From chlorinated transformation products to highly hydrated ions with electrospray ionization mass spectrometry

by

Jennifer Lynn Pape
B.Sc, University of Calgary, 2001

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in the Department of Chemistry

Supervisory Committee

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Abstract

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Pharmaceutical and personal care products (PPCPs) triclosan and nonylphenol, were investigated throughout wastewater treatment in a publicly owned treatment works (POTW). Both compounds react quickly upon chlorination under laboratory conditions, transforming into mono and dichlorinated species. A novel quantitative analytical method employing mass spectrometry was demonstrated on Delaware POTW wastewater samples. Specific transformation products were not detected and the concentration of precursor analytes was not found to be statistically different after treatment. Under tertiary chlorination conditions, transformation products are not produced.

ESI-MS was used to explore triply charged, highly hydrated lanthanide ions and charge reduction was directly observed in the MS collision cell. This process proceeded via proton transfer, proved by a strong correlation between the minimum number of water molecules required to stabilize the Ln^{3+} and the first hydrolysis constant (R^2=0.92). The effect of different solvents on the surface activity of ions under electrospray ionization (ESI) was investigated using dilute ionic liquids and the relative surface activity of a given pair of ions could be reversed by moving from a relatively polar solvent to a relatively non-polar one.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

APCI – atmospheric pressure chemical ionization

APEO – alkylphenol ethoxylate

BIRD – blackbody infrared dissociation

BMIM – 1-butyl-3-methylimidazolium

BOD – biochemical oxygen demand

CID – collision induced dissociation

Da – Dalton

DMF – dimethylformamide

DMSO – dimethyl sulphoxide

DPD – *N*,*N*-diethyl-*p*-phenylenediamine

EDC – endocrine disrupting compound

EDESI – energy dependent electrospray ionization

EDTA – ethylenediamenetetraacetic acid

EI – electron impact

EPA – Environmental Protection Agency

ESI – electrospray ionization

EXAFS – extended X-ray absorption fine structure

FAB – fast atom bombardment

FWHM – full width at half maximum

GC – gas chromatography

HLB – hydrophilic-lipophilic balanced

HPLC – high performance liquid chromatography

IL – ionic liquid

IPR – initial precision and recovery

IUPAC – International Union of Pure and Applied Chemistry

LC – liquid chromatography

LC_{50} – median lethal concentration

LD_{50} – median lethal dose

LDI – laser desorption ionization

m/*z* – mass to charge ratio

MALDI – matrix assisted laser desorption ionization

MAX – mixed-mode anion exchange

MCP – microchannel plate

MDL – method detection limit

MGD – million gallons daily

MRM – multiple reaction monitoring

MS^n – mass spectrometry to the n^{th} stage

NHANES – National Health and Nutrition Examination Survey

NOM – natural organic matter

NP – nonylphenol

NP1EO – monochlorononylphenol

NP2EO – dichlorononylphenol

PCB – polychlorinated biphenyl

POTW – publicly owned treatment works

PPCP – pharmaceutical and personal care product

QQQ – triple quadrupole

RTIL – room temperature ionic liquid

SPE – solid phase extraction

Tf – triflate ($CFSO_3^-$)

ToF – time of flight

UHPLC – ultra-high performance liquid chromatography

USGS – United States Geological Survey

WWTP – wastewater treatment plant

Acknowledgments

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As well, I would like to acknowledge my funding bodies, AXYS Analytical Services Ltd. and Mathematics of Information Technology and Complex Systems (MITACS) for their generous financial support in this undertaking.

Dedication

For my husband Clive and my parents, Eloise & Norman, thank you all for your love and support.

&

For my grandparents, especially those who did not see this work completed but always knew that it would be.

Chapter 1: Introduction to Mass Spectrometry

1.1 The beginnings of mass spectrometry

Mass spectrometry is, as it sounds, a technique fundamentally rooted in the masses of materials at the molecular level. Unlike spectroscopy, this process does not use light, rather, electric and / or magnetic fields are manipulated to accomplish the separation of ions based on their mass to charge ratio (*m/z*). The discipline of mass spectrometry has been evolving for nearly a century with the advances in instrumentation spread primarily between physics and chemistry while its considerable applications extend beyond both of these fields. These objectives vary from fundamental studies to routine quantification with one of the most important and well known functions of mass spectrometry being the determination of molecular weight (allowing elucidation of chemical formulae). As a brief introduction, the roots of mass spectrometry along with selected advances in instrumentation and common techniques currently in use will be explored. This will provide a sound basis for the novel mass spectrometric investigations presented.

In the early 20th century, there was a dearth of information about the elements, certainly much of which is often taken for granted today. The elucidation of the *m/z* ratio of an electron garnered J. J. Thomson the 1906 Nobel Prize[1] and, soon after, in 1913 his continued work resulted in the discovery of the first isotopes, those of neon.[2] The first mass spectrometer, based on the same principles Thomson used to weigh the electron,

was a magnetic sector instrument using directional focusing built by A. Dempster in 1918.[3] This focus on isotopic study was maintained into the mid-1930s[4] but in the twenty years following major developments in instrumentation such as high resolution techniques and the advent of the quadrupole mass filter began. In addition, emphasis on the importance of mass spectrum analysis, centering on hydrocarbons,[5] was established.[6,4] Few major books or reviews were available until this point. However, as mass spectrometry emerged in importance,[7] techniques became increasingly specialized and scientific interest evolved rapidly.

1.2 An overview of instrumental design

Mass spectrometers, regardless of design, include three regions: 1) a source which provides or enhances species ionization, 2) a separation region based on *m/z* and 3) a detector. Multiple pumps, roughing (foreline) pumps and turbomolecular or diffusion varieties, are necessary in these systems to provide the high level of vacuum required. Clearly, for a technique dependent on charged ions, the production of these ions is of the utmost importance and as such there are a great variety of sources available. The style of source used necessarily depends on the physical form of sample to be analyzed (solid, liquid or gas). In particular, these instruments are identified by the source and separation regions as it is this combination which allows fine tuning for the intended applications.

1.3 Ionization sources

Many different ionization techniques have been used in the evolution of mass spectrometry and these sources are generally grouped into two types: those which must be maintained under a vacuum (e.g. electron impact) and those more recently developed which may be operated at atmospheric pressure (e.g. electrospray ionization). Ionization is also a key aspect in the speciation observed in a mass spectrum, consequently these modes of ionization are further separated into a spectrum of hard and soft forms. To understand the importance of this designation, a primary concept is that of the *molecular ion*, which is directly representative of the species being measured. The molecular ion is unfragmented and results from the loss of an electron from the analyte molecule. In some types of ionization a quasi-molecular ion may be formed instead, generally by the addition or loss of a proton. The quasi-molecular ion is still a representative unfragmented species but the *m/z* ratio will demonstrate a +/- 1 unit difference from that of the expected chemical formula. The term hard ionization generally implies extensive fragmentation of the molecular ion. By choosing consistent instrumental settings this fragmentation becomes characteristic across different mass spectrometers of the same type. The fragment data may be compiled into libraries and used for later identification of the molecule. In contrast, in soft ionization techniques the quasi-molecular ion is nearly always observed, sometimes exclusively. Its appearance allows the calculation of the molecular formula from the mass to charge ratio.

1.3.1 Electron impact ionization

The earliest ionization technique in mass spectrometry was electron impact (EI) ionization, first used by A. Dempster[3] and later optimized by A. O. Nier and W. Bleakney[8]. EI is a hard form of ionization, an energetic technique producing a great deal of fragmentation.[4] Requirements for EI are a volatile and thermally stable analyte molecule(s), that are generally introduced into the source perpendicular to the electron beam in the gaseous phase (Figure 1.1.). This region must be under vacuum to avoid the ionization of extraneous gases (elevated background interference). A beam of energetic electrons from a heated tungsten or rhenium filament interacts with the gaseous sample and ionizes it. The optimal beam energy maximizing ionization efficiency is 70eV; this setting may be reduced to minimize fragmentation increasing the likelihood of obtaining a molecular ion. The ions are further acted upon by the ion repeller electrode which forces them away and towards the analysis region of the mass spectrometer travelling through the focusing plates and accelerating into the mass analyzer. It has been estimated that for every 1000 molecules introduced into the source, a single ion is transported into the analysis region of the mass spectrometer.[9] EI is particularly suited to gas chromatography and is often associated with sector mass spectrometers. However, negative ionization is impractical for EI in terms of sensitivity because electron capture is a much less efficient process than ionization. Extensive fragmentation libraries are available for the identification of unknowns due to the long history of EI ionization and its prominence in the GC-MS analysis of mixtures of unknowns.[10,11]

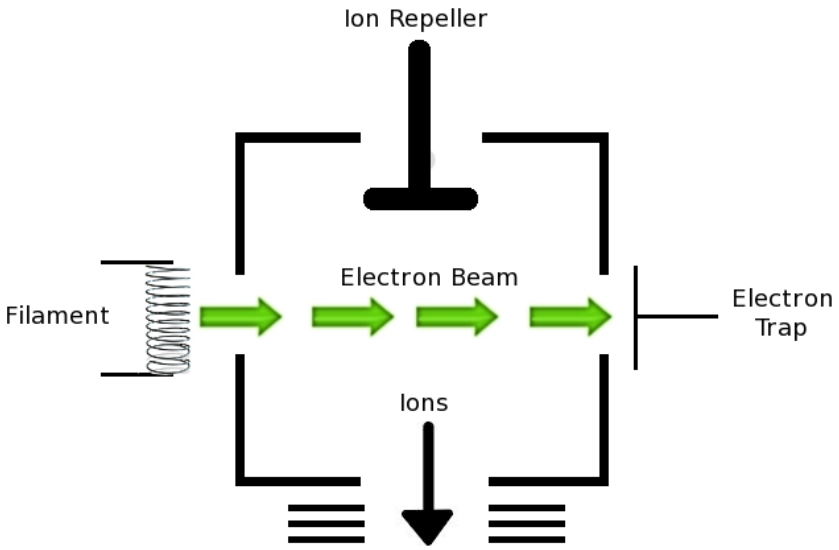

Figure 1.1. An electron impact source used to ionize gaseous samples.[12]

1.3.2 Matrix assisted laser desorption

Matrix assisted laser desorption ionization (MALDI) is a recently introduced (1987) technique.[13,14] It combines the principles behind two older forms of ionization: high-intensity laser desorption/ionization (LDI)[15,16] and fast atom bombardment (FAB)[17]. The first influence, LDI, uses a nanosecond laser pulse to ablate a portion of a sample creating ions and releasing neutral material and often produces highly fragmented signals due to the excess energy provided. In the much improved MALDI a lower fluence laser regime as well as an additional sample preparation step, adopted from FAB, are incorporated. The sample is combined with an appropriate matrix (usually an aromatic acid) in excess and dried, providing a co-crystallized material. The matrix efficiently absorbs much of

the energy from the laser pulse when the desorption/ionization step occurs (Figure 1.2.). This advancement makes MALDI a soft technique with simplified spectra due to the minimal fragmentation and the primarily singly charged species produced. The ionization mechanism for MALDI is not yet well understood and, though both positively and negatively charged ions may be produced,[18] the former species is more commonly analyzed.[19] However, it is the most sensitive laser technique and works well with very large molecules (>100,000 Da) a desirable trait in proteomics.[9] The pulsed ion beam aspect pairs satisfactorily with time-of-flight mass spectrometers. As well, buffers and salts which cause problems in other ionization methods tend to have a lesser impact on MALDI. The benefits of this method have made it very popular and an extension to atmospheric pressure MALDI has been developed.[20]

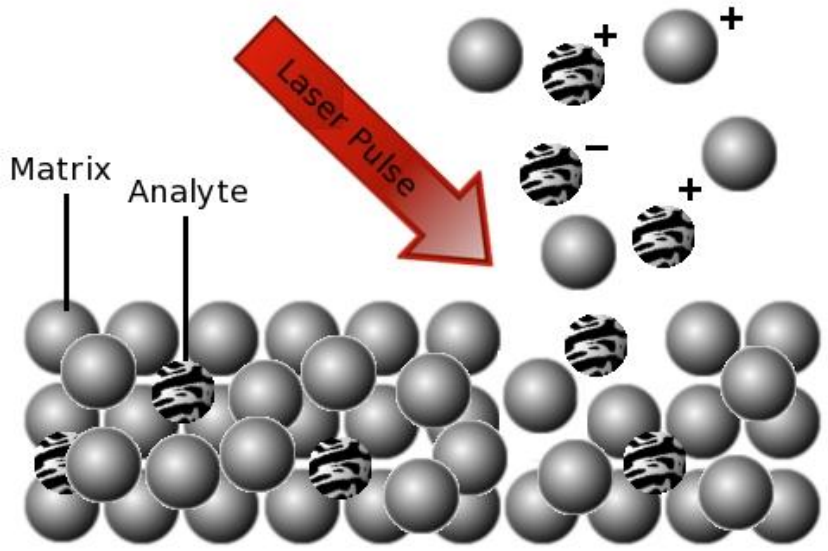

Figure 1.2. Laser desorption of a prepared co-crystallized sample in matrix by MALDI.[21]

1.3.3 Electrospray ionization

Electrospray ionization[22] (ESI) was developed into a preeminent atmospheric pressure technique after the initial work by M. Dole[23] and the significant demonstration by J. B. Fenn in 1989 of the analysis of high mass proteins via multiple charges[24]. Once the ability to identify proteins was evident, acceptance and strong interest came quickly.[25] ESI is now highly commercialized and is currently the most widely used source for mass spectrometry.[26]

In ESI, a sample solution is introduced into the source through a capillary and can therefore be easily coupled to a liquid chromatography system. ESI relies fundamentally on electrochemistry to function and may be regarded as a specialized cell (Figure 1.3.).[27] In the positive ionization mode electrons flow away from the capillary, typically through oxidation of the stainless steel capillary, driven by a power supply. The circuit is completed by ions arriving at the detector or discharged elsewhere in the instrument. Meanwhile positive charge is transmitted through space via the ion flow from the sprayed sample. As well, analytes may be oxidized in solution (or reduced in the case of negative ionization) but only when the analyte is unusually susceptible to this process. The enrichment of the positive charge in the sprayed droplets is due to the high electric field applied at the capillary tip, near 3 kV.[28] There is a minimum conductance required for electrospray to occur; a micromolar concentration of electrolytes is necessary.[29] A charged aerosol is generated, ideally via a jet from a Taylor cone,[30] and the droplets are subjected to further dehydration. At the point where the Rayleigh limit is reached

Coulomb explosion occurs. As an example, given a 1.5 μm diameter droplet from the outset, after the first Coulomb explosion this diameter is reduced to approximately 0.1 μm in the droplets produced and the degree of charge on these smaller droplets increases.[9] Ions are formed in this process, though the actual mechanism is under debate, and those with appropriate characteristics are drawn through orthogonally placed cones into the mass analyzer.

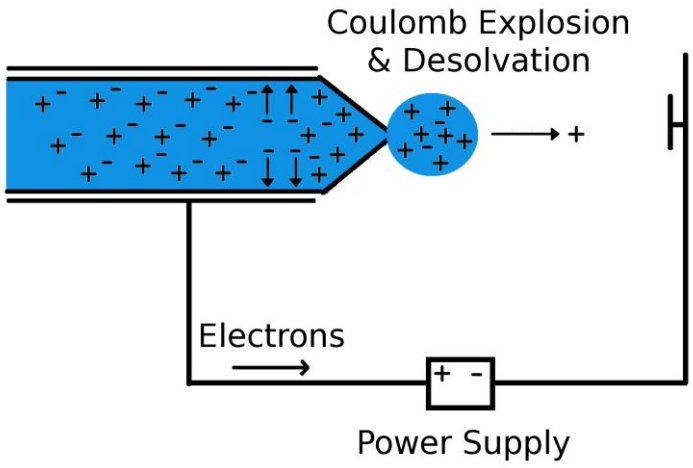

Figure 1.3. The electrospray ionization process "analogy" as a special type of electrolytic cell.[28]

As a successful ionization technique, the ability of ESI to reflect the reality of solution phase chemistry while working with ions in the gas phase has been questioned and explored in a variety of cases.[9,31] While ESI may not always be reflective of the charge state in solution, there is a necessity to understand why these differences exist to obtain the most useful data from this technique. One of the major factors to consider is the mechanism by which ions are produced. There have been two main theories put

forward for the ESI mechanism (Figure 1.4.),[32] the first devised by M. Dole is known as the charged residue method. It states that charges are produced through successive Coulomb explosions and the eventual desolvation of an isolated charge from a tiny droplet (radius < 3 nm). This mechanism has been accepted for large molecules.[33] The second theory proposed by J. V. Iribarne and B. A. Thomson is the ion evaporation model.[34] This approach is similar to the charged residue mechanism with respect to Coulomb explosions. However, rather than having successive charge separation and eventual dehydration this theory proposes that earlier in the process a single solvated charge is ejected directly from a larger, more highly charged droplet (radius = 10 nm) and then desolvated.[35] Ion evaporation of small, protonated aqueous clusters have been directly observed for relatively low mass hydrated lanthanide species by tandem mass spectrometry.[36,37]

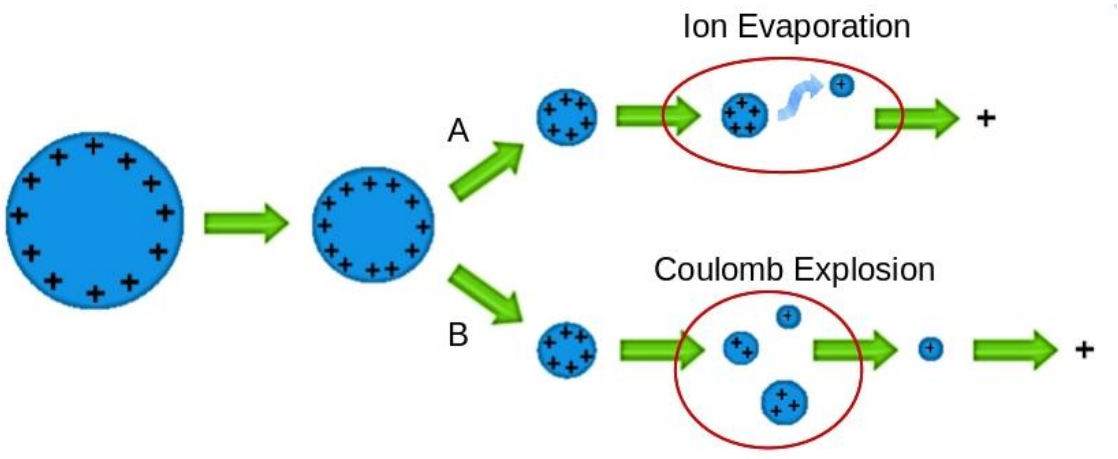

Figure 1.4. Proposed electrospray mechanisms[38]:
(A) Ion Evaporation & (B) Charged Residue Mechanism.

An interesting precursor to the routine electrospray technique capable of handling milliliter per minute flow rates was the use of nanospray ionization[39] for analysis ("nano" referring to the flow rate, i.e. nanolitres per minute). Nanospray essentially moves the electrospray process a step further forward by producing even smaller initial droplets. This results in heightened instrumental sensitivity due to the increased efficiency of ion formation but may be less robust due to easily blocked capillaries.[26,40]

The main issue with ESI, signal suppression or enhancement, is due to the circumstances discussed previously and consequently it is important to understand the impacts of surface activity on a sample.[41] Given two analytes, the one that is the most different from the solvent sprayed would prefer to be the least solvated, increasing its concentration at the surface of a droplet while the other species tends to reside in the bulk solution along with the charge paired species. Due to the mechanism by which smaller droplets are formed during Coulomb explosion, surface analytes are enriched. Ideally all analytes would have a surface concentration proportional to their actual concentration leading to consistent instrumental response. Examples of this non-ideal matrix effect, described as the Achilles' heel of ESI,[42] affecting surface activity and thus quantification have been demonstrated and remediation methods are under debate (refer to Section 2.10 for further discussion).[43,44,45]

Matrix effect is the general term used to describe the phenomenon resulting in a loss of instrumental linearity at high concentration ($> 10^{-5}$ M) as well as non-representative ionization, suppression[46] or enhancement, of the chemical species in a

sample at any concentration. This occurrence in ESI was first described scientifically by L. Tang and P. Kebarle in a 1993 study which compared different analyte concentrations and the resulting ionization. In this case surface activity was found to exert an influence at levels micromolar and below, while ion evaporation became a significant factor at just above micromolar to millimolar levels.[47] Currently, matrix effects in ESI are not well understood[48,49] but with the predominant usage of this technique, clearly any confounding factors are of importance. In one recent analytical study, 164 analytes out of a list of 198 (83%) suffered from matrix effects. These were identified using post-extraction spiked native analytes (avoiding analyte loss due to work-up procedures) and compared to a native standard.[50] Routinely, matrix effects may be evaluated through the use of a standard spiked at known concentration into extracts directly prior to instrumental analysis.

Both gas and solution phase processes are evident in ionization, though it is the solution phase that has been linked to matrix suppression.[51] As the impact of the mass analyzer in this case is of minimal importance (ionization has been accomplished prior to this point), the electrospray interface itself becomes the focus. Competition is responsible, at least in part, for matrix effects and it has been observed that both small and polar analytes tend to be prone to suppression,[52] possibly due to the particular solvents that are amenable to electrospray. A nice demonstration of suppression is provided by R. King comparing two different modes of ionization: APCI and ESI, as well as the use of a dual electrospray source. This study investigates the influence of proteins

in extracted plasma on the ionization of infused pharmaceuticals (including urapidil, caffeine and phenacetin) and exemplifies matrix effects in ESI.[51]

1.4 Mass analyzers for ion selection

Mass spectrometry relies on the ionization of a sample to provide a charged "handle" allowing the sorting of masses by the application of magnetic and / or electric fields and separation to be achieved. Early instruments had poor sensitivity and resolution, but these qualities have long since been optimized through a variety of advancements. There are four major types of mass analyzer: sector, time-of-flight, quadrupole and ion trap and the strengths of each of these designs for ion selection will be discussed.

The earliest mass spectrometers relied on sector technology, introduced in 1940 by A. O. Nier as the magnetic analyzer,[53] and these components are still used today. The development of double focusing techniques, referring to control of both the direction and energy of the ions, in mass spectrometers employing both electric and magnetic sectors (Figure 1.5.) allowed for high mass resolution work just over a decade later. This advancement began the precise identification of unknown species[54] and solidified the sector mass spectrometer as the instrument of choice early in the advancement of the field.

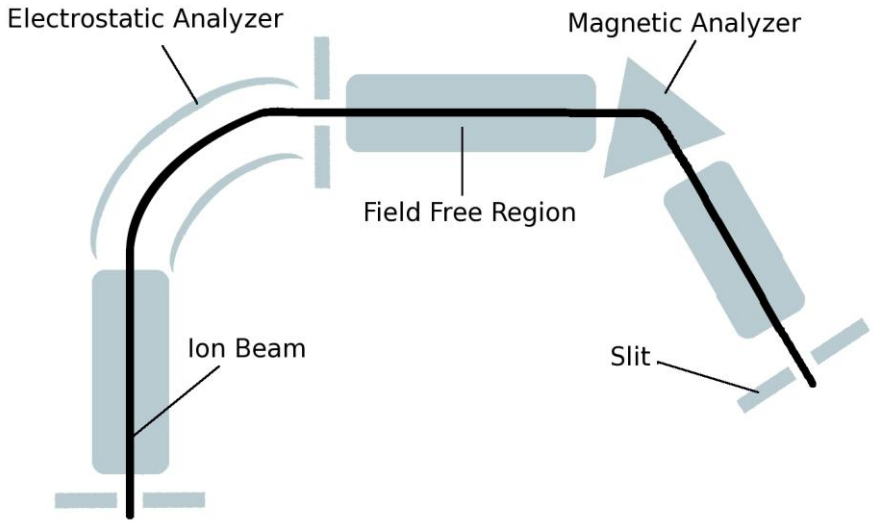

Figure 1.5. A double focusing sector mass spectrometer (Nier-Johnson geometry).[55]

Time of flight analyzers (ToF) were invented by A.E. Cameron and D. F. Eggers in 1948,[56] from theory developed a few years earlier. These instruments initially suffered from very poor resolution due to uneven ion energetics in the flight tube where ion separation occurs.[57] This problem was minimized over twenty years later by the inclusion of a reflectron (Figure 1.6.),[58] a device which revolutionized time of flight mass spectrometry. The reflectron allowed greatly enhanced peak resolution ($m/\Delta m$ ~10,000) by compensating for differing initial energies of ions with the same *m/z* prior to separation.[57] This increased performance caused a surge of popularity for the technique due to the high volume of information achievable, as time of flight provides full spectrum data very quickly and is capable of analyzing a theoretically unlimited mass range (20,000 *m/z* is common in practice though higher values may be reached with specific instrumentation).[59] Routine ToF analysis is well suited to forms of pulsed ionization like

MALDI while continuous ionization like ESI is better paired with orthogonal ToF. In the latter method a group of ions produced by the source are selected as a packet by the periodic application of an accelerating voltage from the pusher electrode. This energetic "kick" provides a small group of ions, which are separated and detected and the cycle is repeated. ToF instruments have become more commonplace with advancements in technology increasing the capacity for data storage and manipulation but they are more expensive than their quadrupole counterparts.

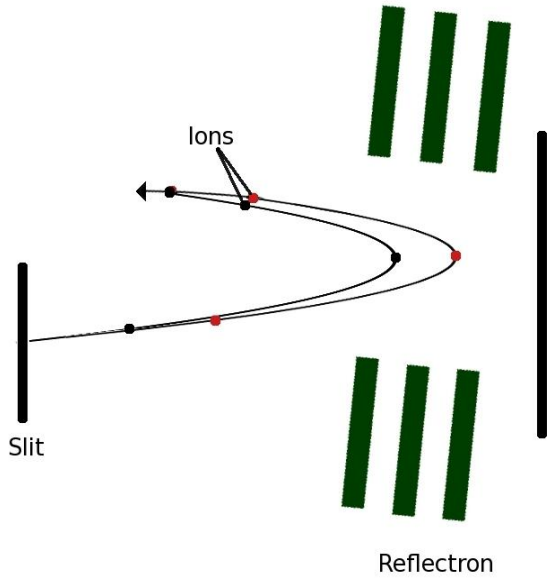

Figure 1.6. The standardization of energies for two ions of equal *m*/*z* provided by the reflectron in the flight tube region of a ToF mass analyzer.[57]

In the early 1950s W. Paul developed the quadrupole mass filter (Figure 1.7.) which quickly became a very useful technology.[60] The original quadrupole mass spectrometer had one set of four charged parallel rods arranged in a square configuration allowing ion

separation in space by the use of both direct and alternating currents. A selected mass would achieve a stable path while traveling between the rods allowing it to pass safely through, while the other ions would be destabilized with increasing oscillation and eventually be discharged directly on the rods themselves.[21] This process and the resulting stability are characterized mathematically by the Mathieu equations.[61] Intrinsically, the quadrupole mass spectrometer is a scanning instrument as ions are permitted through sequentially to acquire a spectrum. These instruments have very good sensitivity and are commonly used for analytical work. Despite lower resolution and mass range (generally <4,000 Da)[62] than other types of mass spectrometers, they are popular commercially due to moderate pricing. As well, with good ion transmission, quadrupoles are often used as an initial mass filter for tandem applications.[59]

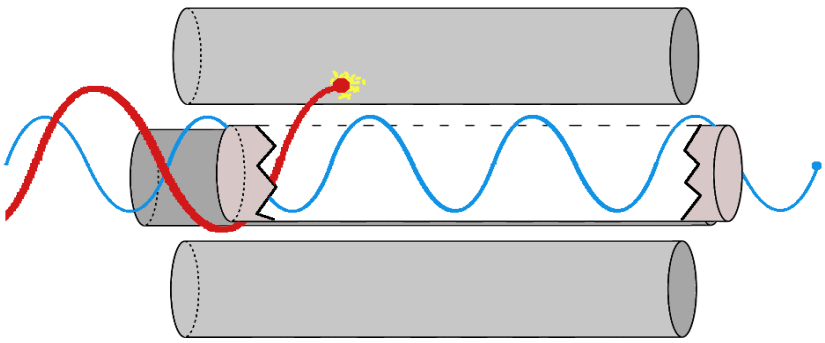

Figure 1.7. Quadrupole rods with alternating charge.[21]

The blue ion, mass selected, has a stable pathway and is passed through while the red ion shows instability discharging (yellow) on one of the rods.

The ion trap mass spectrometer provides a mechanism by which ions may be stored over time then selectively released and monitored. There are many distinct analyzers in this category and a brief overview of selected topics will be provided here, many excellent reviews are available in the published literature. [2,63,64,65,66] Like the quadrupole mass filter, the ion trap is a scanning instrument requiring a small amount of sample. These instruments were first demonstrated in the 1950s though the underlying theory was understood long before this point. To contain the ions three electrodes are used: one ring and two end caps (Figure 1.8.). In contrast to the quadrupole mass filter where ions are passed through the system, here the ions are stabilized within the trapping field then destabilized to eject masses. Ion trapping mass spectrometers have been used for accurate mass applications[2] and the recently invented and commercialized Orbitrap,[67] has been recognized as a promising new pulsed mass analyzer.[68,69] One specialized technique provided with ion traps is the ability to attain MS^n whereby a selected mass is fragmented using multiple stages. After the first generation the "parent" ion breaks apart into numerous fragments, selected fragments from the original *m/z* are then collided with inert gas producing yet further fragments. This step-wise fragmentation process can be very useful in identification when characteristic masses are found.

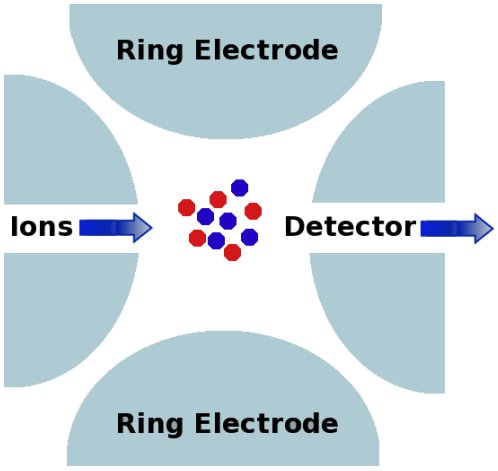

Figure 1.8. A cross section of the trapping region of a quadrupole ion trap mass spectrometer.[2] Ions circulate within this region until selectively ejected for analysis.

1.5 Electron multiplication detectors

Discrete dynode detectors[70] (Figure 1.9.) are a variety of electron multipliers, the descendants of the elementary Faraday cup, a classic detector used in mass spectrometry.[71] These devices are made of materials such as a beryllium / copper alloy that is sensitive to atmosphere. The gain in the system rises with increasing numbers of dynodes, typically 12 to 20, and functions via secondary electron emission.

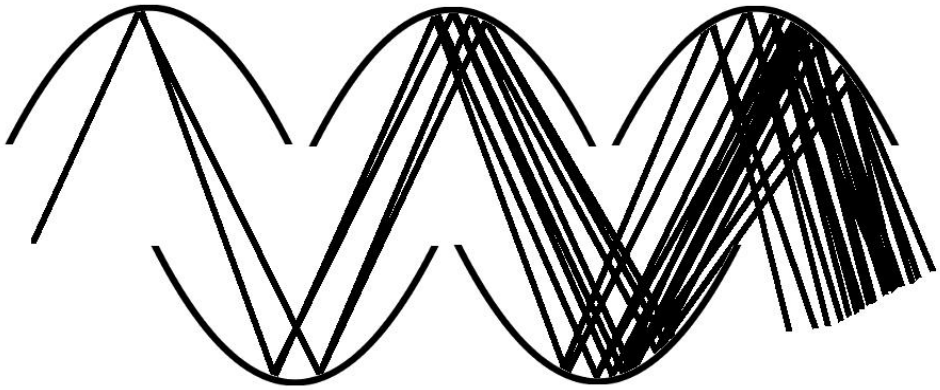

Figure 1.9. A discrete dynode detector.[9] An ion impact releases electrons which begin an electron cascade and results in the overall amplification of the signal.

Channel electron multipliers (or channeltrons), developed around 1960,[72] were an evolution of the discrete dynode detector. They consist of a compact curved lead / silicate glass tube, rather than individual units, and have improved atmospheric stability compared with the discrete dynode detectors. As well, these detectors produce high gain (up to 10^8) and have a large linear range.[73] However, channel electron multipliers are subject to saturation effects at high ion concentrations and have a short 1-2 year lifetime.

A microchannel plate (MCP) detector is a parallel set of hundreds of electron multiplier tubes fashioned out of lead glass with diameters ranging from 10 to 100 μm and a length of at least 40× this (0.4 to 10 mm).[74,75] The channels are nearly parallel to the surface and function as a continuous dynode with the top and bottom of the system as the input and output electrodes. MCPs are very fast, reacting in less than 100 picoseconds

and a single electron is amplified by 4 to 7 orders of magnitude in an electron cascade.[76] Multiple plates (usually two or three) may also be used in conjunction to increase the gain of the detector.[9] After an ion impact there is a short effective dead time, less than 10^{-7} seconds, making these detectors well suited to mass spectrometric techniques although they are expensive and fragile.

1.6 Tandem mass spectrometry

A mass spectrometer may be evaluated based on two major factors: sensitivity and resolution. Sensitivity is a function of the ionization technique selected as well as the type of mass analyzer used and the mode the instrument is operated in. It defines the minimum level at which an analyte can be detected by the system. Resolution is the ability of a system to discriminate between two peaks of similar *m/z* and is defined as $m/\Delta m$ using the full peak width at half maximum response (FWHM).[77]

Mass analyzers are seldom used in isolation, rather the abilities of the different analyzers are operated in combination. The simplest system in this category is the triple quadrupole instrument presented in 1978.[78] The design uses two quadrupole mass analyzers in tandem (Q1 & Q3), separated by a central quadrupole region (Q2) known as the collision cell. Q2 is used to allow interaction of the ion, selected in Q1 and accelerated, with a neutral collision gas, generally argon. The charged fragments created by this process when provided sufficient energy, known as collision induced dissociation (CID),[79] are monitored in Q3. Multiple reaction monitoring (MRM) is a common and more specific use of CID whereby a particular *m/z* is selected in Q1 of the mass

spectrometer, subjected to fragmentation in Q2 and one or more specific fragments are monitored via Q3. The MRM application is of fundamental importance in analytical chemistry for the trace and ultra-trace identification of unknowns because of its specificity (minimizes isobaric interferences) and superior sensitivity. In particular, this level of sensitivity is achieved by effective selected ion transmission, expulsion of extraneous ions and increased dwell time as scanning is focused on the analytes of interest only. In addition to the triple quadrupole other instruments included in the tandem category are the quadrupole-ion trap[59] and quadrupole-time-of-flight (Figure 1.10.).[80] Both of these instruments extend functionality due to the combination of quadrupole (selectivity and ion transmission) and fragment ion monitoring from the ion trap (MS^n) or time-of-flight (full spectrum) analyzers. Additional applications of tandem mass spectrometers including reaction monitoring of systems, such as catalytic cycles, may be accomplished in the collision cell using charged species and altering the gases used in the system to induce reaction.[81]

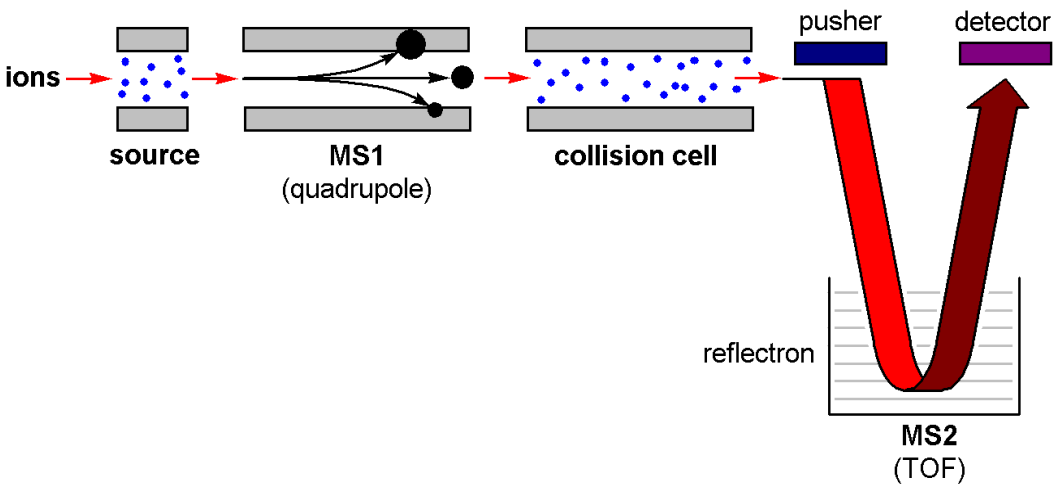

Figure 1.10. A quadrupole / time of flight mass spectrometer, reproduced from Figure 2.13, Henderson et al.[82]

1.7 Further applications

Mass spectrometry applications are as varied as the instruments themselves spanning topics including biological analysis,[83] natural products,[6] proteomics,[84] analytical quantification[85] and inorganic analyses[86,87]. Two dimensional separations have been achieved by both LC[88,89] and GC[90,91] allowing mass spectrometry to be used for the analysis of highly complex mixtures. Recent developments in the speed of electronics and data storage capacity have also allowed mass spectrometers, particularly the ToF variety, to keep pace with new developments. The extremely narrow peak widths (1 to 3 seconds) and fast separation provided by advanced chromatographic methods such as ultrahigh pressure chromatography (UHPLC) require the same number of data points for good peak shape, acquired at ~5 to 10× the rate needed in previous technology.[92,93,94]

1.8 Conclusions

Many types of instrumentation exist in the field of mass spectrometry and a variety of modes of ionization are available for a huge selection of techniques; these continue to be improved in both ruggedness and understanding. While mass spectrometry is not the best solution for all analyses, it is very well suited to studies involving limited amounts of material that may be ionized and will continue to be heavily used particularly in biological and analytical settings. An exploration employing two varieties of mass spectrometer, quadrupole-time-of-flight and triple quadrupole, will be presented. These instruments are used to develop and apply a quantitative analytical method to the current environmental issue of chlorinated transformation products in wastewater. Additionally ESI, presently the most popular ionization source, will be investigated further in terms of the two major issues with the technique: divergent results in comparison with observations drawn from solution phase chemistry and matrix effects related to surface activity.

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Chapter 2: Chlorinated Transformation Products in Wastewater Treatment

2.1 Analytical mass spectrometry

Mass spectrometry is a primary technique for examining trace (roughly parts per thousand to part per million) and ultra-trace (generally parts per billion or lower) analytical systems. There are a variety of mass spectrometers allowing analytical flexibility in combination with chromatographic based separations. One such pairing, high performance liquid chromatography and mass spectrometry (HPLC/MS), is of rising prominence in the environmental analytical field for the analysis of contaminants of emerging concern.[1,2,3] In particular, the triple quadrupole mass spectrometer operating in multiple reaction monitoring (MRM) mode has astonishingly high sensitivity allowing method detection limits in the parts-per-billion to parts-per-trillion range. The quadrupole / time-of-flight instrument has the ability to acquire high resolution *full spectrum* data very quickly providing a large amount of information, even with limited sample. Together these systems allow the identification of unknown contaminants in uncharacterized samples as well as the screening and ongoing monitoring of regulated analytes to be accomplished.

2.2 An introduction to contaminants of emerging concern

As the global population increases, the resulting burden on environmental systems grows rapidly. A variety of waste products including those from industrial, agricultural and household sources are released. Fats, personal care products, medical isotopes and

pharmaceuticals are a few examples of these extraneous and often xenobiotic substances. It is possible for waste products to be harmless or even beneficial to the systems that receive them (e.g. fertilizers for plants), but many have negative impacts on the health and well-being of both humans and the environment. A grave example is the legacy contaminants polychlorinated biphenyls (PCBs). Production of these materials began in 1929, and they were eventually recognized as a far-reaching environmental issue three decades later due to their disturbing tendency to bioaccumulate progressively through the food chain.[4] Modern or contaminants of emerging concern, on the other hand, are more likely to be present in low but constant concentrations due to continuous introduction and are termed "pseudo-persistent". Prior to the late 1990s, few detailed studies on contaminants of emerging concern, particularly pharmaceuticals, were available. Currently, interest in endocrine disrupting compounds such as nonylphenol (Figure 2.1.) or bisphenol A and elevated environmental levels of naturally occurring hormones, such as 17β-estradiol, has risen dramatically. The problem being recognized involves subtle effects, rather than overt toxicity, impacting the ability of a species to procreate successfully as well as the proper development of offspring.[5]

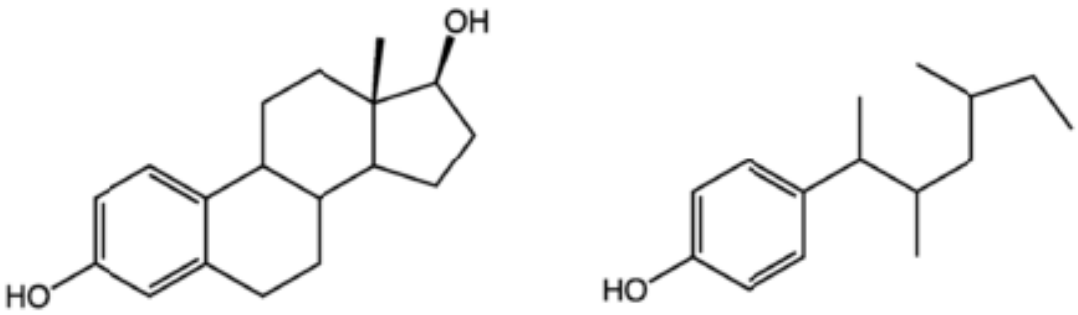


Figure 2.1. Mimicry of the 17β-estradiol steroid backbone by 4-nonylphenol.[151]

To discuss the impacts of particular contaminants, two different types of toxicity must be considered: acute toxicity and chronic effects. Acute toxicity assays provide quantitative information such as the median lethal dose (LD_{50}) or median lethal concentration (LC_{50}), allowing limited comparison between substances. These values are based on the population used for evaluation, rats for instance, and generally vary between species. Acute toxicity however, does not take into account long term effects that may occur after sub-lethal exposure or constant exposure to low concentrations of active substances over time. These chronic effects are more difficult to evaluate due to the large number of variables involved in the proper selection of the optimal testing dose and time span.

One important class of chronic effects is those which impact the endocrine system.[6] Endocrine disrupting chemicals (EDCs), as they are known, often show effects associated with reproduction. The method for monitoring these changes requires the use of biomarkers, specific chemical indicators of change. Generally biomarkers are a particular metabolite or protein that is present where it is not expected. One of the most useful biomarkers in aquatic systems is the production of vitellogenin[7] in male or immature female fish, a sentinel group, in response to estrogens or estrogen mimics. This substance is used by mature females as a component in the production of eggs and should not otherwise be present. When found in male fish, vitellogenin is often accompanied by feminization and may result in a severe impact on the overall population.[8,9] EDCs may affect other species, including humans, and have been controversially postulated as a factor in the declining sperm density in men.[10,11] These considerations have led to the inclusion of EDCs in biomonitoring studies in multiple matrices (e.g. urine, plasma) to

assess the concentrations of representative EDCs found and investigate their impact on human populations.[12,13,14]

Scientific interest in the environmental presence of pharmaceuticals and personal care products (PPCP) as potential endocrine disrupting chemicals commenced in the past two decades. In the 1990s data surrounding the endocrine disrupting ability of chemicals at large was coalescing and the broad scale impacts were recognized.[15,16,17] Beginning with multiple significant reviews pointing out the dearth of information,[18,19] interest in the topic of endocrine disruption[20,21] increased, shifting scientific focus from legacy contaminants and industrial products.[6,16]

The major concern of microbial resistance[22] directed this nascent field firstly towards the analysis of antibiotics. These pharmaceuticals are used in abundance to promote both human and animal health including as medication for veterinary[23] and aquaculture uses, though with time and further method development the analytical scope has expanded. A wide range of pharmaceuticals are routinely used, many to address chronic conditions, and a variety of personal care products are common in households. These PPCPs are discharged (e.g. excreted, improperly disposed medication) into wastewater, proceed through WWTPs and the resulting treated effluent is the predominant source of these chemicals to the environment.[24] In addition, the reuse of biosolid products as fertilizers and the looming issue of recycled drinking water[25,26] provided the drive to understand environmental monitoring data and comprehension of the major issues surrounding PPCP use and disposal. There has been a paucity of regulation in this area, as compared with pesticides, due to the lack of information to date. In the last decade, research in this field has exploded with many papers focusing on

all aspects of PPCPs from occurrence data to toxicological effects and risk assessment. Presently, an understanding of exposure, both human and environmental, to these chemicals is being sought through surveys based on multi-class analytical methods. The incredible variety of structures and properties in this category make even understanding occurrence data a challenge, much less determining the eventual fate of these compounds. Intuitively, substances which demonstrate effects at low levels will also be a challenge analytically and, as such, PPCP method development and optimization is ongoing.[27,28,29,30]

Contaminants of emerging concern are, for the most part, identified as exerting significant impacts at low concentrations. The mechanisms behind these impacts are not always well understood and consequently may be difficult to predict. Additionally, PPCP distribution in particular, is dependent on local usage patterns. These characteristics have caused concern regarding low-dose scenarios in which multiple low concentration chemicals, potentially below detection limits of current methods, may exert a combined impact.[31] In addition, the likely situation where mixtures of xenoestrogens are present has the potential to amplify the overall effects of these components and concentrations compared with isolated species.[32,33] Further the possibility has been raised that non-linear, even non-monotonic, inverted U-shaped, response curves may be observed.[31,34] This response is critical in risk assessment for the extension of predictions from one concentration range to another and non-linearity causes additional cost and complexity.

2.3 Wastewater treatment

Prior to the implementation of wastewater treatment practices in the 18th century much of the population was rural. With the industrial revolution, people began moving

into cities and outbreaks of disease caused by water pollution and the spread of bacteria were rife.[35] To combat this problem, wastewater treatment practices, such as chlorination, were adopted. Today, wastewater treatment is a primary remediation system to enhance the water purification process for waste products disposed of down the drain. Waste materials provided by large populations are now passed through a number of treatments prior to being released into waterways (Figure 2.2.). The extent of treatment procedures used varies with location as well as input and many regulations are maintained. These treatments may be separated into four categories: pre- and primary treatment, secondary and tertiary treatment.

Pre-treatment involves filtering and skimming steps to decrease the size of solid materials and reduce the fats, oils and grease present in the wastewater treatment system as well as passing the influent through screens for additional filtration. In primary treatment the wastewater is allowed to sit in large settling tanks, to allow physical separation of materials by density. This achieves the reduction of suspended solids as well as impacting the biochemical oxygen demand (BOD), the amount of dissolved oxygen required by organisms to break down the organic material.[36] In BOD measurements, decreased levels of dissolved oxygen imply the presence of more organic material.

Secondary treatment is biological in nature, occurs in an aeration basin and is commonly either fixed film (e.g. trickling filter) or suspended growth (e.g. activated sludge). A recent technology, the membrane bioreactor is more costly but appears to have potential in increasing the removal of undesirable organic contaminants.[37] This form of biological treatment uses carbon containing materials as a food source for

microorganisms. Secondary treatment sharply decreases the amount of organic material present, both suspended and dissolved, through breakdown and, ideally, promotes mineralization converting organic matter to CO_2, H_2O and inorganic salts. Notably, the microorganism population varies from plant to plant, allowing acclimatization and, as such, is able to digest different pollutants. This characteristic can increase the variability measured when tracking the efficiency of removal for a particular chemical species between multiple wastewater treatment plants (WWTP). Many WWTP complete their cycle by removing excess sludge and flocculent material using a clarification or filtering step and discharging water at this stage into streams, rivers or wetlands.

Tertiary treatments[38] are those provided in addition to the primary and secondary treatments, incur greater expense and vary from plant to plant. These may include the removal of nutrients or other material, such as phosphorus, or may focus on disinfection. Disinfection treatments such as chlorination, ozonation or exposure to ultraviolet light are generally used to further treat waters which will be released into drinking water sources.

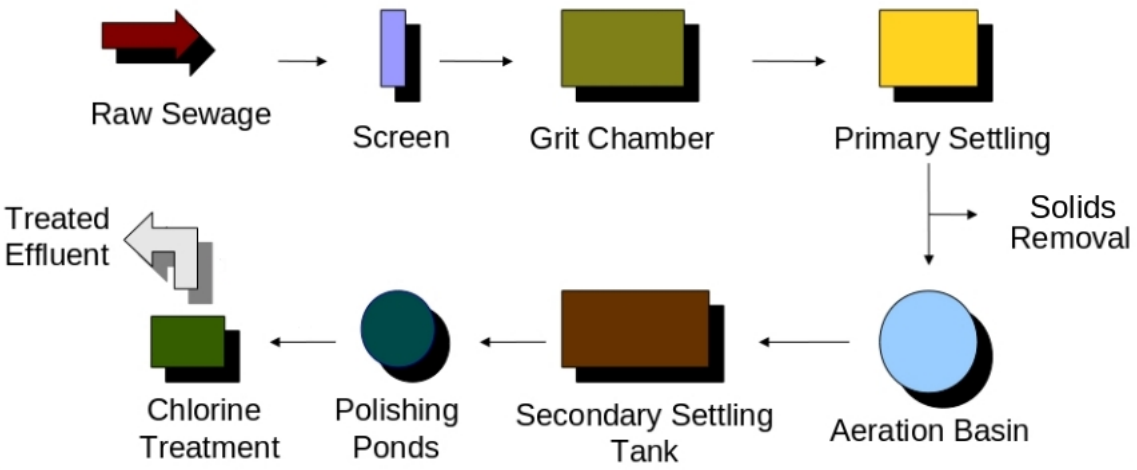


Figure 2.2. A model wastewater treatment plant.[39]

The first step in the regulation of a potentially harmful substance is gathering data characterizing three major areas: occurrence, transport and fate. For endocrine disrupting chemicals it has recently been recognized that there is a presence in the water supply encompassing wastewater,[19,40] surface[41] and ground water.[42,43,44] This was discovered due to observed changes in aquatic wildlife[45], such as feminization, and subsequent testing to identify the major causal agents.[46] This broad occurrence has given rise to concern and the monitoring of drinking water.[47,48] Investigations have traced contamination back to point sources indicating the presence of EDCs in sewage treatment facilities, where they are found in both influent and effluent as well as biosolids. Consequently, it has been recognized that sewage treatment plants are not only a resource for accelerated water purification but also well situated to aid in the control of water quality with respect to contaminants of emerging concern.[49]

The presence of EDCs in surface and ground water, which have clearly survived a variety of treatment processes to be released into the environment, is in itself justification for assessment.[50] The minimization of pollution has routinely been approached through the regulation of point sources in industry. Interestingly, due to the diversification of input to include the general population, these point sources have become the waste treatment facilities themselves. Conventional wisdom has maintained that once a chemical being monitored is not observed in effluent, it has been completely removed, however serious questions have now been raised about these substances. Have they actually been removed and mineralized as assumed, or has a transformation occurred through which relevant chemicals may become invisible to current monitoring strategies?

2.4 Chlorination & transformation products

Reagents such as chlorine gas or sodium hypochlorite are added to wastewater prior to discharge for the purpose of disinfection and the residual levels of chlorine leaving the system are monitored. In the 1970s, disinfection by-products were identified and it was demonstrated that natural organic matter (NOM) could react to produce chloroform when subjected to wastewater treatment chlorination.[51]

The topic of disinfection by-products in drinking water has been well studied and continues to be investigated. Many analytes, such as trihalomethanes, are monitored and treatment systems are carefully designed to minimize the production of these materials.[52] A more recent derivative of this work has been investigation into transformation products from the reaction of anthropogenic or non-naturally occurring contaminants such as pharmaceuticals with the chemicals used to maintain the water supply. These transformations are generally divided into two main categories: 1) microbiological, often concerned with aerobic and anaerobic conditions and 2) non-microbiological such as those caused by chlorination or ozonation. The result is an undesirable alternative to mineralization wherein contaminants are meant to be broken down into environmentally accessible forms.

A transformation product may be defined as a non-isomeric alteration in structure through a reaction, such as hydrolysis, which differentiates a substance from its parent compound. These changes may be reversible as in the case of conjugation (the process by which a xenobiotic compound is bound to an endogenous substance, generally increasing the overall polarity and enabling excretion)[53] or stable such as the chlorination of a phenol. The key aspect is the compositional alteration with the result that the substance is

not immediately recognizable by the techniques commonly used and accepted for monitoring. In consequence, trends that appear unambiguous, such as analyte removal, may actually be confounded. It is apparent that for the already vast field of environmental contaminants transformation products, whose fate it is important to understand, add an additional burden. Naturally, not every transformation product may be important in a regulatory sense (e.g. a benign transformation where activity is lost). However, without the knowledge of the alteration in toxicity it is possible that a transformation product may have an increased effect in comparison with the precursor compound. As a further example, metabolism leading to the conjugation of a substance, usually as a glucuronide or sulfonate, can affect physical properties such as polarity and provide a hidden guise from which the precursor analyte can eventually re-emerge.[54] Clearly, with limited resources available prioritization and thus risk assessment becomes a critical aspect of the solution to maintaining functional water supplies.

After the discovery of PPCPs and other EDCs in surface water, these chemicals were quickly linked to point sources, mainly sewage treatment plants. As the next step in investigation many studies focused on plant influent and effluent[50] to get a sense of the materials which were entering and those that were surviving treatment to later be discharged into surface water and potentially drinking water as well.[55] As each plant has its own combination of treatment strategies as well as biological cultures and input (e.g. diverse pharmaceuticals) these are not straightforward data. A variety of removal efficiencies has been observed from minimal to essentially 100% removal.[56] This immediately shows two facts: firstly that sewage treatment does have an impact on contaminant removal and secondly that there will be materials which survive the

treatment process. This second fact indicates that plant design may need to be optimized as a remediation strategy depending on the environmental impact of substances that are resistant to treatment, particularly in areas that are strongly impacted by effluent (e.g. streams and rivers in regions prone to drought). Monitoring and potential regulation will play an important role in point source management in this area. The question may also be asked: which aspects of treatment are the most effective at removing particular or classes of analytes? By analyzing biosolids, the role of sorption may be seen. Analysis encompassing a treatment process, such as activated sludge or chlorination, indicates the change in mass balance of a substance. Additionally, the mechanism of the process itself may be examined.

Once pharmaceuticals were discovered in the water system,[57,58] the removal efficiency through systems that were not designed for this purpose was questioned. Around the same time an additional factor was presented, polychlorinated phenoxyphenols were demonstrated to form chlorinated dibenzo-*p*-dioxins which spurred interest in the eventual fate of transformation products.[59,60,61] In the past five or six years, chlorination studies of particular analytes[62,63,64,65] have become more commonly available, examining selected groups of interest such as the fluoroquinolones[66] or acidic pharmaceuticals[67]. Chlorination products studied prior to this point tended to be in the context of different systems such as pulp and paper plants where high levels of treatment were used.

There are many difficulties associated with the monitoring of transformation products. In addition to conjugation[68] and metabolism by bodies prior to excretion, the products themselves may not have been previously identified. This complexity portends a

lack of methods, materials and standards. As such, laboratory bench studies are often used to obtain initial information regarding the types of products that may be formed under model conditions and the resulting hypothesis then extended further to more involved systems such as WWTP.

2.5 Project objectives & background

The main objective of this study was to demonstrate whether chlorination, a tertiary wastewater disinfection process occurring following solids removal, has a significant impact on the production of chlorinated transformation products in wastewater. This question was addressed using a two part bench study, reagent water followed by wastewater, to pinpoint well-suited analytes from the initial list for further investigation and determine their likely chlorinated transformation products. A quantitative analytical method was then developed and applied to samples collected from a Delaware publicly owned treatment works (POTW).

The initial analytes of interest (Figure 2.3.) were selected as follows: triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), triclocarban (3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea)), gemfibrozil (5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic acid), carbamazepine (5*H*-dibenzo[*b,f*]azepine-5-carboxamide) and nonylphenol (4-(2,4-dimethylheptan-3-yl)phenol along with its mono and diethoxylates (NP1EO, NP2EO). Selection of these analytes was based on three factors: literature review of previous chlorination reactivity, presence above the sample detection limit in wastewater samples during a prescreening analysis and current scientific and environmental interest. Categorically triclosan, triclocarban, gemfibrozil and carbamazepine are PPCPs while nonylphenols and related compounds are surfactants that may be included in this group.

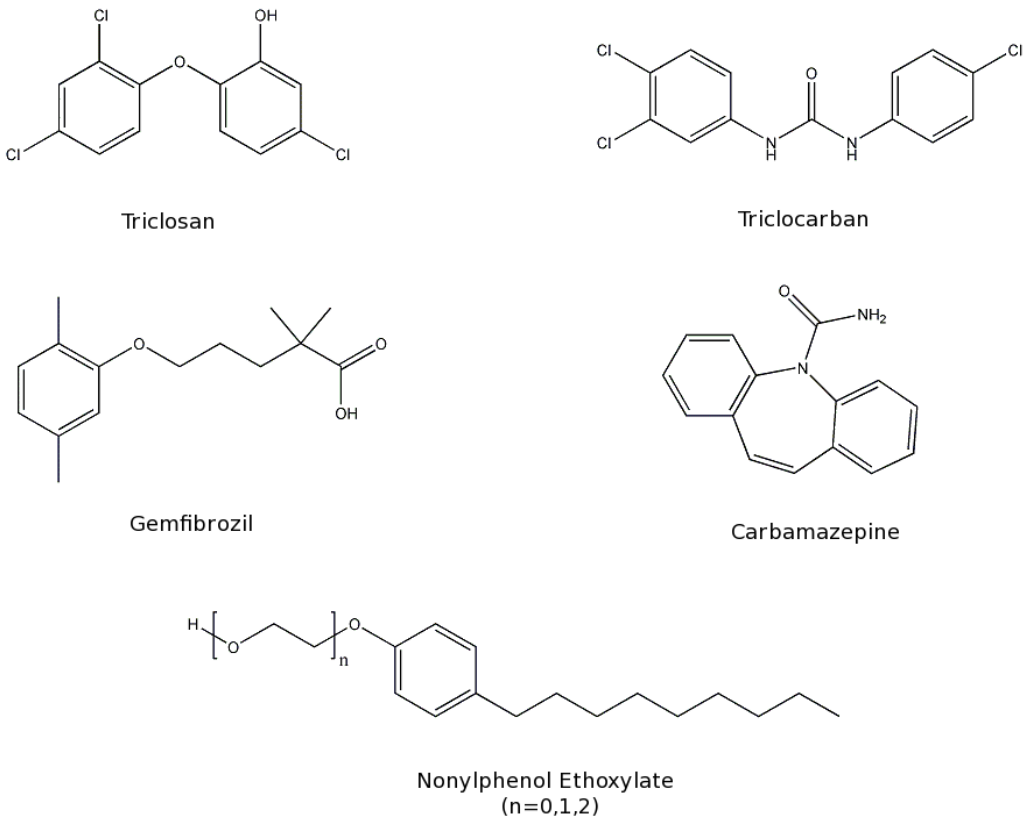


Figure 2.3. Chemical structures for the analytes of interest.

As discussed previously, after the discovery of clofibric acid in water systems, questions were raised about the prevalence and effects of pharmaceuticals and other endocrine disrupting chemicals in the water system. Early monitoring efforts often included the antiepileptic carbamazepine[40,69,70] which is prescribed worldwide,[71] may be found reaching part per billion levels in both effluent and surface waters[19] and, significantly, has been observed in ground water[48] and drinking water[55,72] surveys. Of particular relevance to this study, carbamazepine has survived drinking water treatment[47] involving chlorination[73] and is found as one of the most consistently occurring analytes,

to the point that it has been suggested as an anthropogenic marker.[74,75] This presence is primarily due to low removal efficiency through WWTP, generally below 10%,[76,77] even using advanced technologies such as membrane filtration,[78] though near complete removal has also been observed during wastewater treatment.[79] Notably, carbamazepine is also strongly metabolized in bodies (~1% excreted unchanged).[80] If such a large residual of carbamazepine is being found unaltered it is likely there is an additional burden existing in metabolite forms.[79,81]

Gemfibrozil is a lipid regulating drug found in effluent[40,82] and surface water[41,55] often in the hundreds of parts per trillion range more typical of PPCPs.[27] It is less persistent than carbamazepine; varied removal through WWTPs has been observed during wastewater treatment[83,84,85] Intermediate reaction of gemfibrozil has been observed during chlorination treatment indicating the potential for transformation[73,86,82 ,87] and transformation products have been identified in a recent bench study using high reagent concentrations.[88] Higher chlorine dosage, longer contact time[79] and reduced pH[89] can result in elevated removal, desirable as bioconcentration and adverse effects such as lipoprotein metabolism disruption from gemfibrozil have been observed in fish studies.[90,91]

The concerns around triclosan and triclocarban are slightly more involved than those of the previously described pharmaceuticals and triclosan in particular has been well studied in a variety of areas. Triclosan is a commonly used broad spectrum antibacterial both in medical and more recently consumer products.[92,93] It is not acutely toxic to humans, as an example this material is used in toothpaste,[92] but may be considered a type of chronic exposure[14]. Bioaccumulation of triclosan,[94] and more

significantly, less polar transformation products such as methyl triclosan is currently under investigation.[95,96] The excessive use of triclosan may select for antibiotic resistance[97] and further interest stems from the ability of both triclosan[98,99] and chlorinated triclosan transformation products[100] to be converted photochemically to dioxin structures.[101] Triclocarban is an antibacterial product used in conjunction with triclosan, though it has been studied far less than its counterpart.[102] Wastewater and surface water levels of triclocarban have been measured[103,104] as have biosolids where strong sorption is demonstrated.[105] Triclocarban has also been identified as a potential EDC and it appears that further study of this compound is warranted.[106]

With respect to occurrence, triclosan is seen in effluent generally at part per trillion levels,[55,77,107] though part per billion levels have also been reported[108] as has heavily impacted drinking water.[109] In wastewater treatment, removal of this compound tends to be high, above 90%, a portion of which is partitioned to sludge.[110,111] Triclosan was among the 30 most frequently detected compounds in surface water in a pivotal national United States Geological Survey (USGS) study.[41] It has been assessed by the National Health and Nutrition Examination Survey (NHANES) as part of the body burden and is frequently detected in urine[12] in both free and conjugated forms. Both the glucuronide and sulfonate metabolites are present though the former is more significant.[112] Triclosan has also been found in human plasma and milk,[14] while environmental contamination by this chemical has been tracked back 40 years in sediment cores.[113]

In terms of transformation a triclosan study investigating reactivity with chloramine using high ammonia concentrations found both tetraclosans and pentaclosan. The prediction was made that both the monochloramine and dichloramine species reacted

with the triclosan to produce the transformation products.[114] The chlorinated species, tetraclosan and pentaclosan, monitored in wastewater were seen at low levels.[115] A study in mice also showed that there is different retention of triclosan as compared with its chlorinated derivatives[116] and chlorinated triclosan toxicities have been shown to be elevated when compared with the parent compound.[117] Interest centering on triclosan chlorination has increased and numerous studies are available.[93,114,118,119,120]

Alkylphenol ethoxylates (APEOs) are cost-effective non-ionic surfactants in detergents, as well as having uses in paints, lubricants and pesticides.[121] In structure alkylphenols ethoxylates consist of a ethoxylate chain $(-CH_2CH_2O-)_n$ substituted alkylphenol ring. Global production of APEOs is high, approximately 500,000 tonnes, and the bulk of these substances are manufactured as nonylphenol ethoxylates.[121] APEOs and in particular one degradation product, nonylphenol, have been correlated with anthropogenic environmental contamination and as such are associated with point sources and urban areas.[122] As recognized pollutants[123] and endocrine disruptors,[124] regulations have been put in place banning the use and production of alkylphenol ethoxylates in the European Union and implementing monitoring systems in many other countries. Currently, there are still substantial amounts of alkylphenol ethoxylates passing into waste treatment facilities but there has been a shift in manufacturing towards alcohol ethoxylates. These substances show a lower immediate environmental impact, however, the metabolites have not been thoroughly evaluated. Alkylphenol ethoxylates are known to break down to alkylphenols both in the environment and during the process of sewage treatment.[125] Oxidative decomposition of long chain alkylphenol ethoxylates results in progressive loss of ethoxylate groups and hence shorter chains such as NP1EO and

NP2EO. In particular, the breakdown products of the nonylphenol ethoxylates, a variety of 4-nonylphenol isomers recognized in 1984 during the analysis of sewage sludge, are more toxic than the parent compounds.[122,123]

As well as being antiandrogenic, nonylphenol (NP) is a 17β-oestradiol mimic which can bind to human estrogen receptors (Figure 2.1.).[126,127] NP, first synthesized in 1940, possesses a log k_{ow} ranging from 4.7 to 5.6 indicating a preference for sorption to sediment which is a removal process in wastewater treatment. However, the major source of NP in the environment is the effluent from the sewage treatment plants, particularly those receiving high initial inputs.[122] NP may also be transported in the atmosphere and returned to the earth through wet deposition increasing its propensity to spread.[128] It is the major degradation product found in the environment and the most estrogenic of this analyte group though notably, the activity is still 1,000 to 10,000 times less than that of 17β-oestradiol.[121] Nonylphenol is generally found as the technical mixture, demonstrated to contain a blend of over 100 isomers[129] and it is now possible to synthesize particular isomers aiding in the characterization of technical NP.[130] Approximately 85% of the NP isomers have a quaternary α-carbon on the branched alkyl chain and this feature increases the oxidative resistance of these isomers to biodegradability however, NP is completely degraded by chlorination using hypochlorous acid. Structurally, chlorination occurs ortho to the alcohol substituent and in the trichlorinated transformation product there is para substitution as well. Nonylphenol and its mono and diethoxylate were present in the 30 most frequently detected compounds in streams in a significant national USGS study,[41] while monochlorononylphenol has also been detected in a variety of environmental matrices.[131,132,133]

The concept of free, as opposed to combined, chlorine is critical in understanding analyte reactivity in a wastewater matrix. The speciation of chlorine provides an important factor in limiting or encouraging reaction due to the varying strength of the oxidizing species. Wastewater, unless specifically removed through nitrification, contains ammonia. This ammonia will react rapidly, in less than one second, with the hydrolysed chlorine producing monochloramines. If chlorine is in excess, less stable dichloramines and minor amounts of nitrogen trichloride will be formed[134] and while these species decompose leaving a "nuisance residual", free chlorine will result from additional chlorine added. This may be identified practically when the free chlorine measurement makes up the bulk of the total chlorine result. In wastewater treatment, these processes are described through the use of a breakpoint curve (Figure 2.4.).

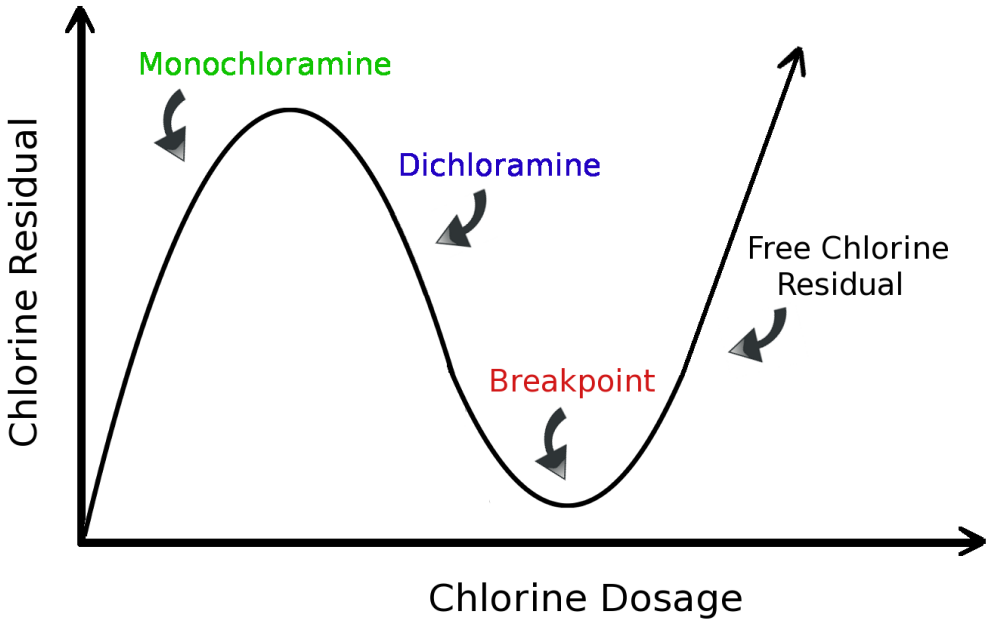


Figure 2.4. A stylized example of a breakpoint curve.[135]

2.6 Experimental procedure – bench chlorination studies

To confirm the literature information available for the transformation products in the system of interest, a bench study was undertaken first in reagent water then expanded to include wastewater matrix. The analytes selected (triclosan, triclocarban, gemfibrozil, carbamazepine, nonylphenol, nonylphenol monoethoxylate and diethoxylate) were tested for reaction with 1 mg/L equivalent of free chlorine (based on the WWTP acceptance criteria of 1-4 mg/L total chlorine), as detailed below, over a two hour time period. This length was chosen based on the specified treatment plant in which the duration of the chlorination process lasted approximately one hour. The most reactive species were then identified, checking both positive and negative ionization modes. Factors such as the addition of chlorine, stability of the parent peak and overall changes in the mass spectrum were examined.

Seastar Ultra-Pure water, to give a final volume of 5000 μL, was transferred by autopipette into a 20 mL clear glass vial protected from sunlight. 50 μL of diluted sodium hypochlorite solution, prepared the day of the chlorination (360 μL of 5% sodium hypochlorite was transferred by autopippet into 200 mL of Seastar Ultra-pure water), was added by autopipette. The vial was shaken for 10 seconds and allowed to equilibrate for a minimum of 2 minutes. This provided a solution at a chlorination level equivalent to 1 mg/L chlorine. AquaChek water quality test strips (reagent water samples) or *N*,*N*-diethyl-*p*-phenylenediamine (DPD) testing using a HACH *Pocket Colorimeter II* (wastewater samples) were used to confirm the chlorination level was between 1.0 and 2.0 ppm total chlorine in a separate solution prepared in parallel. The selected native analyte, prepared in methanol at 50 to 100 mg/L, was then added with an autopipette (at a

volume of 125 to 250 μL), providing an analyte concentration of ~2.5 mg/L. The reaction was sampled at 2, 5, 10, 30, 60, 90 and 120 minute time intervals. Each 250 μL subsample was transferred by autopipette into 750 μL of methanol giving a final solution with a 3:1 methanol:water composition and the analyte present at ~625 μg/L. In addition, a non-chlorinated sample prepared by the same method was sampled to represent 0 minutes. The solution was infused directly into the Micromass Q-ToF *II* ™ using a syringe pump set at 10 μL/min and scans were acquired in continuum mode, storing each individual scan, over a 2 minute time period. The spectra for each time interval were then summed and evaluated overall for evidence of reaction.

2.7 Bench chlorination of analytes in reagent water

The transformation products of triclosan (Figure 2.5.) in reagent water based on representative treatment plant conditions (approximately one hour chlorination resulting in a 1-2 mg/L total chlorine residual) were found to be pentaclosan and tetraclosan as well as dichlorophenol and trichlorophenol based on the measured *m/z* and the calculated isotopic ratio. These are the expected products based on recent literature[118,119,120,136] as well as earlier work.[59,60] Synthetic procedures for tetraclosan and pentaclosan are accessible though at this time the products are not available commercially.[100,137]

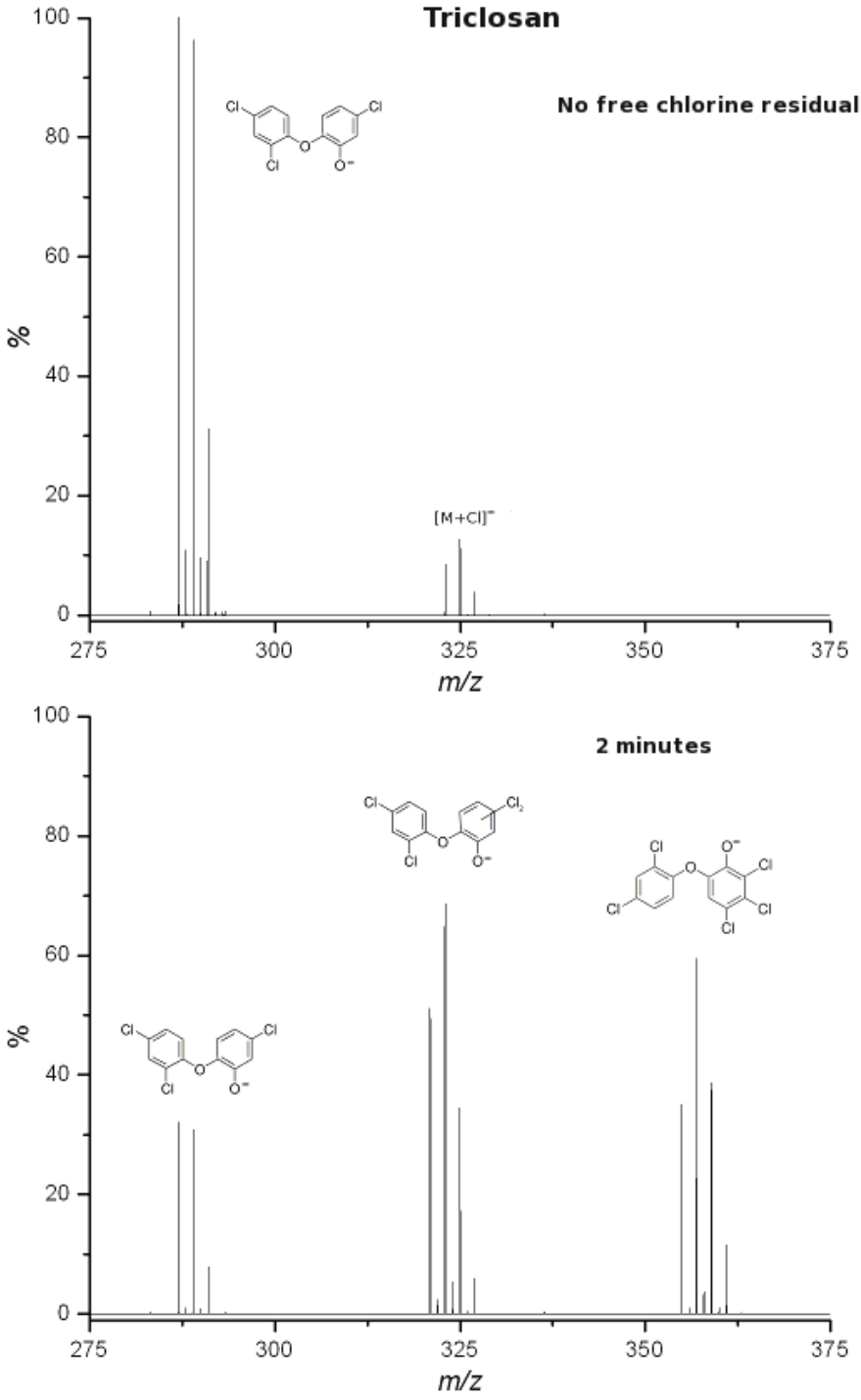


Figure 2.5. Triclosan transformation due to chlorination.

No free chlorine present (top) and reaction products after 2 minutes of chlorination (bottom).

For nonylphenol similar results were observed in reagent water, both mono and dichlorononylphenol were observed as was trichlorophenol based on the measured *m/z* and the calculated isotopic ratio (Figure 2.6.). Again, this result compares well to literature where 2-monochloro-nonylphenol, 2,6-dichloro-nonylphenol and 2,4,6-trichlorophenol have been reported in addition to further chlorinated products occurring at the point the nonylphenol chain was cleaved and the departing fragment chlorinated.[138] Additional products such as dimers have also been reported at high levels of chlorination (100 ppm) representative of industrial cleaning solutions[139] and the kinetics of transformation have been evaluated.[140] Synthetic procedures are available but, like triclosan, analytical standards for the transformation products are not yet commercially available or, in the case of monochlorononylphenol, are cost prohibitive.[141,142]

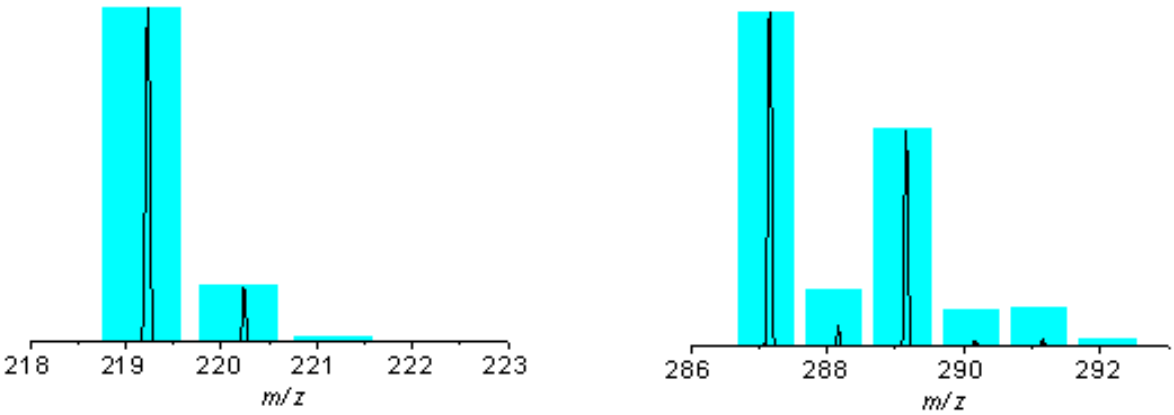


Figure 2.6. Change in isotopic pattern upon addition of two chlorine atoms from nonylphenol (left) to dichlorononylphenol (right).

Carbamazepine appeared to diminish slightly over the complete two hour time period but no corresponding reaction product was observed. For both triclocarban and gemfibrozil under these conditions, no reaction was observed. Upon evaluation of the spectra produced, the phenol moiety appeared to be an important indicator of reactivity and both nonylphenol and triclosan were selected for continued investigation.

The phenol group is an ortho, para directing substituent. It provides a good demonstration of electrophilic substitution and, as such, is well studied.[143] The simplest example of this situation would be the chlorination of phenol (pK_a 9.89)[144] with no additional substituents (Figure 2.7.). As expected mono, di and trichlorophenol are rapidly produced at pH 7.5.[145]

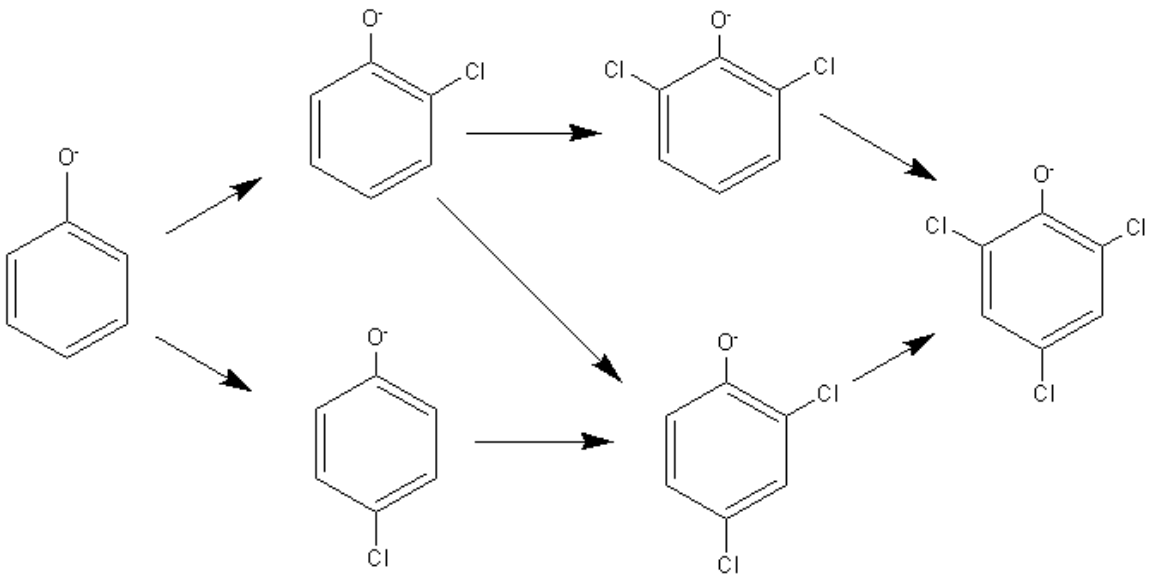


Figure 2.7. The chlorination of phenolate.[143]

When the equilibrium (Equation 2.1.) is considered, HOCl is the most reactive species. Predictably, there tends to be expected sites for reaction such as activated aromatic systems or amines and electrophilic attack is kinetically favoured over the oxidation or addition pathways.[146] Structurally, hypochlorous acid is quite reactive as oxygen has a Pauling electronegativity of 3.44 (on a scale of 0.7 to 4) and chlorine that of 3.16, while hydrogen for instance is much lower at 2.20.[147] This implies that the oxygen is competing strongly with the chlorine atom for negative charge and the isolated chlorine has little to draw from. As well, hypochlorous acid has a pKa of 7.54.[148] At pH 7.5, the equilibrium below demonstrates that 50% of the hypochlorous acid would be converted to the hypochlorite ion, decreasing reactivity, but at a pH of 6.5 over 90% is in the active form.

$$HOCl \rightleftharpoons H^+ + OCl^-$$

Equation 2.1. Equilibrium of hypochlorous acid.

This electrophilic species in conjunction with an activated aromatic system should, and indeed does, result in transformation. The first step is the formation of a cationic "Wheland intermediate" (Figure 2.8.); this is stabilized by electrons donated into the ring. Loss of a proton regains aromaticity and is therefore strongly favoured.

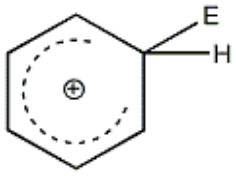


Figure 2.8. A Wheland intermediate caused by electrophilic attack of benzene[149]

2.8 Bench chlorination of analytes in wastewater

To investigate transformation product formation in matrix, chlorination studies were also done using a pre-chlorination, unquenched wastewater sample and diluted sodium hypochlorite solution (~0.01%) prepared fresh daily. Initially, an unspiked wastewater sample with unknown composition was chlorinated (refer to experimental procedure in Section 2.6) to get an estimate of the level required to produce a free chlorine residual in matrix alone. Using a spiked wastewater and an increased level of chlorination (2.5% v/v diluted sodium hypochlorite), it was found that triclosan was stable in wastewater, though reaction was seen as expected in reagent water spiked and prepared in parallel. The chlorination was increased by 7× and the same result was observed. This was unexpected based on the measured free chlorine residual and with further experimentation it was found that due to the high level of total chlorine present upon chlorination (24 mg/L total after 1 hour), an interference was taking place in the free chlorine DPD reading (despite the use of a one minute reaction time).[150,151] At this point, the chlorination level was increased to breakpoint chlorination (60% v/v diluted sodium hypochlorite) resulting in 0.8 mg/L free chlorine and 2.1 mg/L total chlorine residuals after a 1 hour reaction at which point the transformation products tetraclosan and pentaclosan were observed as expected (Figure 2.9.).

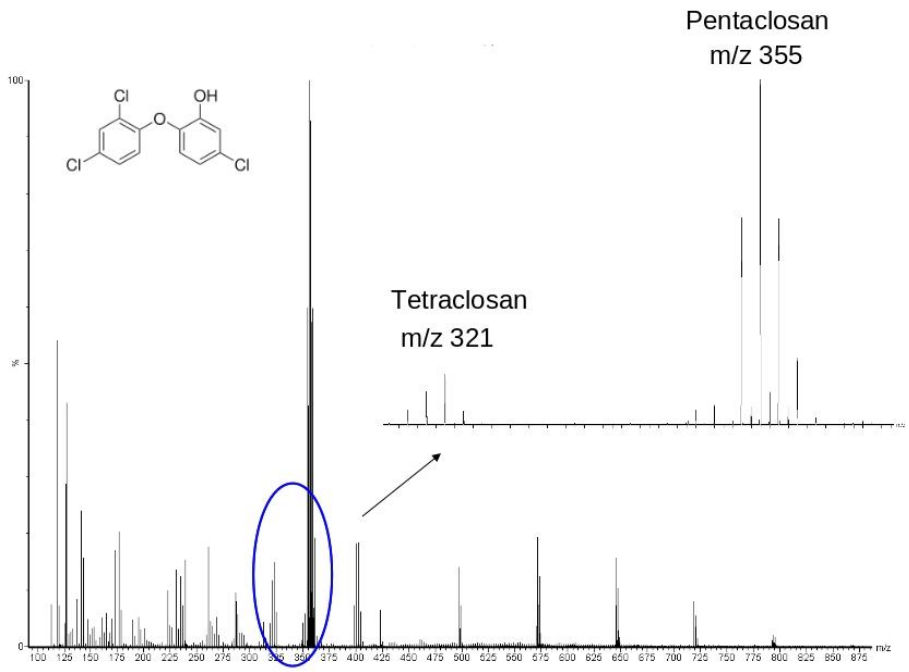


Figure 2.9. Triclosan spiked wastewater after chlorination past the breakpoint.

Wastewater treatment plants do not often practice breakpoint chlorination due to the additional expense as monochloramine has proven to be an acceptable disinfectant when allowed suitable contact time. Additionally, chloramines are less likely to form disinfection by-products due to their decreased oxidative ability and this characteristic makes a sub-breakpoint chlorination approach preferable. Regulation is currently based on the total chlorine residual, eliminating the distinction between free and combined chlorine, and fecal coliform concentrations are monitored to ensure effective disinfection. Bench studies that investigate solely the effect of free chlorine on a particular substance do not properly take the system complexity due to the matrix into account as has been demonstrated.

In literature the differences between chlorination using free chlorine as opposed to monochloramine have been explored and it was demonstrated for numerous

pharmaceuticals, including gemfibrozil, that reaction is typically far slower in the latter case.[89] Triclosan has also been observed to react, though two to four orders of magnitude slower than with free chlorine, if an 8.5× excess of monochloramine is available.[114] In this project triclosan was observed to transform slightly prior to the breakpoint. The total chlorine level was measured at over 10× that of the free chlorine residual and was elevated at about 2.5× the amount where triclosan stability was maintained. Whether this is due to reaction with a sufficient monochloramine excess or the beginnings of a free chlorine residual is uncertain.

Figure 2.10. The Delaware River.[152]

It has been successfully demonstrated that transformation products may be produced in a chlorinated wastewater matrix and that the chemical structure of the products appears to be well represented by the study of reactivity with free chlorine in a reagent water matrix. Using this data, a quantitative method will be developed for triclosan, nonylphenol and their chlorinated transformation products and applied to wastewater samples obtained from a POTW handling over 100 million gallons daily (MGD) and discharging to the Delaware River (Figure 2.10.). The Delaware River is a major waterway in the United States of America running 530 kilometers into the Atlantic Ocean and provides drinking water and industry for nearly 15 million people in Delaware, New Jersey, New York and Pennsylvania.[170]

2.9 Analytical method development

To evaluate and control the impact of substances on the environment, they must first be identified and the levels quantified. By finding an accurate baseline, these levels may be compared, monitored and examined in terms of overall impact. To achieve this goal effectively, rugged analytical methods are required. Undertaking method development is an intricate process incorporating many factors. Both the matrix to be sampled and the suite of analytes play a key role in determining the degree of rigor required for the extraction and cleanup. For practical implementation, the requirements of the laboratory design and equipment are important, as methods are often tailored for particular analysis requirements of the experts collecting and employing the data.

In the present analytical method under development, the liquid portion of wastewater was selected for the analysis of selected chlorinated analytes from the phenol family triclosan, nonylphenol and their chlorinated transformation products. This choice precludes the analysis of sorption effects which remove a portion of the analytes in earlier treatment processes. The results obtained are applied to an evaluation of the production of transformation products in the common wastewater treatment process, chlorination.

The requirements of this project, particularly ultra-trace level sensitivity, lead to the selection of mass spectrometry as a fast and practical analytical technique. A chromatographic interface was included in the system to increase the certainty of identification and separation of isobaric species. A HPLC / QQQ arrangement was selected to optimize both analyte separation and sensitivity. In conclusion, the quantitative measurement of triclosan and nonylphenol as well as their chlorinated transformation products in wastewater from a POTW discharging to the Delaware River,

U.S.A. is discussed.

The analyte suite (Table 2.1.) was selected based on the previously examined chlorination study, representative of the selected POTW. As it is specifically the chlorination process that is of interest, this directs the focus of the sampling regime. Recommendations from the data analysis may tentatively be extended to those systems of similar nature in the area.

Wastewater grab samples were collected on three non-consecutive days at the asterix (*) indicated points (Figure 2.11.) following the Environmental Protection Agency (EPA) Method 1694 protocols. Both sets of pre and post chlorination triplicate samples were then quenched with ascorbic acid to minimize extraneous effects, shipped and stored frozen in amber glass containers until analyzed.[153]

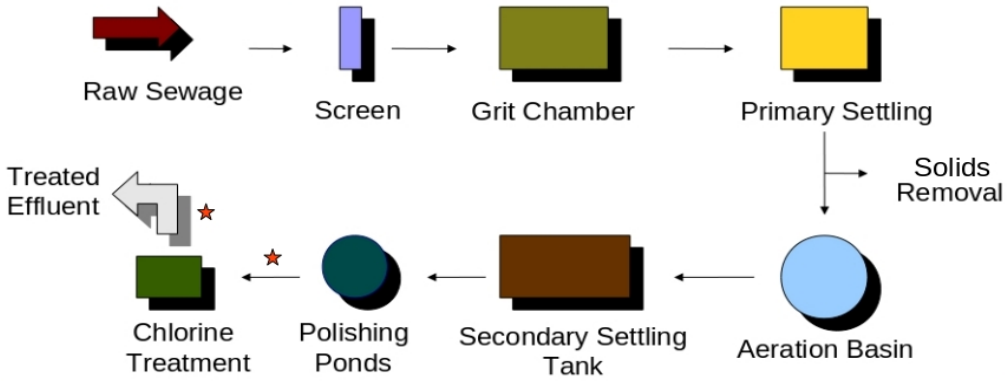


Figure 2.11. POTW sampling locations (indicated by orange stars).[43]

The first step in method development is the elucidation of the analyte transitions on the mass spectrometer and selection of the most favourable ionization mode. Here the analytes are based on the acidic phenol moiety and as such are detected in the negative ionization mode, which has fewer interferences than its positive counterpart. Optimization of the analyte to fragment mass transition for maximum sensitivity was also

pursued and, where possible, a secondary confirmation ion was obtained (Table 2.1.). This was accomplished via infusion (syringe pump) of concentrated 1-2 ppm individual solutions of the analytes (excepting tetraclosan, pentaclosan, monochlorononylphenol and dichlorononylphenol) in methanol into a Micromass *Quattro Ultima*^{TM} mass spectrometer to identify unique analyte to fragment transitions. The additional analytes were prepared by separate chlorination of each parent analyte (for NP the technical mix was used) in water with diluted sodium hypochlorite and the reaction quenched after 5 minutes using ascorbic acid. Each mixed analyte solution was infused in 3:1 methanol:water solution to confirm the calculated transitions for the chlorinated products. Notably, the nonylphenol analytes had only one suitably intense fragment which varied between the ^{13}C label and the native analyte, presumably due to the linear versus technical mix nature of the different standards.[154] 4-Nonylphenol has been previously examined by R. Loos et al. using a 219 → 133 (Δ = 86) fragment while the linear 4n-nonylphenol required a 219 → 106 (Δ = 113) transition.[155] A similar situation occurred here (Figure 2.12.); the native NP analyte was a technical mixture providing a 219 → 133 transition (Δ = 86) whereas the linear ring labeled $^{13}C_6$-NP standard required 225 → 112 (Δ = 113). An earlier retention time can be seen (Table 2.1.) as a consequence of the degree of isomeric branching in the technical NP native while the linear NP elutes the latest, as expected with the use of a reversed phase HPLC column (Figure 2.13.). Once the analyte transitions were determined windowing, grouping the required transitions into separate acquisitions based on the HPLC retention time, was performed allowing increased instrumental scan time for each analyte.

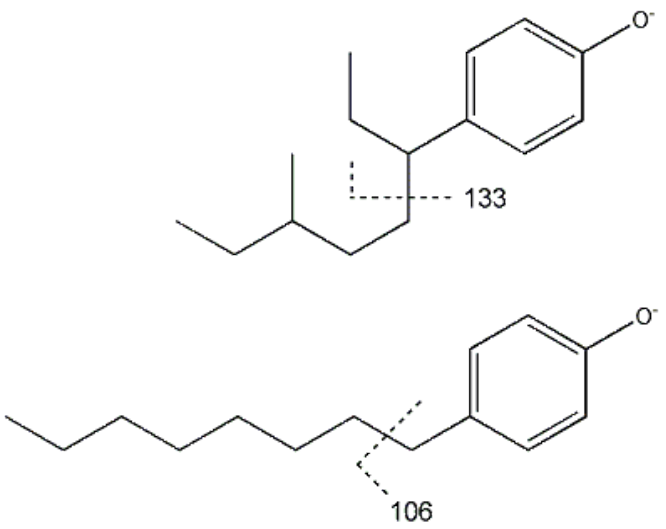


Figure 2.12. The proposed fragmentation of the quasi-molecular ion for two nonylphenol isomers (branched and linear).[198]

Interferences in the form of cross talk between acquisition channels, a response from one MRM appearing as a diminished peak in a different MRM (due to residual ions in the collision cell this effect can be caused by overly short inter-scan times), were evaluated. This was accomplished by injecting each individual analyte at a concentration equal to or exceeding the highest calibration point (Appendix A) on a Waters *2795 Separations Module* (HPLC) / Micromass *Quattro Ultima*^{TM} using the developed program and checking all MRMs acquired for any response at the retention time of the analyte injected. Cross talk was found to be minimal within the range of calibration. Native nonylphenol, on the other hand, is near ubiquitous and B&J methanol, with increased purity, was used in the instrument blank to help minimize the background. If background analytes present in the mobile phase and instrumental lines were determined to be an issue in continued analyses, a C_{18} "trapping" pre-column could be used on-line to displace the analyte retention time away from that of the sample.

Analyte	Parent *(m/z)*	Fragment *(m/z)*	CV† (V)	CE† (eV)	RT‡ (min)
Triclosan 1	287	35	25	30	15.8
Triclosan 2	289	37	25	30	15.8
Tetraclosan 1	323	37	25	45	16.1
Tetraclosan 2	321	35	25	45	16.1
Pentaclosan 1	357	37	25	30	17.2
Pentaclosan 2	355	35	25	30	17.2
Nonylphenol 1	219	133	30	35	17.1
Monochlorononylphenol 1	253	167	30	30	17.6
Dichlorononylphenol 1	287	202	30	30	18.2
Dichlorononylphenol 2	287	35	30	35	18.2
2,4-Dichlorophenol 1	161	35	25	40	12.1
2,4-Dichlorophenol 2	161	125	25	20	12.1
Trichlorophenol 1	195	35	35	45	14.0
Trichlorophenol 2	195	95	35	35	14.0
$^{13}C_{12}$-Triclosan 1	299	35	25	30	15.8
$^{13}C_{12}$-Triclosan 2	301	37	25	30	15.8
$^{13}C_{6}$-Nonylphenol 1	225	112	30	25	18.0
$^{13}C_{6}$-2,4-Dichlorophenol 1	167	131	25	20	12.1
$^{13}C_{6}$-2,4-Dichlorophenol 2	167	35	25	40	12.1
$^{13}C_{6}$-Trichlorophenol 1	201	35	35	45	14.0
$^{13}C_{6}$-Trichlorophenol 2	201	165	35	20	14.0
$^{13}C_{6}$-2,4,5-T	259	201	22	11	9.1

Table 2.1. Analyte masses, transitions & instrumental settings.

† Cone voltage (CV), collision energy (CE), ‡ typical retention time.

Additionally, a reversed phase HPLC method for separation of the analytes was developed (Table 2.2). The inlet for this method is based on an acetate buffered aqueous phase (pH~4) and a combination methanol and acetonitrile organic phase. A long gradient was used to aid in separation and where possible analytes were eluted in the organic phase to optimize response. A 4.5 minute column reconditioning period was allowed after elution for optimal peak shape and retention of early eluting analytes, such as dichlorophenol. A C_{18} stationary phase (4.6 × 30 mm, 3.5 μm, Sunfire, Waters) was used providing reverse phase retention. The utility of the HPLC is demonstrated here as well; it can be seen that the nominal masses for both triclosan and dichlorononylphenol are 287, to ease the burden on the mass spectrometer these species are clearly separated in time (Figure 2.13).

Time	Mobile Phase A	Mobile Phase B	Flow (mL/min)	Curve†
0.00	40.0	60.0	0.200	1
0.50	40.0	60.0	0.200	4
5.00	70.0	30.0	0.150	4
10.0	100.0	0.0	0.150	4
18.5	100.0	0.0	0.150	4
19.5	40.0	60.0	0.200	1
24.0	40.0	60.0	0.200	1

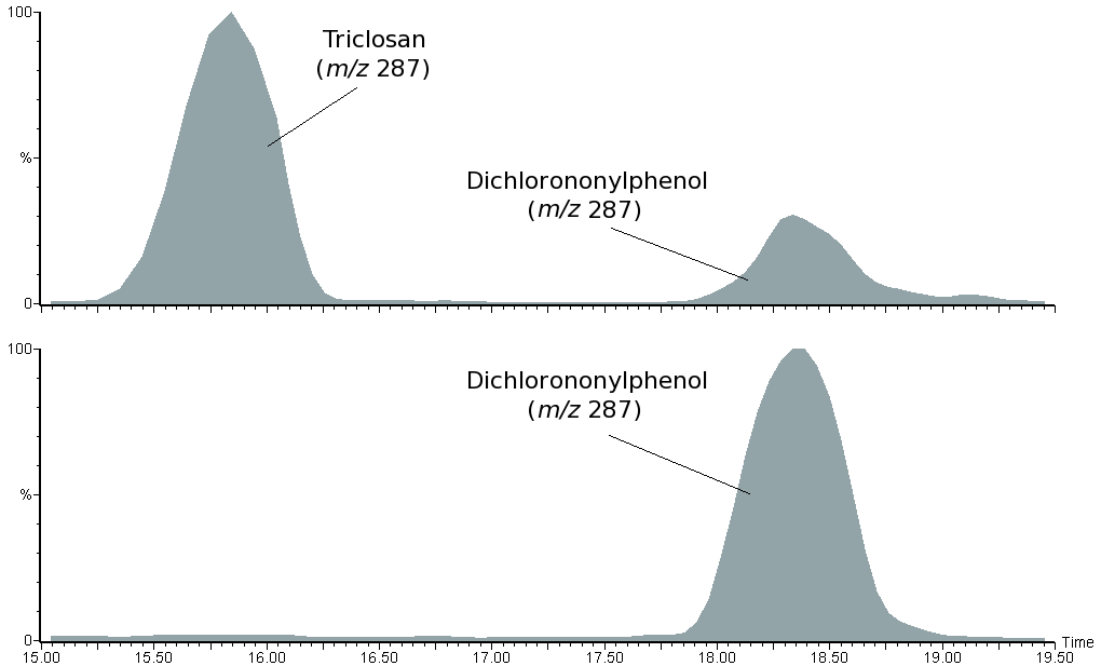
Mobile Phase A: 1:1 methanol:acetonitrile

Mobile Phase B: 0.1% ammonium hydroxide / 0.1% acetic acid aqueous buffer

Table 2.2. HPLC gradient program.

† The curve setting specifies the rate of change of the solvent composition & flow rate to the next time step in the program. The scale runs from 1 to 11, with 1 representing an immediate change.

a)



b)

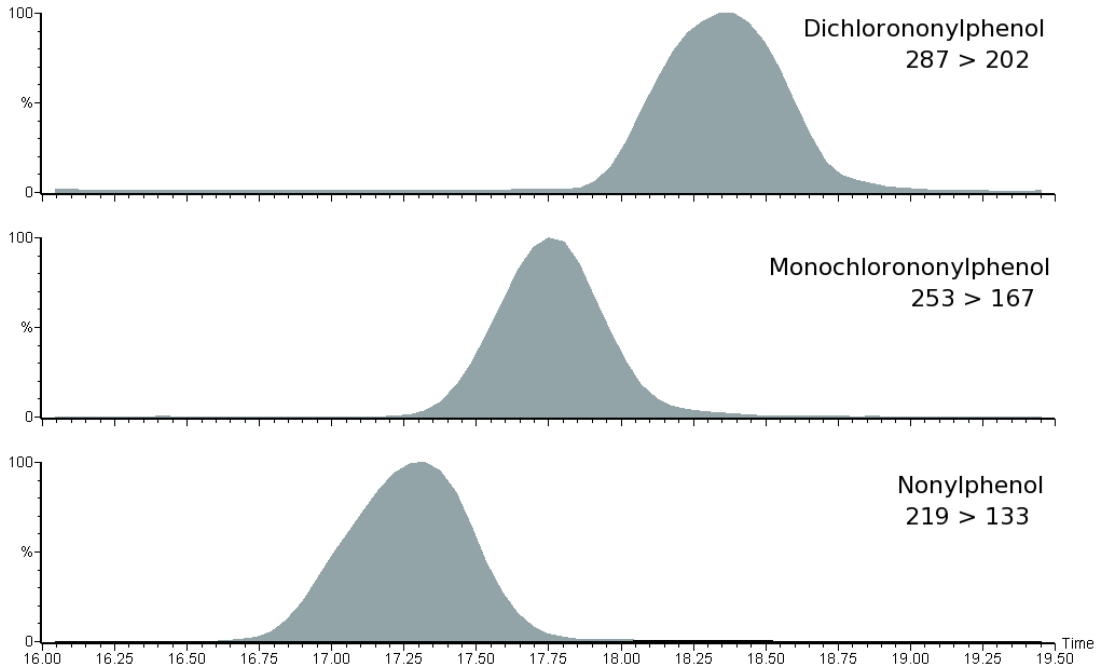


Figure 2.13. a) Cross talk b) HPLC separation of nonylphenol, mono and dichlorononylphenol.

2.10 Combating matrix effects

A variety of approaches have been taken to combat the effects of matrix in ESI analysis.[156,157] Since these issues have been linked to co-eluting interferences[158] it is intuitive that further clean-up may be helpful.[159] This is not straightforward however, depending on the situation. Extensive analyte lists, for example, include chemicals with highly variable properties and may not be conducive to separation of the interferences while maintaining good analyte recoveries. There is also additional cost required in terms of both time and materials, and the possibility that unknown interferences may be difficult to isolate. A second option is to focus on chromatography because shifting the interference away from peaks of interest can mitigate the matrix effects.[160] Once again, the effectiveness of this method is dependent on the similarity of the interference to the analytes. As well, many analytical procedures are limited to clearly stated method protocols to maintain quality objectives.

Additional approaches available to combat matrix effects from an instrumental and method development standpoint would be to select negative ionization preferentially, as fewer substances will be ionized here as compared with the positive ion mode. There are also methods using a variety of calibration types that are effective in correcting for the impact of matrix. A standard addition calibration, done using a particular sample, may be very accurate due to the matching of matrix effects in a sample but it is highly inefficient in terms of time and cost. Matrix matched calibrations are also possible, though sample to sample variability may cause issues due to poor enhancement or suppression matching with the selected matrix. The addition of internal standards is one of the most practical and widely accepted solutions to deal with matrix effects. There are limited situations

where the internal standard does not compensate[161,162] however, in general this is an effective technique.[163,164] Unfortunately, there are only a moderate number of isotopically labeled analogues available, so the selection of an appropriate standard is one of the considerable challenges of method development. Dilution of particularly difficult samples is also used as a remedial technique, simply reducing the matrix effects, but this approach may be impacted by detection limit requirements.[165] Further, it has been noted that there is a disquieting trend of assuming a mass spectrometer can handle a sample "as is" without cleanup. While this may be true in the minority of cases, the majority of samples require further consideration.[166]

2.11 Experimental procedure – quantitative analysis

Once the suite of analytes was determined, a clean matrix, in this case reagent water, was used to test the extraction and cleanup processes. To work with samples at the concentration levels determined by the pre-screen analysis, 500 mL sample sizes were selected and pre-concentration was required prior to instrumental analysis. As the initial reagent water matrix had no solids present, it was not filtered. The sample was acidified to pH~2.5 using concentrated hydrochloric acid and the ^{13}C labeled surrogates were spiked. Based on the characteristics of these analytes both Oasis mixed-mode anion exchange (MAX, 150 mg) and Oasis hydrophilic-lipophilic balanced (HLB, 500mg) solid phase extraction (SPE) cartridges were tested. Upon evaluation of performance and to maintain future flexibility of the analyte list, 500 mg HLB cartridges (Oasis, Waters) were selected for both extraction and clean-up procedures.[167] Ethylenediamenetetraacetic acid, tetrasodium salt dihydrate (EDTA) was used to bind metals, common in wastewater, however an impact elevating the blank nonylphenol levels was observed.

The 500 mL sample was pH re-adjusted (as required) after the addition of the EDTA to bring the sample into the pH range 3 to 4 prior to the SPE procedure. The SPE cartridge (500 mg HLB) was conditioned with 3× 6 mL of 1:1 methanol:acetone followed by 2× 6 mL of pH 2 Seastar Ultra-pure water. The samples were loaded on the SPE cartridge and rinsed with 2× 6 mL Seastar Ultra-pure water followed by 3 mL of 5% methanol in Seastar Ultra-pure water. The cartridge was dried for 2 minutes under vacuum, then eluted with 8 mL of 1:1 methanol:acetone. This extract was reduced to 300-500 μL under nitrogen and diluted to a final volume of 1 mL with methanol for a final composition of ~3:1 methanol:water. The instrumental standard ($^{13}C_6$-2,4,5-trichlorophenoxyacetic acid) was added at this point, prior to instrumental analysis on a Waters *2795 Separations Module* (HPLC) / Micromass *Quattro Ultima*^{TM}. Absolute recovery of the analytes was corrected for by the use of surrogate standards. The surrogate standards themselves were corrected against the instrumental standard to account for matrix suppression. For field samples, vacuum filtration of the unaltered sample using a millipore apparatus was done to discard any residual solid material in the sample prior to spiking and analysis.

2.12 Method evaluation

When studying emerging analytes, it is perfectly plausible that an exact labeled analogue may not be available and indeed this is the case for the specific transformation products considered here. This requires some decisions around quantification and for this method it was decided that the specific transformation products would be treated in the same manner as their respective parent materials by assuming the same response factor. The chlorinated nonylphenol transformation products are quantified against carbon-

labeled nonylphenol and the higher chlorinated triclosan transformation products are quantified against carbon-labeled triclosan. Notably, trichlorophenol is analyzed using a 1:1 mix of the 2,4,5 and 2,4,6 isomers (for both native and labeled). Nonylphenol has many isomers and may be present either as a technical mix or a linear chain, in this case the native analyte is represented by a technical mixture while the internal standard is a linear chain. This could impact the accuracy of experimental results if the nonylphenol isomers recovered differently than the linear surrogate through the work-up procedure but, because the isomer composition of samples is variable, selecting a consistent reference is a reasonable approach. Accuracy is demonstrated by the initial precision and recovery study (Table 2.4.). The experimental procedure developed was evaluated for performance by two statistical methods, a method detection limit study for precision and a mid-level spiked recovery study indicating accuracy.

Precision is critical to the ability to replicate a measurement consistently. The method detection limit (MDL) calculation requires a batch of representative matrix samples, reagent water in this case, to be spiked at a level approximating the detection limit.[168] Essentially, this involves a statistical analysis of multiple samples spiked at low levels demonstrating statistical difference from a blank. The result requires the rejection of the null hypothesis, that the concentration of the sample is equal to that of a blank, above the MDL (Table 2.3.). The presence of an analyte cannot be stated in absolute terms if it is below the MDL, it may or may not be present, and is referred to as a non-detect (N.D.). It is only at levels above the MDL can a meaningful quantitative statement be made.

For the MDL, the samples were processed routinely through the procedure (Section 2.11). The results are calculated against a bracketing point (a mid-level calibration solution injected multiple times, run prior to and post sample injections with the opening injections used as the quantification standard) and analyzed statistically based on the number of replicates (8 replicates are used, Student's t-value = 2.998 at 99% confidence, one-tailed). The MDL value itself is a representation of the variability in the lowest analysis region with a detectable signal. This calculation minimizes the probability of type I errors (i.e. the rejection of the null hypothesis, the sample concentration being equal to that of a blank, when it is true)[169], or false positive results, to 1% at the MDL level. Numerically it is the standard deviation of the data set multiplied by the Student's t-value. Type II errors (the incorrect acceptance of the null hypothesis) however, are not minimized using the MDL procedure.[170]

Analyte	Standard Deviation (ng/L)	MDL (ng/L)
Triclosan	1.7	5.0
Nonylphenol	15	45
2,4-Dichlorophenol	33	100
Trichlorophenol	1.8	5.4

Table 2.3. Precision analysis – calculated method detection limit values (n=8).

The MDL result provides a statistical lower limit when considering the available calibration range of the instrument, and in this way sets the best achievable detection limits as well as giving context to comparisons between different methods. A detection limit must necessarily be based on the instrumentation used as noise and sensitivity are variable. Indirectly, low detection limits allow the use of smaller sample sizes beneficial in the majority of environmental analysis. However, the sensitivity of a method encompasses both the sample size and complexity which may be ultimately be limiting factors in the sample detection limit even if the instrumentation has the ability to detect lower levels in a less involved matrix.

An additional step in method characterization to determine accuracy, known as an initial precision and recovery (IPR) study, involves spiking blank samples, reagent water, with the suite of analytes at a mid-point calibration level (Table 2.4.). Ideally the sample levels fall in this range near the middle of the calibration curve as well. Once processed through the method (Section 2.11), these samples provide information regarding the recoveries of both the analytes and the standards used for recovery correction.

Analyte	IPR 1 (%)	IPR 2 (%)	IPR 3 (%)	IPR 4 (%)	IPR 5 (%)	Mean	SD
Triclosan	105.3	106.6	92.5	105.4	112.7	100	7.4
Tetraclosan	99.4	87.7	88.2	90.0	80.8	89	6.7
Pentaclosan	85.0	75.2	69.1	70.2	70.6	74	6.6
Nonylphenol	140.8	120.8	103.2	135.5	130.0	130	15
Monochlorononylphenol	48.5	46.2	34.8	48.9	57.3	50	8.1
Dichlorononylphenol	46.9	77.1	93.7	80.3	83.1	76	18
2,4-Dichlorophenol	88.4	103.2	117.2	96.4	93.4	100	11
Trichlorophenol	93.0	96.2	109.5	94.9	154.6	110	26
$^{13}C_{12}$-Triclosan	80.0	98.7	93.1	88.0	90.3	90	6.9
$^{13}C_{6}$-Nonylphenol	38.5	47.7	52.1	39.9	41.8	44	5.7
$^{13}C_{6}$-2,4-Dichlorophenol	26.6	60.8	64.3	78.3	84.0	63	22
$^{13}C_{6}$-Trichlorophenol	55.4	92.3	83.9	91.7	59.6	77	18
$^{13}C_{6}$-2,4,5-T	93.9	92.2	100.8	101.4	100.7	98	4.4

Table 2.4. Accuracy analysis – initial precision and recovery results (n=5).

It can be observed that the nonylphenol recoveries are slightly elevated (Table 2.4.). This may be explained by the background presence of the ubiquitous nonylphenol also seen in the blank quality control sample. Monochlorononylphenol is observed to track relatively poorly when recovery corrected against the carbon-labeled nonylphenol. This indicates that the absolute recovery of this analyte through the method is about half that of its nonylphenol surrogate. The internal standard carbon-labeled nonylphenol recovery

is low at ~45%, but consistent as may be seen by the standard deviation of 5.7%. An unusual result is seen for trichlorophenol in IPR #5. Using the Q-test, this data point would be valid to reject as an outlier at n=5, α=95% and the standard deviation would be reduced from 26% to 7.5% while the mean would improve from 110% to 98%.

2.13 Sample data and discussion

Quality control was exercised throughout the analysis. A minimum five point calibration (R^2>0.985) was run along with opening and closing mid-level points (native 70-130%). Each batch processed through the laboratory contained a method blank sample to determine background levels. As well, a clean water matrix spiked with all analytes, the method spike, was used to evaluate accuracy through the particular workup recovery and a clean solvent extract spiked with the standards used was included. The sample batches also contained duplicate sample workups to further evaluate method precision and wastewater matrix spiked with analytes to evaluate the role of matrix on analyte recovery. As well, field blanks were analyzed and show slightly elevated nonylphenol concentrations compared with the method blank.

The blank sample contained low amounts of background nonylphenol but the sample levels were above 100× in excess of this concentration so there is a negligible impact on the data. Native recoveries based on internal standard correction in the method spike were 75-120% for all analytes excepting monochlorononylphenol which was recovered at ~20%. This was not due to matrix suppression (tested by dilution) but rather is due to poor tracking against the $^{13}C_6$-nonylphenol surrogate which also had a low and consistent recovery at ~35% similar to the IPR study (Table 2.4.).

Duplication was <45% difference for hits over 2× the A level calibration standard (Appendix A). For the precursor analyte detects, triclosan and nonylphenol, the percent difference dropped to <15%. Two separate matrix spikes showed good recoveries on the whole, monochlorononylphenol was determined to be low at ~55%, a similar trend to the method spike and dichlorinated nonylphenol was not recovered, though it was seen in the method spike. Nonylphenol showed slight over-recoveries in one matrix spike due to the high level of analyte present in the sample prior to spiking (~25×) compared to the amount of analyte spiked. Ideally the amount of analyte added to the matrix spike would be equal to or larger than the background to obtain accurate results. The sample sizes used were based more strongly on the triclosan levels of the samples as these were closer to the detection limit in the initial pre-screen.

The original sample data demonstrated a high degree of matrix suppression, observed in the recoveries of the instrumental standard carbon-labeled 2,4,5-trichlorophenoxyacetic acid (^{13}C-2,4,5-T) at ~10 to 30%. To address this, the samples were reanalyzed at a 3× dilution and the majority of the data, excepting that for dichlorophenol, is reported from the dilution data (Table 2.5.). Final values for the three measured triclosan and nonylphenol concentrations in the pre and post-chlorination wastewater were tested for statistical significance at $\alpha = 0.05$ using a F test for variance followed by a t-test of the means using s_{pooled}. Statistical differences were not found between these concentrations as both the F and t tests were passed indicating that the 95% confidence interval contained both values.

Analyte	Pre-Chlorination Mean (ng/L)	Post-Chlorination Mean (ng/L)	R. L.† (ng/L)	Significant Difference?
Triclosan	390	340	30	No
Nonylphenol	30,000	31,000	300	No
2,4-Dichlorophenol	130	320	100	No
Trichlorophenol	N.D.‡	N.D.	30	
Tetraclosan	N.D.	N.D.	30	
Pentaclosan	N.D.	N.D.	30	
Monochloro-nonylphenol	N.D.	N.D.	300	
Dichloro-nonylphenol	N.D.	N.D.	300	

Table 2.5. Wastewater chlorination sample data.

† Reporting limit (R.L.), ‡ non-detect (N.D.).

2.14 Conclusions

The Delaware POTW samples analyzed demonstrate that chlorination does not significantly contribute to the production of chlorinated transformation products in wastewater. For the precursor analytes triclosan and nonylphenol, detected at an average of 320 ng/L and 31,000 ng/L respectively, no significant change in concentration across the chlorination treatment was found. This is consistent with the fact that transformation products at levels above the reporting limit of the developed method were not observed.

These results are supported by the two part bench study where, provided the same level of chlorination, triclosan was shown to react rapidly in reagent water while simultaneously maintaining its stability in the wastewater matrix. Based on the absence of a nitrification step at the selected POTW, ammonia would be expected in the

wastewater, as discussed previously, and would be able to react preferentially with the free chlorine provided. Further, it can be postulated that plants which use nitrification to minimize or eliminate ammonia would be more likely to produce transformation products if provided a phenolic input. POTW using elevated chlorination levels resulting in breakpoint chlorination, though uncommon, would also be at risk for these consequences.

Overall, transformation products have been demonstrated to form in a variety of instances, including a wastewater matrix, and situations allowing free chlorine or elevated contact time with chloramines should be the focus of further risk evaluation studies to elucidate regulation strategies.

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Chapter 3: ESI-MS of Highly Hydrated Lanthanide Trications

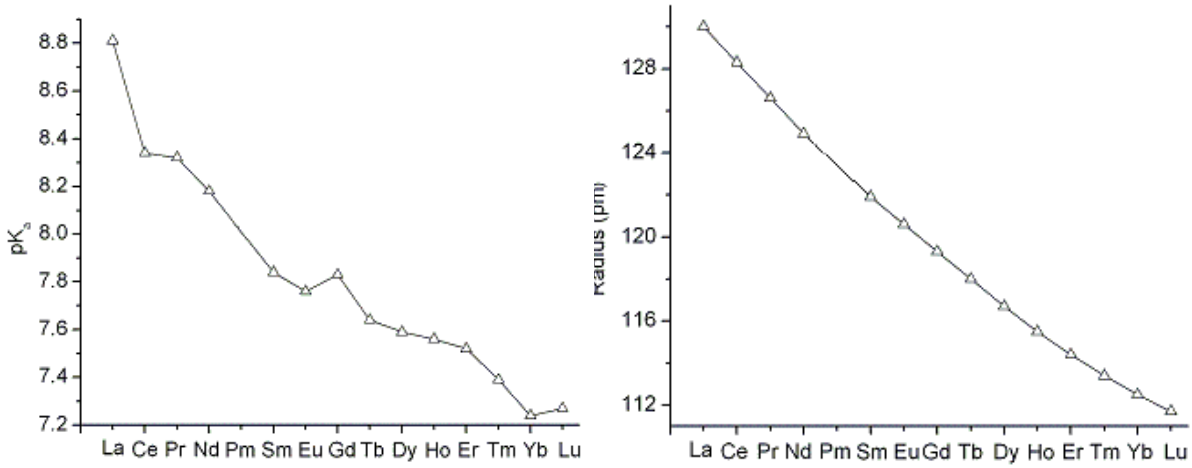
3.1 Trends of the lanthanide series

The lanthanides, also known as lanthanoids (IUPAC) or the “rare earths”, are formally the elements from lanthanum to ytterbium (Z=57 to 70). The group 3 elements scandium (Z=21) and yttrium (Z=39), are often included in discussions of the lanthanides because of their similar physical properties. These stem from the dearth of oxidation states available to these elements due to their lack of d-orbital electrons. The production of a 3^+ ion is favourable for both scandium and yttrium as it leads to a closed shell structure and the lanthanide elements also tend to exist as 3^+ ions, because removal of three electrons results in the 4*f* orbitals essentially becoming a core set. The lanthanides are well-known in the periodic table for the lack of influence exerted by the 4*f* electrons which comprise their valence shell,[1] as compared with the 3*d* electrons of the transition metals which provide strong crystal field effects.[2] As a system, the lanthanides allow, almost exclusively, insight into size effects largely free from electronic considerations (all the lanthanides and Group 3 metals have a stable 3^+ oxidation state). The well-known phenomenon of the lanthanide contraction, a reduction in the atomic radius across the row of 18.3 pm for Ln^{3+} arises due to imperfect shielding of the nuclear charge.[3,4]

The description of the lanthanides as “rare earths” is a misnomer originating in the difficulty of obtaining these elements in pure form. The abundance in the earth's crust

reaches 60 ppm for cerium (compare iodine, 0.5 ppm) while only one lanthanide, promethium, is not naturally occurring.[5] By the Oddo-Harkins rule even atomic numbered elements are more abundant than their odd numbered counterparts and the latter are split into fewer isotopes as well, possessing a maximum of two.[6] There is also a division in occurrence patterns between the light (La to Gd) and heavy (Tb to Lu) lanthanides with the lighter lanthanides found in greater abundance. Yttrium is generally grouped in with the heavy lanthanides based on its atomic radius (1.159Å) which lies between that of dysprosium and holmium.[7] All of the lanthanides are paramagnetic excepting La^{3+} and Lu^{3+}. The trivalent cations in this series are also increasingly hard Lewis acids (by virtue of their high charge and decreasing size).[8] The third ionization energy shows a similar but inverted pattern (Figure 3.1.).[9]

a) b)



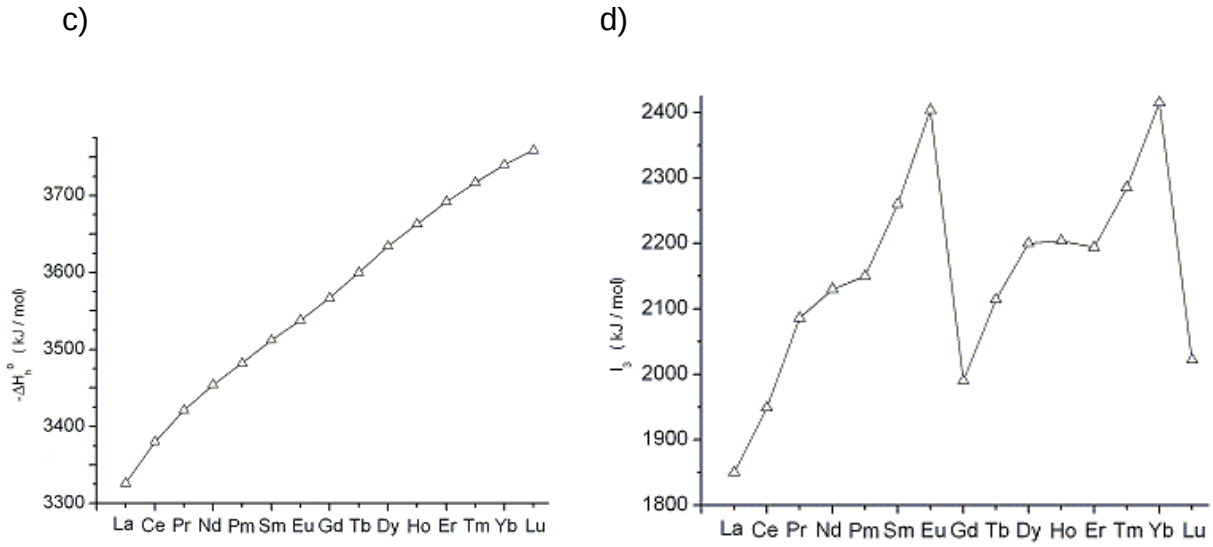


Figure 3.1. Trends of the lanthanide series.

a) Lanthanide series pK_a. b) Eight co-ordinate lanthanide 3^+ oxidation state radius.[3]

c) Lanthanide series hydration enthalpy ($-\Delta H$).[2] d) Lanthanide third ionization energy.[10]

The gadolinium break is a term used to describe an abrupt change in physical properties along the lanthanide series occurring in the region of gadolinium. Observations began in the 1930s, however, limited accuracy in measurement hindered the results as well, hence they were difficult to interpret.[11] The pioneering research in the gadolinium break came from a 1953 observation by F. H. Spedding while studying EDTA complexes of the lanthanides in aqueous solution. This research showed the gadolinium complex to be less stable than expected from the overall trend (Figure 3.2.). An explanation was proposed in terms of steric hindrance due to the decreasing ionic radius across the

lanthanide series eventually resulting in a change of coordination number.[12] This discovery fueled interest in the physical property trends along the lanthanide series.

In the 1960s, many physical chemistry studies were undertaken and various discrepancies were found. The steric aspect of the original idea was nullified by complex studies of a non-sterically bulky ligand, nitrilotriacetic acid, which was found to demonstrate the same effect.[13] An excellent review of the current knowledge at this time is provided by T. Moeller who notes two important ideas: the recognition that crystal field effects were not the primary factor (for example, there is no crystal field stabilization for gadolinium) and the fact that a sudden change in coordination number as predicted by the gadolinium break was not feasible based on the evidence at that time.[14] A coordination equilibrium demonstrating a gradual inner sphere coordination change from 9 to 8 ligands beginning after neodymium and completing around terbium was next proposed by F. H. Spedding based primarily on studies of molal volume and enthalpy.[15,16]

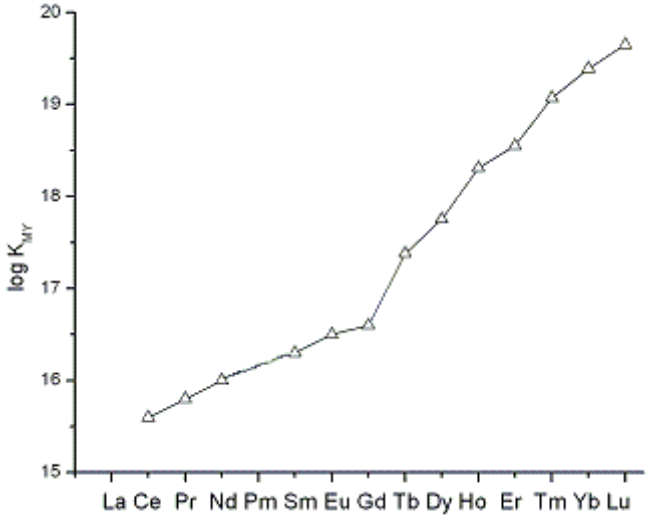


Figure 3.2. The gadolinium break demonstrated with respect to the stability of lanthanide EDTA complexes.[12]

As more results became available, around 1970 controversy arose about the existence of a coordination change in the series.[17,18,19] An additional interpretation of the data, deemed the tetrad effect, also known as the double-double effect,[20,21] was postulated in 1969 by Peppard.[22] The tetrad effect is an alternative way of splitting the lanthanide series based on properties: from lanthanum to neodymium, promethium to gadolinium, gadolinium to holmium and lastly erbium to lutetium. This proposal has also been examined for the actinides series[23] and a theoretical explanation of these occurrences based on quantum mechanical electron repulsion (spin pairing) energy of the *4f* or *5f* electrons was put forward.[24] More recently, papers in the late 1970s looked at the different interpretations that had been advanced and formulated a more inclusive

interpretation whereby the gadolinium break, attributed to the element's half filled f-shell, is a simplified version of the tetrad effect.[25,26] Support was provided for the gradual coordination change hypothesis by X-ray diffraction study[27] and entropic data[28] as well as the microcalorimetry of nitrate complexes.[29,30]

The electronic structure of the lanthanides has also been a topic of interest in many spectroscopic studies.[1] Electronic transitions for this series come in three varieties: two high energy, allowed transitions and one forbidden transition. The first allowed transition is the *4f* to *5d* which produces a broad band but is frequently observed only for cerium, praseodymium and terbium. The second allowed transition is charge transfer between the ligand and metal centers, once again this is a high energy process and is most common for europium and ytterbium. The last and possibly most useful transition is the narrow and Laporte forbidden f→f (not observed for trivalent lanthanum or lutetium). Notably, when the f→f intensity responds to the chemical environment it is known as a hypersensitive transition.[2,31] Luminescence or phosphorescence is observed for many lanthanides, though when hydrated this effect is diminished.

Common uses of the lanthanides include the metals themselves which are strong reducing agents. A great deal of study has focused on gadolinium as a MRI contrast agent.[32] Dysprosium and thulium both have applications as NMR shift reagents. Europium, terbium and ytterbium have a variety of uses as luminescent probes, generally with the aim of selectively imaging cancer cells.[33] The trivalent lanthanides are palely coloured (the absence of ligand field splitting means that selection rules forbidding f-f

transitions are not relaxed in the way that d-d transitions are), at best, pastel shades of yellow or pink unaffected by the choice of ligand. Further applications may be found as superconductors as well as filters in sunglasses and phosphors in fluorescent lighting.

3.2 Lanthanide coordination

The coordination behavior of the lanthanides has long been a provocative question and as late as 1988 the question of a change in coordination along the series was still being reviewed.[34] The consensus reached slightly prior to 21st century was that a definite transition in the lanthanide inner sphere coordination occurred starting from nine water molecules for the earlier lanthanides and dwindling to eight for the heavier lanthanides with an intermediate equilibrium between eight and nine for the mid-series samarium and europium. The accepted structures were based primarily on modeling studies and demonstrated a shift from square antiprismatic to tricapped trigonal planar.[35,36,37,38] This suggested structural shift has been demonstrated to be inaccurate by a recent EXAFS study which found that the hydrated lanthanides maintain a tricapped trigonal prismatic structure across the series with capping positions becoming water deficient. This leads from the initial nine coordinate structure at lanthanum through to bond lengths representative of the eight coordinate species at holmium and increasing further for the smallest lanthanides.[39]

3.3 Hydrated clusters & gas phase reactivity

As opposed to the inner sphere coordination discussed above, outer sphere

coordination is more difficult to study due to the lesser degree of influence of the metal center on the ligands. Outer sphere coordination has been accessed via studies of metal-solvent clusters, which are interesting systems because their stability is dependent on the degree of solvation provided.[40] Many lanthanide studies have focused on the physical properties of clusters using spectroscopic techniques. The application of mass spectrometry to this topic, however, is an area of growth. In particular, for work involving ESI, solvated cluster studies can help address questions such as how representative the technique is of the solution phase.

Singly charged, protonated aqueous clusters $[H(H_2O)_n]^+$ have been well investigated using various ionization techniques and particularly by mass spectrometry.[41] Unusual stability has been observed for the n = 21 cluster, recognized in 1973.[42] These structures which exhibit elevated stability have been termed magic number clusters. Notably, the n = 21 cluster has been described as a clathrate hydrate type structure involving a dodecahedral cage, 20 pentagon faces with one central entity,[43] based on modeling as well as experimentation (Figure 3.3.). The identity of the central species, hydronium ion or water molecule, is as of yet still under debate. Remarkably, the water cluster series is sufficiently easily generated to have been used for mass spectrometry calibration, with the obvious advantages of being non-contaminating and easily available.[44] The step to multiply charged species from singly charged species however, was elusive.

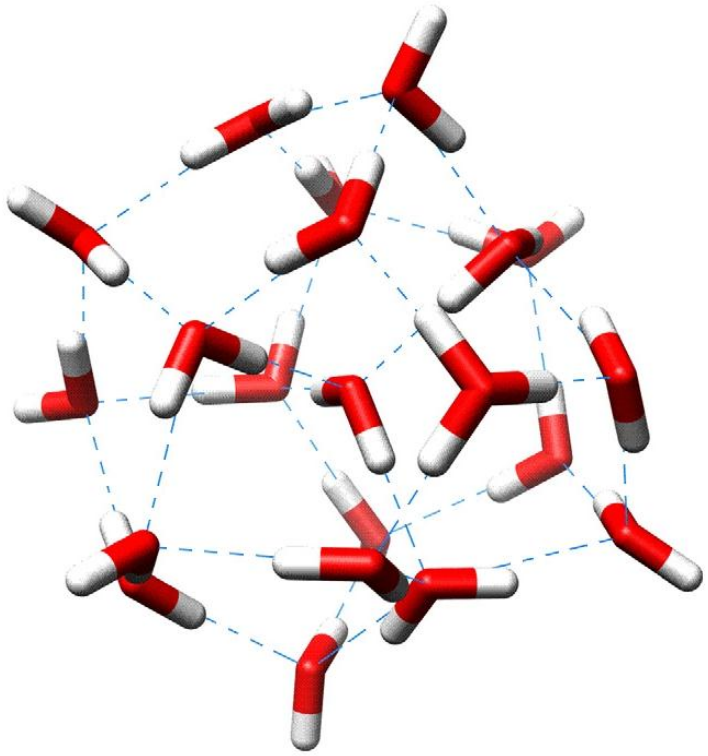


Figure 3.3. The magic cluster $[H_2O]_{21}^{+}$, a dodecahedral cage adapted from Figure 1 in McQuinn et al.[45]

With the development of ESI, the gas phase chemistry of water clusters became conveniently accessible.[46,47] Gas phase studies focused on hydrated metals[48] where interest centered on the structure of these clusters with respect to the position of the metal ion. Doubly charged metal ions such as Mg^{2+},[49] Ca^{2+},[50,51] Ba^{2+}, Cu^{2+} [52] and Pb^{2+} [53] have been investigated[54,55] and multiple solvents, both protic and aprotic, including water, methanol and acetonitrile have been used. One study, on the group 2 elements and the first row transition metals,[56] focused on the minimum attainable solvation prior to charge transfer. Indeed, it was found that all the divalent species studied with the exception of

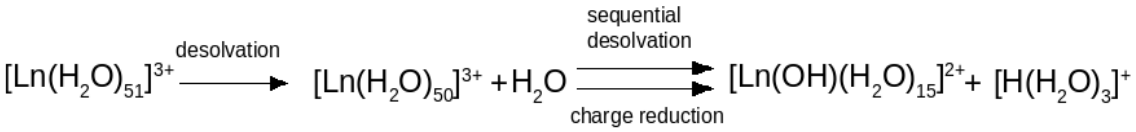
beryllium could exist in a doubly charged, singly solvated state.[57] Similar to many other situations in chemistry, two choices are available when considering how to obtain clusters of the appropriate size for investigation: working from something large and breaking it down (top down) or starting with small individual units and expanding (pick-up).[58] The pick-up method in this case begins with neutral clusters which are then ionized via electron impact[59] while the top down approach is the realm of ESI where large droplets are reduced to the desired size. In contrast, an additional ESI technique using addition of solvent to the cone gas has also been demonstrated to produce solvated clusters.[60]

Once the gaseous divalent species were identified, interest in triply charged gas phase metal ions was imminent. The triply charged clusters, solvated by dimethyl sulphoxide (DMSO)[61] or dimethylformamide (DMF), of the lanthanides yttrium, lanthanum, cerium, neodymium and samarium, were first observed 20 years ago by A. T. Blades et al.[62] However only recently, as of 2006, has solely aqueous solvated M^{3+} been detected for the lanthanides M = Ce, La, Eu through the use of nanospray and a modified mass spectrometer.[63] Additional work involving protic solvents soon followed[64] and further aqueous studies were subsequently done for the lanthanides.[65] Recently, aqueous solvated lanthanide clusters have also been subjected to CID in the collision cell of a mass spectrometer[66] and aqueous hydration trends across the lanthanide series have been modeled.[67]

3.4 Lanthanide acidity

Charge transfer is a mechanism by which Coulombic stress can be relieved from a

molecule. It proceeds as follows (Equation 3.1.):



$$[Ln(H_2O)_{51}]^{3+} \xrightarrow{\text{desolvation}} [Ln(H_2O)_{50}]^{3+} + H_2O \underset{\text{charge reduction}}{\overset{\text{sequential desolvation}}{\rightleftharpoons}} [Ln(OH)(H_2O)_{15}]^{2+} + [H(H_2O)_3]^{+}$$

Equation 3.1. Generalized charge reduction of a lanthanide

This propensity for the overall reduction of the charge in the hydrated species via solvated proton transfer (rather than electron transfer) limits the stability of triply charged lanthanide ions. A minimum coordination sphere is required to stabilize the highly charged metal center or products such as $[M(OH)(H_2O)_n]^{2+}$ may be solely observed. It has been found that for the most acidic lanthanide, ytterbium, it is more difficult to stabilize the non-charge reduced species.[65] It is possible that beyond this point, creating stable aqueous M^{3+} clusters is not feasible and similar observations have been made for systems using acetonitrile as a solvent.[68]

3.5 ESI-MS of hydrated tricationic clusters

Though known to be stable in solution, solvated lanthanide clusters easily experience charge separation, resulting in the observation of the divalent species only. In the past 5 years, ESI has been demonstrated to be an effective technique for the study of aqueous triply charged clusters, though the initial work involved a modified mass spectrometer. As there have been other situations where ESI has been found to be non-representative of solution phase,[58] to aid in the resolution of this issue and contribute to

the understanding of multiply charged gas phase ions, further study of aqueous, triply charged lanthanide systems has been undertaken. The final results will be related to the gadolinium break postulate.

The technique of "cold flooding" has been successfully used to produce trivalent ions without further instrumental modifications.[69] Hydrated lanthanide clusters have been observed for La^{3+}, Tb^{3+} and Lu^{3+}.[66] Cold flooding requires unusual tuning of the mass spectrometer involving a high cone voltage in conjunction with low temperature and gas flow settings. The instrument, which is generally optimized to produce gas phase ions, thus allows solvated droplets to be introduced into the mass analyzer. By selecting a solvated ion in the first mass analyzer, the sequential desolvation process of that specific ion can be directly observed in the collision cell. The primary instrumental adjustments are simple, consisting of an elevated solvent flow, elimination of the cone gas and a high cone voltage setting.

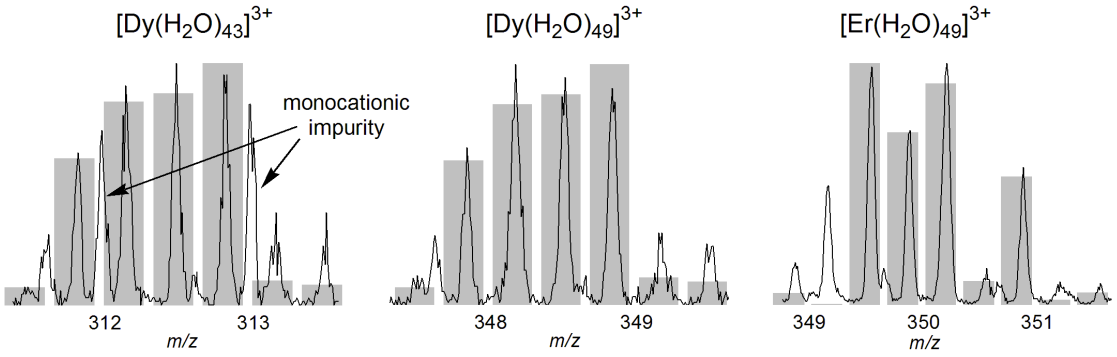
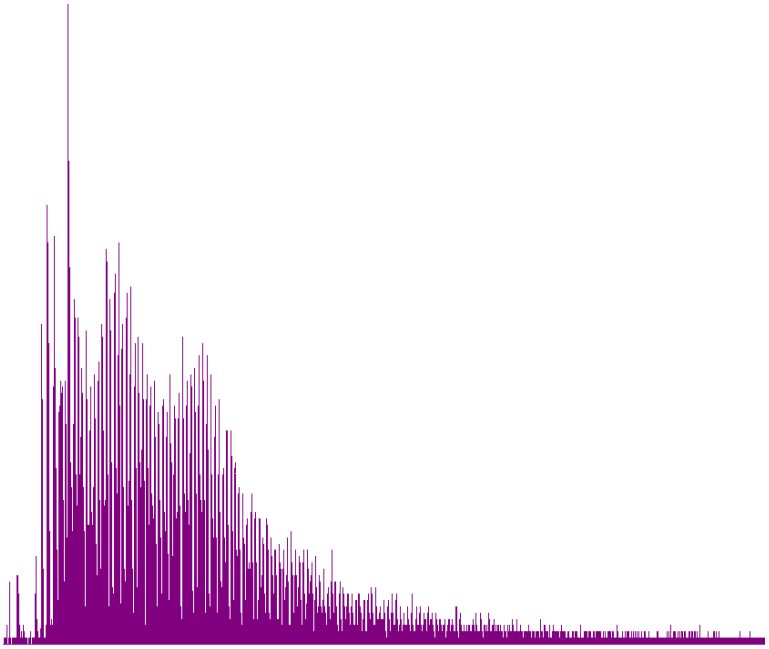


Figure 3.4. Triply charged lanthanide clusters with the calculated isotopic pattern overlaid.[70]

a)



b)

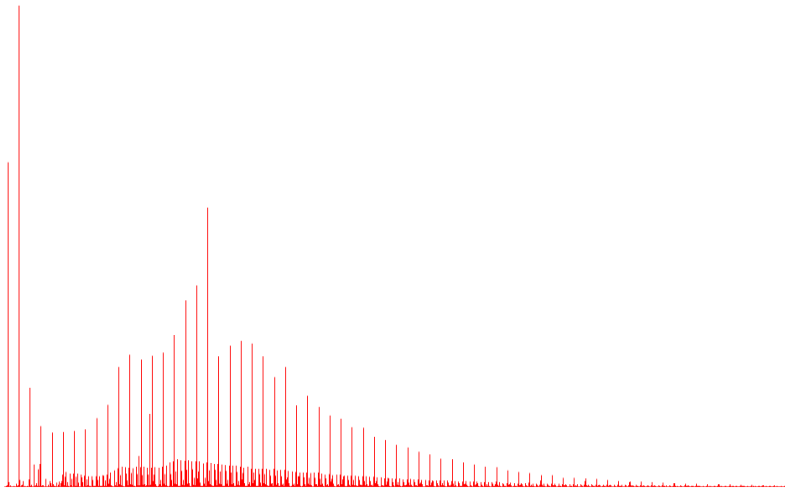


Figure 3.5. a) Full scan spectrum demonstrating dominant 3^+ hydrated neodymium clusters. b) Full scan aqueous yttrium 3^+ cluster spectrum dominated by 1^+ water clusters.

The full spectrum data for triply charged clusters is complex due to multiple series and elucidation requires the specificity of a tandem mass spectrometer. The initial quadrupole analyzer allows one cluster of specified *m*/*z* (represented by a single peak) to be selected then interacted with argon in the collision cell. As the accelerating energy prior to the collision cell is increased, the stability of the cluster structure is tested and the sequential loss of water molecules is directly observed, eventually to the point at which the triply charged species becomes unstable and charge reduction occurs. All of the charged fragments produced are separated in the flight tube of the ToF analyzer and monitored, each resulting from the initial *m*/*z* selected. For lanthanides with significant isotope patterns the signal intensity is reduced further, spread between numerous peaks (Figure 3.4.), and the intrinsic multitude of peaks contribute to dense spectra (Figure 3.5.).

3.6 Experimental procedure

The metal ions were investigated as nitrates and chlorides and were purchased from Aldrich. Experimental data was collected on an unmodified Micromass Q-Tof *micro*™. Voltage settings ranged from 180 V to 200 V for the cone voltage and 2600 to 2900 V for the capillary. The cone and desolvation gas flows were 0 L/hr and 100 L/hr respectively, while desolvation and source temperatures were generally maintained at 20°C and 60°C. Resolution settings and scan times were varied based on the quality of the signal obtained. Typical values for the low and high mass resolution were 12.0 while 2 minutes

was the routine scan time. The ion energy was kept constant at 1V and adjustment of other instrumental parameters showed minimal impact. Aqueous solutions were 5 to 15 mM and flow rates of 20 to 50 μL/min were used. Energy dependent electrospray ionization spectra were obtained through MS / MS fragmentation of a selected mass using a collision energy step of 1V per scan.

A hydrated lanthanide cluster with acceptable intensity and minimal interference was selected (n = 47 to 53) giving a nominal *m/z* between 335 and 375. This was evaluated based on the intensity of the cluster, the natural isotopic distribution and a preliminary MS/MS spectrum. The technique of EDESI was used and may be described as follows: the desired cluster mass was selected via a quadrupole filter and directed into the collision cell. Sequential increases of collision energy were provided and the cluster was accelerated into argon gas residing in the cell. Full spectrum data was acquired at each collision energy step through the time-of-flight section of the mass spectrometer. The data for each step was plotted as mass to charge ratio (*m/z)* against collision energy using a contour plot (providing the third dimension of signal intensity). This data was obtained for yttrium and the even atomic number lanthanides then analyzed in combination with data from K. McQuinn (published[66] and unpublished) for the rest of the lanthanide series.

As may be observed (Figure 3.6.), the difference between a hydrated Ln^{3+} ion and a charge reduced $Ln(OH)^{2+}$ cluster may be seen from both the *m/z* ratio and the spacing between the lines in the pattern, of 6 and 9 *m/z* respectively. There is a minimal hydration

number for the Ln^{3+} species after which point only charge reduced $Ln(OH)^{2+}$ species are observed. It should be emphasized that this is the *direct* observation of the charge reduction process in the collision cell, further supported by the presence of the Eigen cation $[H(H_2O)_4]^+$ which may also be seen as a product of the reaction. Clearly, the minimum hydration value for Ln^{3+} is larger than the eight or nine water molecules expected to form the inner coordination sphere and, rather, is expected to at least partially encompass the secondary solvation shell. By sequentially removing water molecules, charge reduction to the hydrated $Ln(OH)^{2+}$ species could be induced. The overall abundance of each species during the process may be seen in the total intensities plotted at the top of the diagram. The vertical length of each contour also gives an indication of the range of collision energies over which a species may exist with a longer line implying greater stability.

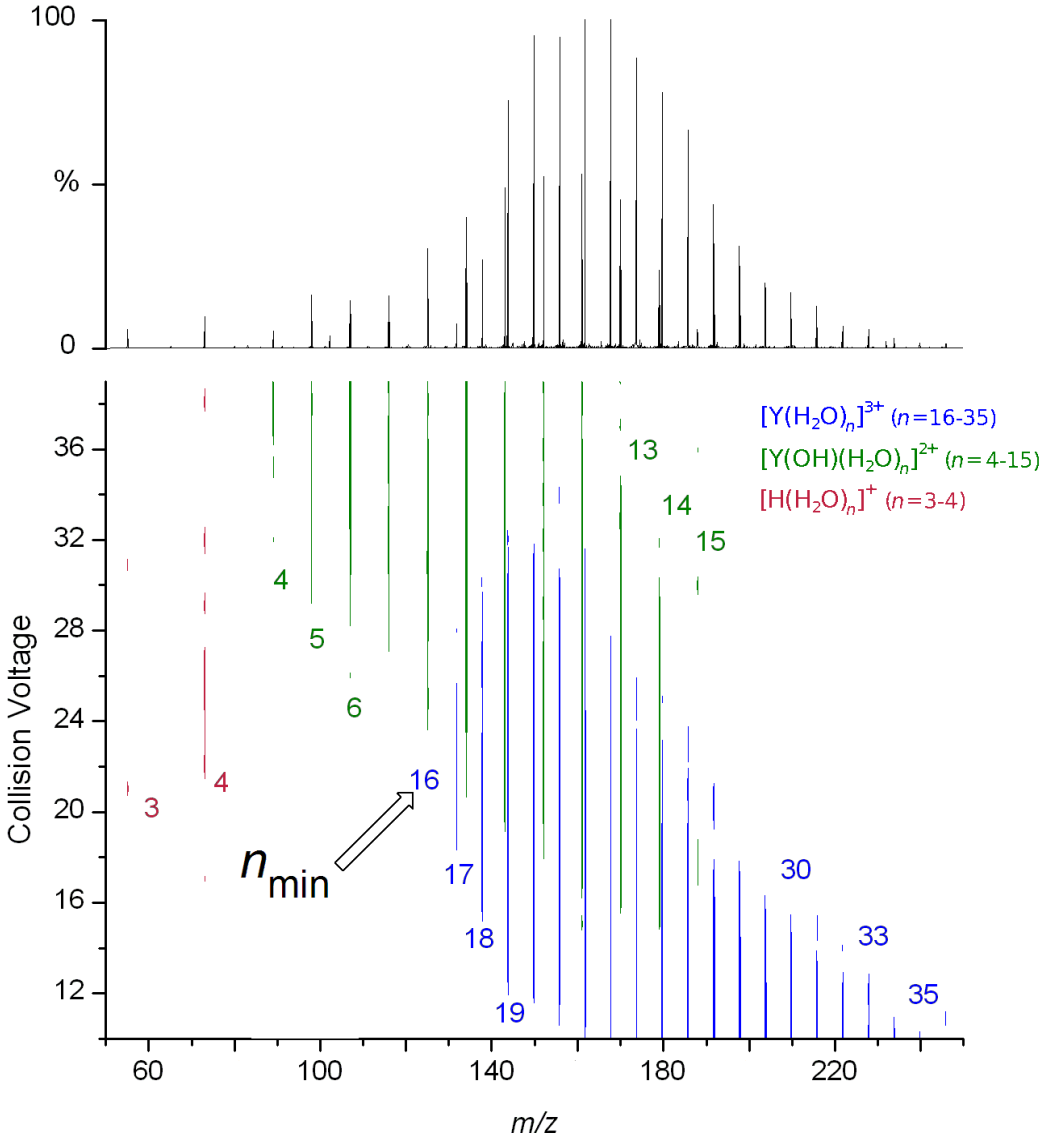


Figure 3.6. EDESI plot of the charge reduction of an aqueous Y^{3+} cluster.[70]

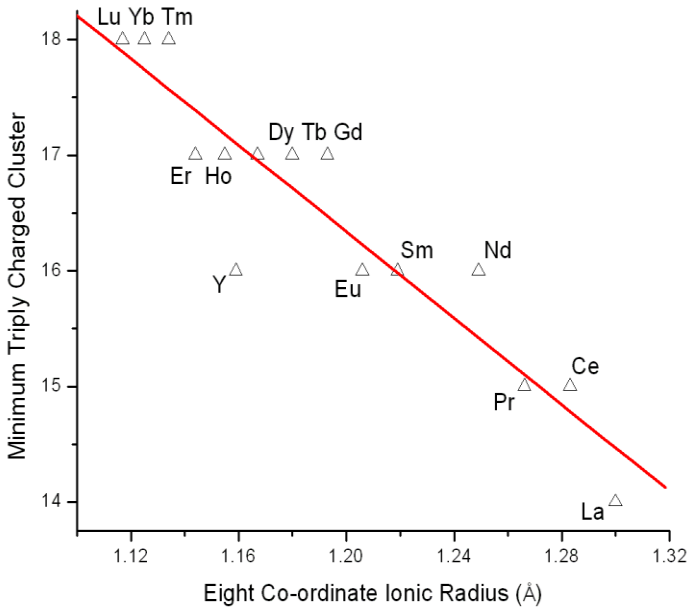
3.7 Discussion

Data for the minimum Ln^{3+} solvated cluster for each element of the lanthanide series is presented (Figure 3.7.). When the minimum solvation (n = 14 to 18) is plotted against the lanthanide ionic radii, it can be seen that there is a reasonable linear correlation (absolute R^2=0.93). The largest lanthanides, those which are early in the series and have lower charge density, are stable with less coordinated water. Yttrium may be identified as an outlier on the basis of ionic radius alone.

Interpreting the data in terms of pK_a is also instructive. In Figure 3.7b, the absolute R^2 of the line is 0.96, an improvement over the fit where the ionic radii are used. The improvement is primarily due to the better representation of the properties of yttrium. The good correlation here demonstrates that ESI-MS can be used accurately in this case to probe the details of the system under examination, such as the relative acidity of a group of compounds, and is representative of the solution phase. Of the lanthanides, ytterbium displays the lowest pK_a, significant as difficulties have been noted when attempting to gather data for this triply charged species.[65]

When the data are considered in relation to the gadolinium break hypothesis, the lack of an abrupt discrepancy in the gadolinium region (diagnostic of an instantaneous change in coordination) is evident. If, instead, the more generalized tetrad effect is considered, it would be possible to interpret a series beginning at samarium and ending at holmium. However, the resolution limitations of this approach (+/-1) must be considered given that, in either interpretation, these effects are subtle.

a)



b)

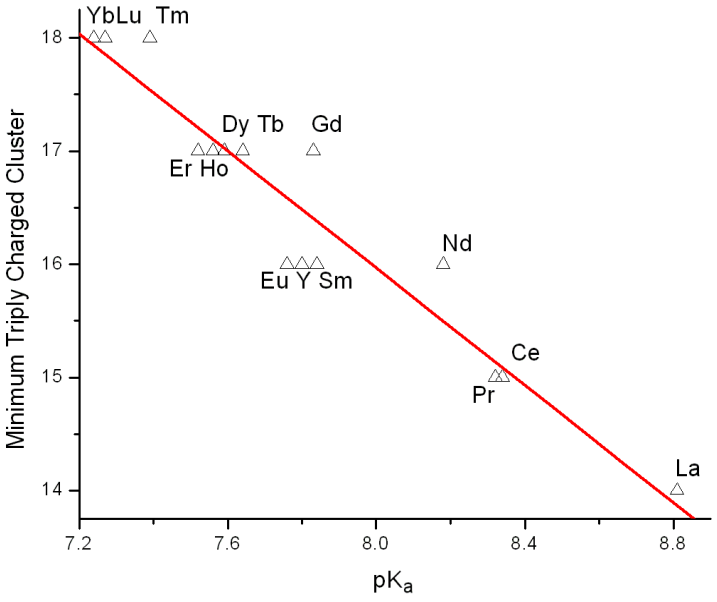


Figure 3.7. a) Lanthanide 3^+ minimum solvation correlated with ionic radius

b) Lanthanide 3^+ minimum solvation correlated with pK_a

Focusing on yttrium, rather than grouping with the early lanthanides, due to its increased ionic radius (1.159 Å) this triply charged ion falls between dysprosium and holmium well into the latter half of the lanthanide series. For the naturally occurring lanthanide series, lanthanum to lutetium inclusive, R^2 is 0.97 for the ionic radius and 0.96 for the pK_a. This contrasts with the yttrium included values of R^2 of 0.93 and 0.96 respectively. This prompts the question: why are radius and pK_a not similar influences on hydrolysis for yttrium despite its many similarities to the lanthanides? The third ionization energy of yttrium is most similar to gadolinium and aqueous coordination studies of yttrium have demonstrated that it has a coordination number of eight which is also consistent with the lanthanides.[71,72] Yet yttrium is less acidic than expected based on its ionic radius requiring less water molecules, 16 rather than 17, to be stabilized as a triply charged ion.

The lanthanide n_{min} data collected may be compared with published work detailing the n_{crit} values of the charge reduction process. In this case, n_{crit} has been measured by Bush et al. based on the turnover size (the point at which the rate of water loss equals that of charge reduction), correlated with the hydrolysis constant and the data interpreted in support of the gadolinium break (Figure 3.8.).[65] A modified nano-spray ionization mass spectrometer operated at 220 K and employing blackbody infrared dissociation (BIRD) was used in this investigation. The overall trend in n_{crit} values is higher than those of n_{min}, likely due to the length of time involved in the dissociation process (CID occurring faster than BIRD and potentially allowing the acquisition of meta-stable species).

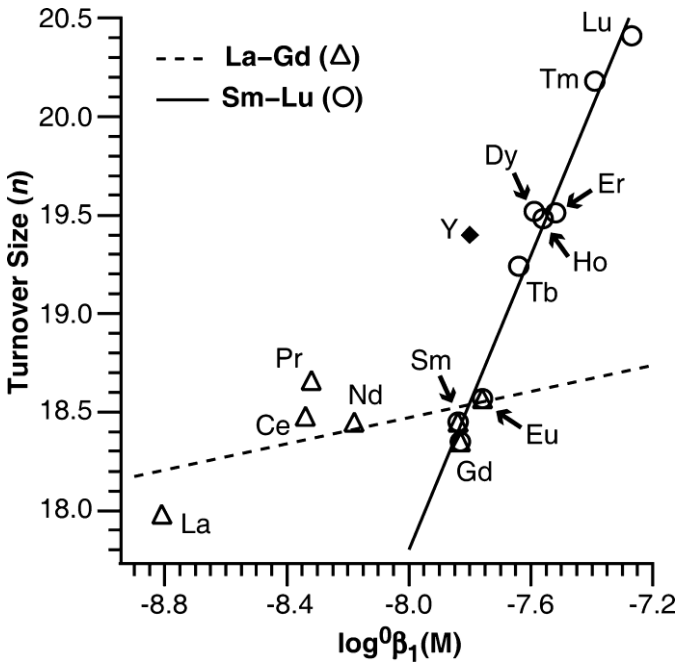
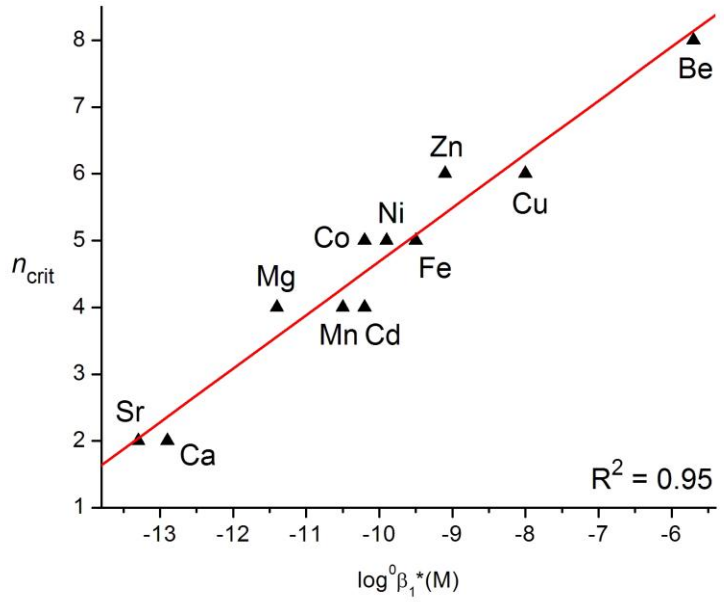


Figure 3.8. The gadolinium break related to turnover size replicated from Figure 5, Bush et al.[65]

These trends may also be examined for existing n_{crit} data related to divalent ions (Figure 3.9.). Similarly, n_{min} represents the minimum triply charged cluster seen prior to charge reduction. Consistent with the data obtained for the lanthanides, the fit of the trendline using the log of the hydrolysis constant ($-pK_a$) demonstrates an improved R^2 over that of the radius. An alternative to the process of proton transfer would be charge reduction through electron transfer. If the latter were responsible, the n_{min} (or n_{crit}) values would be expected to relate well to the second or third ionization energy for 2^+ and 3^+ ions respectively. When plotted, however, the R^2 fit is poor, particularly for trivalent ions (R^2=0.34), (Appendix B).

a)



b)

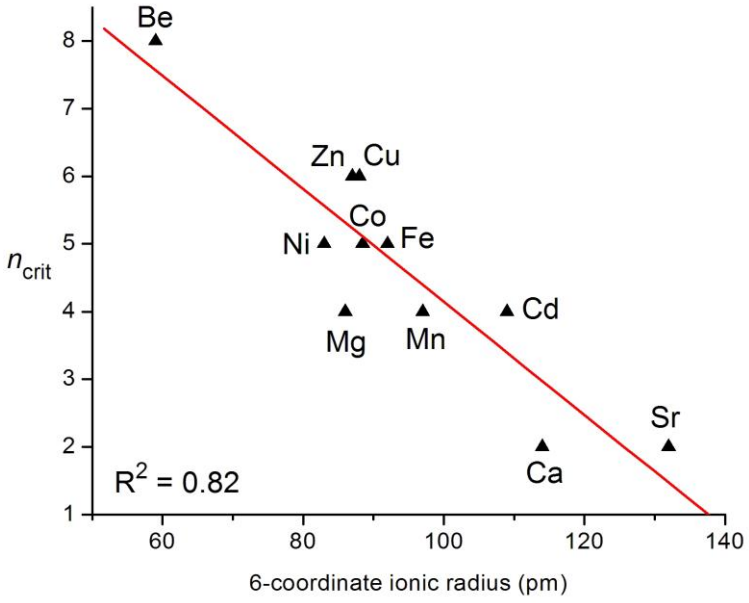


Figure 3.9. a) Divalent ion hydrolysis constants and b) divalent ionic radius, correlated to n_{crit}.[70]

3.8 Conclusions

Highly hydrated, triply charged lanthanide clusters were successfully produced on an unmodified ESI-MS. The Ln^{3+} series was investigated through directly observed charge reduction determined to occur by proton transfer based on the strong correlation to acidity and ionic radius. This conclusion was supported further by the scanty relationship to the third ionization energy. Published data on divalent species was examined in this context and found to exhibit the same overall trend. ESI was effectively demonstrated to be representative of the solution phase and the lanthanide results were related to the on-going discussion of the gadolinium break hypothesis.

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Chapter 4: The Effect of Solvent on the Surface Activity of Ions in ESI

4.1 Surface activity in ESI

Surface activity is a broad term encompassing many fields of chemistry including catalysis, adsorption, host-guest interactions and nanoparticles. In ESI, surface activity is intricately involved with the mechanism by which charged ions are produced. Consider the case where two ions, A and B, are present in a solution. If the solvent selected is different in nature from A, this ion will prefer to be present at an interface which minimizes its overall solvation. If the container in question is a semi-spherical electrospray droplet, the ion A will partition as much as possible to the outer layer becoming surface active, while ion B resides preferentially in the core of the droplet. When ions A and B are similar in structure, the gaseous ionization may be considered an equilibrium with equal chance of either species existing at the droplet boundary. Essentially, ions that are the least well solvated and / or ion paired are most likely to be found on the surface of a droplet rather than buried in the interior, and so are over-represented in the spectrum as they are the ions most likely to evaporate from the droplet first. For ions of similar properties, ESI provides a good match between concentration and abundance but, for ions that differ greatly in size or polarity, the results obtained may become distorted from those of the original solution analyzed. The ESI solvent polarity (Table 4.1.) will affect the absolute instrumental response of the ions as well. The situations in which bias occurs must be understood and accounted for in order that any ESI-MS data have real quantitative meaning.[1]

Solvent	Polarity Index (P')
Water	10.0
Methanol	6.6
Acetonitrile	6.2
Dichloromethane	3.4

Table 4.1. Polarity index of selected ESI solvents.[2]

4.2 Introduction to ionic liquids

Room temperature ionic liquids (RTILs) have been known for nearly a century, the first, ethylammonium nitrate, was recognized in 1914 with a melting point reported in the range of 8-16°C.[3,4] In the past decade however, these materials have attracted renewed interest as a component of the green chemistry movement both as an alternative to volatile solvents in synthesis[5] and by increasing industrial efficiency through the minimization of unrecyclable, solvent burdened waste.[6] ILs consist of poorly matched ions generating low lattice energies and often resulting in a liquid salt at low temperatures (generally < 100°C). RTIL are a convenient class of ionic liquids, an example of which is 1-butyl-3-methylimidzolium hexafluorophosphate.[7,6] As of 2009, it has been estimated that more than 10^6 different compounds exist in the IL family and there is ample room for growth in this field, particularly involving task-specific IL[8]. In general, recent systems tend to be stable to air and water, based on the 1,3-dialkylimidazolium cation (Figure 4.1.) and a good deal of research has focused on this area, however, an increasing number of alternatives are being explored.[9,10,11]

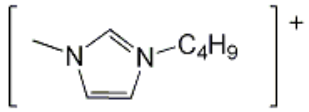

Figure 4.1. 1-Butyl-3-methylimidazolium (BMIM).

Ionic liquids (IL) are easily prepared from commercially available reagents and with tunable properties they have been deemed "designer solvents". The anion of the IL tends to be inorganic while the cation is organic and usually bulky with low symmetry. Both the anion and cation (Figure 4.2.) affect properties of ionic liquids, such as the melting point (Table 4.2.). Anion effects tend to be explained in terms of electron delocalization and hydrogen bonding or the lack thereof[6] while the cation effects are rationalized in terms of structural fit and packing.[12] ILs to date have primarily been used in electrochemistry during redox investigations as an alternative solvent and earlier as a molten salt electrolyte in batteries, [13] however, the number of applications in synthesis, analytical chemistry and catalysis[14,15,16] is rising.[17]

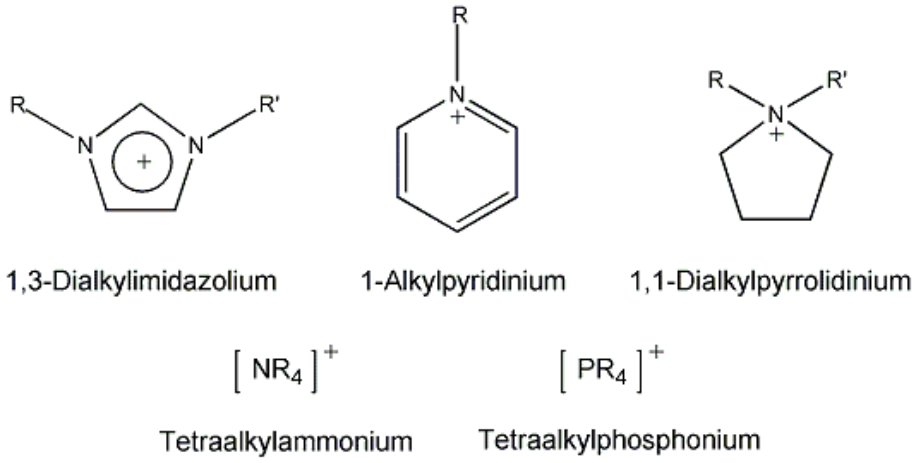

Figure 4.2. Common cations used in ionic liquids.

Substance	Surface Tension (dyn cm^{-1} at 25°C)	Melting Point	Density (g mL^{-1} at 25°C)	Dipolarity / Polarizability (40°C)	Molar Mass (g/mol)
Water	73	0.0	0.997	n/a	18.0
$[BMIM]^+Cl^-$	n/a	41†	1.08	2.247	174.7
$[BMIM]^+I^-$	54.7	-72†	1.44	n/a	266.1
$[BMIM]^+[BF_4]^-$	46.6	-81†	1.12	1.647	226.0
$[BMIM]^+[PF_6]^-$	48.8	10†	1.368	1.914	284.2
$[BMIM]^+[Tf_2N]^-$	37.5	-25‡	1.436	1.889	419.4

Table 4.2. Physical properties of various solvents.[6,18,19]

† Dried, ‡ water equilibrated.

4.3 Ionization study approach

To test the effects of the ESI mechanism on the production of ions, dilute solutions, on the order of 10^{-5} M, containing a series of ionic liquids based on the BMIM cation were prepared in a variety of solvents (dichloromethane, acetonitrile, methanol, 1:1 acetonitrile:water) at similar concentration. Using the same solvent, two different anions were combined and the instrumental response monitored. The difference in intensity observed provides a comparison of ionization efficiency and solvent polarity effects.

4.4 Preparation of $[BMIM]^+[NTf_2]^-$

$$[BMIM]^+Cl^- + Li^+[N(SO_2CF_3)_2]^- \longrightarrow [BMIM]^+[NSO_2CF_3)_2]^- + LiCl$$

Equation 4.1. Metathesis forming $[BMIM]^+[NTf_2]^-$

Preparation of $[BMIM]^+[NTf_2]^-$ (Equation 4.1.), was based on literature procedures[20] via a metathesis reaction as follows. Lithium bis(trifluoromethanesulfonyl) imide (0.8 g, 0.003 mol, Aldrich) and 1-butyl-3-methylimidazolium chloride (0.5 g, 0.003 mol, TCI America) were each dissolved separately in 50 mL of deionized water. The lithium solution was added to the $[BMIM]^+Cl^-$ solution with stirring and the previously clear, colourless solution became milky immediately and was allowed to sit for 45 minutes. The combined solution was warmed to 55°C and observed to return to clear and colourless. Heating was applied for 15 minutes after which point small oily droplets could be observed in the bottom of the Erlenmeyer. This material was extracted with dichloromethane (3 × 15mL). The organic layer was then exhaustively washed with deionized water (5 × 10mL) to remove any residual lithium chloride and starting material. The dichloromethane was removed via rotary evaporation resulting in 1 mL of solution. The material was dried under vacuum on a Schlenk line for 48 hours prior to use.

4.5 Experimental procedure

For each test a stable signal of a diluted IL was obtained (concentrations were 0.04mM, 4×10^{-5} M, +/- 15%). Once a suitable number of scans were acquired, a second solution containing a different IL (in the same solvent) was added in equal volume. The

peaks monitored in this experiment were [$BMIM_2$ + anion]$^+$ and for comparison a response ratio, defined as the peak area of the IL of interest to the second IL peak area, was calculated. This procedure was expected to result in halving of the signal for the initial IL ions present while a secondary peak should also be observed at equal intensity for the IL added. If one signal is obviously favoured, it can be surmised that the ion solvent interaction is less favourable resulting in surface enrichment and suppression of the secondary ion. The clusters were examined using positive ionization mode and the full scan MS function on a Micromass Q-Tof *micro*^{TM} mass spectrometer (Table 4.3. and Appendix C). The unpublished raw data for the $[BMIM]^+Cl^-$, $[BMIM]^+I^-$, $[BMIM]^+[BF_4]^-$ and $[BMIM]^+[PF_6]^-$ combinations was collected by University of Victoria undergraduate students during Chem 361: Analytical Chemistry Laboratory, Spring 2010 under partial supervision of the author as a Laboratory Instructor using standard solutions prepared by the author.

a)

$[BMIM]^+Cl^-$	1:1 Water : Acetonitrile	Methanol	Acetonitrile	Dichloromethane
$[BMIM]^+I^-$	0.14	3.5	1.8	5.4
$[BMIM]^+[BF_4]^-$	1.5	0.17	0.75	1.5
$[BMIM]^+[PF_6]^-$	0.049	0.026	0.74	3.9
$[BMIM]^+[NTf_2]^-$	0.021	0.0069	1.9	6.3

b)

$[BMIM]^+[NTf_2]^-$	1:1 Water : Acetonitrile	Methanol	Acetonitrile	Dichloromethane
$[BMIM]^+Cl^-$	47	150	0.52	0.16
$[BMIM]^+I^-$	40	56	0.81	0.39
$[BMIM]^+[BF_4]^-$	20	25	1.1	0.34
$[BMIM]^+[PF_6]^-$	2.4	2.7	0.80	1.0

Table 4.3. ESI-MS response ratio (species in bold used as the numerator) for selected dilute ILs. a) $[BMIM]^+Cl^-$ and b) $[BMIM]^+[NTf_2]^-$ in varied solvents.

4.6 Discussion

If the calculated intensity ratio is >> 1 or << 1, enhancement or suppression respectively of the selected anion is evident while a ratio of ~ 1 indicates that both analytes are ionized with equal likelihood demonstrating similar levels of surface activity in the selected solvent. Regarding the concentration of the solutions, 4×10^{-5} M is on the borderline between high and low concentration as previously discussed however, based on the literature it is expected that at this level the majority of the observed effects would be due to the surface activity of the analyte.[21] Given the largest possible concentration difference between solutions of 25%, it may be seen that 82% of the response ratios exceed the 0.75 to 1.25 expected range. In acetonitrile this values drops to 75% with an overall average (all solvents tested) of 1.1 +/- 0.61.

Anion	Ionic Radius (pm)	Surface Area† ($Å^2$)	Volume† ($Å^3$)	$\Delta_t G^{\circ}$‡ (kJ/mol)
Cl^-	184	39.9	23.7	7.0
I^-	220	51.5	34.8	2.6
$[BF_4]^-$	228	76.7	54.6	0.6
$[PF_6]^-$	254	100.7	73.1	-0.7
$[NTf_2]^-$	n/a	195.5	156.1	n/a

Table 4.4. Anionic properties (radii[22,23,24], surface area, volume).

† Values calculated using Hartree-Fock at the 3-21G level of theory.

‡ Standard molar Gibbs transfer energy for anions from water to 0.6x methanol in water.[25]

Comparing the least similar anions, Cl^- and $[NTf_2]^-$ it may be observed that starting in the most polar solvent, 1:1 acetonitrile:water, strong enhancement of the Cl^- anion is seen. This continues on in methanol but completely inverts in acetonitrile where the enhancement of $[NTf_2]^-$ begins to occur becoming even larger in dichloromethane. In practical terms, this means when examining a solution with approximately equal concentrations of anion, a result ranging across two orders of magnitude and demonstrating a change in the primary species produced could be found by simply adjusting the solvent selected.

In polar solvents the response is overwhelmingly due to the less polar $[NTf_2]^-$. This anion favours the droplet surface as there is decreased solvation at the gas-liquid interface and, as such, it is more easily ionized. This is supported by the trend in the Gibbs energy of transfer ($\Delta_t G$) which is favourable (i.e. a lower value) for the transfer of non-polar anions away from pure water and into a mixed methanol/water phase resulting in lowered aqueous solvation (Table 4.4.). In terms of hydration, relatively small anions like those used in this study are expected to have a ΔG value approximately linearly proportional to volume (contrary to larger anions which would be more accurately related to surface area).[26] The $[NTf_2]^-$ anion has a relatively large volume, 6.6× that of Cl^-, and, consequently a large ΔG is expected indicating a propensity to avoid aqueous solvation.

A comparison of the response ratios obtained in acetonitrile shows that 90% of the results fall into the range of 2× enhancement to 1.5× suppression. When this is compared with 1:1 acetonitrile:water as a solvent only 10% of the values are present in this range. Further, it may be observed, in the 1:1 acetonitrile:water system, the largest and most non-polar species, $[NTf_2]^-$, was enhanced greatly in each anion pairing as were the majority of the results for $[PF_6]^-$ (Figure 4.3.). Somewhat surprisingly, the anion behavior in methanol appears to parallel that of the 1:1 actetonitrile to water system. Dichloromethane on the other hand, is reasonably similar to acetonitrile, with a stronger enhancement impact observed for the smallest anion, Cl^-.

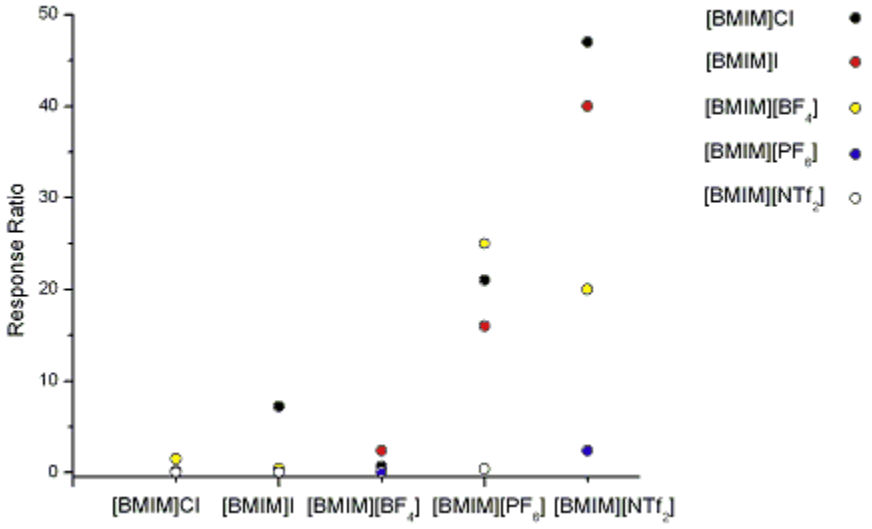

Figure 4.3. Enhancement of the ESI-MS response ratio for varied anions in 1:1 acetonitrile:water. Divergent species, such as $[BMIM]^{+}[PF6]^{-}$ & $[BMIM]^{+}Cl^{-}$, provide an example of this distortion.

4.7 Conclusions

Overall, to minimize both ion enhancement and suppression acetonitrile, a moderate polarity solvent, would be the optimum choice for ESI analyses. This selection would minimize surface activity effects by moderating the interactions between ions at extreme polarities and the solvent. This study also provides evidence against the simultaneous analysis of systems with extremely different physical properties using ESI without careful consideration, as matrix effects are much more likely and, at minimum, remedial work may be required to achieve accurate results representative of the original solution.

4.8 References

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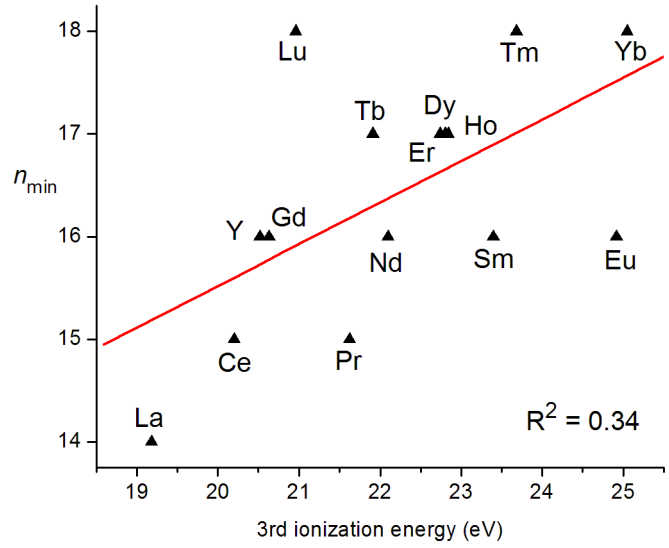
Appendix A

	Level A (ng/mL)	Level B (ng/mL)	Level C (ng/mL)	Level D (ng/mL)	Level E (ng/mL)	Level F (ng/mL)	Level G (ng/mL)
Triclosan	5.0	25	50	100	200	1,000	2,500
Tetraclosan	5.0	25	50	100	200	1,000	2,500
Pentaclosan	5.0	25	50	100	200	1,000	2,500
Nonylphenol	50	100	500	1,000	5,000	15,000	25,000
Monochloro-nonylphenol	50	100	500	1,000	5,000	15,000	25,000
Dichloro-nonylphenol	50	100	500	1,000	5,000	15,000	25,000
2,4-Dichlorophenol	50	250	500	1,000	2,500	n/a	n/a
Trichlorophenol	5.0	25	50	100	250	1,000	2,500
$^{13}C_{12}$-Triclosan	250	250	250	250	250	250	250
$^{13}C_6$-Nonylphenol	250	250	250	250	250	250	250
$^{13}C_6$-2,4-Dichlorophenol	100	100	100	100	100	100	100
$^{13}C_6$-Trichlorophenol	200	200	200	200	200	200	200
$^{13}C_6$-2,4,5-T	50	50	50	50	50	50	50

Table A 1. Calibration levels based on a 500 mL sample size

Appendix B

a)



b)

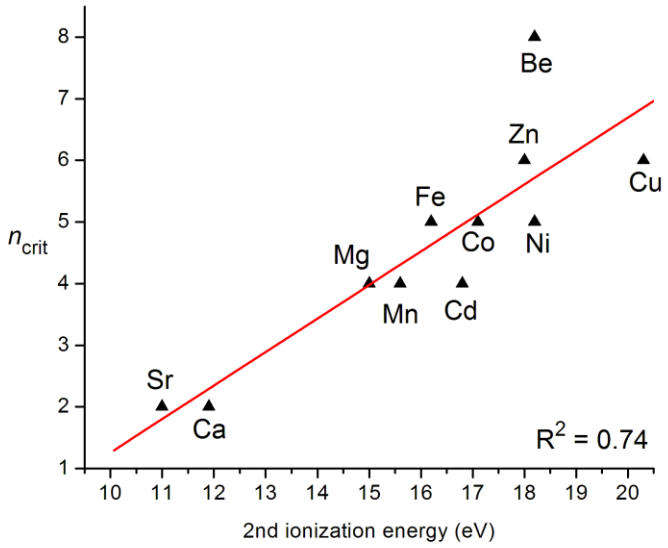


Figure B 1. Minimum solvation of: a) Divalent ions related to IE_2, b) Ln^{3+} related to IE_3.

Appendix C

a)

$[BMIM]^+I^-$	1:1 Water : Acetonitrile	Methanol	Acetonitrile	Dichloromethane
$[BMIM]^+Cl^-$	7.2	0.28	0.55	0.19
$[BMIM]^+[BF_4]^-$	0.41	0.69	0.88	0.92
$[BMIM]^+[PF_6]^-$	0.061	0.023	1.6	1.4
$[BMIM]^+[NTf_2]^-$	0.025	0.018	1.2	2.6

b)

$[BMIM]^+[BF_4]^-$	1:1 Water : Acetonitrile	Methanol	Acetonitrile	Dichloromethane
$[BMIM]^+Cl^-$	0.66	6.0	1.3	0.66
$[BMIM]^+I^-$	2.4	1.5	1.1	1.1
$[BMIM]^+[PF_6]^-$	0.040	0.14	0.34	1.1
$[BMIM]^+[NTf_2]^-$	0.051	0.040	0.88	3.0

c)

$[BMIM]^+[PF_6]^-$	1:1 Water : Acetonitrile	Methanol	Acetonitrile	Dichloromethane
$[BMIM]^+Cl^-$	21	39	1.4	0.25
$[BMIM]^+I^-$	16	44	0.62	0.70
$[BMIM]^+[BF_4]^-$	25	7.1	3.0	0.89
$[BMIM]^+[NTf_2]^-$	0.41	0.37	1.3	0.96

Table C 1. Response ratios for: a) $[BMIM]^+I^-$, b) $[BMIM]^+[BF_4]^-$ and c) $[BMIM]^+[PF_6]^-$.