperscript{30,31,32} set a major cornerstone in mass spectrometry history. These methods enabled the formation of gas-phase ions from nonvolatile molecules, thereby radically expanding mass spectrometry applications to different and diverse fields. Compounds that had been characterized by other platforms started becoming analyzable and more precisely identifiable by mass spectrometry (MS). The potential of this analytical technique was rapidly recognized by polymer scientists, conscious of the great contribution that it can offer to synthetic polymer and materials studies.\textsuperscript{33,34} The growing need for new, inexpensive, and green synthetic routes to both widely used and newly designed polymers has made MS the tool of choice compared to other alternative and less sensitive or selective techniques, such as IR and NMR.\textsuperscript{35,36} MS can rapidly provide the molecular weight distribution (MWD) of the polymer, (co)monomer compositions, and end-group identification. The unique dispersive character of MS allows for the detection of a single component within convoluted polymer distributions arising from different end groups, varied structural arrangements, unpredicted byproducts, or degradation products.\textsuperscript{35}
Because of these benefits, MS applications to synthetic polymers have increased enormously.\(^\text{36,37,38,39,40,41,42}\)

Single-stage mass spectrometry, also referred to as one-dimensional MS, provides the mass-to-charge ratio \((m/z)\) of each polymer component, from which the corresponding mass can be obtained depending on the size of the polymer and the mass accuracy of the instrumentation used. For new or known polymers prepared via established synthetic procedures, the mass information is often sufficient to derive the elemental composition of the product’s constituents and predict their structure. If new synthetic concepts are evaluated or the origin of the sample is unknown, mass data alone may not permit unequivocal compositional or structural assignments. In such cases, two-dimensional or tandem mass spectrometry \((\text{MS/MS or MS}^2)\) offers a means to collect additional analytical information, so that the problem can be solved. In \(\text{MS}^2\), ions of the same \(m/z\) ratio corresponding to a specific \(n\)-mer are first isolated and then energetically activated in order to undergo structurally diagnostic fragmentations. Detection and interpretation of the resulting fragment ions allows one to reconstruct the primary structure (connectivity) of the selected \(n\)-mer. The successive isolation and fragmentation events can take place either in physically separated regions of the mass spectrometer \((\text{MS}^2\ \text{in space})\) or in the same location at different times \((\text{MS}^2\ \text{in time})\), depending on the type of mass spectrometer available. \(\text{MS}^2\ \text{in space}\) is performed with beam instruments, while the ions travel from the source to the detector; inversely, \(\text{MS}^2\ \text{in time}\) is performed in ion traps (ITs), while the ions are stored in the trapping region. The \(\text{MS}^2\ \text{in-space}\) experiment requires the coupling of at least two mass analyzers having a collision cell or different excitation section in between. The first analyzer is set to transmit only ions of a specific
After exiting this analyzer, the selected ions (precursor or parent ions) enter the excitation section, where their internal energy is perturbed by collisions with an inert gas, or other reactive species (for example, ions of opposite charge), by collisions with a surface, or by photons. This process increases the ions’ internal energy, so that they undergo unimolecular fragmentations. Consecutively, the newly formed fragments and any residual precursor ions travel to the second mass analyzer, which deconvolutes and transmits them to the detector according to their m/z ratio. Instruments performing MS^2 in space may contain quadrupole (Q), time-of-flight (ToF), or IT mass analyzers. Different instrument manufacturers have assembled diverse combinations of these analyzers to achieve enhanced transmission, sensitivity, and resolution of both the selected ions and the fragments generated from them; the most common arrangements have been QqQ, Q-ToF, ToF/ToF, and IT/ToF (q designates a quadrupole used as collision cell).

In the case of mass spectrometers containing trapping analyzers, all ions generated in the source are injected and stored inside the trapping device by electric or magnetic fields. The isolation of a specific precursor ion, its fragmentation, and the dispersion of the resulting fragment ions are executed in time by varying one of these two fields. Examples of such analyzers are the quadrupole (3D) IT, the linear (2D) IT, the orbitrap, and the ion cyclotron resonance (ICR) trap. With quadrupole or linear ITs, the trapped ions are ejected sequentially by m/z value for detection. In contrast, ions stored in an orbitrap or ICR trap are detected nondestructively, based on the image currents induced by the orbiting ions on specific trapping elements; the currents of all ions can be detected simultaneously and converted to mass spectra by Fourier transformation (FT). An advantage of the trapping instruments, compared to the beam instruments, is that the
isolation/fragmentation processes can be iterated on the fragment ions in triple-stage MS experiments (MS/MS/MS or MS³). Theoretically, $n$ stages (MS$^n$) can be carried out in the same trapping analyzer, provided a sufficient number of ions remain trapped to yield measurable signals. On the other hand, each tandem mass spectrometry stage in beam instruments requires one mass analyzer. To minimize pumping volume and ion losses due to scattering and maximize sensitivity, the vast majority of in-space tandem mass spectrometers contain only two mass-analyzing devices.

2.2 Ionization techniques

The core of the mass spectrometry experiment is the ionization process. In order for a sample or a mixture to be analyzed, it must be ionized first and transferred from the liquid or solid phase into the gas phase before its component can be deconvoluted according to their different $m/z$ ratios by the mass analyzer. The formation of gas-phase ions may involve transfer of charged species to the gas phase as well as electron ejection, electron capture, protonation, deprotonation, or cationization of neutral (volatilized) species in the ion source. Most of the time it is the type of sample that dictates the best ionization technique to apply and the type of information that scientists can derive. Hard ionization techniques such as impact electron (EI), chemical ionization (CI), and field ionization (FI), break the sample partly or completely into fragment ions during the ionization. On the other hand, soft ionization techniques such as fast atom bombardment (FAB), field desorption (FD), MALDI, and ESI, do not dissociate the sample and provide
$m/z$ information on the intact sample. The last two soft ionization methods are the most widely used today for the analysis of molecules having a broad molecular weight range. In the case of small and volatile compounds, EI is the favorable technique. The techniques used in this dissertation, ESI and MALDI, will be briefly described below.

2.2.1 Electrospray Ionization (ESI)

The ability of producing gaseous ions by spraying a solution through a capillary needle held at high voltage was first investigated by Malcolm Dole in 1968 and then advanced by John Fenn who in the late 1980s reported the first ESI applications to biological macromolecules.\textsuperscript{29} For this accomplishment Fenn was awarded the 2002 Nobel Prize in Chemistry.

In the ESI process, the sample solution is introduced in the ion source through a metal needle that is connected either to a syringe pump or the exit of an LC column. The application of a strong electric field at the needle tip (2-6 kV) induces charge accumulation on the surface of the analyte solution resulting in highly charged droplets as the solution is being sprayed. A nebulizing gas, generally nitrogen, is used to assist this process. The repulsive forces between the accumulated charges on the droplet are usually higher than the surface tension, causing the droplets to take the shape of a Taylor cone and release smaller droplets. This droplet decomposition starts when the repulsive forces become equal to the droplet surface tension (Rayleigh limit).\textsuperscript{43,44} The droplets formed at
the ESI needle tip are mechanically deformed due to the electric field. This deformation leads to decomposition before the Rayleigh limit.

Further evaporation of the solvent is assisted by heated nitrogen flowing in opposite direction of the charged droplets’ motion. This promotes the evaporation of the solvent from the droplets which start shrinking leading to an increase of their charge per unit volume. The decrease in droplet size results in an increase of Coulombic repulsion of the charges in the droplets which leads to a fast separation into much smaller droplets (Coulomb explosion). Repeated solvent evaporation and Coulombic explosions continue until the charge density on the droplet surface becomes so high that single ions desorb from the droplet or until only a single ion remains. These ions are ultimately sent to the mass analyzer and the detector for determination of their \( m/z \) values.

2.2.2 Matrix-Assisted Laser Desorption Ionization (MALDI)

MALDI, which is closely related to the soft laser desorption method developed by Tanaka\(^45\) in the 1980s, was first introduced by Karas and Hillenkamp\(^46,47\) in the late 1980s. Similar to the ESI technique, the MALDI ionization method generates intact gas-phase molecular ions. But unlike in the ESI process, the ions generated by MALDI bear only a singly charge, which makes the molecular weight determination straightforward especially for high molecular weight analytes which in ESI would give rise to a charge distribution of ions. Another advantage of this method over ESI is its high tolerance towards contaminants such as detergents, buffers, and salts. The ESI process is driven by
the evaporation of the solvent from the sample solution; those impurities may change the colligative properties of the overall solution such as its boiling point. This phenomenon affects the ionization and can cause signal suppression.43

The sample preparation for the MALDI analysis requires a dilute sample solution to be mixed with a more concentrated matrix solution. The matrix is a small organic molecule with absorbance capacity at the laser’s wavelength. The large molar excess of matrix not only disperses the sample molecules but also prevents cluster formation and protects the sample molecules from photo-induced decomposition when the laser beam hits the solid solution of the sample in the matrix. A few microliters of the resulting mixture solution of sample and matrix solutions is deposited on a sample plate and allowed to dry so that the matrix crystals encapsulate the analyte molecules. Absorption of laser energy leads to evaporation and ionization of the matrix creating a gas-phase plume, in which sample molecules are also trapped, thus reaching the gas phase. It is believed that the sample molecules are ionized by gas phase proton transfer reactions with matrix ions. In the case of samples that do not protonate easily, a salt solution is added to the mixture of matrix and sample to promote ionization by formation of metal ion adducts, for example, of Na⁺, Li⁺ or Ag⁺ ions. The nature of the salt is strictly dependent on the particular functional groups present on the molecules under investigation.
2.3 Activation methods

The most widely used ionization techniques, namely MALDI and ESI, are characterized as soft, because they generate prevalently molecular ions (M⁺ or M⁻) or quasimolecular ions ([M+H]+, [M-H]−, [M+X]+ where X=metal), with little or no fragmentation. These species represent the precursor ions that are mass-selected and energetically activated in MS² experiments in order to generate fragments indicative of the precursor ion structure. Since ion fragmentation is the core of tandem mass spectrometry, many different methods for promoting this process have been developed during the past few decades.

2.3.1 Collisionally activated dissociation (CAD)

Collisionally activated dissociation (CAD), also known as collision-induced dissociation (CID),⁴⁸ is the most common fragmentation method in use nowadays. Activation of the mass-selected precursor ions is effected by accelerating them, so that they undergo energizing collisions with gaseous targets, commonly argon or helium atoms. During the collision, a fraction of the kinetic (translational) energy of the precursor ion is converted into internal energy, which is redistributed rapidly among the rotational–vibrational degrees of freedom of the ion before fragmentation occurs (ergodic process). As a consequence, the weakest bonds in the precursor ion have a higher predisposition for cleavage. Also, unimolecular rearrangement mechanisms are triggered, because they tend to produce stable fragment ions and, hence, have favorable energy requirements.
CAD can be performed in both beam and trapping mass spectrometers. The maximum amount of kinetic energy that can be converted into internal energy after a single collision depends on the masses of both the precursor ion and the collision gas atom or molecule involved, as well as on the kinetic energy of the ion before the collision. This relationship is expressed as follows:

\[ E = \frac{N}{m_p + N} E_k \]  

(2.1)

\( N \) identifies the atomic/molar mass of the neutral collision gas, \( m_p \) is the molar mass of the selected precursor ion, and \( E_k \) is the laboratory-frame kinetic energy of the precursor ion before the collision. \( E \) is often termed the center-of-mass kinetic energy (\( E_{\text{com}} \)). It is the type of instrument and analyzer that set the initial kinetic energy, \( E_k \), according to which CAD can be classified as “high energy” or “low energy.”

High-energy CAD is generally performed with ToF-type instruments or older instruments containing magnetic and electric sectors. The kinetic energies of the ions subjected to CAD are in the order of several keV, and collisions take place at relatively low pressure (10^{-2} mbar). Under these conditions, each precursor ion experiences on average 1–10 collisions\(^{49} \) that can deposit high enough energy to cause electronic excitation; generally, this process is followed by randomization of the excess energy over the rotational–vibrational degrees of freedom of the precursor ion, which ultimately leads to fragmentation, as mentioned above. With large precursor ions (for example, from polymers), direct excitation of rotational–vibrational modes is more probable at keV
collision energies, because the corresponding center-of-mass kinetic energies are too low to access excited electronic states.

Low-energy CAD involves ions with much lower initial kinetic energies (usually \( \leq 200 \text{ eV} \)) that are required with quadrupole and trapping analyzers for efficient precursor ion selection and fragment ion dispersion. One consequence of using low-energy CAD, compared to high-energy CAD, is that the internal energy transferred during one collision is not enough to cause fragmentation. Multiple collisions are needed, which can be achieved by increasing the pressure of the collision gas (mbar range) or the time of excitation (in ITs). For example, a He pressure of 1 mbar can cause up to \( 10^6 \) collisions in a quadrupole IT within tens of milliseconds.\(^{49}\) Low energy CAD can only cause rotational–vibrational excitation of the precursor ion.

2.3.2 Surface-Induced Dissociation (SID)

The amount of internal energy that can be transferred to a precursor ion in a collision with a gaseous target depends on the mass of the target atom (or molecule), cf. Eq. (2.1). If the collision gas is substituted with a solid surface, more energetic collisions result because of the much higher mass of the surface compared to that of the gas atom (or molecule). The precursor ion undergoes strictly one collision during SID, gaining a narrower internal energy distribution than upon CAD. Moreover, lower background pressures are maintained, as no collision gas is required, which enhances the reproducibility of the fragmentation process. The first SID experiments were carried out
on sector-type instruments. More recently, SID has been adapted to mass spectrometers containing quadrupole, ToF, and ICR analyzers. Since SID deposits higher average internal energies than CAD, but CAD allows for multiple activating collisions, these two activation methods tend to yield comparable fragmentation patterns. SID has not yet been employed to the analysis of synthetic polymers.

2.3.3 Photodissociation Methods

Alternative activation methods that do not require collision(s) of the precursor ion with atomic or larger entities have been considered and developed. The absorption of photons by isolated, mass-selected ions can easily induce excitation that ultimately causes fragmentation. Here, the wavelength of the exciting photons can be varied in the UV, vis, and IR regions, providing different levels of molecular excitement.

IR photons are most widely used to activate mass-selected ions. This radiation has frequencies that fall in the range of molecular vibrational frequencies; hence, the absorption of IR photons increases the vibrational energy. Multiple photon absorption is required to trigger fragmentation due to the low energy transferred by a single photon. Low-power, continuous-wave (cw) CO$_2$ lasers have served most frequently as the source of radiation, which must intercept the precursor ions for moderately long times (milliseconds or higher) in order to produce fragments with measurable intensities. Trapping instruments are most suitable for such IR multiphoton photodissociation (IRMPD). Note that the background pressure must be maintained low during the
absorption window to minimize collisional relaxation. For this reason, IRMPD is performed mainly with FT-ICR mass spectrometers (which operate at ultrahigh vacuum; ~ $10^{-9}$ bar) and electrostatic trapping instruments that require very low He bath gas pressure inside the trap device. IRMPD applications have so far focused on biological analytes.\textsuperscript{52}

2.3.4 Electron Capture Dissociation and Electron Transfer Dissociation (ECD/ETD)

Activation methods that exploit the interaction between isolated precursor ions and thermal electrons were first investigated by McLafferty et al.\textsuperscript{53} The process, named electron capture dissociation (ECD), involves the capture of thermal electrons (< 0.2 eV), emitted from heated tungsten filaments, by isolated multiply charged cations. This reaction gives rise to incipient radical cations that dissociate rapidly via radical-induced decompositions and rearrangements at the site of electron attachment, before energy redistribution occurs (nonergodic process).\textsuperscript{54} Thus, ECD is site-specific, whereas CAD (ergodic process; vide supra) induces bond cleavages that require low activation energy. ECD is almost exclusively performed in FT-ICR instruments, where thermal electrons can be trapped over long periods to produce detectable fragments and where the background pressure is sufficiently low to suppress competitive CAD pathways. The necessity to have multiply charged precursor ions makes ESI particularly suitable for ECD studies.
The first ECD applications concerned biological analytes, especially proteins and peptides, and documented considerable differences between ECD and CAD fragmentation pathways. The major fragments from ECD of proteins and peptides are c/z-type ions from cleavages at the N-C$^{\alpha}$ backbone bonds, while CAD on the same species mainly gives rise to b/y-type ions from cleavages at the C(=O)-N backbone bonds. An important feature of ECD is that it generally preserves labile posttranslational modifications at the protein/peptide side chains during fragmentation due to the rapid, nonergodic nature of energy transfer. ECD has been applied on select synthetic polymers, including polyestereamide, poly(alkene glycol), and polyamidoamine samples.55,56,57,58

An alternative, closely related activation method, is electron transfer dissociation (ETD).59,60,61 The inability to store thermal electrons in trapping instruments utilizing strong radiofrequency (rf) fields, such as the 3D and 2D quadrupole IT, has been overcome by employing ion–ion reactions between reagent radical ions (for example, from anthracene or similar substances) and multiply charged precursor ions of opposite charge, both confined in the same trapping device. It is during such reactions that electrons are transferred. Most widely used are ion–ion reactions between reagent anions and multiply charged cations. The negatively charged radical reagents are generated in a separate negative chemical ionization (nCI) source and transmitted to the IT where they are left to react with the mass-selected precursor ions for the necessary time interval. The fragment ions arising from ion–ion reactions are very similar to the ones observed in ECD; an important feature of both methods is that, unlike CAD, they preserve posttranslational modifications in peptides and proteins.62 Although no ETD application to synthetic polymers has yet been published, preliminary studies on polyethers63 and
polyesters (vide intra) have been reported, based on which increased future use is anticipated.

2.3.5 Post-Source Decay (PSD)

This type of fragmentation takes place in reflectron ToF or ToF/ToF instruments equipped with a MALDI source. A high laser power is utilized to generate ions with enough internal energy to decompose spontaneously after exiting the source but before reaching the detector (“metastable ions”). PSD experiments were first demonstrated on reflectron ToF mass spectrometers, in which the fragments formed from a particular precursor ion in the field-free region between the MALDI source and the reflectron were dispersed by appropriate scanning of the reflecting lens potentials. Precursor and fragment ions have the same velocities due to the principles of conservation of mass and momentum; hence, they travel as a packet to the reflectron. Selection of the desired precursor ion (together with its coherently moving fragments) was achieved with an ion gate that allowed only packets within a specific velocity window, corresponding to a precursor ion resolution of $\sim 20 \text{ m/z}$ units, to pass through.

The use of the reflectron as mass-analyzing device became obsolete with the introduction of commercial MALDI-ToF/ToF mass spectrometers, in which a short linear ToF analyzer is axially interfaced with a reflectron ToF device. Here, the first (linear) ToF analyzer and an ion gate are used for selection of the precursor ion (and its fragments), while mass analysis of the fragmentation products occurs in the second
(reflectron) ToF analyzer. MALDI-ToF/ToF mass spectrometers have significantly improved precursor ion resolution (<5 m/z units below m/z 3000). Additionally, ToF/ToF instruments may be equipped with collision cells along the beam path from the ion source to the second ToF analyzer to combine PSD with CAD and enhance the fragmentation yield.  

Mainly biological samples, such as proteins peptides and oligosaccharides, have been analyzed by MALDI PSD. Investigations on select polymer classes have also been reported. MALDI-ToF/ToF instrumentation improved markedly the quality of MALDI MS² spectra, as compared to PSD spectra acquired with a simple reflectron ToF mass spectrometer, leading to increased applications of MALDI-ToF/ToF MS² to synthetic polymers.

2.4 Instrumentation

In trapping instruments, the isolation and fragmentation processes take place in the same section (analyzer) of the mass spectrometer but in temporal sequence, whereas in beam instruments, isolation, fragmentation, and deconvolution of the resulting fragments occur in different parts of the mass spectrometer, while the ions travel from the source to the detector. This section provides brief descriptions of the trapping and beam instrument setups which, at the present, experience widespread use in synthetic polymer analyses.
2.4.1 Quadrupole Ion Trap (QIT) Mass Spectrometers

Quadrupole ion traps (QITs) are storage devices. They are comprised of a ring electrode and two end caps having small holes for injection of the ions produced in an external ion source or ejection of the stored ions to a detector (Figure 2.1).\textsuperscript{84} Trapping is achieved by grounding the end caps and applying an rf field of fixed frequency ($\nu$) to the ring electrode, $\Phi_0 = V \cos \omega t$, where $V$ is the amplitude of the applied rf potential ($\leq 30$ kV) and $\omega$ the angular frequency ($\omega = 2\pi \nu$; $\nu \approx 0.75$-1.2 MHz). The trappable $m/z$ range is determined by the rf amplitude. QITs are normally filled with helium bath gas ($\sim 10^{-3}$ mbar), which cools down the ions with collisions and forces them to move to the center of the trap, so that they are not accidentally ejected due to the kinetic energy acquired during the injection step or due to the repulsive forces between ions of the same charge.

![Schematic view of a QIT](image-url)

Figure 2.1. Schematic view of a QIT (Bruker HCT ultra). Reproduced from reference 84 with permission.
The ion motion inside the trap is described by the dimensionless parameter 
\[ q_z = 8\pi e V / m (r_0^2 + 2z_0^2) \omega^2, \]
which is derived from the Mathieu equation and represents a measure of the rf amplitude \( V \); \( m \) and \( z \) are the mass and charge of the ion, and \( r_0 \) and \( z_0 \) the radial and axial dimensions of the trap, respectively.\(^8\) Since modern IT instruments do not utilize dc fields for trapping (\( U = 0 \)), the parameter \( a_z \) (also derived from the Mathieu equation and representing \( U \)) is equal to zero. In rf-only traps, the ions are confined in the \( q_z \)-axis, as shown in Figure 2.2 for four ions differing in mass. Only ions with \( q_z \leq 0.908 \) have stable trajectories inside the trap and can be stored. Figure 2.2 illustrates the pseudo-potential well that defines the stability region; ions outside this region have unstable trajectories and are ejected. Scanning \( V \) successively causes ions of increasing \( m/z \) to reach \( q_z > 0.908 \), at which point they are ejected through the end caps for detection (mass-selective axial instability mode).\(^8\)

The limit value \( q_z = 0.908 \) determines the low-mass cutoff of a QIT; ions below this mass cannot be stored and analyzed. In addition to the mass-selective axial instability mode (vide supra), stored ions can be ejected and detected via resonant ejection. Inside the trap, ions oscillate with a secular frequency (\( \omega_s \)) that is lower than the main rf field frequency (\( \omega \)) and inversely proportional to their \( m/z \) value. By applying an auxiliary rf voltage to an end cap that has the same angular frequency as a trapped ion (\( \omega_s \), this ion will come into resonance and will be ejected from the trap.
Figure 2.2. Stability diagram for a QIT, showing four ions along the $q_z$ axis; the three ions residing inside the stability region ($q_z \leq 0.908$) are trapped, while the one outside this region ($q_z > 0.908$) is ejected. Reproduced from reference 84 with permission.

Scanning the frequency of the auxiliary field allows for the successive ejection (and detection) of all ions stored in the QIT. Instead of a single frequency, an rf signal composed of multiple frequencies can be generated and applied on an end cap to excite and eject many different ions at the same time; this principle is used to isolate specific precursor ions for MS$^2$ experiments.

Once the precursor ion has been chosen, ions with lower $m/z$ values can be ejected by a rapid scan and ions with higher $m/z$ values by resonance ejection; alternatively, all but the selected ion can be ejected by resonance ejection. The isolated precursor ions are accelerated by an auxiliary rf field, applied to the end caps, which increases their kinetic
energy via resonance excitation. The amplitude of the auxiliary field is kept small (~1 V) to avoid ejection of the mass-selected ions from the trap. The excitation time usually is in the range 20–60 ms; during this time, the precursor ions undergo activating collisions with the He bath gas inside the QIT analyzer. Because ion kinetic energies are low in QITs, compared to beam instruments, a higher number of collisions is required to promote efficient CAD. The main fragments arise from dissociations that are associated with low activation energies. Since the auxiliary rf field is in resonance with the selected precursor ions but not their fragments, consecutive fragmentations proceed inefficiently unless they have lower energy requirements than competitive pathways. After the excitation time has elapsed, the CAD products are scanned by resonant ejection to render the corresponding MS\(^2\) spectrum. The individual steps of an MS\(^2\) experiment are generally preprogrammed into a scan function, as shown in Figure 2.3, which sets the order and duration of the events taking place during the acquisition of an MS\(^2\) spectrum.

A specific MS\(^2\) fragment can be isolated and excited to undergo further CAD by repeating the isolation, excitation, and ejection/detection steps outlined above. This procedure leads to the respective MS\(^3\) spectrum; further MS\(^n\) cycles are possible, depending on the efficiency of fragmentation and the amount of ions remaining in the trap.\(^{12,85,86}\)
Figure 2.3. QIT MS\textsuperscript{2} scan process for precursor ions produced in an external ESI source. 1: clear trap (all stored ions are ejected); 2: accumulation time (ions are injected); 3: isolation delay (cooling time); 4: precursor ion isolation; 5: fragmentation delay (cooling time); 6: fragmentation; 7: scan delay; 8: mass analysis. Reproduced from reference \textsuperscript{84} with permission.

A shortcoming of CAD experiments in QITs is that fragment ions with m/z ratios smaller than \(~1/3\) of the precursor ion m/z are not efficiently retained in the trap because the resonance excitation step moves their m/z ratios below the low-mass cutoff. By modulating the QIT electronics, the cutoff can be reduced to \(< 1/4\) of the precursor ion m/z, the trade-off being lower sensitivity. Alternatively, an MS\textsuperscript{2} method that does not accelerate the precursor ions, such as ETD may be used (see below).

QITs can also be adapted to carry out ETD experiments. Instruments with this capability are equipped with two external ion sources, one ESI source to produce multiply charged precursor ions and one chemical ionization (CI) source to produce reagent ions of opposite charge.\textsuperscript{59} In the Bruker HCT model, the CI source is located above the octapole lens used to transfer ESI-generated ions to the IT, cf. Figure 2.4.\textsuperscript{87,88}
Figure 2.4. QIT with external ESI and CI sources for ETD experiments; the most widespread mode involves ETD between multiply charged cations produced by ESI and reagent anions produced by nCI, as shown. Reproduced from reference 88 with permission.

In the vast majority of ETD experiments, the CI source is operated in negative mode (nCI), furnishing anions that react with multiply charged cations (as shown in Figure 2.4). Inside the nCI source, 70 eV electrons are emitted from a tungsten filament in a chamber filled with methane at a pressure of 2.0–2.6 bar. The methane gas acts as a mediator that cools down the electrons to thermal energies, so that they attach to the reagent molecules to form intact radical anion species. Different reagents have been tested and utilized for ETD experiments, including 9-anthracenecarboxylic acid, 2-fluoro-5-iodobenzoic acid, 2-(fluoranthene-8-carbonyl)benzoic acid, and fluoranthene.

Although the ESI and nCI sources operate continuously, the respective ions are transmitted to the QIT alternately. During the transfer of the ions from the ESI source to the IT, the reagent radical anions from the nCI source are blocked at the gate lens by the application of a voltage (cf. Figure 2.4). After a specific ion accumulation time, the ion flow from the ESI source is stopped at the skimmer and the QIT optics start operating...
partially in the negative mode to move the radical anions formed in the nCI source to the trap. Ion–ion reactions between the two species follow for a specific time interval (ms), which is generally sample-related.

2.4.2 Quadrupole/time-of-flight (Q/ToF) Mass Spectrometers

A tandem mass spectrometer composed of quadrupole and ToF mass analyzers offers the high mass accuracy and resolving power of double focusing sector mass spectrometers. Moreover, unlike sector instruments, which do not function optimally with pulsed ionization methods (MALDI) or ionization methods requiring high voltages (ESI), Q/ToF instrumentation can be interfaced with almost any ionization technique, including MALDI, ESI, atmospheric pressure chemical ionization (APCI), atmospheric solid analysis probe (ASAP),\textsuperscript{90} and desorption electrospray ionization (DESI).\textsuperscript{91} The instrument set up takes advantage of the mass selection and transmission properties of the quadrupole and the resolution and mass accuracy capabilities of the ToF analyzer. Generally, the ToF part is orthogonal to the quadrupole, a geometry imposed by the different features of these devices: a quadrupole transmits ions continuously, whereas a ToF analyzer functions in pulsed mode, by resolving packets of ions having the same initial kinetic energy.\textsuperscript{65}

A Q/ToF mass spectrometer equipped with a MALDI source and a collision cell between the two mass analyzers is depicted in Figure 2.5.\textsuperscript{92} After passing the quadrupole and the collision cell, precursor and fragment ions are pushed down the ToF tube with an
acceleration voltage of ~10 kV, provided by a pusher, which is synchronized with the detector in order to measure accurately the time elapsing after every push until the ions reach the detector.

Figure 2.5. Schematic of a MALDI-Q/ToF tandem mass spectrometer (the Waters Q-ToF Ultima MALDI). Reproduced from reference 92 with permission.

For the acquisition of regular mass spectra, the quadrupole is set in the rf-only mode to transmit all ions generated in the ion source to the ToF segment, where they are accelerated and dispersed by their m/z ratios. Conversely, in the MS² mode, both dc and rf voltages are applied to the quadrupole and tuned to transmit only the desired precursor ion, which is fragmented in the ensuing collision cell. The collision cell is an rf-only quadrupole or hexapole, filled with a collision gas (typically Ar) to promote CAD. For this, the potential energy of the collision cell, which sets the laboratory-frame collision energy, is raised to several tens of eV (up to ~200 eV), so that the entering precursor ions
undergo energizing collisions and dissociate. The resulting fragments can be mass analyzed in the ToF segment with mass accuracies of <10 ppm if the mass scale is calibrated immediately before mass analysis.

The precursor ion isolation window can be tuned by adjusting the dc and rf voltages applied to the quadrupole rods. This makes it possible to isolate either the complete isotope cluster or a single isotope of the precursor ions. Keeping the isolation window large enough to transmit the entire isotope cluster of the precursor ion is advantageous for the identification of polymers containing elements with unique isotope distributions, such as halogens and sulfur. In these cases, the isotope pattern of the resulting fragment ions unveils not only the presence of a specific atom, but also how many of these atoms are contained in the fragment, which significantly facilitates MS$^2$ spectral interpretation.

Recently, Q/ToF instrumentation has become available that enables the combination of MS or MS$^2$ experiments with ion mobility spectrometry (IMS). This capability is offered by the Waters Synapt HDMS™ mass spectrometer, which contains a traveling wave (T-wave) section at the interface of the Q and ToF analyzers, consisting of three cells in the order trap cell, ion mobility (IM) cell, and transfer cell (Figure 2.6). By activating the ion mobility device, ions can be separated first according to their size/shape within the IM chamber and, later, according to their mass-to-charge ratio in the ToF mass analyzer. If a precursor ion is mass-selected by the quadrupole, it can be fragmented in the trap cell, which is operated as a normal collision cell using, typically, Ar as collision gas.
When the IM device is also activated, the fragments formed by CAD remain trapped for a short period of time and then are released in the adjacent ion mobility cell. This trapping and release process is synchronized with the pusher located at the entrance of the ToF analyzer, so that the drift time of an ion through the ion mobility cell is matched with the corresponding $m/z$ ratio. Once the ion packet released from the trap cell enters the ion mobility region, the ions move under the influence of a traveling wave electric field in the presence of a drift gas ($N_2$) which flows in the opposite direction of the ions’ motion. Isobaric or isomeric fragments are separated according to their size, shape, and charge state and displayed in a 2D ion mobility diagram of $m/z$ versus drift time.\textsuperscript{96} Alternatively, the mass-selected precursor ions may first be separated in the IM cell before entering the transfer cell for CAD; this way the individual MS\textsuperscript{2} spectra of overlapping isobaric or isomeric components can be acquired.\textsuperscript{86,96,97}
2.4.3 ToF/ToF Instruments

Tandem ToF mass spectrometers are comprised of two axially interfaced ToF analyzers (vide supra). Several models are commercially available; all are equipped with a MALDI source attached to a linear ToF tube, which in turn is interfaced with a reflectron ToF device (cf. Figure 2.7). Mass selection of the desired precursor ion is effected within the linear ToF segment with the help of an ion gate for timed ion selection (TIS). Only fragments formed in the linear drift tube, namely after the precursor ion has left the ion source, are transmitted; since these fragments move with the same velocity as their precursor ion, TIS can take place anywhere within the linear ToF part. The TIS device consists of two deflection gates, which are opened in a synchronized manner for a narrow time window, to allow for passage of only the desired precursor ion and its fragments. Usually the complete isotope distribution is transmitted, as precursor ion selection at higher resolution is accompanied by significant sensitivity losses. ToF/ToF instruments contain dedicated collision cells for CAD, which may be located anywhere in the linear ToF segment. Fragmentation is induced by raising the laser intensity, to cause laser-induced dissociation, as well as by CAD.
In the design shown in Figure 2.7, ions formed by MALDI are accelerated to 8 keV and decompose within the linear ToF, primarily due to laser-induced dissociation. Adding CAD increases the yield of low-mass fragments, but also causes scattering losses and, thus, is useful only if the low-mass fragments provide irreplaceable structural insight, as in de novo peptide sequencing studies. After the desired precursor ion and its fragments are separated from all other ions by the TIS gates, they enter a reacceleration region that post accelerates (“lifts”) them by +19 keV for mass analysis by the reflectron ToF analyzer. Shortly after the “lift” device, a postlift metastable suppressor (PLMS) is located, which is timed to deflect the remaining precursor ions, so that fragmentation is stopped after postacceleration.

In a different design, a time-selected 8-keV precursor ion (or precursor/fragment packet) are decelerated to 1–2 keV as they enter a floated collision cell for CAD. After exiting this cell, precursor and fragments reach a postacceleration region that raises the kinetic energy to 15–20 keV for mass analysis by the reflectron ToF analyzer. In the two designs discussed, the reacceleration step reduces the spread of kinetic energies of
precursor and fragment ions, so that all can pass the reflectron and reach the detector. In a third design, the ions leave the MALDI source at 20 keV and undergo CAD at this high kinetic energy; the resulting larger range of kinetic energies is accommodated with a curved-field reflectron.\textsuperscript{99,103}

The ToF/ToF instruments described can be used to acquire regular mass spectra by grounding the TIS gates and (if present) the deceleration/postacceleration lenses. Further, ToF/ToF equipment can provide information about the absolute MW and MWD of synthetic polymers. Q/ToF or QIT instrumentation is less suitable for such measurements because quadrupolar fields skew the MWD. On the other hand, Q/ToFs and QITs permit the selection of much narrower \( m/z \) ranges in MS\(^2\) experiments and offer better control of the energy deposited in CAD than ToF/ToF instruments. Nevertheless, the superior MW and MWD data accessible with ToF/ToF instrumentation and the capability to perform MS and MS\(^2\) analyses within the same experimental setup (vide supra) make this type of tandem mass spectrometer essential for the compositional and structural characterization of synthetic polymers.\textsuperscript{76,77,82,104}
3.1 Background

Phosphazenes are characterized by an alternating phosphorus-nitrogen bond sequence, in which the phosphorus atom carries two other side groups. They can form small molecules, long chains or rings, having different properties. The phosphorus-nitrogen bond is formally depicted as double bond for electron counting reasons. These compounds are neither “unsaturated”, like nonconjugated organic olefin species, nor “aromatic”, like benzene. The backbone bond lengths are approximately the same along the chain, which was confirmed by different structural measurements, but there is no long-range electronic conjugation typical of organic polyunsaturated molecules. Although the N–P bond is still poorly understood, some models have been proposed that better fit the unique characteristics of these compounds. In traditional bonding descriptions of \((\text{PCl}_2\text{N})_3\), a \(\pi\)-system can be formed by the overlap of p-orbitals on N and d-orbitals on P. The more modern bonding descriptions do not use extensive d-orbital character on the P atom for bonding. The multiple bonds are rather formed by the attraction between the formal positive charge on the P and negative charge on N. These bonds are about 85% ionic and 15% negative hyperconjugation interactions. The ionic bond nature arises from the different electronegativities of the nitrogen and phosphorus
atoms, nitrogen being more electronegative than phosphorus. The zwitterionic model, combined with a negative hyperconjugation enhanced by the nature of the side groups on the P atoms, is the most widely accepted bond model for the P-N bond in phosphazenes (Figure 3.1).105

![Figure 3.1. P–N zwitterionic bond representation.](image)

When heated at high temperature, the small chloro-substituted cyclic compounds (R = Cl) can generate elastomeric materials.106 It has been noticed that this process can be reversed by increasing the temperature and decreasing the pressure, which give back the cyclic starting materials.

The insolubility of the elastomeric material, defined as “inorganic rubber”, in any solvent and its hydrolytic instability discouraged its applications until the mid-1960s, when Allcock, Kugel and Valan107,108,109,110 showed potential applications of these compounds. They proved that the solubility issue could be overcome by controlling the time, temperature and purity of the cyclic phosphazenes and by stopping the reaction before it reaches 70% of polymer formation.7 The main reason of the insolubility was that this polymer appears to be crosslinked. Accurate control of the factors mentioned above yielded instead uncrosslinked, high molecular weight presumably linear polymers, which are soluble in solvents like benzene, toluene, cyclohexane and tetrahydrofuran.
A variety of applications have been developed for polyphosphazenes by using alkoxide side groups, polyphosphazenes can be used as elastomers, films, coatings, fire retardants, fibers for optical, electro-optical materials, solid battery electrolytes, fuel cell components and a variety of different membranes. The use of amino side groups gives water unstable polyphosphazenes that are suitable for biomedical applications.

The most common synthetic methods to phosphazenes are: ring-opening polymerization (ROP), involving small cyclic phosphazene molecules; condensation polymerization of starting materials that are usually non-cyclic phosphazene species; or by mixing NH$_4$Cl and PCl$_5$ under aerobic conditions. Each of these synthetic routes gives rise to a mixture of products having different morphology, chain length and undesired byproducts.

The major common synthetic route utilized up to date to generate the polymer is the thermal “ring-opening polymerization” (ROP) developed by Allcock,$^{108,109,110}$ which involves hexachlorocyclotriphosphazene, (PCl$_2$N)$_3$, as starting material. In the most commonly accepted mechanism, the high temperature utilized ($\sim 250 \, ^{\circ}\mathrm{C}$) triggers the separation of a chloride ion from the phosphorus. The resulting phosphazenium cation can interact with the nitrogen lone pair of a different ring which induces sequential opening of the ring and transfer of the positive charge on the terminal phosphorus, which can react with another ring (Figure 3.2). Iteration of this process increases the polymer chain length. The polymerization ends either when there is no starting material available or when a chloride ion from another ring or chain is abstracted which neutralizes the positive charge on the phosphorus atom.
Figure 3.2. Initiation step of the thermal “ring-opening polymerization” reaction of (PCl\(_2\)N)\(_3\). (a) Dissociation of a chloride ion from the phosphorus atom. (b) Attack of the phosphazenium cation by a lone pair of a nitrogen on a different trimer and consecutive opening of the ring, leaving a positive phosphorus atom which can react further.

Because each cyclic trimeric species involved in the polymerization is made up of three phosphazene units, (PCl\(_2\)N)\(_3\), the polymer chain should increase linearly by three units: (PCl\(_2\)N)\(_n\) where \(n = 3, 6, 9, 12\ldots\) Analyses showed instead that the resulting polymer has a regular polymer distribution, having all the possible oligomers. This discrepancy is justified by the occurrence of a concurrent process which involves a ring-ring equilibration, converting small rings to bigger and big rings to smaller ones. Moreover, short linear chains can cyclize giving rise to big rings of different size which can ultimately take part in the polymer reaction as well.

Characterizations of polyphosphazenes aim at deriving information about their chain length, molecular weight distribution, end groups and of course the presence of possible byproducts. For these particular polymers, the degree of substitution on the P atom is also important to know. For the latter information, elemental microanalysis has been one of the basic techniques utilized so far. It can detect the type of elements that are present in the polymers, such as P, N, Cl, C, H. Once this information is obtained the
degree of substitution on the phosphorus can be rapidly deduced just by determining the amount of chlorine atoms present. Techniques such as NMR and X-ray spectroscopy provide structural information, particularly the application of $^{31}$P NMR as the phosphorus chemical shift depends on the ring size, chain length and side groups.

Very few investigations by mass spectrometry have been reported thus far about these compounds, either small cyclic species or long chain polymers, in particular for the chloro-substituted ones, which are the starting materials for the different arrays of functionalized phosphazenes. The major reason has been their intrinsic physical and chemical properties which make chlorophosphazenes very reactive and unstable at the working conditions of most mass spectrometers. Their high tendency to hydrolyze in presence of water molecules and their fast degradation when exposed to air, have made the phosphazene analyses by new soft mass spectrometry approaches also very challenging.

In this dissertation, soft ionization techniques, such as matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) are employed for the investigation of these compounds.
3.2 Characterization of polydichlorophosphazene oligomers arising from NH₄Cl and PCl₅ by ESI mass spectrometry

3.2.1 Experimental section

The polydichlorophosphazene oligomers investigated in this study were synthesized by David I. Bowers in Professor Claire A. Tessier’s laboratory using the following procedure: NH₄Cl (5.0 g, 0.1 mol), PCl₅ (15.0 g, 0.07 mol), anhydrous chlorobenzene (30 mL), and a ½” stir bar were added to a 100 mL Schlenk flask. The flask was fitted with a reflux condenser, topped with a drying tube (Drierite®), and refluxed for 24 hours. The pale yellow solution was filtered to separate unreacted NH₄Cl. The volatile components were removed under reduced pressure at 0.1 Torr. The product is a yellow slurry which supposedly consists of cyclic and linear oligomeric phosphazenes. The small cyclics (PCl₂N)₃ and (PCl₂N)₄ were purified by sublimation at 60 °C for 6 days with a vacuum line whose ultimate vacuum was ~10⁻⁴ Torr. Percent yields of (PCl₂N)₃ and (PCl₂N)₄ were 30% and 12%, respectively. The remaining mixture composed of larger rings and linear oligomers, was treated by liquid-liquid extraction with hexane and chlorobenzene solvents to separate the larger rings (PCl₂N)₅₋₁₂ from the hexane-insoluble linear oligomers. The resulting two phases, chlorobenzene and hexane, were investigated by mass spectrometry.

The sampling, for the MS analysis, of the highly reactive oligomers in the chlorobenzene phase was performed in a glove box in order to avoid, or at least reduce, the exposure to air. The yellowish oil was dissolved in dry THF at a concentration of 0.2
mg/mL. Always inside the glove box, the resulting solution was loaded in a 250 µL syringe already pre-loaded with dry THF so to further dilute the original polymer solution to 1/10. The syringe was sealed in two glass tubes held together with an air and vacuum tight joint before being taken out of the glove box. This procedure allowed a lower exposure time of the sample solution to air. In the case of the fraction containing the rings, hexane was used as solvent. The compounds extracted with hexane were prepared for the MS analysis outside the glove box as they were found to be relatively air stable.

ESI spectra were acquired in both positive and negative mode, to detect the positive or negative ions, respectively, generated at the source. The mass spectrometer utilized in this study was a SYNAPT HDMS™ hybrid quadrupole/time-of-flight (Q/oa-ToF) mass spectrometer (Waters, Beverly, MA) equipped with a Z-spray electrospray source operated in both positive and negative ion mode. The instrumental settings were tuned in order to optimize both signal and resolution as follows: capillary voltage 3.5 kV, cone voltage 35-40 V, sampling cone voltage 3-4 V. The source temperature and the desolvation gas temperature were held to 90-60 °C and 240-110 °C, respectively. The sample solution was electrosprayed at a flow rate of 15 µL/min. For tandem mass spectrometry (MS²) studies, a specific oligomer or ring was first mass-selected by the quadrupole, fragmented in the trap collision cell (vide infra) using argon as collision gas, and then the resulting fragment ions and residual of intact precursor ions were mass measured in the ToF analyzer. The collision energy applied to fragment the isolated ion species was varied in the range from 35 to 50 eV, depending on the oligomer or ring under investigation. The isolation window was set in order to isolate the whole isotope envelope. Ion mobility mass spectrometry (IM-MS) experiments were performed on the
same instrument by activating the traveling wave (T-Wave) separation section located between the two analyzers. This section consists of three cells in the order trap cell, ion mobility cell, and transfer cell. Once the ions generated in the source or by fragmentation in the trap cell (vide supra) enter the ion mobility cell, they move under the influence of a travelling wave electric field, in the presence of a drift gas (N₂) which flows in the opposite direction of the ions’ motion. Separation takes place according to the size, shape, and charge state of the ions. These parameters determine the drift time of the ions, which is the time they have spent to traverse the ion mobility cell. This value can be measured and converted to an experimental collision cross-section which reflects the ion shape and size. The electric field used in the ion mobility experiments was generated by tuning the travelling wave velocity and the travelling wave height at 280 m/s and 5 V, respectively, while the nitrogen gas flow rate was set at 10 mL/min. The data acquisition, data processing and theoretical isotope distribution generation were performed by using Waters’ MassLynx 4.1 software.

3.2.2 Result and discussion for the characterization of polydichlorophosphazene oligomers arising from NH₄Cl and PCl₅ by ESI mass spectrometry

ESI was the method of choice for the characterization of these species due their high reactivity. MALDI analysis would have involved mixing of the sample with a molar excess of matrix. This step could potentially give rise to different side-products from the interaction with the matrix molecules. Furthermore, the evaporation of the solvent from
the resulting mixture, which is required for the slow co-crystallization of the matrix with the sample before the MALDI analysis, would trigger the formation of degradation products.

Figure 3.3 shows the ESI mass spectrum of the chlorobenzene fraction, acquired by operating the instrument in positive mode (“positive ESI mass spectrum”).

![Figure 3.3. ESI mass spectrum of the chlorobenzene soluble fraction (insoluble in hexane), acquired in positive mode with a Q/ToF mass spectrometer.](image)

The spectrum clearly contains only one polymer distribution. The difference in mass between two consecutive peaks belonging to the same series is equal to 114.9 Da, which is the mass of one polymer repeat unit (PCl$_2$N). This polymer is identified as the ionic linear polymer with the molecular formula [Cl$_3$PN(PCl$_2$N)$_n$PCl$_3$]$^+$, which has also been reported in the literature as one of the products arising from this synthesis.$^{111}$ Its
assignment was facilitated by the high accuracy of the \( m/z \) value measured for each oligomer, which agrees with the corresponding theoretical \( m/z \) value within a few ppm, and also by the comparison of the theoretical and experimental isotope distributions of each specific oligomer, which, in analogy to fingerprints, confirms not only the presence of specific elements having a characteristic isotope distribution but also their exact number, reflecting in this way the elemental composition, Figure 3.4.

![Figure 3.4. Comparison of theoretical (top) and experimental (bottom) isotope distributions of the \([\text{Cl}_3\text{PN(PCI}_2\text{N)}_3\text{PCI}_3]^+\) species.](image)

A fresh solution of the same sample solution was also investigated by negative ESI in order to detect any other, not pre-ionized component in the hexane fraction, cf. Figure 3.5.
Figure 3.5. ESI mass spectrum of the chlorobenzene soluble fraction acquired in negative mode with a Q/ToF mass spectrometer.

The spectrum shows one major (A) and one much smaller (B) polymer distribution. The difference in mass between two consecutive peaks belonging to the same distribution is equal to 114.9 Da, which is the mass of one polymer repeat unit.

In order to determine the number of monomers present in a given oligomer, the measured \( m/z \) value of an oligomer is divided by the exact mass of the repeat unit (114.915 Da for PCl\(_2\)N). The integer number of the quotient gives the maximum number of monomer units. The decimal part of the quotient is multiplied by the mass of the repeat unit to provide the mass of the nominal end groups plus the mass of the ionizing agent, if any. This procedure is illustrated for the oligomer of the distribution A observed at \( m/z \) 477.655:

\[
477.655 / 114.915 = 4.157 \\
0.157 \times 114.915 = 18.042
\]
Hence, this oligomer ($m/z$ 477.7) may contain up to 4 repeat units. If 4 repeat units are present in $m/z$ 477.7, the remaining components of the oligomer (i.e. its end groups and any moiety added during the ionization) have a mass of 18Da. Alternatively, $m/z$ 477.7 may contain only 3 repeat units, while the remaining components contribute $18.042 + 114.915 = 132.957$ Da, etc. For $m/z$ 477.7, 18 Da is the most reasonable residual mass. The extra 18 Da are attributed to the addition of one water molecule to the oligomer during either the sampling or the ionization process. Hence, the major series has no nominal end groups. The ionization process and water addition are rationalized in the Scheme 1.1; either of these processes can occur first.

Scheme 3.1. Proposed ionization process of the polydichlorophosphazene detected in the chlorobenzene soluble fraction during the acquisition in negative mode.

As reported earlier, the P=N double bond is best described as a $\sigma$ bond with a significant ionic interaction between the two atoms, resulting from the formal positive charge on the P atom and the formal negative charge on the N atom. During the ESI
process, electrochemical reactions take place at the working electrode-solution interface in the ion source. These reactions are promoted by both the high voltage applied to the ESI capillary needle and the electrochemical circuit used to maintain the flow of charge on the tip of the needle as the droplets form and leave (Figure 3.6).\textsuperscript{112,113} Because the instrument was operated to generate and detect negative ions, the reaction occurring at the contact surface of the metal needle with the sample solution is a reduction (M + e\textsuperscript{−} → M\textsuperscript{−}).

![Figure 3.6. Electrochemical reaction process occurring during the ESI process.](image)

The more electropositive phosphorus atoms in the polymer, which have a formal positive charge and possess also unoccupied antibonding molecular orbitals, can easily accommodate the extra electron added in the reduction reaction. This step gives rise to a negative radical species having the radical on the phosphorus atom and the negative charge localized on the adjacent nitrogen atom. Because the ionization process is not carried out under vacuum but at atmospheric pressure, as the charged droplets leave the needle tip to travel through different vacuum stages inside the mass spectrometer, the highly reactive radical anion can easily react with the moisture present in the ionization chamber adding a water molecule. The negatively charged nitrogen atom can abstract a
proton while the remaining hydroxide attacks the adjacent positively charged phosphorus atom, which reaches a stable penta-coordinate state, Scheme 3.1. Water addition next to the reduced P atom is favored because of the higher negative charge on the N atom adjacent to the radical site.

It is also possible that the water addition is the first step. This could take place during the extraction or ESI sample preparation due to traces of water in the solvent used. Upon ESI, the P atom next to the protonated nitrogen should be preferably reduced because of its higher positive charge density. Consequently, the same radical anions are generated irrespective of the sequence of water addition and radical formation.

Figure 3.7. Magnified view of the inset in the negative ESI mass spectrum of polydichlorophosphazene shown in Figure 3.5.

The less intense polymer distribution labeled as B (Figure 3.7) appears 36.9 Da lower than the major polymer distribution A. Such difference indicates that B contains one chlorine and two hydrogen atoms less than A, representing polyphosphazene oligomers in which one Cl atom has been replaced by an O⁻ anion (see structure in Figure
This composition was again confirmed not only by exact mass measurement but also by comparing theoretical and experimental isotope distributions, as shown for the 6-mer in Figure 3.8.

Figure 3.8. Comparison of theoretical (top) and experimental (bottom) isotope distributions of the [(PCl$_2$N)$_6$(PClON)]$^-$ species, which is labeled B in Figure 3.7.

Distribution B is most likely formed by OH$^-$ addition and sequential HCl elimination from oligomers that were not reduced during ESI. Based on the $m/z$ values of its members, distribution B does not present any nominal end groups, as found for A (vide supra). The lack of end groups in polymeric molecules usually implies a cyclic structure, where the two ends of the polymer chain are connected to each other. Alternative possible configurations that do not posses nominal end groups are the “tadpole” structure, composed of a head, which is generally a small cyclic, and a polymeric tail, or branched structures.
Nothing has been reported in the literature about the architecture of these specific oligomers detected in the chlorobenzene phase under negative ESI conditions.

ESI analysis in negative mode of the hexane soluble fraction gave a mass spectrum which is dominated by three different series, labeled as A, B and C. The difference in mass between two consecutive peaks in each series is 114.9 Da, the mass of one phosphazene unit ($\text{PCl}_2\text{N}$), Figure 3.9.

![ESI mass spectrum](image)

**Figure 3.9.** ESI mass spectrum of the hexane fraction acquired in negative mode with a Waters Synapt Q/ToF mass spectrometer.

The oligomers belonging to the A series do not carry any nominal end groups. They are all comprised of an exact number of phosphazene repeat units ($n > 5$); the $m/z$ values calculated for such a composition and those measured agree excellently, $\Delta \approx 3$ ppm. This evidence reveals that the oligomers in series A are radical anions, formed by addition of one electron to the phosphorus atom during the ionization process.
The B series is 18.97 Da lower than the A series which is consistent with replacement of one chlorine atom with one oxygen atom. On the other hand, the C series which is 34.97 Da lower that the A series, arises from a loss of one chlorine atom. The replacement of one chlorine with one oxygen atom in series B and the lack of one chlorine atom in series C were confirmed by the isotope distributions. The same n-mers of B and C series showed similar isotope distributions as they both have the same number of chlorine atoms, Figure 3.10. The structure of these oligomers has been reported to be cyclic.\textsuperscript{111} No ions were detected in positive ESI mode from the hexane fraction.

Figure 3.10. Magnified view of the inset in the negative ESI mass spectrum of the hexane fraction.
3.3 Tandem mass spectrometry and ion mobility mass spectrometry (IM-MS) studies for the elucidation of the polymer architectures

In order to gain insight on the structure of the oligomers detected in both fractions under negative-mode ESI-MS conditions, which did not identify any end groups, tandem mass spectrometry experiments (MS\textsuperscript{2}) were carried out. Ions belonging to the same n-mer were first mass-selected and then energetically excited in order to dissociate into structurally indicative fragments by collisionally activated dissociation (CAD). During this process the selected precursor ions collide with inert gas molecules; in this specific case, argon was used as inert gas. These collisions increase the internal energy of precursor ions, which promotes bond cleavage, bond formation or molecule rearrangement that lead to dissociation.

MS\textsuperscript{2} experiments were carried out on the 4-mer, 5-mer and 7-mer from the main oligomer distribution A observed in the chlorobenzene soluble fraction as well as on different oligomers of the A series of the hexane fraction.

The MS\textsuperscript{2} spectra of oligomers from the chlorobenzene fraction will be discussed initially. The molecular formula of each major fragment ion was extrapolated not only by accurate mass measurements and by accounting for the difference in mass between parent ion and fragment ion (neutral loss $\Delta$), but also by comparing theoretical and experimental isotope distributions. The CAD tandem mass spectrum of the 4-mer, $m/z$ 477, is illustrated in Figure 3.11. A Cl\textsubscript{2} loss is observed which generates the fragment ion at $m/z$ 407 ($\Delta = 70$ Da). This loss is also confirmed by the isotope distribution of $m/z$ 407 which confirmed that this ion contains two chlorine atoms less than the precursor ion. The
fragment ion detected at \( m/z \) 325, which is the base peak of the \( \text{MS}^2 \) spectrum, is 151.9 Da lower in mass than the precursor ion. The moiety lost is identified as a radical containing one repeat unit (114.9 Da), two hydrogen atoms and one chlorine atom (cf. Scheme 3.2). Accurate mass measurements and the isotope distribution of the fragment ion corroborate the mechanism shown in scheme 3.2 for the dissociation pathway leading to \( m/z \) 325.

Figure 3.11. CAD tandem mass spectrum of the ion at \( m/z \) 477 corresponding to the polydichlorophosphazene 4-mer from distribution A in the chlorobenzene soluble fraction. The spectrum was acquired in negative mode on an ESI-Q/ToF mass spectrometer at a collision energy of 30 eV.

The other fragment ion at \( m/z \) 271 is accounted from a loss of \( \text{PCl}_5 \) (\( \Delta = 205.8 \text{ Da} \)), cf. Scheme 3.3.
Scheme 3.2. Proposed mechanism for the dissociation of [(PCl$_2$)$_4$+H$_2$O]$^+$ (m/z 477), the 4-mer from the distribution A in the chlorobenzene soluble fraction, to form the most abundant MS$^2$ fragment ion at m/z 325.

Scheme 3.3. Proposed mechanism for the dissociation of [(PCl$_2$)$_4$+H$_2$O]$^+$ (m/z 477), the 4-mer from the distribution A in the chlorobenzene soluble fraction, via PCl$_5$ loss, leading to the fragment ion at m/z 271.

Figure 3.12 illustrates the MS$^2$ (CAD) spectrum of the 5-mer, m/z 592. Its fragmentation pathways follow the same pattern observed for the 4-mer. The loss of a Cl$_2$ molecule from the parent ion ($\Delta = 70$ Da) leads to the fragment ion at m/z 522. The fragment at m/z 440 arises from the loss of 151.9 Da, through a mechanism (Scheme 3.4) analogous to the one in scheme 3.2; in both cases, the radical site is on the P atom at the chain end and the OH group on the next P atom down the chain (cf. Scheme 3.2 and scheme 3.3). This loss gives rise to the base peak. The peak at m/z 325 appears exactly
one polymer repeat unit lower than the peak at m/z 440, suggesting the occurrence of a similar fragmentation mechanism, as outlined in Scheme 3.4; here, the radical and hydroxyl group are attached to the P atoms located one repeat unit down the chain.

Figure 3.12. CAD tandem mass spectrum of the ion at m/z 592 corresponding to the polydichlorophosphazene 5-mer (P₅N₅Cl₁₀H₂O) from the distribution A in the chlorobenzene soluble fraction. The spectrum was acquired in negative mode on an ESI-Q/ToF mass spectrometer at a collision energy of 35 eV.

PCl₅ loss (Δ = 205.8 Da) from the parent ion also take place, producing the fragment at m/z 386; this reaction was also observed in the MS² spectrum of the 4-mer. A fragment having the composition P₂Cl₄NO, presumably ⊀O-PCl-N=PCl₃, is also detected, at m/z 231.8. The isotope distributions of all mentioned fragment ions were also investigated and conclusively support the given molecular compositions.
Scheme 3.4. Proposed mechanism for the major dissociations of the 5-mer (m/z 592.58) and 7-mer (m/z 822.42) from distribution A in the chlorobenzene soluble fraction. Depending on the P atom carrying the OH group, an n-mer from this dissociation loses up to n-3 radicals with the composition H₂N-P₃Cl₂-(N=PCl₃)ₓ-Cl, x = 0 to (n-4).

Based on the MS² data, the only architecture that can reconcile the fragment ions observed is the tadpole arrangement, having a head composed of three repeat units and a linear tail. All fragments observed in the MS² spectrum of the 4-mer carry at least three repeat units. Also all major fragments from the 5-mer contain at least three repeat units; here, the MS² spectrum includes a fragment with two repeat units, resulting from cleavage of the tail which, in this length, is absent in the 4-mer. A cyclic arrangement for
the A oligomers in the chlorobenzene fraction would have given rise, after ring opening, to fragments with less than three repeats units.

In order to add more corroborating evidence to the previous results, an additional MS\(^2\) experiment was carried out on the 7-mer, whose spectrum is shown in Figure 3.13.

![Figure 3.13. CAD tandem mass spectrum of the ion at \(m/z\) 822 corresponding to the polydichlorophosphazene 7-mer (P\(_7\)N\(_7\)Cl\(_{14}\)H\(_2\)O) of from distribution A in the chlorobenzene soluble fraction. The spectrum was acquired in negative mode on a ESI-Q/ToF mass spectrometer at a collision energy of 45 eV.](image)

The most intense and structurally diagnostic fragment ions observed in the spectrum belong to the fragmentation series labeled as *, which arises from losses of (151.9 + 114.9 x) Da (x = 0-3), where x indicates the number of repeat units. As it has been explained earlier, the 151.9-Da loss contains one repeat unit (114.9 Da), two hydrogen atoms and one chlorine atom (cf. Scheme 3.2 and 3.4). This fragmentation breaks the P-N bond to which H\(_2\)O was added (Scheme 3.2), leaving an oxygen atom on the phosphorus atom that carries the negative charge. This series ends with the fragment ion at \(m/z\) 325,
which contains three repeat units, not only for the 7-mer (Figure 3.13 and Scheme 3.4) but also for the 4-mer (Figure 3.11) and the 5-mer (Figure 3.12). A completely linear oligomer, or a cyclic after opening of the ring, would have also produced fragment ions with two repeat units within the * series, which are instead lacking in all the MS\(^2\) spectra analyzed. The fragment series * derives from P-N bond dissociations in the linear tail up to the trimeric head, as indicated in Figure 3.13.

The different fragment ions observed in the MS\(^2\) spectra can be rationalized by a combination of radical-induced and charge-induced dissociations, accompanied by atom rearrangements, as shown in Scheme 3.2 and 3.3 for the losses of a \(\text{P}^+\text{Cl}_3\)-NH\(_2\) radical and a PCl\(_5\) molecule, respectively, from the polychlorophosphazene 4-mer with the composition [(PCl\(_2\)N)\(_4\) + H\(_2\)O]\(^+\). One further mechanistic rationalization, explaining the Cl\(_2\) loss, is presented in Scheme 3.5.

![Scheme 3.5. Proposed mechanism for Cl\(_2\) loss from the polydichlorophosphazene n-mers in distribution A of the chlorobenzene fraction.](image)

ESI ionization generates mainly radical anions as discussed above (cf. Scheme 3.1). During CAD these ions undergo dissociations promoted by the unpaired electron or
the negative charge, giving rise to different fragment ions. The more electronegative nitrogen atom is the primary charge location (Scheme 3.2-3.5). The main fragmentation pathway involves heterolytic cleavage of the P-N bond, induced by the anion, and concomitant transfer of the proton of the hydroxyl group to the nitrogen, which leaves as a neutral radical (cf. scheme 3.2). The resulting fragment ion carries the negative charge on the oxygen atom. Because the initial radical formation can occur at any phosphorus atom of the polymers, a series of fragment ions differing in mass by a repeat unit is generated. This specific dissociation can result in fragments only if it occurs at the N-P bonds of the tadpole tail, justifying that the fragments of this series contain at least three repeat units. Fragment formation by the same mechanism from the head of the tadpole would have required the cleavage of two bonds and it is therefore not observed (energetically not feasible). The loss of Cl₂ from the parent ion occurs when the radical is formed on the second phosphorus atom from the end of the linear tadpole tail, Scheme 3.5. Instead the loss of PCl₅ appears to be initiated by an initial radical formation at the last phosphorus atom of the tadpole tail (Scheme 3.3). This dissociation is absent from the longer oligomers (Figure 3.13), presumably because it requires interaction of the terminal -PCl₃ group with the tadpole head, as shown in Scheme 3.3.

The oligomers belonging to the distribution A of the hexane soluble fraction in negative ESI mode did not undergo structural indicative fragmentation at any collision energy. At very high collision energies, the only reaction observed was the loss of just one chlorine atom. This finding implies a higher stability for series A from the hexane soluble fraction compared to series A from the chlorobenzene soluble fraction. The different fragmentation chemistries can be reconciled by the presence of one water
molecule in the oligomers of the chlorobenzene phase, which apparently facilitates fragmentation. The MS² spectra of the oligomers in the chlorobenzene soluble phase are consistent with a tadpole arrangement for this product; on the other hand, no conclusion can be unambiguously deducted for the polymer observed in the hexane phase from the MS data discussed so far, although the literature suggests that these species are cyclic.\textsuperscript{111}

In order to determine if the different polymers in the two distinct phases have similar architecture or not, ion mobility mass spectrometry (IM-MS) has been utilized. IM-MS can differentiate isobaric and isomeric constituents of complex mixtures as well as macromolecular architectures such as linear, cyclic, or branched.\textsuperscript{96,97} IM-MS exploits the motion of ions in a confined region (ion mobility cell), in which the ions travel under the influence of an electric field in the presence of a drift gas flowing in the opposite direction of the ions’ motion. During this process, separation of the ions takes place according to their size, collision cross section (shape) and charge states. All ions experience the same gas pressure and friction. Ions with an extended shape, and thus a longer collision cross section (CCS), travel more slowly than ions with a smaller collision cross section. The larger the CCS the longer the drift time, which is the time an ion spends in the ion mobility section.

The traveling wave IM-MS variant was used for the analysis of the polydichlorophosphazenes;\textsuperscript{94} this capability is available in the Q/ToF mass spectrometer used for the MS and MS\textsuperscript{2} experiments described so far. Ion mobility separation was achieved by tuning the traveling wave velocity and the traveling wave height to 280 m/s and 5 V, respectively. Nitrogen served as IM drift gas at a flow rate of 10 mL/min. All the other parameters were kept the same as for the regular MS acquisition. For this
investigation, two isomeric ions, having the same $m/z$ value, from distribution B of the two phases were considered. The selected ions represent the 6-mers in both samples ($m/z$ 670). As mentioned earlier, these ions have the composition $[(\text{PCl}_2\text{N})_n - \text{Cl} + \text{O}^-]$ and formally arise by substitution of one Cl atom in the polydichlorophosphazene frame by an O$^-$ anion. The drift times measured by IM-MS analysis are different for the two isomers. The ion derived from the B oligomer in the hexane phase shows a lower drift time (3.34 ms) compared to its B homologue in chlorobenzene (4.24). This information by itself indicates that these species have two completely different atomic rearrangements. Furthermore, the longer drift time of the isomer from the chlorobenzene fraction indicates that this species has a more extended architecture than the species from the hexane fraction. Molecular modeling was utilized with the intent to obtain further information about the actual structure of these two compounds. The software used for this modeling was Accelrys Material Studio 5.0. This program performs simulated annealing on 3-D hypothetical structures to optimize their geometry and minimize their energy; this process is then iterated on the products of each cycle. For each oligomer under investigation the software was programmed to perform 100 annealing cycles, with 20 heating ramps per cycle and the initial temperature set at 300 K. The theoretical collision cross section (CCS) each one of the low-energy conformers obtained during each annealing step is calculated and then averaged. The experimental CCS results from the ion-buffer gas scattering process in the IM region; these integrals are difficult to evaluate when dealing with nonspherical systems but, in many cases, simple projection cross sections provide an excellent approximation to the collision integrals. The projection approximation (PA) approach was used to derive the theoretical CCSs. This method has
been shown to yield reasonable CCS predictions for polyatomic systems between 10-200 atoms.\textsuperscript{114} Calculated CCS and experimental drift time data were utilized to unambiguously deduce the right architecture. In the modeling, the tadpole architecture was considered for the compound in chlorobenzene, as also suggested by MS\textsuperscript{2} analysis (vide supra); whereas the cyclic structure was hypothesized for the oligomer in hexane. The results obtained from the molecular modeling and PA calculations showed a higher CCS value for the tadpole architecture (200.046 Å\textsuperscript{2}) compared to the homologous cyclic species (171.714 Å\textsuperscript{2}). A larger CCS corresponds to a more extended architecture. Hence, this result is in tune with the experiment which showed a higher drift time for tadpole molecules (more extended species) and a lower drift time for the cyclic compounds (more compact species). Moreover, investigation of the 7-mer (m/z 785) showed a similar trend between drift time and theoretical CCS. The drift time of the species in hexane was 4.05 ms, while the theoretical CCS associated with the cyclic structure attributed to this ion was 196.016 Å\textsuperscript{2}; on the other hand, the drift time and the theoretical CCS associated to a tadpole structure for the analogous 7-mer in the chlorobenzene soluble fraction were significantly higher, viz. 5.42 ms and 218.327 Å\textsuperscript{2}, respectively, Figure 3.14.
Figure 3.14. Extracted ion mobility chromatograms for the 6-mer and 7-mer from distribution B of the two species detected in the chlorobenzene soluble (right) and hexane soluble (left) phases.

The dependence of drift time/CCS on oligomer shape (architecture) is clearly demonstrated by a comparison of the 6-mer and the 7-mer. The 6-mer tadpole results in a higher drift time than the 7-mer cyclic species, despite its smaller mass; this trend confirms that the tadpole 6-mer occupies more 3D space than the cyclic 7-mer, because of a more extended geometry, as also predicted by the corresponding theoretical CCS difference.

The information gathered from both the MS² analysis as well as the IM-MS investigation provides convincing evidence that the species detected in the chlorobenzene soluble phase in negative ESI mode has a tadpole architecture composed of a trimeric ring as head and a polymeric tail carrying an additional chlorine atom at the terminal P atom. Note the absence of a 3-mer, which would be a trimeric cyclic species (the tadpole
head), as trimeric and tetrameric rings were sublimed out of the reaction products before the liquid-liquid separation. On the other hand, the MS$^2$ and IM-MS results confirm that the dichlorophosphazene species detected in the hexane phase in ESI negative mode possess a cyclic structure.

3.3 Characterization of polyphosphazenes with azolylphenoxy side groups by MALDI mass spectrometry

Hundreds of different polyphosphazenes have been synthesized.$^{111}$ These hybrid inorganic-organic polymers with inorganic elements in the backbone and organic side-groups can be designed to have different properties by changing the organic side groups. They have been employed in the production of new proton conducting membranes for fuel cells, where polyphosphazenes serve as alternative polymer electrolyte membranes. Some of the most thermally and chemically stable polyphosphazenes are aryloxy derivatives. Aryloxy side groups can be modified to be sites of proton transportation in the proton-conducting membranes.

The general synthetic route to this class of phosphazenes is depicted in Figure 3.15. Poly[bis(aryloxy) phosphazenes] are obtained from (PCl$_2$N)$_a$ and phenol derivatives by using a strong base, such as KH, in THF.
Figure 3.15. Different aryloxy derivatized phosphazenes obtained by SN$_2$ reactions at the P-Cl site. Poly[bis(aryloxy) phosphazene] (1); poly[bis(4-imidazol-1-yl)phenoxy) phosphazene] (2); poly[bis(4-(1,2,4-triazol-1-yl)phenoxy) phosphazene] (3); poly[bis(4-cyanophenoxy) phosphazene] (4); poly[bis(4-tetrazolylphenoxy) phosphazene] (5).

3.3.1 Experimental section

The hybrid polyphosphazenes studied in this dissertation were synthesized by Dr. Supat Moolsin in the research group of Professor Wiley J. Youngs (The University of Akron). The starting polydichlorophosphazene was synthesized by adding LiN(SiMe$_3$)$_2$ (30.0 g, 179 mmol) and dry toluene (150 mL) to a 500-mL Schlenk flask under a N$_2$
purge. The clear solution was stirred at 0 °C. By a cannula, a the solution of PCl₃ (15.6 mL, 179 mmol) and dry toluene (30 mL) was added dropwise to the stirred flask containing LiN(SiMe₃)₂ over a period of 1 hour at 0 °C. The resulting solution mixture was continuously stirred at 0 °C for 30 minutes and at room temperature for 1 hour. Then, the solution of SO₂Cl₂ (14.7 mL, 183 mmol) in dry toluene (30 mL) was added dropwise by a cannula at 0 °C. After stirring for 1 hour, PCl₅ was used to initiate condensation polymerization at 0 °C. The temperature of 100 °C was applied to accelerate the polycondensation overnight. The solution mixture was filtered through Celite® and washed with dry toluene. Evaporation of the solvent gave a yellow viscous liquid. Dry THF (10 mL) was added to the viscous liquid. By cannula, the solution was transferred to another Schlenk flask containing dry hexane. The polymer, which is insoluble in dry hexane, stuck to the bottom of the flask whereas the small molecule impurities and byproducts dissolved in hexane. The polymer was redissolved in dry THF. The insolubility in hexane is an indication of the non cyclic structure of the polymer, as has been discussed previously for the products arising from the reaction of NH₄Cl and PCl₅. Evaporation of volatile components gave the polymer as a yellow viscous liquid.

The different polyorganophosphazenes were synthesized in a Schlenk flask by using the respective substituted phenol and KH in THF. The solution containing (PCl₂N)ₙ (0.50 g, 4.3 mmol) and dry THF (40 mL) was transferred to the flask by a cannular. The resulting clear yellow solution was stirred and refluxed under N₂ for several days, depending on the phenol used. Filtration, evaporation and washing with water after the reaction was completed yielded the substituted polyorganophosphazene.
The mass spectrometry analysis of this less stable and more reactive poly(dichlorophosphazene) was carried out by using a quadrupole ion trap (QIT) mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an electrospray ionization (ESI) source operated in negative ion mode. The sample investigated was completely dissolved in dry CHCl₃ at a final concentration of 0.5 mg/mL and directly infused in the mass spectrometer at a flow rate of 3 µL/min.

The different poly(organophosphazene)s, which are air stable, were characterized by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The MALDI time-of-flight (ToF) mass spectra were recorded on two different platforms, either a Bruker Reflex-III ToF (Bruker Daltonics, Bellerica, MA) or a Bruker Ultraflex III ToF/ToF (Bruker Daltonics, Inc., Bellerica, MA), equipped with a LSI model VSL-337ND pulsed 337 nm nitrogen laser (3 nm pulse width) or a Nd:YAG laser emitting at a wavelength of 355 nm, respectively. Both instruments contain a single-stage pulsed ion extraction source and a two-stage gridless reflectron. All analyses were performed by operating the mass spectrometers in negative mode in order to detect the negative ions generated in the source. The samples designated for the MALDI analysis were dissolved in the appropriate solvents at a final concentration of 10 mg/mL. The MALDI matrix used was α-cyano-4-hydroxycinnamic acid, which was dissolved in ACN/H₂O (70:30 v:v) at a concentration of 20 mg/mL. Both solutions were mixed in the ratio matrix:polymer (10:2). No ionizing agent was added for the analysis. 0.5 µL of the resulting mixture was deposited onto a MALDI sample target and allowed to dry.
3.3.2 Result and discussion for the Characterization of polyphosphazenes with azolylphenoxy side groups by MALDI mass spectrometry

The analysis by ESI of poly(dichlorophosphazene) shows different polymer distributions, each one having a difference in mass between two consecutive oligomers of 114.9 Da, which is representative of the mass of one PCl$_2$N monomer unit (calculated mass 114.91 Da) Figure 3.16.

![Figure 3.16. Mass spectrum of polydichlorophosphazene synthesized from LiN(SiMe$_3$)$_2$ and PCl$_3$, acquired with a Bruker Esquire ESI QIT.](image)

The main polymer distribution observed in the mass spectrum, labeled as β, arises by the substitution of one chlorine atom with an oxygen atom that carries negative charge ( -Cl => -O$^-$). This substitution could occur either during the exposure of the polymer to air before the analysis or upon the ionization process. The other two minor distributions...
are generated by the addition of one water molecule to the polymer (M) via radical anion formation (α), [M+H₂O]⁻, and by a loss of one chlorine atom from the major polymer distribution (γ). The m/z values of the oligomers in each polymer distribution do not indicate any nominal end groups. This result is in agreement with what was observed for the polydichlorophosphazene oligomers synthesized by mixing NH₄Cl and PCl₅. The only difference observed is that this polymer contains an additional distribution (γ), which may originate either from the synthesis or from the exposure of the polymer to air during the sampling or analysis. It can also be a byproduct generated inside the ion trap due to the moisture present in the instrument. This aspect will be examined investigated later in this chapter with the analysis of hexachlorocyclotriphosphazene.

Analysis of poly[bis(phenoxo) phosphazene] by MALDI gave rise to the mass spectrum shown in Figure 3.17.

![Figure 3.17. Mass spectrum poly[bis(phenoxo) phosphazene] acquired with a Bruker Reflex III MALDI ToF mass spectrometer in negative mode.](image-url)
The different polymer distributions detected have a repeat unit of 231 Da, which is equal to the mass of one monomeric unit (calculated monoisotopic mass = 231.05 Da). The major polymer distribution (β) originates by the addition of a hydroxyl anion after a hydrolysis reaction at the phosphorus atom that replaces one lateral chain group (-OR) with a hydroxyl group (-OH), Δ=76 Da. The polymer distribution α arises from the addition of one hydroxyl anion to the intact polymer during the ionization, [M+OH]⁺; whereas the distribution γ is formed by deprotonation of the hydrolyzed product or by the loss of one lateral chain (ROH) from the distribution α, Δ = 94 Da. No radical ions are observed from the polyorganophosphazenes as compared to polydichlorophosphazenes due to the different ionization mechanism.

MALDI analysis of poly[bis(4-(imidazol-1-yl)phenoxy) phosphazene] gave two major distributions with repeat units of 363 Da, which match the mass of one monomer unit (calculated monoisotopic mass 363.09 Da), Figure 3.18. The distribution β is generated by a hydrolysis reaction at the phosphorus atom followed by deprotonation. The second distribution γ results from an additional hydrolysis reaction. The difference in mass between the two polymer distributions is 142 Da, reflecting the mass difference between one –OR group (159 Da) with the -OH functionality (17 Da).
Figure 3.18. Mass spectrum of poly[bis(4-(imidazol-1-yl)phenoxy) phosphazene] acquired with a Bruker Ultraflex MALDI ToF/ToF mass spectrometer in negative mode.

The MALDI mass spectra obtained from poly[bis(4-(1, 2, 4-triazol-1-yl)phenoxy) phosphazene] (Figure 3.19), and poly[bis(4-cyanophenoxy) phosphazene] (Figure 3.20), display polymer distributions having repeat units of 365 Da and 281, respectively, which are indicative of the masses of their monomeric units (calculated masses: 365.08 Da and 281.04 Da, respectively).

Figure 3.19. Mass spectrum of poly[bis(4-(1,2,4-triazol-1-yl)phenoxy) phosphazene] acquired with a Bruker Ultraflex MALDI ToF/ToF mass spectrometer in negative mode.
For both polymers, the main distributions (α) are generated by cluster formation with a hydroxyl anion, \([M+OH]^{-}\), whereas the second distributions (β) are derived by the hydrolysis reaction at the phosphorus atom already observed for the other polyorganophosphazenes investigated, followed by OH⁻ addition.

The cyano groups of poly[bis(4-cyanophenoxy) phosphazene] can be transformed into tetrazole groups using NaN₃ in the presence of NH₄Cl in DMF, followed by the addition of 3 N HCl, which gives rise to poly[bis(4-tetrazolylphenoxy) phosphazene]. The MALDI-ToF mass spectrum of this polymer is shown in Figure 3.20. Two distributions are discerned, having a repeat unit of 367 Da, which is equal to the mass of one monomer unit (calculated monoisotopic mass 367.07 Da). Following the same trend observed for the previously discussed polymers, one distribution (β) arises from oligomers ionized by OH⁻ addition after one lateral chain (-OR) was hydrolyzed to a hydroxyl group (-OH). A further hydrolysis reaction gives rise to the second polymer
distribution (γ). The difference in mass between the two distributions (144 Da) confirms the occurrence of this reaction.

Figure 3.21. Mass spectrum of poly[bis(4-tetrazolylphenoxy) phosphazene] acquired with a Bruker Ultraflex MALDI ToF/ToF in negative mode.

Tandem mass spectrometry characterization of some of these polymers provided additional evidence on the tadpole structure already established for polydichlorophosphazenes. This is not surprising, considering that the substitution reaction replaces only the chlorine atoms without affecting the polymer backbone. For this MS² study, the 4-mer and the 5-mer of the main polymer distribution (α) of poly[bis(4-(1,2,4-triazol-1-yl)phenoxy) phosphazene] were isolated and fragmented in the Waters Ultima MALDI Q/ToF mass spectrometer using argon as collision gas. The resulting tandem mass spectra are reported in Figure 3.22.
Figure 3.22. Tandem mass spectra of the 4-mer (A) and 5-mer (B) of the $\alpha$ distribution from poly[bis(4-(1, 2, 4-triazol-1-yl)phenoxy) phosphazene], obtained with a MALDI Q/ToF mass spectrometer operated in negative mode, at a collision energy of 55 eV.

Both spectra are characterized by consecutive lateral chain losses, labeled with a diamond, and repeat unit evaporation from the precursor ion, labeled with a cross. The lateral chains are cleaved either in the form of X-Ph-OH phenols (161 Da) or as X-Ph' radicals (144 Da). Dissociation products that are diagnostic for that tadpole architecture are observed in the spectra of both oligomers; these are the fragment ion at $m/z$ 950.5 from the 4-mer and fragment ions $m/z$ 1315.4 and $m/z$ 950.6 $m/z$ from the 5-mer. Pertinent mechanistic rationalizations are presented in Scheme 3.6.
Scheme 3.6. Proposed mechanism for the charge-induced fragmentation in the tail of poly(organophosphazene)s with tadpole architecture, promoted by attachment of the hydroxyl anion to one of the tail P atoms; from the poly[bis(4-(1,2,4-triazol-1-yl)phenoxy phosphazene], this pathway produces the fragments at \( m/z \) 950 and 1315. From the 4-mer, only \( m/z \) 950 can be generated.

There is no electrochemical reaction involved the MALDI process; hence, \([M+OH]^-\) the quasi-molecular ions are generated by cluster formation with hydroxyl anion, which are formed from the matrix during the MALDI. Most likely, the hydroxyl anion attaches to a P atom on the polyphosphazene leaving the negative charge on the adjacent N atom. A very similar process was proposed to rationalize the addition of a water molecule to polydichlorophosphazene. The only difference in this case it is that there is no radical formation.

The fragment ions at \( m/z \) 950 in both spectra and \( m/z \) 1315 in the spectrum of the 5-mer are derived from precursor ions that carry the hydroxyl attached to a P atom of the tail of the tadpole architecture. Abstraction of the hydroxyl proton by the adjacent negatively charged nitrogen atom induces cleavage of the P-N bond and concomitant formation of a neutral molecule having a P=O double bond (cf. Scheme 3.6). Depending
on the size of the n-mer and the position of the OH$^-$ addition, (RO)$_3$P=O or (RO)$_3$P=N-(RO)$_2$P=O molecules are lost to yield [P(OR)$_2$N]$_3$-(P(OR)$_2$N)$_n$-NH$^-$ (n = 0, 1) fragments which diagnose the tadpole structure and size. Addition of the OH$^-$ anion to one the the P atoms in the tadpole head, on the other hand, promotes P-N bond cleavages between the tail and head (Scheme 3.7, top) or within the tadpole head (Scheme 3.7, bottom, and Scheme 3.8) which ultimately lead to the other backbone fragments observed in the MS$^2$ spectra, viz. m/z 524.8 and the fragments generated by nominal monomer evaporations.
Scheme 3.7. Proposed mechanism for the charge-induced fragmentation in the tadpole head of the 5-mer from poly[bis(4-(1,2,4-triazol-1-yl)phenoxy phosphazene], promoted by the attachment of the hydroxyl anion to the ipso atom. Depending on the bond broken at the ipso P atom, this pathway leads to the elimination of the head (m/z 890) and head plus monomer (m/z 525) or to the elimination of one and two monomers (m/z 1477 and 1112).
Scheme 3.8. Proposed mechanism for the charge-induced fragmentation in the tadpole head of the 5-mer from poly[bis(4-(1,2,4-triazol-1-yl)phenoxy phosphazene], promoted by attachment of the hydroxyl anion to the head P atom; this pathway produces \( m/z \) 747 and 383, corresponding to losses of overall three or four monomer units, respectively.

3.4 Investigation of Lewis and Brönsted acid adducts of hexachlorocyclotriphosphazene

An advantage of polyphosphazenes over classical organic polymers is the simplicity of their functionalization. The highly reactive chlorinated polymer \((\text{PCl}_2\text{N})_n\), which is the common starting material of an array of polyphosphazenes, can easily be functionalized by replacement of the chlorine atoms with specific functional groups. These substituents can be organic, organometallic or inorganic. The overall properties of the resulting polymer are tuned by varying the functional groups. In the case of organic polymers, on the other hand, a monomer with the desired properties needs to be first
synthesized and then polymerized.\textsuperscript{115} Although during the last decades a variety of polyphosphazenes have been synthesized and applied to different fields, these polymers are still not used in industry. The reasons that make the industrial production of this polymer inadequate compared to other organic polymers are the inefficient, irreproducible and expensive synthesis of the polydichlorophosphazene, which is the starting polymer, and the high tendency of the latter polymer to degrade upon storage. Different synthetic routes have been investigated with the aim to develop a more reproducible method to prepare this polymer with a higher yield, but up to date ROP appears to be the only synthetic pathway that can generate a high molecular weight polymer. The evidence that certain Lewis acids seem to help in some aspect the ROP and the fact that small traces of water have been observed to be necessary for the ROP have motivated scientists to investigate the acid-base properties of the dichlorophosphazene compounds.\textsuperscript{116} This information would help to understand the role of this chemistry in both the synthesis and storage of chlorophosphazenes and determine how the development of protonic and acidic impurities, whose origin is unclear since the only known acidic “impurities” (HCl and NH\textsubscript{4}Cl) are removed during the synthesis, affect these compounds. It is known that the basicity of the nitrogen atoms on the phosphazenes backbone is influenced by the side groups attached to the phosphorus atoms by a hyperconjugation effect.\textsuperscript{111} The halide substituted phosphazenes are very weak bases, in fact their N atoms do not protonate even in the presence of strong acids such as hydrochloric acid. Their protonation is believed to occur only in the presence of a superacid. Water-sensitive, metal-halide Lewis acids can react with water and become strong Brønsted-Lowry acids.\textsuperscript{117} Water can hydrolyze the metal-halogen bond in the
Lewis acid MX\textsubscript{n} to form the Brønsted acid HX, which in turn can react with a second molecule of the Lewis acid MX\textsubscript{n} to form HMX\textsubscript{n+1}. This species (HMX\textsubscript{n+1}) is a stronger Brønsted-Lowry acid than the parent HX because the MX\textsubscript{n+1} anion is more weakly coordinating than X\textsuperscript{-}. Sometimes superacids are described as HX/MX\textsubscript{n} because these systems are complex and different cations and anions can be present depending on the concentration of MX\textsubscript{n} in solution. It has been proposed that metals in Lewis acids might coordinate with cyclodichlorophosphazenes at the N atom\textsuperscript{118}, Figure 3.23, but under less anaerobic conditions this system could easily convert to conjugate Brønsted-Lewis superacids as described above.

![Figure 3.23. Proposed adduct formation between Lewis acids and hexachlorocyclotriphosphazene followed by Cl\textsuperscript{-} abstraction from the cyclophosphazene by the acid.](image)

Different attempts of forming these very air sensitive complexes between (PCl\textsubscript{2}N)\textsubscript{3} with Lewis acids have been reported. Unfortunately, the lack of characterization by modern analytical techniques has led to contradicting interpretations about their identity.

For more information on this issue, the reactivity of different Lewis acids, such as GaCl\textsubscript{3}, AlCl\textsubscript{3}, SbCl\textsubscript{5} and AlBr\textsubscript{3}, with hexachlorocyclotriphosphazene have been
investigated under less strict anaerobic conditions by ESI mass spectrometry in order to examine both the cluster and superacid formation. Because of their high tendency to degrade in air, \((\text{PCl}_2\text{N})_3\) and the Lewis acids were both kept and mixed in a glove box using dry solvents. The different phosphazene-acid mixtures were prepared for mass spectrometry analysis following the procedure used for the sampling of polydichlorophosphazene as described previously, using CHCl₃ as solvent. The analysis was carried out on a SYNAPT HDMS™ Q/ToF mass spectrometer (Waters, Beverly, MA). The instrument was operated at an ESI capillary voltage of 3.5 kV, sample cone voltage of 30 V and extraction cone voltage of 3 V; the desolvation gas flow was 500 L/h (N₂); the source temperature and desolvation gas temperature were 40 °C and 50 °C, respectively, in order to avoid or minimize any thermal degradation of the sensitive products. The sample flow rate was set at 10 µL/min. Both positive and negative modes were investigated.

Mass spectrometry analysis of each different system did not provide evidence for the formation of a stable adduct between the phosphazene species and the Lewis acid as proposed in Figure 3.23. The spectrum indicates instead the occurrence of an interaction between the two species via protonation of the N atom on the phosphazene, Figure 3.24

![Figure 3.24. Protonation reaction at the N on the phosphazene triggered by proton donor impurities.](image)

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This process is confirmed by detection of the protonated \([\text{PCl}_2\text{N}]_3\) when the instrument is operated in positive mode with all systems investigated, as showed in Figure 3.25.

Evidence for the protonated compound is provided not only by the high accuracy of the mass measurement (≈ 15 ppm or better) but also by comparing the unique isotope distribution of the observed ion with the theoretical isotope distribution expected for the \([\text{M+H}]^+\) composition.

![Graph showing mass spectrum with experimental and theoretical data](image)

**Figure 3.25.** Partial ESI mass spectrum of hexachlorocyclotriphosphazene (M) mixed with \(\text{GaCl}_3\). The experimental isotope distribution (bottom) is compared to the expected for the \([\text{M+H}]^+\) ion (top) for conclusive compound identification. The same spectrum is obtained with all Lewis acids examined.

The protonation at the N atom of the dichlorophosphazene can only be justified if a superacid is generated during the reaction.

Mass spectrometry analysis of the same systems in negative mode revealed the presence of the respective counterion species of the superacids, \(\text{GaCl}_4^-\), \(\text{AlCl}_4^-\) and \(\text{SbCl}_6^-\).
but not AlBr$_4^-$. Furthermore, a comparison between the theoretical and the experimental isotope distribution of these anions was also performed to confirm the identity of different species detected (Figure 3.26-3.28).

Figure 3.26. Theoretical isotope distribution (top) and experimental isotope distribution (bottom) for GaCl$_4^-$, obtained with a Waters Synapt ESI Q/ToF mass spectrometer operated in negative ion mode.
Figure 3.27. Theoretical isotope distribution (top) and experimental isotope distribution (bottom) for $\text{AlCl}_4^-$, obtained with a Waters Synapt ESI Q/ToF mass spectrometer operated in negative ion mode.

Figure 3.28. Theoretical isotope distribution (top) and experimental isotope distribution (bottom) for $\text{SbCl}_6^-$, obtained with a Waters Synapt ESI Q/ToF mass spectrometer operated in negative ion mode.

Although the anion for the system containing AlBr$_3$ as Lewis acid could not be detected by mass spectrometry, the presence of protonated $[\text{PCl}_5\text{N}]_3$ suggested that the
respective Brønsted-Lewis superacid was formed. Unlike the other systems, the superacid originating from AlBr$_3$ has a higher tendency to degrade under ESI mass spectrometry conditions.

With all systems investigated, the intact adducts Lewis acid-[PCl$_2$N]$_3$ were not detected due their very labile characters; instead, the adducts probably react with water to give protonated species. It is also possible that the clusters containing the Lewis acid attached directly to the N on the chlorocyclophosphazene (Figure 3.23) were also present in the mixtures, but decomposed to the detected products either during their sampling or the MS analysis.

Nevertheless, the data presented in Figure 3.25-3.28 provide strong evidence for the formation of superacids from dichlorophosphazenes.

3.5 General observations on the analysis of phosphazenes by mass spectrometry

As described in the previous sections of this chapter, dichlorophosphazenes have a higher tendency to form negative ions in ESI mass spectrometry experiments. This typical ionization involves either radical anion formation, with the radical centered at the P atom, which may be followed by the addition of a water molecule, or replacement of a chlorine atom with an oxygen atom that bears negative charge. Due to the fast degradation of the radical anions, the species carrying O$^-$ becomes the prevalent ion at long lifetime. In experiments carried out on an ion trap mass spectrometer which probe
long-lived ions, the most abundant species always are those bearing O\(^-\) substitution, i.e. [M-Cl+O\(^-\)]. The radical anions resulting by water addition (from the tadpole architecture) are depleted by dissociation, while the radical anions from the macrocycles (M\(^+\)) are most probably depleted by reaction with residual water in the ion trap which converts them to [M-Cl+O\(^-\)]. Thus, for the (PCl\(_2\)N)\(_3\) trimer, the only ion detected in the ion trap in negative ESI mode corresponds to phosphazene species having one chlorine atom replaced by an oxygen anion. Obviously, no ion with this composition is observed by switching polarity to positive mode. As described in the previous section, dichlorophosphazene compounds are observed in positive ESI mode only in the presence of a superacid, cf. Figure 3.29.

Figure 3.29. Mass spectra on top of (PCl\(_2\)N)\(_3\) acquired in (a) negative ESI mode and (b) positive ESI mode after mixing (PCl\(_2\)N)\(_3\) with GaCl\(_3\) under less anaerobic conditions. Both mass spectra were recorded on a Bruker Esquire QIT mass spectrometer.
Tandem mass spectrometry experiments on the protonated trimeric cyclodichlorophosphazene performed on the ion trap illustrate its high reactivity with the very small amount of water vapor present in the analyzer, Figure 3.30. The initial loss of HCl from the parent ion is followed by addition of a water molecule, and resulting species follows the same iterative process. Alternatively, H2O addition to the parent ion may occur first to yield a transient adduct that loses HCl, forming a product ion that undergoes a new addition/elimination. These reaction sequences cause up to four Cl/OH substitutions within the milliseconds residence time of the ion in the QIT.

Figure 3.30. MS² (CAD) spectrum of protonated (PCl₂N)₃ acquired with the Bruker Esquire QIT, indicating that the fragment ions react the water present inside the trap.
Once the Cl substituents in dichlorophosphazenes have been replaced with OR groups, ionization to positive ions is facile, either by protonation or adduction of metal ions such as Na⁺, Li⁺ or Ag⁺, depending on the specific functional group in the side chains. In the following examples a cyclic organophosphazene trimer carrying substituted phenoxy groups as lateral chains was cationized by sodium ion addition using ESI on a Bruker Daltonics Esquire ion trap mass spectrometer. The charge is presumably attached to one of the oxygen atoms of the lateral chains, as Na⁺ has a high sodium ion affinity. If a tandem mass spectrometry experiment is performed on this specific sodiated quasi-molecular ion \([\text{M+Na}]^+\) at \(m/z\) of 1196.4, the resulting MS² spectrum shows, along with different fragment ions, a peak that is 18 Da higher than the precursor ion. This behavior is in tune with what has been stated above about the interaction of phosphazenes cations with water molecules present in the ion trap during their activation for MS² experiments. Most of the other MS² fragment ions are generated by eliminations of the phenyl substituent in the lateral chain (68 Da) or the entire lateral chain (174 Da). These reactions do not occur in a regular, repetitive manner to provide straightforward information about the sample structure (Figure 3.31, top spectrum). By switching the polarity of the instrument, in order to detect the negative ions generated in the ion source, an ion at \(m/z\) 1016.3 is observed which is identified as the phosphazene compound under investigation with one of its lateral chains substituted by an O atom bearing negative charge. This behavior is very similar to that encountered in the analysis of dichlorophosphazene compounds with an ion trap mass spectrometer. Tandem mass spectrometry experiments on this specific anion generate a different MS² spectrum, as compared to the MS² spectrum arising from the sodiated ion in positive mode.
Structurally indicative fragments are generated by losses of the pirazolyl moiety (68 Da), or the total side group (174 Da) or one complete repeat unit (391 Da), cf. Figure 3.31, bottom. The latter reaction points out that the oxygen anion can induce ring opening which triggers the loss of a repeat unit via charge-induced dissociation. No addition of water molecules to the precursor ion is detected, but water addition to the fragments that have lost the lateral chain does occur. Interestingly, H$_2$O is added to the fragment missing one lateral chain (m/z 842.2), while the fragment that lost two lateral chains (m/z 668.1) accepts up to two H$_2$O molecules.

Figure 3.31. Tandem mass spectra of a substituted-phenoxy trimeric cyclophosphazene acquired with a Bruker HCT Ultra II ESI-QIT mass spectrometer in both positive (top) and negative (bottom) ESI modes.
Since collisionally activated dissociation (CAD) of the positively charged (sodiated) organophosphazene did not provide any particularly useful information, electron transfer dissociation (ETD) was considered and applied. ETD, which is an alternative tandem mass spectrometry technique to the classical CAD, involves the transfer of an electron from a reagent radical anion (fluoranthene anion) to the isolated multiply charged precursor cations. The ion selected for these investigation was the doubly charged sodiated organophosphazene \([\text{M+2Na}^2+]\), \(m/z\) 609.7. CAD on this precursor ion produces only the singly charged sodiated ion, \(m/z\) 1196.3, and no fragment, cf. Figure 3.32, top spectrum. On the other hand, ETD on the same precursor ion gives rise not only to the respective singly charged ion, as observed in the CAD spectrum, but also to a fragment at \(m/z\) 1062.3. Two isobaric reasonable structures can be assigned to this fragment ion as depicted in the MS\(^2\) (ETD) spectrum, Figure 3.32, bottom. One structure contains a salt bridge between one oxide anion, formed by the loss of the organic piece of the lateral chain, and a sodium cation, the second sodium cation providing the charge. The other isobaric structure is instead accounted for by the elimination of two pyrazolyl radicals (2 x 67 Da) from two lateral chains, induced by the ion-ion reaction with the reagent fluoranthene radical anions inside the trap. This dissociation leaves two unpaired electrons on two distinct 4-methylenephenoxo moieties, which are stabilized by resonance delocalization into the aromatic ring. These radicals can be formed at any of the substituents. The charge of the resulting fragment is provided by a sodium cation.
Figure 3.32. CAD (top) and ETD (bottom) tandem mass spectra of the $[\text{M+2Na}]^{2+}$ ion from the pyrazolyl-phenoxy trimeric cyclophosphazene, acquired using a Bruker HCT Ultra II ESI-QIT mass spectrometer.

In order to determine the correct structure of this fragment, an additional fragmentation step was performed in an MS/MS/MS or MS$^3$ experiment. Basically, the fragment resulting from ETD was selected and excited for further fragmentation via CAD, Figure 3.33.
Figure 3.33. MS$^3$ spectrum of the fragment at $m/z$ 1062 arising from ETD of [M+2Na]$^{2+}$ from the pyrazolyl-phenoxy trimeric cyclophosphazene. The mass spectrometer used was a Bruker HCT Ultra II ESI-QIT.

The MS$^3$ spectrum shows three major losses from the precursor ion: the loss of one lateral chain RH, 174 Da, the loss of one pyrazolyl group ($\Delta = 68$ Da) and a 106-Da loss which gives rise to the base peak. Whereas the repeat unit and the pyrazolyl losses can take place from both structures proposed for the parent ion at $m/z$ 1062, the loss of 106 Da, which is equal to the mass of the 4-methylenecyclohexadienone moiety, can be reconciled only from the structure arising after dissociation of two pyrazolyl radicals, cf. Figure 3.33. In the other, salt-bridge containing structure proposed, this 106-Da loss would be possible only after an initial loss of 68 Da.
3.6 Conclusion

Different phosphazene compounds, both chloro- and organo- substituted, have been successfully investigated by mass spectrometry. A systematic approach aimed at deriving information not only about end groups and polymer composition but also about architecture has been applied and validated. The distinct geometries of two different isomeric polydichlorophosphazene oligomers that were chromatographically separated have been unambiguously assigned as tadpole and cyclic. The tadpole architecture was detected for the first time among the products of the reaction between NH₄Cl and PCl₅. MALDI analysis of the more stable polyorganophosphazenes resulted in a conclusive assignment not only of the end groups of the polymers but also of degradation products arising from hydrolysis reactions at the lateral chains promoted by the presence of water. The formation of superacids by reacting [PCl₂N]₃ with MXₙ under less strict anaerobic conditions or in the presence of HX to yield [PCl₂N]₃HMXₙ₊₁ species was also investigated by ESI mass spectrometry. The soft ESI ionization technique was able to reduce the degradation of the very air-sensitive anions [MXₙ₊₁]⁻, which were successfully detected and identified not only by accurate mass measurements but also by isotope distribution analyses. The observation of the protonated form of the very weak base hexachlorocyclotriphosphazene was an additional proof for the formation of these very strong acid systems. Meanwhile, a palette of different studies to better understand the behavior of phosphazene compounds under mass spectrometry conditions have been carried out. The overall results indicate that the formation of negative ions and their investigation by tandem mass spectrometry provide information about the backbone
structure and end groups of the species, while analogous analysis on the homologous positive ions gives information on the constituents of the lateral chains.
4.1 Introduction

The increasing concern about petrochemical plastics, which are known to persist in the environment for many years after their disposal, has promoted the search for alternative synthetic materials, prepared by environmentally friendly or green chemistry methods. Among the polymers evaluated, aliphatic polyesters are considered to be the best candidates for biodegradable and renewable resources. They have been thoroughly studied and their biodegradation properties are well understood. Some of them, like poly(lactide), exhibit satisfactory biocompatibility, which allows them to be used for implants and other biomedical applications in living organisms.

The characterization of these polymers by mass spectrometry has significantly improved after the discovery of soft ionization techniques, such as matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI), which enable the detection and analysis of each component of the polymeric analyte. The development of instruments with the capability of performing tandem mass spectrometry analysis has also allowed to obtain detailed information related to the polymer connectivity and architecture. The most common technique used to perform tandem MS analysis of polymers is collisionally activated dissociation (CAD). In the last decade other
fragmentation techniques, such as electron-transfer dissociation (ETD)\textsuperscript{14} and closely related electron capture dissociation (ECD),\textsuperscript{126,127,128} which employ ion-ion or ion-electron reactions, respectively, as ion excitation method rather than collisions, have been developed. Although the precise ETD or ECD fragmentation mechanism is not fully understood, the basic process involves the transfer (or capture) of an electron to (from) isolated parent ions, which generates exited radical species that rapidly undergo radical induced cleavages.\textsuperscript{127} This type of fragmentation is very bond specific and non-ergodic, compared to the vibration-induced dissociations that occur in CAD.\textsuperscript{126}

Here, ETD was investigated for the first time as potential new tandem mass spectrometry approach for the characterization of synthetic polymers. The study focused on the application of ETD to polyesters, and the comparison of the resulting MS/MS spectra with those generated by the classical CAD method on the same set of precursor ions. A major goal was to gain a better understanding of the ETD mechanism associated with polymer fragmentation and see if the information obtained can be used for the identification of polymer end groups, architectures and copolymer sequences; such applications would broaden the usefulness of this new technique, which since its discovery has practically been restricted to the analysis of proteins, peptides and their post-translational modifications.\textsuperscript{129}

4.2 ETD vs. CAD fragmentation of polyesters

The polymers investigated were: poly(lactide), purchased from Polymer Source Inc., and poly(ethylene adipate) and poly(butylene adipate), synthesized by the group of Professor Mark Soucek at the University of Akron. All polymers were of low molecular
weight. For the MS analysis, each sample was completely dissolved in methanol:THF (Sigma) 1:1 (v:v) at a final concentration of 0.2 mg/mL. Sodium trifluoroacetate (Fluka), used as cationizing agent, was dissolved in THF at a concentration of 0.2 mg/mL. Sample and the salt solutions were mixed together in a ratio 100:2 (v:v), respectively.

The experiments were performed using a HCT Ultra II quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an electrospray ionization (ESI) source. The sample solutions were injected into the instrument by direct infusion at a flow rate of 3 µL/min. The cone voltage was held at 3.5 kV, the nebulizer gas pressure at 10 psi, and the drying gas flow rate and temperature at 8 L/min and 300 °C, respectively.

After the acquisition of single-stage mass spectra (MS), both CAD and ETD experiments (MS²) were carried out on the same doubly charged precursor ions, [M+2Na]²⁺, from each polyester investigated. The selection of doubly charged ions arose from the nature of the ETD process. ETD involves the transfer of an electron from a radical anion reactant to the isolated precursor ions, inducing basically charge reduction; hence, the requirement of using multiply charged ions, unlike CAD, which can be carried out on precursor ions of any charge state.

CAD experiments were carried out using helium as collision gas and setting the excitation amplitude value in the range between 0.15 and 0.25 (arbitrary units), depending on the precursor ion isolated. For the experiments involving ETD, the anion reagent species were generated in a negative chemical ionization (nCI) source, which was tuned to maximize the generation and transmission of the ETD reagent ions (fluoranthene radical anions) as follows: reagent ion ICC 100.000, ionization energy 70 eV, emission
current 2.0 µA, reactant remove cut off $m/z$ 210 and methane as buffer gas. After the accumulation of both precursor ions and ETD reagent radical anions inside the ion trap, the reaction time was set in the range between 100-150 ms, depending on both the polymer investigated and the precursor ion selected.

The mass spectrum of each sample analyzed showed several distributions of oligomers, all of approximately Gaussian shape, Figure 4.1. The nominal end groups of each polymer distribution present in the spectra were extrapolated first, and the exact structure was then confirmed by tandem mass spectrometry.

The main distribution of each polymer is comprised of linear chains with the following end groups: glycol units at both chain ends for poly(ethylene adipate) and poly(butylene adipate), hydroxyl and methoxyethyl groups for poly(lactide).
Figure 4.1. ESI mass spectra of poly(lactide), poly(ethylene adipate) and poly(butylene adipate); top to bottom respectively. Spectra acquired with the Bruker HCT Ultra II ESI-QIT mass spectrometer.
Other, minor polymer distributions in the spectra are derived most likely from both hydrolysis reactions at the terminal ester groups and cyclization processes. The doubly charged sodiated species of the main polymer distributions, $[\text{M+2Na}]^{2+}$, are also observed. Figure 4.2 shows the $m/z$ regions of the mass spectra of the polymers containing the doubly charged ions selected for tandem mass spectrometry experiments; these were the doubly sodiated ions of the 16-mer from poly(lactide), the 9-mer from poly(ethylene adipate) and the 8-mer from poly(butylene adipate), observed at $m/z$ 637, 828 and 868, respectively.
Figure 4.2. Expanded traces of the ESI mass spectra of poly(lactide), poly(ethylene adipate) and poly(butylene adipate), top to bottom respectively, showing the distributions detected in the analyzed samples. A, main distributions (structures given in Figure 4.1); B, distribution with H- and –OH end group; C, cyclic species (no end group). All ions are sodiated species, either [M+Na]$^+$ or [M+2Na]$^{2+}$. The † label indicates doubly charged sodiated ions.
The CAD spectrum of poly(lactide), Figure 4.3 (top spectrum), shows two distinct fragment ion series having repeat units of 72 Da, the mass of one monomeric unit.

Figure 4.3. CAD versus ETD MS\(^2\) spectra of [M+2Na]\(^{2+}\) (m/z 637) from the 16-mer of poly(lactide). The notation \(l\) indicates linear fragment structure. The superscripts in the fragment notation give the end groups (\(H\), hydroxyl; \(V\), vinyl; \(A\), acid). The * and † labels indicate doubly sodiated monocations and doubly charged fragments, respectively.

The nomenclature used here to classify the different fragments is aimed at providing both their structure, either linear or cyclic, and their end groups. In the case of poly(lactide), all fragment ions observed in the CAD spectrum have linear structure, labeled as \(l_n\). The end groups are given as superscripts, where \(A\) designates the carboxylic acid functionality, \(H\) stays for hydroxy or alkyl capped polyester groups and \(V\) for vinyl or alkene moieties.\(^{12}\) Essentially all fragments are reconciled by random charge-remote
fragmentation along the polymer chain, involving 1,5-H rearrangement via a six
membered ring over the ester group, scheme 4.1. This dissociation mechanism gives rise
to two sets of linear fragments carrying one of the original end groups (H) plus a
carboxylic acid (A) or vinyl (V) functionality, viz. $I_n^{AH}$ (90 Da end group mass) and $I_n^{HV}$
(130 Da end group mass), respectively. Both singly and doubly charged (labeled as $^+$)
fragment ions are observed in the CAD spectrum.

![Scheme 4.1. CAD fragmentation mechanism of poly(lactide).](image)

The ETD experiment performed on the same precursor ions, Figure 4.3 (bottom
spectrum), resulted in a spectrum with completely different fragment ion series, which
confirms the occurrence of distinct fragmentation pathways, initiated at the location of
the electron attachment, Scheme 4.2. The ion-ion reaction of the ETD process involves
the transfer of an electron from the anion radical reagent to the precursor ion; this
electron is most likely transmitted randomly to a carbonyl carbon atom of the ester
functionality. The C-centered radical generated this way is stabilized by delocalization of
the electron along the carbonyl group and by formation of a salt bridge with a sodium
ion, ($O'^-Na^+$).
Scheme 4.2. ETD fragmentation mechanism of poly(lactide): a) radical-induced fragmentation, b) nucleophilic addition/fragmentation.

The added electron promotes radical-induced dissociation of the (CO)O-C bond of the ester group, Scheme 4.2 (a). The two sets of fragments arising from this radical dissociation are labeled as $l_n^{AH+}$ and $l_n^{HV+1}$ or +2, following the same nomenclature used to classify the CAD fragments. The asterisk on the superscript notation of the end groups indicates doubly sodiated singly charged fragments that carry a sodium carboxylate chain end, (CO)O$^-$Na$^+$. On the other hand, the notation +1 identifies a fragment as a radical ion having one more hydrogen atom than the respective $l_n^{AH}$ fragment observed in the CAD spectrum; +2 refers instead to fragments that have accepted a further H$^-$ radical, presumably from the ETD reagent, to form even electron ions, a behavior observed also upon ETD of peptides and proteins. This type of
fragmentation pathway is in tune with the one proposed for ETD of proteins/peptides, where radical formation at the carbonyl carbon atom of the amide group leads to homolytic cleavage of the N-C\textsubscript{\(\alpha\)} bond.

Additional fragment ion series, all with a repeat unit of 72 Da, were generated during the ETD process. The one labeled as l\textsubscript{n}\textsuperscript{AH} is derived by a proton-sodium ion exchange in the l\textsubscript{n}\textsuperscript{AH}\textsuperscript{*} products. The other fragment ions, which do not appear as complete series, are doubly sodiated truncated polymer chains having two hydroxy end groups, l\textsubscript{n}\textsuperscript{HH}*. Most likely these fragment ions arise from a nucleophilic reaction, where the oxy anion created during the ETD step attacks the adjacent carbonyl carbon atom via a cyclic intermediate, which ultimately leads to l\textsubscript{n}\textsuperscript{HH}\textsuperscript{*} ions and a lactone (L) leaving group, Scheme 4-2 (b). The lactone fragment is not observed, most likely because it can undergo facile sequential elimination of methyl ketene radical, 'CH\textsubscript{2}-CH=\textsuperscript{C}=O, to produce the corresponding l\textsubscript{n}\textsuperscript{AH} fragment.

The other two systems investigated, poly(ethylene adipate) and poly(butylene adipate) showed analogous CAD and ETD fragmentation pathways to those of poly(lactide), Figures 4.4 and 4.5. These two polyesters are copolymers made up of alternating adipic acid and ethylene glycol or butylene glycol units. CAD on these two polymers generated fragment ion series, both singly and doubly charged, that are accounted for by random charge-remote 1,5-H rearrangements along the chain, Scheme 4.3.
The resulting two fragment ion series are labeled with the same nomenclature used so far, viz. \( l_n^{HV} \) and \( l_n^{AH} \). Both fragment series have repeat units of 172 Da for poly(ethylene adipate) and 200 Da for poly(butylene adipate). Consecutive elimination reactions are observed from these fragments via the same mechanism. This additional dissociation route gives rise to fragment ion series having acid/vinyl and acid/acid end groups, \( l_n^{AV} \) and \( l_n^{AA} \). The product ions \( l_n^{AV} \) are isomeric with the cyclic fragments \( c_n^{AH} \) that arise from an intramolecular addition/elimination transesterification involving a hydroxy end group and a backbone carbonyl group.\(^\text{12}\) Such cyclic ion species are also present in the mass spectra of both poly(ethylene adipate) and poly(butylene adipate), see distribution C in Figure 4.2.
Figure 4.4. CAD versus ETD MS$^2$ spectra of [M+2Na]$^{2+}$ (m/z 828) from the 9-mer of polyethylene adipate. The notation $l$ and $c$ indicate linear and cyclic structures, respectively. The superscripts in the fragment notation give the end groups ($H$, hydroxyl; $V$, vinyl; $A$, acid; $L$, lactone). The *, o, l labels indicate doubly sodiated monocations, proton/sodium exchange and doubly charged fragments, respectively.
Figure 4.5. CAD versus ETD MS² spectra of [M+2Na]²⁺ (m/z 886) from the 8-mer of polybutylene adipate. The notation \( l \) and \( c \) indicate linear and cyclic structures, respectively. The superscripts in the fragment notation give the end groups (\( H \), hydroxyl; \( V \), vinyl; \( A \), acid; \( L \), lactone). The * and † labels indicate doubly sodiated monocations, proton/sodium exchange and doubly charged fragments, respectively.

ETD on both poly(ethylene adipate) and poly(butylene adipate) induces similar fragmentation pathways as ETD of poly(lactide), Scheme 4.4. The electron transferred to the carbonyl carbon atom triggers fast, radical-induced dissociation at the (CO)O-C bond of the ester group, Sheme 4.4 (a), resulting in the linear fragment ion series \( l_n^{AH^+} \) and \( l_n^{HV+1} \) or \( l_n^{+} \); superscripts and symbols maintain the same meaning as for the ETD fragment ions of poly(lactide), vide supra. The additional fragment ion series \( l_n^{HH^+} \) and \( l_n^{HL} \) are accounted for by nucleophilic attack of the oxy anion at the adjacent carbonyl carbon atom, Scheme 4.4 (b). The fragments \( l_n^{HL} \), that were missing in the ETD spectrum
of poly(lactide), are observed in the ETD spectra of both poly(ethylene adipate) and poly(butylene adipate), validating the mechanism proposed. The consecutive dissociation that depleted $l_n^{HL}$ from poly(lactide) is not feasible with the copolymer structures, explaining the different reactivity.

Scheme 4.4. ETD fragmentation mechanism of poly(ethylene adipate), $n=0$; and poly(butylene adipate), $n=1$: a) radical-induced fragmentation, b) nucleophilic addition/elimination.

The radical ion fragments $l_n^{HL}$ tend to lose a H instead of abstracting one from the reagent. This behavior can be rationalized by taking into consideration the steric hindrance at the radical site. Their poor tendency to retain the charge, compared to their complementary fragments, $l_n^{HH^+}$, is an additional reason for their low intensity or
complete absence, as in the case of poly(lactide). The high intensity of the products of the nucleophilic displacement mechanism in the ETD spectra of both poly(ethylene adipate) and poly(butylene adipate) compared to the ETD spectrum of poly(lactide) could be related to steric factors. In order for this specific reaction to occur both the attacking nucleophile and the electrophilic site, viz. the oxy anion and the carbonyl carbon atom respectively, must be physically close to each other and in the right configuration. This appears to be more favorable for poly(ethylene adipate) and poly(butylene adipate), due to their longer and more flexible aliphatic chains in between two consecutive carbonyl groups, than for poly(lactide).

4.3 Conclusions

The results obtained from this study validated ETD as a suitable complementary tandem mass spectrometry technique for the characterization of polyesters, opening the door to its application in the analysis of these and other synthetic macromolecules. One of the advantages observed over the classical CAD was that ETD did not trigger any significant consecutive fragmentation reaction. During CAD of both poly(ethylene adipate) and poly(butylene adipate), the first generation of fragment ions series $I_n^{HV}$ and $I_n^{AH}$, underwent sequential 1,5-H rearrangements at the glycol chain ends, generating the fragment ion series $I_n^{AV}$ and $I_n^{AA}$, obscuring the identity of the original end groups. A further practical characteristic of ETD versus CAD was the formation of fragment ions with charge states lower than the one of their respective precursor ions. This charge reduction is promoted by the transfer of electrons during the ion-ion reaction inside the
ion trap. CAD of doubly charged sodiated precursor ions resulted in fragment ions that were both singly and doubly charged, which convolutes the MS$^2$ spectrum and obscures spectral interpretation. In contrast, the ETD spectra of the same precursor ions gave only singly charged species.

These features lead to less congested ETD spectra compared to the analogous CAD spectra. Their interpretation is facile and straightforward, rendering conclusive information about both polymer architecture and end group assignment.
CHAPTER V

POSS-SORBITOL COMPLEXES STUDIED BY MASS SPECTROMETRY

5.1 Introduction

In the last decades, the effort to invent new materials that possess superior qualities has challenged synthetic chemists. One of the main goals has been to provide inorganic properties to the classical organic polymers that we are using in our everyday lives. Thermal properties, reduced flammability and electric conductivity are some of the qualities in which inorganic compounds are superior compared to organic molecules. Among the different inorganic molecules that have been considered to connect or blend with organic polymers, POSS stands out. POSS molecules have similar size with most of the organic polymer segments, and additionally their isotropic shape provides uniformity of their properties in tridimesional architectures. Another important characteristic is that POSS molecules can self-assemble into 20-100 nm particles. Incorporation of POSS units into the chains of several polymer systems has initially been performed via copolymerization and grafting reactions by functionalizing the POSS molecules so that they can reac to help the polymerization processes.\textsuperscript{132,133,134} One major issue associated with these types of reactions for the development of POSS-polymer composites is that they deteriorate mechanical properties.\textsuperscript{132} A better and simpler alternative to grafting and copolymerization reactions is the melt blending of non-reactive POSS molecules with the
host polymers for the preparation of POSS-polymer nanocomposites. One advantage of the latter approaches is that non-reactive POSS molecules are easier to synthesize, as no functionalization process is required. The mixture with the host polymer can be blended by using an array of extruders and internal mixers. Nevertheless, it is the nature of the side groups of POSS and their compatibility with the polymer matrix that dictates the degree of dispersion of the melt-blended composite. If POSS and the polymer are incompatible, the dispersion does not uniformly occur and the resultant compounds usually exhibit inferior properties. In this case, microscopic POSS aggregates act as defects. On the other hand, a highly compatible POSS may undergo nanoscale dispersion in the polymer, although, such molecularly dispersed composites do not show the desired improvements in mechanical properties. Hence, there is a trade-off between the extent of dispersion and the prospect of mechanical properties improvement.

Controlling the level of dispersion of POSS in the polymer in order to achieve POSS aggregates of 50-100 nm in size is the ideal process so that the overall mechanical properties can be positively affected. In theory, synthetic chemists can achieve the desired molecular dispersion of POSS at the time of melt compounding, which substantially reduces the viscosity, allowing for the self-assembly of the polymer products into nanoparticles during the cooling process of the polymer products.

Recent work in the research group of Professor Sadham C. Jana (The University of Akron) has demonstrated that POSS molecules, which were not compatible with isotactic polypropylene (iPP) could easily be dispersed in molten iPP when mixed with a phenyl-substituted sorbitol nucleating agent. In this dissertation, the POSS and
sorbitol units employed, as well as their non-covalent complexes, have been investigated by a palette of mass spectrometry methods. Figure 5.1 depicts examples of the sorbitol and POSS molecules examined and also shows a cartoon illustrating how the components may be dispersed in the iPP matrix.

Figure 5.1. Cartoon illustrating the dispersion and interaction of sorbitol and POSS components in the polypropylene matrix.

The free -OH groups in sorbitol can form both intramolecular and intermolecular self-assembled superstructures via hydrogen bonding. On the other hand, the hydrophobic aromatic rings in sorbitol are known to promote compatibility with polyolefins. Sorbitol molecules become completely dissolved and homogeneously dispersed in molten iPP. Upon cooling, a transient gel is formed with endless, thin, twisted fibrils of sorbitol which nucleate the crystallization of iPP chains. It was reported that homogeneous melt mixtures of di(benzylidene)sorbitol (DBS) and iPP, upon cooling, result in elementary
DBS fibers with diameter of $\sim 10$ nm. A similar phenomenon occurs with di-(dimethylbenzylidene)sorbitol (DMDBS). The self-aggregation of sorbitol molecules such as DBS is believed to be the result of intermolecular hydrogen bonding and $\pi-\pi$ interactions between the adjacent phenyl rings. Highly organized fibrillar bundles of DBS are formed up to a concentration of 1-2 wt %, beyond which DBS remains insoluble in molten iPP.$^{26,27,28}$

Previous work reported that dispersion of nonreactive trisilanol phenyl-POSS (tri-POSS, see Figure 5.1) in iPP can be improved in the presence of DBS. These studies also reported that in the presence of tri-POSS the fibrillar networks of DBS did not form easily when compounds were cooled from a homogeneous melt state.$^{26,27,28}$ This was attributed to stronger interactions between silanol-POSS and DBS than between DBS molecules. In the previous work, indirect evidence of the occurrence of interactions between tri-POSS and DBS molecules was gleaned from rheological data, and such interactions were attributed to hydrogen bonding. However, it has not been determined whether the number of Si-OH groups or the nature of organic substituents in the silanol-POSS molecules would have any impact on such interactions. It has also remained unclear whether the POSS-sorbitol interactions would lead to non-covalent complex formation or if such complexes were indeed chemical compounds (i.e. covalently bonded). These questions will be addressed in this dissertation by ESI mass spectrometry.

5.2 Experimental
Several samples of POSS were obtained from Hybrid Plastics (Hattiesburg, MS). The POSS molecules under consideration were tetrasilanol phenyl POSS (tetra-POSS, SO 1460), molecular weight (Mw) of 1069.5 g/mol and Tm of 260 °C, trisilanol phenyl POSS (tri-POSS, SO 1458; Mw = 930.07 g/mol and Tm = 230 °C), trisilanol cyclopentyl POSS (cyclo-POSS; SO 1430; Mw = 874.7 g/mol and Tm = 250 °C), trisilanol isobutyl POSS (tri-iso-POSS; SO 1450; Mw = 791.42 g/mol and Tm = 195 °C), disilanol isobutyl POSS (di-iso-POSS; SO 1440; Mw = 891.62 g/mol and Tm = ~100 °C), octaisobutyl POSS iso-POSS; MS 0825; Mw = 873.6 g/mol and Tm = 270 °C), and octaphenyl POSS (phenyl-POSS; MS 0840; Mw = 1033.53 g/mol and Tm = 173 °C and 426 °C). The above mentioned POSS materials were available in the form of white powder. Figure 5.2(a-g) presents the chemical structures of the POSS molecules. Note that tri-POSS, cyclo-POSS, tri-iso-POSS have three silanol groups, but they differ in the nature of the side groups, e.g., phenyl in the case of tri-POSS, cyclopentyl in the case of cyclo-POSS, and isobutyl in the case of tri-iso-POSS. Tetra-POSS has four and di-iso-POSS has two silanol groups, with side phenyl and isobutyl groups, respectively. The phenyl-POSS and iso-POSS molecules do not have silanol groups, but they contain eight side groups, in the form of phenyl and isobutyl substituents, respectively. Note that the POSS molecules were received in crystalline state. The sorbitols chosen for this study were di(benzylidene)sorbitol (DBS; Millad 3905; Mw = 358.4 g/mol and Tm = 225 °C) and di(dimethylbenzylidene)sorbitol (DMDBS; Millad 3988; Mw = 414.49 g/mol and Tm = 275 °C), Figure 5.3. Both DBS and DMDBS carry two free –OH groups and two phenyl substituents and have been used as clarifying agents of iPP, although DMDBS is more frequently used due to its higher melting temperature. DMDBS molecules contain two
methyl substituents on each phenyl ring and melt at a 50 °C higher temperature than DBS. These materials were obtained from Milliken Chemicals (Spartanburg, SC) in the form of white powder.

Figure 5.2. POSS molecules investigated; (a) trisilanol phenyl POSS (tri-POSS), (b) tetrasilanol phenyl POSS (tetra-POSS), (c) trisilanol cyclopentyl POSS (cyclo-POSS), (d) trisilanol isobutyl POSS (tri-iso-POSS), (e) disilanol isobutyl POSS (di-iso-POSS), (f) octaisobutyl POSS (iso-POSS) and (g) octaphenyl POSS (phenyl-POSS).
DBS or DMDBS and POSS were intimately mixed at different weight ratios in tetrahydrofuran (THF). The solvent was evaporated, and the resultant materials were ground and vacuum-dried. One part of the mixture was kept in the oven at 200 °C for 5 min to imitate the thermal history experienced in typical polymer compound preparation and melt spinning experiments. The resultant materials were cooled to room temperature and ground into powder form for further analysis. The samples designated for mass spectrometry (MS) and ion mobility mass spectrometry (IM-MS) analysis were completely dissolved in THF. An aliquot of MeOH was added to these solutions in order to obtain a final concentration of 0.1 mg/mL in 1:1 (v:v) THF/MeOH.

All MS measurements were carried out using a SYNAPT HDMS™ hybrid quadrupole/orthogonal acceleration time-of-flight (Q/oa-ToF) mass spectrometer (Waters, Beverly, MA) equipped with a Z-spray electrospray source. The instrumental settings were optimized to minimize dissociation of the complexes due to the energy provided during the ionization. The instrument was operated in positive mode using the following settings: capillary voltage 3.5 kV, cone voltage 35 V, sampling cone voltage 3.2 V, source temperature 60 °C, and desolvation gas temperature 100º C. The sample solutions were electrospayed by direct infusion at a flow rate of 15 µL/min.
The ion mobility mass spectrometry (IM-MS) experiments were performed on the same instrument by activating the traveling wave (T-Wave) separation section located between the two analyzers. This section consists of three cells in the order trap cell, ion mobility cell, and transfer cell. Once the ions generated in the source or by fragmentation in the trap cell (vide infra) enter the ion mobility cell, they move under the influence of a travelling wave electric field, in the presence of a drift gas (N\textsubscript{2}) which flows in the opposite direction of the ions motion. Separation takes place according to the size, shape, and charge state of the ions. These parameters determine the drift times of the ions through the ion mobility cell, which can be measured and converted to experimental collision cross-sections that reflect ion shape and size. The electric field used in the ion mobility experiments was generated by tuning the travelling wave velocity and the travelling wave height at 350 m/s and 15 V, respectively, while the nitrogen gas flow rate was set at 22.7 mL/min. For tandem mass spectrometry (MS\textsuperscript{2}) studies, with or without TWIM separation, a specific non-covalent complex was first mass-selected by the quadrupole and then fragmented in the trap collision cell using argon as collision gas. The collision energy applied to disrupt the complexes under study was varied in the range from 6 to 20 eV, depending on the type of complex, POSS-POSS or POSS-sorbitol, and its stoichiometry. The fragments were mass-analyzed by the ToF analyzer either without or with prior TWIM separation.
5.3 Results and Discussion

The self-assembled, non-covalent complexes between POSS and nucleating agent molecules were investigated by ESI mass spectrometry. This gentle ionization method is able to retain the non-covalent interactions intact during the transfer of the analytes into the gas phase. The resulting mass spectra identified the composition of the complexes formed, either POSS-POSS or POSS-sorbitol, and their stoichiometry, whose determination is not always straightforward in solution.

Each single material was investigated first separately, in order to determine its behavior before and after heating at 200 ºC. Several combinations of POSS-sorbitol molecules, mixed at different molar ratios and at different temperatures (room temperature and 200 ºC), were then characterized.

The ESI mass spectra of the sorbitol samples, viz. benzylindene sorbitol (DBS) and di(dimethylbenzylindene) sorbitol (DMDBS), individually examined, show the quasi-molecular ions [M+Na]⁺ as base peaks as well as the respective dimeric species [2M+Na]⁺, Figure 5.4. Although no salt was added to the sample solutions, all ions observed were adduct with sodium ions, Figure 5.4 (a), or sodium and potassium ions, Figure 5.4 (b). The Na⁺ and K⁺ ions were supplied from the glassware and/or were present in the samples because of the synthetic methods used to prepare them. Polyethylene glycol (PEG) impurities, probably originating from the synthesis of the samples, were also detected in both sorbitol compounds analyzed.
After the same sorbitol molecules were heated at a temperature of 200 °C for 5 minutes and allowed to cool down to room temperature, no change or degradation was evident in the respective mass spectra, indicating reasonable thermal stability.

ESI analysis of the individual silanol-POSS materials (trisilanol phenyl POSS, tetrasilanol phenyl POSS and trisilanol cyclopentyl POSS) confirms that such molecules can form non-covalent complexes, figure 5.5. Note that the stoichiometry of the complexes detected is up to [4M+Na]+ for trisilanol phenyl POSS, [3M+Na]+ for tetrasilanol phenyl POSS, and just [2M+Na]+ for the trisilanol cyclopentyl POSS.
Figure 5.5. ESI mass spectra of (a) trisilanol phenyl POSS, (b) tetrasilanol phenyl POSS and (c) trisilanol cyclopentyl POSS.

For trisilanol cyclopentyl POSS, Figure 5.5(c), which does not carry any aromatic substituents, the binding interactions in the observed complexes must be the result of hydrogen bonding. The higher degree of complexation observed for the other POSS
systems can be rationalized by the additional π-π stacking interactions derived from the phenyl functionalities, which improve the overall stability of the resulting complexes. When thermally treated at 200 °C for 5 minutes, the same set of POSS molecules underwent some degradation. The mass spectra of both trisilanol phenyl POSS and trisilanol cyclopentyl POSS, Figure 5.6(a) and 5.6(c), indicate consecutive water losses, pointing out the occurrence of condensation reactions. Note that the number of water losses increased with the complex order, as follows: one water loss from the monomer \([M+Na]^+\), two water losses for the dimer \([2M+Na]^+\), and four water losses from the trimer \([3M+Na]^+\). Additional minor fragments in the spectra, labeled as *, arose from consecutive elimination of SiPhO and H₂O units. Conversely, no condensation or degradation product was detected for tetrasilanol phenyl POSS, Figure 5.6(b).
Figure 5.6. ESI mass spectra of POSS samples that were heated at 200 ºC; (a) trisilanol phenyl POSS, (b) tetrasilanol phenyl POSS and (c) trisilanol cyclopentyl POSS.

Tandem mass spectrometry (MS$^2$) studies of the ion species arising from water losses confirmed that the condensation reactions occurred in the single POSS molecules via intramolecular condensation rather than between two POSS units, which would have
led to covalently bounded POSS units. This is exemplified in Figure 5.7, which illustrates the MS\textsuperscript{2} spectra of the sodium adducts of 2M-H\textsubscript{2}O and 2M-2H\textsubscript{2}O from trisilanol phenyl POSS heated at 200 °C.

![Figure 5.7. MS\textsuperscript{2} spectra of the condensation products from trisilanol phenyl POSS heated at 200 °C: (a) [(2M-H\textsubscript{2}O)+Na]^+ (m/z 1865) fragmented at a collision energy of 17 eV; (b) [(2M-2H\textsubscript{2}O)+Na]^+ (m/z 1847) fragmented at a collision energy of 20 eV.](image)

Consistent with non-covalent bonding between the POSS units, relatively low collision energies were necessary to disrupt the complexes, resulting in the loss of a dehydrated POSS unit, (M-H\textsubscript{2}O), from both condensation products investigated. In the case of (2M-2H\textsubscript{2}O), Figure 5.7(b), the loss of a (M-H\textsubscript{2}O) unit to generate a sodiated (M-H\textsubscript{2}O) fragment ion, indicates that each of the POSS molecules in the complex had undergone one intramolecular condensation reaction. In the same vein, the loss of (M-
H₂O) from sodiated (2M-H₂O) to produce [M+Na]⁺, Figure 5.7(a), confirms that only one of the two POSS molecules had undergone condensation.

With all different mixtures of POSS and sorbitol inspected by ESI, POSS-sorbitol complexes were detected, along with POSS-POSS complexes. The observation of trisilanol cyclopentyl POSS-DBS complexes confirms that hydrogen bonding brings upon the binding interaction between the complex constituents. The POSS-sorbitol complexes with 1:1 stoichiometry were always the dominant products, Figure 5.8. Note that by increasing the molar ratio of POSS and sorbitol to 30:1, the intensity of the POSS-sorbitol complexes decreases, Figure 5.8(b). This expected behavior is a consequence of the lower amount of sorbitol molecules available to interact with the POSS molecules, which can instead self-assemble with themselves. The POSS-sorbitol complexes detected in the ESI mass spectra did not contain more than 2 sorbitol or 3 POSS molecules per complex, Figure 5.8(c).
Figure 5.8. ESI mass spectra of (a) tetrasilanol phenyl POSS + DBS, (b) tetrasilanol phenyl POSS + DMDBS, (c) trisilanol phenyl POSS + DMDBS, and (d) trisilanol cyclopentyl POSS + DBS. POSS (M) and sorbitol (L) were mixed in the molar ratio (a,c,d) 2:1 or (b) 30:1.
The investigation of the POSS-sorbitol systems by ion mobility mass spectrometry (IM-MS) unveiled the existence of multiply charged higher order complexes, which could not be identified by traditional MS analysis due to the overlapping of their \( m/z \) values with those of other complexes at a different charge state, for example \([(\text{M+L})+\text{Na}]^+\) and \([(\text{2M+2L})+2\text{Na}]^{2+}\), Figure 5.9. IM-MS has been developed and applied as a method to differentiate isobaric and isomeric constituents of complex mixtures as well as macromolecular architectures such as linear, cyclic, or branched. It can also differentiate species with the same m/z value but different charge state, which results in a shorter or longer drift time through the ion mobility cell, respectively.\(^{148, 149,150,151}\)

![Figure 5.9. Ion mobility diagram of m/z versus drift time for trisilanol phenyl POSS–DMDBS mixed in a ratio 2:1. Band 1 is composed of singly charged complexes (higher drift time), while band 2 represents doubly charged complexes (lower drift time).](image)

The IM-MS diagram of trisilanol phenyl POSS-DMDBS shows two distinct bands, each containing a different charge state of the POSS-sorbitol complexes: singly charged complexes (band 1) and doubly charged complexes (band 2). Separate
integration of each band provides the corresponding 2D mass spectra, Figure 5.10. Figure 5.10(b) illustrates the mass spectrum derived from band 2, which confirms the presence of complexes with a higher stoichiometry compared to the singly charged complexes observed in the spectrum of the band 1, figure 5.10(a). The higher charge state of the complexes with a higher number of POSS or sorbitol components arises from the high sodium affinity of the oxygen atoms present on both the sorbitol and POSS molecules.

Figure 5.10. Mass spectra extracted from the different bands of the 2D ion mobility diagram shown in figure 5.9. Spectrum (a) corresponds to the band 1 of singly charged complexes; spectrum (b) results from the band 2 of doubly charged complexes.

Additional evidence for the formation of higher order complexes was obtained by tandem mass spectrometry analysis coupled with ion mobility separation (IM-MS²).

Figure 5.11 shows the ion mobility diagram obtained by isolation of the complex [(M+2L)+Na]^+ (m/z 1781.8) from trisilanol phenyl POSS-DMDBS, collisionally activated dissociation (CAD) in the following trap cell to disrupt the complex, IM
separation of the fragments and remaining precursors, and mass analysis of the ion mixture with the ToF device. Along with the [(M+2L)+Na]⁺ ion and its fragments, the isobaric [(2M+4L)+2Na]²⁺ and its fragments were also detected. The singly charged species [(M+2L)+Na]⁺ resulted having a longer drift time than the isobaric [(2M+4L)+2Na]²⁺ complex because of its lower charge. The losses of ligands from the isobaric parent ions generated isobaric fragment complexes as well, viz. [(M+L)+Na]⁺ and [(2M+2L)+2Na]²⁺. The charge states of precursor ions and fragments are readily determined from the corresponding isotope patterns, revealed by integration of the respective bands.

![IM-MS² diagram](image)

Figure 5.11. IM-MS² diagram of the overlapping complexes [(M+2L)+Na]⁺ and [(2M+4L)+2Na]²⁺ from trisilanol phenyl POSS-DMDBS (2:1). The trap collision energy applied was 12 eV.

When heated at 200 ºC, the POSS-sorbitol samples showed different behavior, depending on the type of sorbitol molecules involved, either DBS or DMDBS. The mixtures POSS-sorbitol having DBS as nucleating agent underwent extensively
condensation reactions. Thus, thermal degradation is observed not only in the POSS-POSS complexes, but also in the POSS-DBS complexes, Figure 5.12.
Figure 5.12. ESI mass spectra of (a) trisilanol phenyl POSS + DBS, (b) tetrasilanol phenyl POSS + DBS, (c) trisilanol cyclopentyl POSS + DBS. The samples were heated at 200 ºC before the analysis.
The nature of the condensation products in the POSS-DBS complexes was tested by tandem mass spectrometry. The analysis revealed that no bond formation was created between the POSS and the DBS molecules, Figure 5.13. Losses of both intact sorbitol (L) and either POSS or dehydrated POSS molecules confirmed that the condensation occurred in the POSS molecules by intramolecular H$_2$O elimination, as seen before.

Figure 5.13. MS$^2$ spectra of condensation products in the tretrasilanol phenyl POSS-DBS mixture heated at 200 °C: (a) [(M+L-H$_2$O)+Na]$^+$ (m/z 1431.4) fragmented at a collision energy of 12 eV; (b) [(2M+L-H$_2$O)+Na]$^+$ (m/z 2499.7) fragmented at a collision energy of 12 eV.

The POSS-DMDBS systems heated at a temperature of 200 °C did not decompose the same way as the homologous complexes having DBS as nucleating agent. The mixture of trisilanol phenyl POSS and DMDBS in the molar ratio of 2:1 gave [M+Na]$^+$ as the most abundant ion, however, this peak is 14 Da higher than the expected m/z value.
(m/z 953), which suggests that methylation of one OH group in POSS took place. No complex was observed, Figure 5.14(a). When the POSS:sorbitol molar ratio was increased to 30:1, thermal degradation products were detected from both POSS-POSS as well as POSS-sorbitol complexes, Figure 5.14(b).

![Figure 5.14. ESI mass spectra of trisilanol phenyl POSS + DMDBS heated at 200 °C for 5 minutes. Molar ratio POSS: sorbitol (a) 2:1 and (b) 30:1. The superscripts * and # indicate +14 Da (methylation) and + 2 Da (reduction), respectively.]

On the other hand, the tetrasilanol phenyl POSS-DMDBS systems gave rise to different spectra depending on the POSS:sorbitol molar ratio. With a mixing ratio of 2:1, non-covalent complexes and their degradation products were detected, Figure 5.15(a). Inversely, the same sample with a combining molar ratio of 30:1, POSS:sorbitol, did not show any condensation or degradation product, Figure 5.15(b). Intact complexes were,
however, detected. Singly charged POSS-POSS complexes were observed at lower \( m/z \) values, while higher-order POSS-sorbitol complexes appeared as doubly charged species in the higher mass range.

Figure 5.15. ESI mass spectra of tetrasilanol phenyl POSS + DMDBS heated at 200 ºC for 5 minutes. Molar mixing ratio POSS: sorbitol (a) 2:1 and (b) 30:1.

Similar analyses were performed with a wider range of silanol and non-silanol POSS molecules in order to establish the determinants of self-assembly between the POSS molecules themselves and also between the POSS and sorbitol molecules. For this purpose, non-silanol POSS molecules with and without phenyl substituents were also
investigated to confirm if they are capable of forming supramolecular architectures or not. The following POSS structures were taken under consideration: cyclo-POSS and tri-iso-POSS which have three silanol groups but differ in the nature of the side groups, cyclopentyl in the case of cyclo-POSS, and isobutyl in case of tri-iso-POSS; di-iso-POSS which has two silanol groups with isobutyl side groups; and phenyl-POSS and iso-POSS which do not carry any silanol groups, just eight phenyl or isobutyl side groups, respectively.

Higher order complexes were observed for the POSS-sorbitol systems involving POSS molecules having both silanol and phenyl substituents (vide supra). This finding indicated that both hydrogen bonding and $\pi$-stacking interactions take place; both these contributions lower the complex energy and in turn increase the complex stability.\textsuperscript{151}

The second set of POSS molecules, having different substituents, have been investigated as non-covalent partners of DBS in order to correlate complex formation and the nature and chemical properties of the POSS molecules.

Using the same procedure, the ESI analysis of each single species is reported first, followed by the investigation of the different POSS/DBS combinations. Among the different POSS molecules chosen, only disilanol isobutyl POSS (di-iso-POSS), trisilanol isobutyl POSS (tri-iso-POSS) and octaphenyl POSS (phenyl POSS), provided clear evidence of non-covalent complex occurrence with the same species (homocomplexes), Figure 5.16. In all the spectra the quasi-molecular ions detected were either sodium, [M+Na]$^+$, or ammonium, [M+NH$_4$]$^+$, adducts. These cations are believed to be present in both the glassware and solvents used during the sampling and or in the sample.
Figure 5.16. ESI mass spectra of di-iso-POSS (top), tri-iso-POSS (center), and phenyl-POSS (bottom).
On the other hand, the analysis of the octaisobutyl POSS (iso-POSS) did not show any complex formation, Figure 5.17. This different behavior can be rationalized by taking into consideration the nature of the substituents on the different POSS molecules. Both di-iso-POSS and tri-iso-POSS have silanol functionalities which can be involved in hydrogen bonding. Phenyl-POSS has only phenyl groups that can promote π-stacking interactions with phenyl groups on other POSS molecules. In contrast, iso-POSS has only isobutyl groups as substituents which cannot be engaged in any type of measurable interaction among the same species.

Figure 5.17. ESI mass spectra of iso-POSS.

No significant change was observed in the ESI mass spectra arising from the analysis of the same set of POSS molecules after their thermal treatment. Non-covalent interactions between the same species, homocomplexes, were detected for di-iso-POSS, tri-iso-POSS, and phenyl-POSS, Figure 5.18. Tri-iso-POSS appeared to undergo condensation reaction, as indicated by water loss from its dimeric species [2M+Na]⁺.
Figure 5.18. ESI mass spectra of di-iso-POSS (top), tri-iso-POSS (center), and phenyl-POSS (bottom) after thermal treatment at 200 °C.
This water loss, which was previously investigated, occurs by intramolecular condensation reactions in the POSS molecules having silanol groups, before these undergo complex formation. Expectedly, no peak related to non-covalent complex formation was detected for the iso-POSS molecules.

When mixed with DBS, each POSS/DBS combination behaved differently toward complex formation, depending on the POSS component involved. Di-iso-POSS and tri-iso-POSS (M), which are similar in composition, gave rise to analogous spectra when mixed with DBS (L), Figure 5.19. Along with dimer formation between two POSS molecules, \([2M+Na]^+\), the complex POSS-sorbitol, \([M+L+Na]^+\), was also observed in both systems. Note that the highest order complex in both systems has 1:1 stoichiometry.
Figure 5.19. ESI mass spectra of di-iso-POSS/DBS (top) and tri-iso-POSS/DBS (bottom). M and L indicate the POSS and DBS units, respectively.

The phenyl-POSS/DBS mixture gave rise to completely different results. Non-covalent interactions between the two components were observed along with the homocomplexes, Figure 5.20. Note the intensity and complex order of the heterocomplexes compared to the other POSS/DBS combinations. Only π-stacking interactions among the phenyl rings of POSS and DBS are possible in these complexes, as no hydroxyl group is present in phenyl-POSS. Moreover, the presence of phenyl-POSS
in the mixture seemed to promote the formation of high order DBS homocomplexes $L_n$, as is evident in the spectrum.

Figure 5.20. ESI mass spectra of phenyl-POSS/DBS. M and L indicate the POSS and DBS units, respectively. The difference in mass between two consecutive DBS homocomplexes is 179 Da, which is half value of the mass of one DBS molecule.

No interaction between the two components was detected for the iso-POSS/DBS system. The only non-covalent interaction observed in the resulting spectrum was among the DBS molecules themselves, which formed high order doubly charged sodiated homocomplexes ($L_n$), Figure 5.21.
The same analysis was performed on the same set of POSS/DBS combinations after thermal treatment at 200 °C. Tri-iso-POSS/DBS and phenyl-POSS/DBS gave rise to both types of complexes, homocomplexes (POSS-POSS, DBS-DBS) and heterocomplexes (POSS-DBS), Figure 5.22. Peaks indicating intramolecular condensation reactions were observed in the spectrum of the tri-iso-POSS/DBS system, in which the POSS units carry three silanol functionalities. The nature of these condensations was already investigated by tandem mass spectrometry which confirmed the occurrence of intramolecular condensation reactions in the POSS part before the same molecule underwent complex formation. Note the high intensity of the heterocomplex in the phenyl-POSS/DBS mixture compared to those arising from the tri-POSS/DBS combination, which implies a superior stability after the thermal perturbation.
No interaction between POSS and DBS was instead detected in the di-iso-POSS/DBS and iso-POSS/DBS systems after heating them up at 200 °C, figure 5.23. Peaks arising from homocomplex formation and some complex degradation are observed in the spectrum of di-iso-POSS/DBS, while just the singly charged sodiated DBS species appeared in the iso-POSS/DBS spectrum.
Figure 5.23. ESI mass spectra of di-iso-POSS/DBS (top) and iso-POSS/DBS (bottom) after thermal treatment at 200 °C. M and L indicate the POSS and DBS units, respectively.
5.4 Conclusion

This study showed that the molecules of silanol-POSS and a sorbitol derivative were capable of forming several complexe molecular adducts. Such complex formation occurred due to noncovalent interactions, such as hydrogen bonding and $\pi-\pi$. Tandem mass spectrometry investigation revealed the absence of covalent bond formation between silanol-POSS and sorbitol, although tri-POSS and cyclo-POSS underwent intramolecular condensation of silanol groups. A trend of POSS-sorbitol complex formation and complex stability was also derived by changing the functional groups on the POSS molecules. The complex formation was more abundant with higher number of silanol groups per molecule of POSS and significantly more abundant with POSS molecules carrying both phenyl and silanol groups. The latter finding points out that cooperativity between $\pi-\pi$ stacking and hydrogen bonding enhances complex stability and promotes the self-assembly process.
CHAPTER VI

SUMMARY

The material presented in this dissertation illustrates how innovations in the mass spectrometry field help in the analysis of a variety of synthetic compounds, ranging from inorganic light and water sensitive materials to the detection and identification of extremely weak interactions between two different materials. The attempt was not only to achieve ionization and detection of the resulting ions but also to provide new information about the different analytes under inspection. Polymer end groups, architectures and polymer sequences are shown to be easily deducted with the help of new techniques such as ion mobility mass spectrometry (IM-IM) and electron transfer dissociation (ETD), which along with the classical mass spectrometry methods can unveil the whole picture of the different compounds analyzed.

Chapter III in this dissertation focused on the characterization of phosphazene compounds, either the chloro- or organo-substituted, by both ESI and MALDI mass spectrometry. The dichlorophosphanenes obtained from the reaction of NH₄Cl with PCl₅ resulted in a mixture of linear ionic, linear tadpole and macrocyclic polymers. The tadpole structure, which is isomeric with cyclic structures, was confirmed and thoroughly characterized by MS² and IM-MS methods. ESI was most gentle for the analysis of
chlorophosphazenes. MALDI was used instead for the characterization of the more stable organophosphazenes. The reaction of [PCl₂N]₃ with MXₙ under less strict anaerobic conditions or in the presence of HX to yield [PCl₂N]₃HMXₙ₊₁ superacids was also investigated by ESI mass spectrometry. Their formation was proved not only by the observation of protonated [PCl₂N]₃, which is a very weak base, but also by the detection of the complementary, extremely labile anion species [MXₙ₊₁]⁻. This chapter also describes the different approaches employed to better understand the behavior of these materials under mass spectrometry conditions, in particular their ionization and fragmentation mechanisms.

Chapter IV reports an evaluation of ETD as a complementary technique to collisionally activated dissociation (CAD) for the characterization of three important polyesters, viz. poly(lactide), poly(ethylene adipate) and poly(butylene adipate). The application described in this dissertation was a breakthrough for polymer analysis as ETD has mainly been utilized in the biological field. This dissociation method involves a site specific, non-ergodic ion-ion reaction and is capable of preserving labile groups, such as those present in post-translation modifications or non-covalent complexes, during the MS² process. For polyesters, the selectivity of ETD can lead to less congested MS² spectra which are easy to interpret. An additional advantage is the charge reduction typical of this dissociation process. With the polymers investigated, ETD on the doubly charged cations resulted in only singly charged fragment ions, while CAD on the same set of precursor ions gave rise to both singly and doubly charged fragment ions. The dissociation pathways leading to the fragment ions observed in the ETD spectra were elucidated and confirmed that such spectra contain sufficient information for a complete
characterization of polymer backbone structure and end groups. Furthermore, ETD fragment ions do not undergo consecutive dissociation while the first generation of fragment ions from CAD have enough internal energy to dissociate by successive rearrangements which not only increases the amount of fragment ions observed in the MS\(^2\) spectrum but also produces confusing information since parts of the molecule, such as an end group or a particular substitution, are lost.

In chapter V, the non-covalent interaction between polyhedral oligomeric silsesquioxanes bearing multiple polar silanol groups (Si-OH) and different sorbitol-type nucleating agent, such as di(benzylidene)sorbitol (DBS) and di-(dimethylbenzylidene)sorbitol (DMDBS), were investigated to aid the development of polymer nanocomposites. By controlling instrumental settings not only their detection was possible, but also the resolution and the yield of complexes that survive during the ionization were kept high. Noncovalent complex formation was observed with all the different POSS-DBS combinations. Strong evidence for the noncovalent nature of the POSS/DBS complexes was also provided by tandem mass spectrometry (MS\(^2\)) analysis with and without mobility separation (IM-MS\(^2\)). It has also been found that the yield and stoichiometries of these complexes, which reflect their stability and self-assembling behavior, respectively, depend on the substituents and the number of silanol groups per molecule of POSS. Separation via ion mobility (IM) prior to mass analysis was essential for the detection of the higher order complexes, containing multiple POSS and sorbitol molecules. These were most abundant if the POSS unit is also substituted by phenyl, as in such case self-assembly is promoted by both hydrogen bonding as well as \(\pi-\pi\) interactions.
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APPENDIX

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Vincenzo Scionti <vs25@zips.uakron.edu>

Permission request
2 messages

Vincenzo Scionti <vs25@zips.uakron.edu>
To: Brian J. Murphy@waters.com

Sun, Apr 15, 2012 at 7:38 PM

Dear Mr. Brian Murphy,

I am a graduate student at the University of Akron and I am getting my Ph.D. under the supervision of Dr. Chris Wesdemiotis. I emailed you to ask the permission to use the figures from Q-ToF Ultima MALDI User’s Guide: Atlas Park, Manchester; Micromass UK Limited M12 5PP. Waters Synapt High Definition Mass Spectrometry System, Operator’s Guide. 7.000612666 Revision A. These figure are going to be in my Ph.D. thesis.

Thank you in advance for your reply.

Sincerely,

Vincenzo Scionti

Brian J. Murphy@waters.com <brian_j_murphy@waters.com>
To: Vincenzo Scionti <vs25@zips.uakron.edu>

Mon, Apr 16, 2012 at 3:40 AM

Hello Vincenzo,

Thank you for your email message.

You have our permission. Please use the following copyright notice with each figure. The year of publication will be found in the operator’s manual.

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Best of luck completing your thesis.

Brian J. Murphy
Manager, Public Relations

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WHATS POSSIBLE™

Waters Corporation
34 Maple Street
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Permission request
2 messages

Vincenzo Scionti< vs25@zips.uakron.edu>
To: vgf@total.com
Sun, Apr 15, 2012 at 7:27 PM

Dear Mr. Victor Fursey,

I am a graduate student at the University of Akron and I am getting my Ph.D. under the supervision of Dr. Chrys Kostoglou. I emailed you to ask the permission to use the figure from Understanding ion trap mass spectrometry, Bruker Eksigent HCT Series User Manual, version 1.1, p. 1-3; ETU and PTR with the HCT ultra (July 2008) Bruker Compass Application Tutorial, version 1.3, p. 9; MALDI ToF/ToF Ultraflex III user manual. These figures are going to be in my Ph.D. thesis.

Thank you in advance for you reply.

Sincerely,

Vincenzo Scionti

Fursey, Victor < vgf@total.com>
To: Vincenzo Scionti < vs25@zips.uakron.edu>
Cc: ns@total.de
Sun, Apr 15, 2012 at 5:56 PM

Hi Vincenzo

This should be fine and I copy our EVP Michael Schubert from our factory for info as well.

Good luck with the Thesis

Vic Fursey

Bruker Daltonics

Victor Fursey,
Vice President, Sales – North America
Bruker Daltonics Inc.