THE USE OF IONIC LIQUID BASED SALTS IN GAS CHROMATOGRAPHY AND ELECTRO-SPRAY IONIZATION MASS SPECTROMETRY

by

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ABSTRACT

THE USE OF IONIC LIQUID BASED SALTS IN GAS CHROMATOGRAPHY AND ELECTRO-SPRAY IONIZATION MASS SPECTROMETRY

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This dissertation focuses on two uses for Room Temperature Ionic Liquids (RTILs). First, RTILs will be used to produce novel Gas Chromatographic (GC) stationary phases. The use of new phosphonium based RTIL GC columns for one-dimensional and two-dimensional GC will be discussed. The high thermal stability and unique orthogonality of these phases will be demonstrated. Additionally, the development of the first tricationic RTIL based GC stationary phases will be presented. The exceptional peak shape for polar compounds and thermal stability of these stationary phases are examined. Furthermore, the solvation properties of these novel solvents will be described *via* a linear solvation energy relationship.

The second use for RTIL based salts presented herein, will be their use as ion-pairing agents for the complexation and successive detection of anions in the positive ion mode Electrospray Ionization Mass Spectrometry (ESI-MS). Dicationic, tricationic, and tetracationic salts will be used for the detection of mono-, di-, and tri-valent anions. The use of this method always results in the more sensitive detection of anions. The structural motifs of successful ion-

pairing agents will be discussed. Furthermore, the use of these ion-pairing agents for Liquid Chromatography (LC) MS and Capillary Electrophoresis (CE) MS experiments will be presented. The penultimate chapter will discuss the mechanism(s) which allow this ion-pairing methodology to yield ultra-high sensitivity. Through this dissertation, a better understanding of the uses and capabilities of RTIL based materials in analytical chemistry will be attained.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction to Ionic Liquids

Ionic Liquids (ILs) are a novel class of solvents that consist entirely of ions. This differs greatly from typical solvents consisting exclusively of covalently bound molecules. The generally accepted definition of an IL is a pure ionic compound that has a melting point below 100℃. Furthermore, Room Temperature Ionic Liquids (RTILs) are ILs that are liquids at ambient temperatures. This deviates immensely from the traditional viewpoint that salts generally have very high melting points. Sodium chloride, for example, has a melting point of 801℃. The melting point of molecular solvents mainly depends on relatively weak intermolecular forces (i.e. London-dispersion, dipole-dipole, and hydrogen bonding). In contrast, ionic compounds interact electrostatically, which is a relatively stronger attraction than the aforementioned forces. Thus, it is sensible to believe that ionic compounds would have higher melting points than covalent compounds.

This being the case, RTILs are a very interesting class of compounds and have caught the attention of many researchers in recent years. The seminal report for RTILs came nearly 100 years ago, when Paul Walden introduced ethylammonium nitrate (mp 12℃) in 1914.[1] However, the study of these low melting point salts was more or less ignored for the better part of a century until Wilkes et. al. reported several stable RTILs based on imidazolium cations in 1992.[2] Since this time, researchers have obtained a better understanding of how RTILs can be formed. Typically, at least one (if not both) of the ions (i.e. cation or anion) of a RTIL possesses some degree of organic nature in its structural make-up. Additionally, charge delocalization within the ionic species is found to be an important feature in producing RTILs, such that the electrostatic interactions between ionic counterparts is weaker. Likewise, having a sufficient

number of degrees of freedom in the RTIL structure will also aid in the formation of the low melting salts. Figure 1.1 shows the general structures of some typical cations and anions that are employed to produce RTILs.

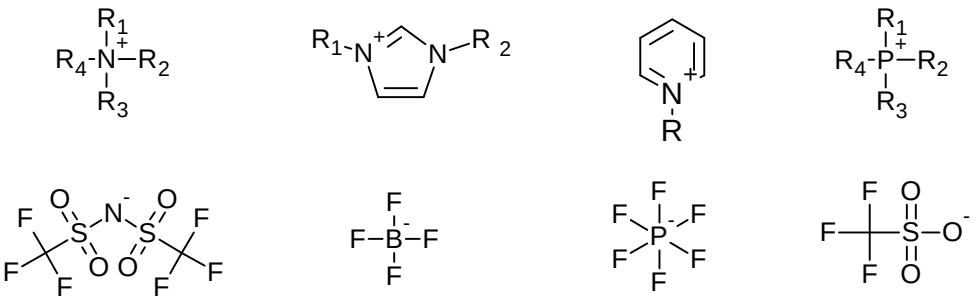


Figure 1.1 Structures of commonly used cation and anions in the production of RTILs

As shown in Figure 1.1, ammonium, imidazolium, pyridinium, and phosphonium cations are commonly used for the production of RTILs. The anions shown which are frequently used include bis(trifluoromethanesulfonyl)imide (NTf_2^-), tetrafluoroborate (BF_4^-), hexafluorophosphate (PF_6^-), and trifluoromethylsulfonate (TfO^-).

Of course, these are not the only cations and anions that can be used to produce RTILs. A plethora of cations and anions have been combined to form RTILs.[3-19] Indeed, the ability to change cations and anions with ease allows these compounds to be highly tunable, such that they can be formed specifically for a particular task. In addition, geminal dicationic, unsymmetrical dicationic, and tricationic ILs have also been reported.[20-23]

RTILs posses a number of properties that make them attractive solvents. First, they typically have very low or no vapor pressure. Many of them show very high thermal stabilities; greater than 400℃ in some cases.[20] They often have a wide liquid ranges. Some RTILs can remain in their liquid state from 0° to 300℃.[24] Most RTILs are flame retardant, though they can also be made combustible if so desired.[25] They can be custom synthesized to be either miscible or immiscible in aqueous. Lastly, RTILs are capable of undergoing virtually every type

of intermolecular interactions.[9,20] For these reasons, research in all areas of chemistry has been affected by the advent of these novel salts.

1.2 The Use of Ionic Liquids in Separations

1.2.1 Ionic Liquids in Liquid-Liquid Extractions

Ionic liquids have been found to be useful solvents in several types of analytical separations. One such example is the use of RTILs as solvents in liquid-liquid extractions. The fact that RTILs can be custom synthesized to be either water miscible or hexane miscible is one advantage when using them as solvents for liquid-liquid extractions. The ability for an IL to be hydrophobic or hydrophilic is typically dictated by the anion. RTILs with halide, BF_4^-, or TfO^- anions are usually water soluble, while those RTILs with PF_6^- or NTf_2^- anions are typically used as water immiscible solvents.

In some cases, an additive can be dissolved in a RTIL and used a co-extractant. Chun et. al. showed that dicyclohexyl-18-crown-6 could be used as an additive to a RTIL phase for the extraction of alkali metal chlorides from aqueous media.[26] Other examples show that just using the RTIL as the extractant can have favorable outcomes. Visser et. al. showed that certain RTILs could be used to coordinate mercury^{2+} and cadmium^{2+} and extract those metals from water.[8] Lo et. al. have shown that RTILs can be used as extraction media for the continuous removal of sulfur containing compounds from diesel fuels.[27] In any case, the tuneability of RTILs have proven to be advantageous in their use as new solvents with capabilities in liquid-liquid extractions.

1.2.2 Ionic Liquids in Capillary Electrophoresis

RTILs also have been found to be fascinating additives for use in capillary electrophoresis (CE). In this case, the RTILs are dissolved in the run buffer and are no longer ILs, but rather are simply novel salts from which the electroosmotic flow (EOF) can be interestingly modified. The RTILs can be custom synthesized to either slow, stop, or reverse

the EOF. Additionally, the IL based salts may have some interaction with the analytes being separated by CE and can be used also as selectivity inducing agents.

The use of RTIL additives in CE has been successfully applied to the separation of polyphenols, proteins, and drug molecules.[28-30] In the later case, an additional benefit of using the RTIL additive was found. It was observed that drug molecules, which previously had too strong of an affinity for the bare capillary walls, were easily separated. This was attributed to the coating of the IL on the inner wall of the fused silica capillary.[30] Recently, some new phosphonium based RTILs were used as buffer additives for the separation of inorganic and small organic anions.[31] The separation of these analytes is not at all possible under normal CE operating conditions and requires cationic additives that can reverse or suppress the EOF. After studying several different monocationic and dicationic phosphonium RTILs as background electrolyte additives, Křížek et. al. were able to unveil an unprecedented separation of these small anionic compounds.[31] The unending combinations of cations and anions in ILs has lead to a revolution in the number and character of available buffer additives.

1.2.3 Ionic Liquids in Gas Chromatography

RTILs posses many of the qualities that must be satisfied in order to use them as gas chromatographic (GC) stationary phases. Namely, their high thermal stability, large liquid temperature range, and their ability to undergo multiple solvation interactions. Additionally, they are tunable materials that allow a large number of combinations of cations and anions to be used to produce an optimized GC stationary phase. Also, the viscosity of RTILs often allow them to be easily coated on fused silica capillary walls creating highly efficient columns. Considering that this topic is directly related to the early chapters of this dissertation, more time will be spent in this section discussing the background.

Until recently, GC was considered a mature separation technique and the production of new GC stationary phases has stagnated. The advent of RTILs as novel GC stationary phases has lead to resurgence in the development of new liquid GC media. There are three main

niches which RTIL GC stationary phases may fill. First, they may be manipulated to give identical selectivities to existing GC stationary phases, but with a much higher maximum operating temperature. Second, they may be synthesized so that they offer entirely different selectivities than existing commercial columns. Lastly, some RTILs may be used for extremely high temperature GC studies that exceed the maximum safe operating temperatures of most commercial columns.

Though the aforementioned properties of RTIL GC stationary phases are generally regarded as true, there were some exceptions to these properties in the early generations of RTIL phases. Poole et. al. attempted to use alkylammonium and alkylpyridinium nitrates and bromides.[32-33] In this initial work, they found these RTIL phases suffered from small liquid ranges as well as low plate counts. Subsequently, they examined a plethora of anions in combination with tetra-n-butyl ammonium and phosphonium cations.[34-35] Again, he found these phases to both lack efficiency and broad liquid ranges. Later, Armstrong et. al. experimented with using monocationic imidazolium based RTILs as GC stationary phases.[36] These phases provided greater efficiencies as well as liquid operating ranges.

Though the difficulties concerning efficiencies and liquid temperature ranges had been solved, another obstacle presented itself. The previously mentioned monocationic imidazolium salts possessed rather low thermal stabilites. For example, 1-butyl-3-methylimidazolium chloride degraded at temperatures above 145℃.[9] Anderson et. al. addressed this problem by testing a variety of new monocationic imidazolium RTIL GC stationary phases.[9] Also, a vast number of anions were used to examine their effect on the thermal stability. Figure 1.2 shows the thermal stability plots for some of these newly synthesized RTILs.

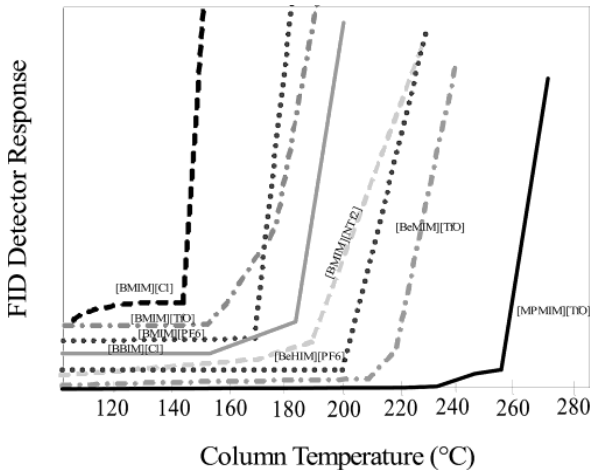


Figure 1.2 Thermal decomposition plots for various monocationic RTILs.

In the above figure, [BMIM][Cl], [BMIM][TfO], [BMIM][PF6], [BBIM][Cl], [BeHIM][PF6], [BMIM][NTf2], [BeMIM][TfO], and [MPMIM][TfO] represent the RTILs 1-butly-3-methylimidazolium chloride, 1-butly-3-methylimidazolium triflate, 1-butly-3-methylimidazolium hexaflurophosphate, 1-butly-3-butylimidazolium chloride, 1-benzyl-3-hexylimidazolium chloride, 1-butly-3-methylimidazolium NTf_2, 1-benzyl-3-methylimidazolium triflate, and 1-(4-methoxyphenol)imidazolium-3-methylimidazolium triflate, respectively.
This figure was reproduced with permission from reference 9.

As can be seen in Figure 1.2, the thermal stability of monocationic RTIL GC stationary phases was increased up to 260℃.[9] However, there already exists many commercial GC stationary phases that can also be used at these temperatures. Hence, they extended their work to the synthesis and use of novel geminal dicationic RTILs as GC stationary phases.[20] Figure 1.3 shows the structures of the newly synthesized dicationic RTILs.

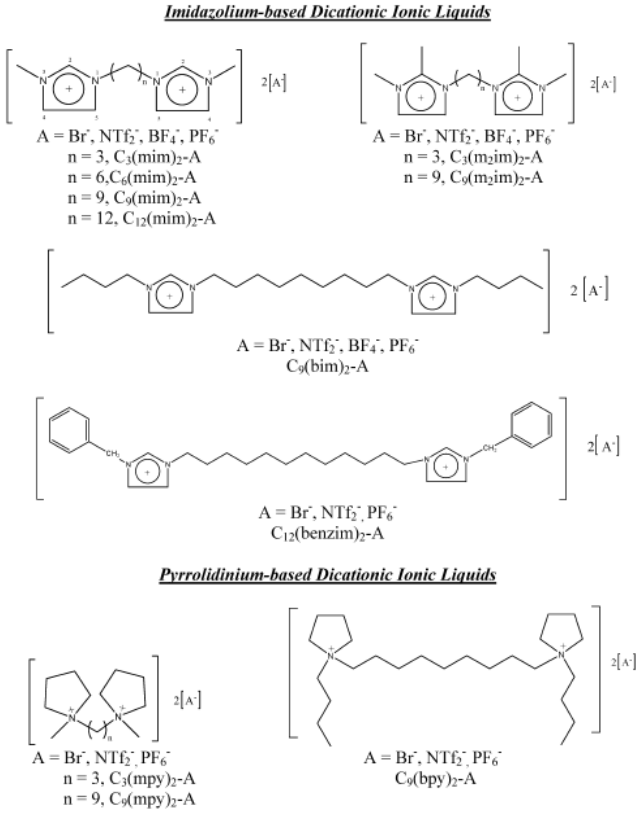


Figure 1.3 Structures of the newly synthesized dications. Reprinted with permission from reference 20.

The most important finding in studying these novel dicationic RTILs was that they had extremely high thermal stabilities. Many of them were stable to above 300℃ and one ($C_9(bpy)_2^{2+}$ $2NTf_2^-$) was stable above 400℃. The thermal stability di agrams for these dications are shown in Figure 1.4.

As can be seen by Figure 1.4, the thermal stability of the geminal dications was found to be at least twice that of the previously mentioned monocationic RTILs. In fact, the stability of some rivaled the upper operating conditions of the GC itself, as well as, the stability of the polyamide coating that is regularly found on the exterior of the fused silica capillary.

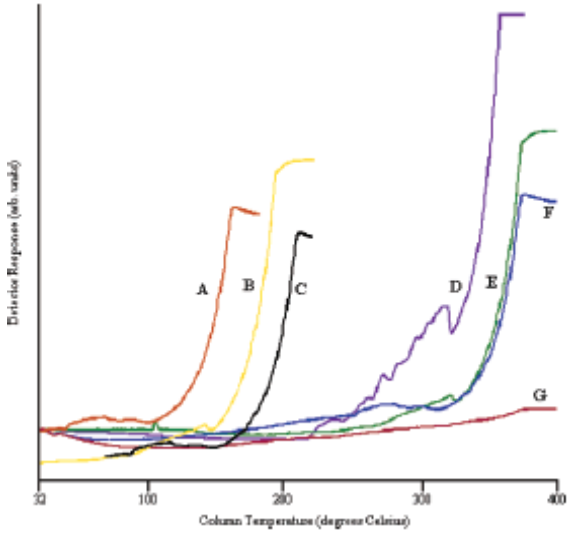


Figure 1.4 Thermal stability curves for geminal dicationic RTILs.

The plot illustrates the fact that the geminal dicationic ionic liquids (D-G) have thermal stabilities much higher than those of conventional ionic liquids (A-C). (A) 1-Butyl-3-methylimidazolium chloride (BMIM-Cl); (B) BMIM-PF6; (C) BMIM-NTf2; (D) C9(bpy)2-NTf2, **38**; (E) C9(mim)2-NTf2, **10**; (F)C12(benzim)2-NTf2, **29**; (G) C9(mpy)2-NTf2, **35**. Reprinted with permission from reference 20.

For the most part, studies using RTILs as GC stationary phases has been limited to monocationic and dicationic nitrogenous based ILs. It is well known that phosphonium RTILs are more thermally stable than nitrogen based RTILs.[35] For this reason, Chapters 2 and 3 will take a further look at the use of newly synthesized dicationic phosphonium based RTILs for GC stationary phases. Furthermore, Chapter 4 will describe the use of tricationic ILs as stationary phases for GC.

Perhaps the most interesting feature of RTIL based GC stationary phases is their dual-nature. That is to say that they are able to retain polar compounds as if they are polar phases and retain non-polar compounds as if they are non-polar phases. This is due to the fact that RTILs are able to undergo all types of solvation interactions, and as such are very unique solvents. Understanding the polarity of these solvents is of upmost interest when using them as new GC phases. Initial attempts to define the polarity of RTILs employed the use of

solvatochromic dyes.[37-38] The results of these studies indicated that all ILs have similar polarities and that they were all comparable to the polarity of propanol. Considering the vast solvating differences noted from IL to IL, it was difficult to believe that they all had similar polarity. For this reason, Armstrong et. al. used Rohrschneider-McReynolds inverse GC to further understand the solvation interactions of some monocationic RTIL stationary phases.[35-36] In this technique, five probe molecules, each with different abilities to interact with the RTIL intermolecularly, were used to examine the RTILs. Using these probe molecules, it was demonstrated that the separate interaction types could be examined and differences, in terms of solvation, between ILs could be observed.

An additional step was taken to further and more accurately elucidate the different solvation qualities of RTILs. These studies utilized a Linear Solvation Energy Relationship (LSER).[9,20,39-40] LSERs have been extensively reviewed.[41-42] LSERs are theoretically similar to the use of Rohrschneider-McReynolds inverse GC, but are far more accurate. In short, several probe molecules ($\geq$ 25) are used to interrogate the RTIL stationary phase. These probe molecules have known solute descriptors. By collecting the retention factors for all the probe molecules and using their solute descriptors, multiple linear regression analysis can deconvolute the contributions of each type of interaction to the retention mechanism of the RTIL stationary phases. Using the Abraham LSER, these interactions are broken down to five parameters: i) interactions through pi- or non-electrons; ii) dipolar interactions; iii) hydrogen bond basicity; iv) hydrogen bond acidity; v) interactions through dispersion forces.

The use of LSERs have allowed the elucidation of the individual interactions that allow RTILs to have different solvating capabilities. The use of LSERs seems to be the best way to examine new RTIL GC stationary phases and has been applied to several new RTIL phases in Chapters 2 and 4.

Lastly, it should be noted that since these RTIL GC phases have such unique solvation properties, they can often be thought of as having orthogonal selectivities when compared to

existing commercial columns. This being the case, RTIL phases may offer advantages when used in comprehensive two-dimensional GC (GC x GC), where orthogonality is the key to obtaining successful multi-dimensional chromatography.[43] For this reason, Chapter 3 examines the use of a novel phosphonium based RTIL GC column for use in GC x GC.

1.3 The Use of Ionic Liquids in Mass Spectrometry

1.3.1 The Use of Ionic Liquids in Matrix Assisted Laser Desorption Ionization Mass Spectrometry

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is a soft MS ionization technique. In simple terms, MALDI uses a laser beam to ablate and ionize a matrix which contains analytes. The ionized matrix molecules can then pass their charge on to the analytes of interest, which are in turn carried into the mass spectrometer. The success of MALDI relies heavily on the effectiveness of the matrix being used. Conventional MALDI matrices are solids. This means that the analytes that are contained in them are not distributed evenly throughout the matrix. Hence, several laser shots are required to find a region of the matrix that contains a sufficient amount of analyte molecules. This is considered the "hot-spot."

Since the ionization process occurs in vacuum, conventional molecular solvents cannot be used as MALDI matrices. However, RTILs are a new class of solvents that have negligible vapor pressure, and thus can be used as liquid matrices in MALDI. The main advantage of using a liquid matrix, over a traditional solid matrix, is that the analytes will be dispersed homogenously throughout the matrix, making every spot within the liquid matrix a "hot-spot." Such an experiment was first demonstrated in 2001 by Armstrong et. al.[44] In this work it was determined that the RTIL matrices had excellent solubilizing properties and great vacuum stability. Also, it was observed that the ability for the IL matrix to aid in the ionization of the analytes varied greatly from IL to IL. For this reason, Crank et. al. studied over 100 new IL matrices in search of a more universal matrix.[45] They found new IL matrices that had a wide

mass detection range (1000Da to 270,000Da) for proteins and peptides with greater S/N ratios than solid matrices.

1.3.2 The Use of Ionic Liquids in Electrospray Ionization Mass Spectrometry

To fully understand the application of RTIL based salts in Electrospray Ionization Mass Spectrometry (ESI-MS) it is first necessary to briefly review the working fundamentals of this ionization technique. Also, since a major portion of this dissertation is related to this topic, extra time will be spent reviewing this technique.

ESI-MS is a soft ionization source that gained great popularity and wide acceptability for its ability to charge biomolecules. It is also a choice method of ionization when interfacing to High Performance Liquid Chromatography (HPLC). Figure 1.5 shows a simple diagram that will be used to help explain the ionization process in ESI.

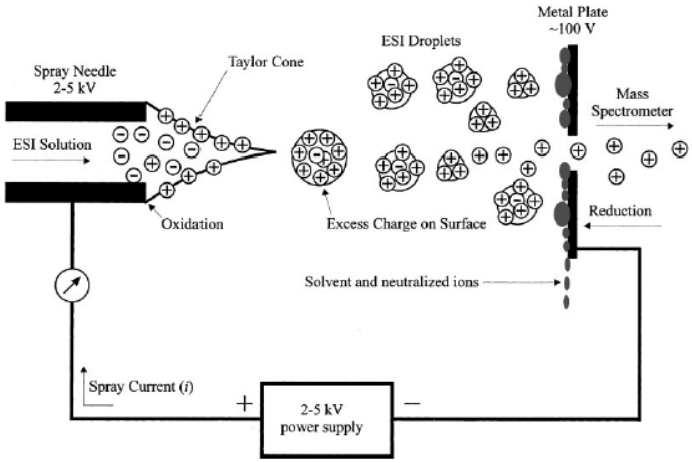


Figure 1.5 General schematic for the ESI process in positive ion mode. Reprinted with permission from reference 46.

During the ESI process, solutions containing dissolved analytes are pumped through a spray needle. A large voltage is applied to this needle (2-5 kV in the positive ion mode). In the positive ion mode, this high voltage causes oxidation to occur at the tip of the spray needle. This in turn, causes anionic species to be attracted to the spray needle and cationic species to

be repelled from the spray needle. When this occurs, a Taylor cone will form which contains excess positive charge on its surface. When the Coulombic repulsion of like charges at the surface of the Taylor cone exceeds the Rayleigh limit of the spray solution, droplets with excess positive charge are ejected from the cone.

The means by which these droplets containing excess charge are desolvated to form naked ions is a matter of debate. There are two generally accepted models that are used to describe this process.[47-48] The ion evaporation model suggests that as the droplets are dried (usually with the aid of a nebulization gas such as N_2) the Rayleigh limit of the droplet is exceed and smaller droplets are ejected. This process then continues until naked ions are formed. In contrast, the charged residue model suggests that as the droplets dry and shrink, the Coulombic repulsion of the ions becomes great enough to cause a Coulombic explosion, from which naked ions are projected. In either case, desolvation of ions is of the upmost importance to the ESI process. The ability for an ion to become desolvated is typically referred to as its ionization efficiency. The ionization efficiency is directly related to the ions surface activity within the droplet.

The formation of ions can take place by one or more of four different mechanisms.[46] First, the analytes may already be in their ionic state in solution. Second, the analytes may form adducts with ionic species. Third, gas phase reactions can occur during the ESI process. Lastly, electrochemical oxidation or reduction can take place to form ions.

Negative ion mode ESI-MS works identically to the previously outlined scenario for the positive ion mode. The only exception is that the polarity of the ionization source is changed so that a large negative voltage is applied to the spray needle, creating a reductive process. For reasons that will be discussed later, the negative ion mode is generally less sensitive and less stable than operation in the positive ion mode.

In addition to understanding ESI-MS basics, it is also important to briefly review the analysis of anions so that the use of IL based salts in ESI can be described. The analysis of

anions is important to many fields of chemistry. The separation of anions is typically achieved using Ion Chromatography (IC). This technique has been extensively reviewed.[49] Reversed phase HPLC can also be used in some cases where the anions of interest are organic slats that have some hydrophobic character. Detection of the anionic species is routinely performed via conductivity or ion-selective electrodes.[49-54] One major drawback of these detection techniques is that they are either universal and lack specificity or they are highly specific and thus are used for only a single species.

The advent of ESI-MS has allowed it to become a popular choice for the analysis of ions. MS is both a universal detection technique and a specific technique. That is to say that it can detect all ions as well as provide structural information regarding the analytes. Additionally, anions are inherently negatively charged in solution, so it would bare to reason that negative ion mode ESI-MS would be an excellent choice for the analysis of anions. However, as alluded to previously, the negative ion mode does have some drawbacks that hinder its use for sensitive detection of anions. The major problem associated with the negative ion mode is the prevalence of corona discharge. The frequency of corona discharge in the negative ion mode is a result of the large negative voltage applied which causes the ionization of gas molecules surrounding the spray needle. This plasma region (corona discharge) can induce arcing to occur. The net result is an unstable Taylor cone and decreased sensitivity. Yamashita and Fenn actually noted this in their seminal paper concerning the use of ESI in the negative ion mode.[55] Corona discharge can be controlled to an extent with the addition of halogenated solvents or electron scavenging gases.[56-57] However, a sensitive method for the detection of anions in ESI which used more typical reversed phase HPLC-ESI solvents (i.e. water, methanol, acetonitrile) was still desired.

Recently, a new technique has been developed for anion analysis that circumvents the corona discharge related problems and the accompanying poor sensitivities associated with the negative ion mode. This technique employs the post column addition of IL based dicationic ion-

pairing reagents, which are allowed to bind singly charged anions in solution, such that a cationic complex consisting of the ion-pairing reagent and the anion can be detected in the positive ion mode. The following relationship describes this process:

$$D^{2+} + A^{-} \rightarrow DA^{+}$$

Here, D^{2+} represents the dicationic ion-pairing reagent and A^{-} represents the anion of interest.

The seminal work introducing this technique was narrowly focused toward the use in the detection and quantitation of perchlorate and iodide. Kirk et. al. were the first to account for the use of this methodology in a study that examined trace levels of perchlorate and iodide in dairy and breast milk.[58] Shortly after this account, Martinelango and co-workers provided the first in depth look at the use of IL based dicationic ion-paring reagents for the detection of perchlorate in the positive ion mode.[59] Figure 1.6 shows a schematic for the complexation of one dicationic ion-pairing reagent and perchlorate.

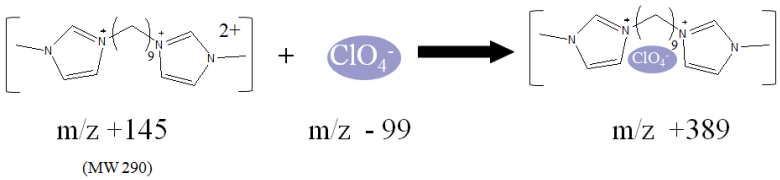


Figure 1.6 Schematic showing the pairing of a dicationic ion-pairing reagent with perchlorate.

Using the ion-pairing technique as show in Figure 1.6, the Limit of Detection (LOD) for perchlorate was determined to be 25 ng/L. This sensitivity was unprecedented and was subsequently used for the detection and quantitation of perchlorate in milk, urine, and seaweed.[60-62]

Soukup-Hein et. al. extended the use of this technique for the detection of a plethora of anions in the positive ion mode.[63] The dicationic IL based salt used in this work contained two

methylimidazolium cationic moieties bound together by a nonyl linkage chain. The LODs that were determined in this study are listed in Table 1.1.

Table 1.1 LOD for a plethora of monovalent anions using the ion-paring technique.

anions	SIM mass	SIM LOD (ng)	SRM mass	SRM LOD (ng)
perfluorooctanate (PFOA)	703	1.22×10^{-4}	289	7.32×10^{-5}
nitrate (NO_3^-)	352	1.84×10^{-3}	289	1.38×10^{-3}
tetrafluoroborate (BF_4^-)	376	1.96×10^{-3}	289	3.90×10^{-1}
thiocyanate (SCN^-)	348	2.00×10^{-3}	289	2.00×10^{-3}
benzenesuflonate (BZSN)	447	2.06×10^{-3}	289	4.12×10^{-4}
trifluoromethanesulfonimide (NTF_2^-)	570	2.26×10^{-3}	289	2.26×10^{-3}
hexafluorophosphate (PF_6^-)	435	4.28×10^{-3}	289	2.14×10^{-3}
iodide (I^-)	417	6.00×10^{-3}	289	2.00×10^{-1}
perchlorate(ClO_4^-)	389	1.02×10^{-2}	289	1.02×10^{-2}
dichloroacetate (DCA)	417, 419	1.50×10^{-2}	289	2.00×10^{-2}
monochloroacetate (MCA)	383, 385	1.50×10^{-2}	289	1.90×10^{0}
bromochloroacetate (BCA)	461, 463	1.54×10^{-2}	289	1.54×10^{-2}
periodate (IO_4^-)	481	4.48×10^{-2}	289	1.12×10^{0}
bromate (BrO_3^-)	417, 419	5.00×10^{-2}	289	5.00×10^{-2}
iodate (IO_3^-)	465	6.00×10^{-2}	289	1.39×10^{-2}
bromide (Br^-)	369, 371	6.00×10^{-2}	289	6.00×10^{-2}
bromooctanoate (BOA)	511, 513	6.00×10^{-2}	289	6.00×10^{-2}
trifluoromethanesulfonate (TFO^-)	439	1.98×10^{-1}	207	1.98×10^{-3}
trifluoroacetate (TFA)	403	2.00×10^{-1}	289	2.00×10^{-1}
malate	423	2.12×10^{-1}	289	6.36×10^{-2}
monobromoacetate (MBA)	427, 429	2.22×10^{-1}	289	1.11×10^{-2}
benzoate	411	3.88×10^{-1}	289	9.72×10^{-1}
monomethylarsonate (MMA^v)	429	6.00×10^{-1}	289	4.02×10^{-2}
nitrite (NO_2^-)	336	6.24×10^{-1}	289	2.10×10^{-1}
permanganate (MnO_4^-)	409	6.84×10^{-1}	N/A	N/A
arsenate ($H_2AsO_4^-$)	431	1.00×10^{0}	289	4.12×10^{-2}
chloride (Cl^-)	325	1.77×10^{0}	289	1.77×10^{0}
formate ($HCOO^-$)	335	4.40×10^{0}	289	2.20×10^{0}
dimethylarsinate (DMA^v)	427	5.56×10^{0}	289	1.00×10^{2}
trichloroacetate (TCA)	452	6.42×10^{0}	289	1.96×10^{0}
cyanate (OCN^-)	332	6.42×10^{1}	289	1.93×10^{1}
arsenite ($H_2AsO_3^-$)	415	1.00×10^{2}	289	2.02×10^{1}
acetate (CH_3COO^-)	349	$>2.00 \times 10^{3}$	289	$>2.00 \times 10^{3}$
cyanide (CN^-)	316	$>2.00 \times 10^{3}$	289	$>2.00 \times 10^{3}$

Reprinted with permission from reference 63.

As can be seen from Table 1.1, the LODs that were found using this IL as an ion-pairing reagent were quite low. In fact, a comparison of these LODs to the LODs found using other analytical techniques was made. It was determined that the many of the LODs reported in Table 1.1 were lower than any other reported analytical technique.

Also listed in Table 1.1 are the LODs for each anion found using Selected Reaction Monitoring (SRM). Using the SRM technique requires the use of a trapping type mass spectrometer. The procedure for doing such an experiment if as follows: i) the mass-to-charge (m/z) of the IL based dicationic ion-pairing reagent-anion complex is isolated within the ion trap;

ii) the complex is excited and undergoes collision induced dissociation (CID); iii) a fragment of the complex is monitored. When doing such an experiment, the actual detected ion is not the anion, but rather is some remnant or portion of the dication-anion complex. As can be seen by Table 1.1, SRM experimentation was used to dramatically lower the LODs for the given anions. Figure 1.7 shows the typical fragmentation patterns for the SRM dissociations.

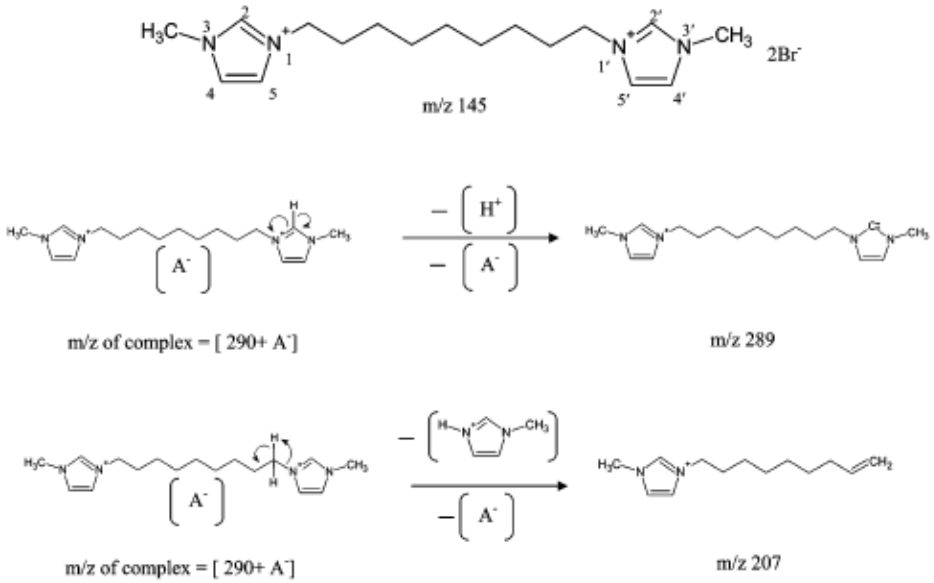


Figure 1.7 Representation of the typical fragmentation patterns observed in SRM. Reprinted with permission from reference 63.

In addition to the ultra-high sensitivity observed in this study, several other key advantages of using ion-pairing reagents for the detection of anions in the positive ion mode have been outlined. First, only a small amount of ion-pairing reagent is required for the analyses. Second, the dicationic ion-pairing reagent can be selected to bring the anion to a higher m/z range where there is inherently less chemical noise. Lastly, the complexation with the dications will allow some low mass anions which fall below the low mass cut-off of the MS to be detected.

Finally, the ease of use, ultra-high sensitivity, and applicability towards coupling this method with HPLC experiments has been examined.[63] Figure 1.8 shows the results of such an experiment. When comparing the chromatograms in Figure 1.8, it is easy to see the increase in sensitivity when using the ion-pairing reagent as a post-column additive for the detection of anions in the positive ion mode. For example, thiocyanate was detected with a signal-to-noise ratio (S/N) of 138 in the positive ion mode, while it is barley observable in the negative ion mode, where ten times more analyte was injected.

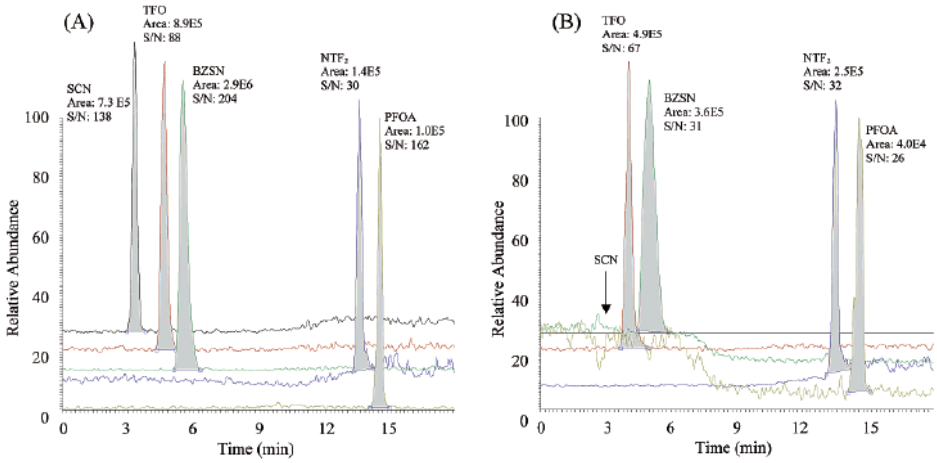


Figure 1.8 A comparison of the chromatographic separation and sensitivity of five anions in the (A) positive ion mode using ion-pairing and (B) using the negative ion mode.

The mass injected in B is 10x that of A for SCN, TFO, and BZSN; 5x for PFOA; and the same for NTF2. The mass injected in A is 1.43 ng SCN, 9.92 ng TFO, 1.16 ng BZSN, 0.68 ng NTF2, and 1.30 ng PFOA. The Cylodbond I column was equilibrated for 15 min with 100% water with a linear gradient to 100% MeOH beginning at 3 min and complete at 9 min. The flow rate was 300 µL/min. In A, the dicationic salt solution (40 µM in MeOH) was added postcolumn at 100 µL/min whereas in B it is methanol only. SCN, thiocyanate; TFO, triflate; BZSN, benzenesulfonate; PFOA, perfluorooctanoic acid; NTF2, trifluoromethanesulfonimide.

The later chapters of this dissertation describe work that was done to extend the use of IL based salts as ion-pairing reagents. The use of tricationic and tetracationic ion-pairing

reagents for the detection of multi-valent anions will be described. The final chapter will take an in-depth look at the mechanism by which these ion-paring reagents are able to produce such low LODs for anions.

1.4 Organization of Dissertation

The first portion (Chapters 2-4) of this dissertation will be related to the use of RTILs as GC stationary phases. Specifically, Chapter 2 examines the use of newly synthesized monocationic and dicationic phosphonium based RTILs as GC phases. A LSER is used to describe the solvation properties of these novel salts. Chapter 3 will investigate the use of a new phosphonium RTIL GC column as a primary column for comprehensive two-dimensional GC (GC x GC). Chapter 4 further extends the use of RTILs as GC stationary phases by examining the first tricationic IL based GC phases. Again, a LSER will be used to understand the solvating properties of these new ILs. The new tricationic phases were used to separate various complex mixtures.

The later portions of this dissertation (Chapters 5-11) will concern the use of IL based salts as ion-pairing reagents for the detection of anions in the positive ion mode. Chapter 5 will investigate the use of a plethora of dicationic ion-paring agents to find the optimum structural make-up required for a good pairing agent. Chapter 6 will describe the first use of tricationic ion-paring reagents for the detection of some di-valent anions. Chapter 7 will discuss new tricationic pairing agents that yield higher sensitivity than those reported in Chapter 6. Chapter 8 will evaluate the best tricationic ion-pairing regents discovered in Chapters 6 and 7 for efficacy in pairing with a plethora of di-valent anions. In Chapter 9, the dicationic ion-pairing technique will be coupled with CE. Chapter 10 further extends the work on ion-paring reagents to the use of newly synthesized tetracationic IL based salts for the detection of tri-valent anions. The penultimate chapter (Chapter 11) describes the mechanism by which these ion-paring reagents are able to produce such low LODs for anions. Lastly, a general summary will be presented in Chapter 12.

CHAPTER 2

CHARACTERIZATION OF PHOSPHONIUM IONIC LIQUIDS THROUGH A LINEAR SOLVATION ENERGY RELATIONSHIP AND THEIR USE AS GLC STATIONARY PHASES

2.1 Abstract

In recent years, room temperature ionic liquids (RTILs) have proven to be of great interest to analytical chemists. One important development is the use of RTILs as highly thermally stable GLC stationary phases. To date, nearly all of the RTIL stationary phases have been nitrogen-based (ammonium, pyrrolidinium, imidazolium, etc.). In this work, eight new mono-cationic and three new di-cationic phosphonium based RTILs are used as gas-liquid chromatography (GLC) stationary phases. Inverse gas chromatography (GC) analyses are used to study the solvation properties of the phosphonium RTILs through a linear solvation energy model. This model describes the multiple solvation interactions that the phosphonium RTILs can undergo and is useful in understanding their properties. In addition, the phosphonium based stationary phases are used to separate complex analyte mixtures by GLC. Results show that the small differences in the solvent properties of the phosphonium ILs compared to ammonium based ILs will allow for different and unique separation selectivities. Also, the phosphonium based stationary phases tend to be more thermally stable than nitrogen-based ILs, which is an advantage in many GC applications.

2.2 Introduction

Recently, the interest in ionic liquids (ILs) has extended to many areas of chemistry.[3,9,20,26,36,44,64-81] ILs are salts that have melting points below 100°C and room temperature (RTILs) are those salts that exist as liquids at ambient temperatures. RTILs are essentially an entirely unique class of solvents consisting only of ions, making them very different from conventional molecular solvents. RTILs often possess properties such as:

negligible vapor pressures, high thermal stabilities, tunable viscosities, and both hydrophobic or hydrophilic natures. These reasons, along with the fact that RTILs can undergo multiple solvation interactions[9,20,79-81], have placed RTILs at the forefront of many research programs.

Uses for ILs have spaned a wide range of applications. They have been used as alternative solvents for many synthetic and catalytic reactions[3,64-72], as well as, solvents for liquid extractions.[26,73-74] ILs also can be used as MALDI-MS matrices,[44,75-76] surfactants[77], and as stable, high temperature gas-liquid chromatographic (GLC) stationary phases.[9,36,78-80] Also, since there is a wide range of cation and anion combinations to choose from, chemists are able to select or "fine tune" these solvents for a variety of specific uses.

Although there are published synthetic procedures for over 200 different ILs[82-84], there has been an overwhelming preponderance of papers and an apparent preference to produce and study ILs that have nitrogen based cations. In contrast, the amount of research that has been conducted on phosphonium or sulphonium ILs is comparatively minimal.[35,85-92] In part, this may be the result of an abundance of commercially available amines compared to the small number of commercially available phosphines. This is unfortunate since phosphonium ILs are known to posses some rather interesting, and often advantageous properties. When compared to the popular imidazolium-based ILs, phosphonium cations lack an acidic proton which can lead to carbene formation. This has obvious advantages when employing an IL solvent in organic syntheses. Interestingly, phosphonium ILs often are less dense than water, whereas, nitrogen based ILs are usually more dense than water.[91-92] In liquid-liquid extractions, this allows researchers to be able to choose whether the IL phase is the upper or lower phase. Lastly, it is well known that most phosphonium ILs are more thermally stable than many nitrogen based ILs.[35] This is anticipated to be a major advantage when using phosphonium ILs as GLC stationary phases.

Given the known properties of phosphonium ILs, it is surprising that they haven't been more extensively characterized as GC stationary phases. Also, there still seems to be a

significant amount of information that can be uncovered by studying new variations of these ILs. For example, before phosphonium ILs can start to replace nitrogen based ILs in some of the former applications, researchers will first need to know the exact solvation properties of these new materials. Only after effectively characterizing phosphonium ILs will their full potential be realized and understood.

Accurately characterizing the solvent properties of liquids has been an aim of chemists for more than 40 years.[93-94] One of the earlier approaches for classifying solvents, involved the use of a polarity scale based on solvatochromatic dyes, such as, Nile Red or Reichardt's dye.[37-38,94-95] This approach simply measured the shift in absorption maxima for the dyes in different solvents and used this empirical data to form a relative ranking of polarity. However, when this type of approach was used to characterize ILs, they all appeared to have the same or similar polarities, i.e., that of short chain alcohols.[38] It was postulated that these empirical results were either incorrect or misleading as far as ILs were concerned since different ILs produced very different products (when used as reaction solvents) and different partitioning behavior (when used as extraction solvents).[9,20,79-80] Consequently, a different approach was needed to characterize the solvation properties of ILs. This approach would have to account for the multiple solvent-solute interactions possible with ILs.[79]

The Rohrschneider-McReynolds GLC process has been used to examine and characterize GLC stationary phases.[96] This method uses just five probe analytes to interrogate the IL and discriminate five different types of solvation interactions that the ILs can undergo. Although, this method divided the interactions parameters into different groups, its ability to accurately define each parameter suffers from its small number of probe analytes.

More recently, the method of choice for the characterization for IL solvation interactions has been an inverse GLC approach using the Abraham linear solvation energy relationship, which is described by the following equation.[9,20,41-42,79-80]

$$\log k = c + eE + sS + aA + bB + lL$$

Here, E, S, A, B, and L are solute descriptors (Table 2.1 gives these known values for the solutes used in this analysis) that represent the excess molar refraction, dipolarity, H-bond acidity, H-bond basicity, and gas-hexadecane partition coefficient, respectively. Whereas, e, s, a, b, and l are a measure of the ability for the solvent to interact with the solute through pi/non-bonding electrons, dipole-dipole interactions, H-bond basicity, H-bond acidity, or dispersion forces, respectively. When using an inverse GLC method of analysis, the solvent refers to the IL stationary phase, which is interrogated with a large number of diverse solutes. By experimentally measuring the retention factor (k) for each probe solute and subjecting the resulting data set and solute descriptors to multiple linear regression analysis (MLRA), the solvent descriptors can be found and the multiple solvation interactions of the IL are easily determined.

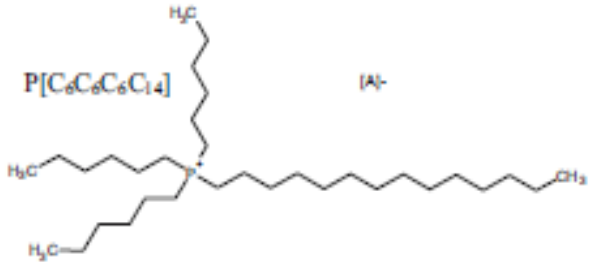
In this analysis, we present a comprehensive study of the solvation properties for eight mono-cationic and three newly synthesized di-cationic phosphonium based RTILs. Their physiochemical properties and thermal stabilities are examined as well. The phosphonium ILs will be compared to analogous imidazolium-based ILs, and in the case of GC applications, to conventional commercial stationary phases. It is hoped that these studies will reveal the potential of phosphonium-based ILs for a broad range of applications.

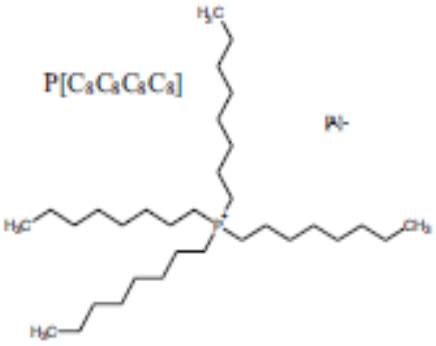
2.3 Experimental

2.3.1 Materials

Figure 2.1 gives the structures of all the phosphonium-based ILs used in this study. Mono-cationic RTILs **A1**, **A2**, **A5**, **B1**, and **C1** were graciously donated by CYTEC (West Paterson, NJ, USA). All commercial RTILs were dried in vacuum over phosphorous pentoxide before any use. Silver triflate, lithium bis(trifluoromethane)sulfonamide, saccharin sodium salt, tripropylphosphine, 1,10-dibromodecane, 1,12-dibromododecane, phosphorous tribromide, tetra(ethylene glycol), and all probe analytes (listed in Table 2.1) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). All were of reagent grade and were used without further

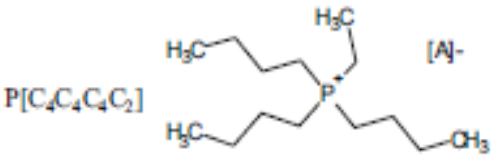
Mono-cationic Phosphonium Ionic Liquids





A1 = $[A]^-$ = $[Cl]^-$ **A4** = $[A]^-$ = $[NTf_2]^-$
A2 = $[A]^-$ = $[BF_4]^-$ **A5** = $[A]^-$ = $[NCNCN]^-$
A3 = $[A]^-$ = $[TfO]^-$ **A6** = $[A]^-$ = $[SAC]^-$

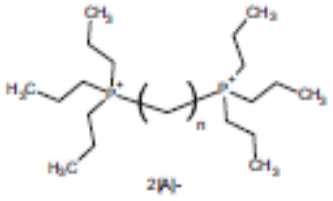
B1 = $[A]^-$ = $[NTf_2]^-$



C1 = $[A]^-$ = $[DEP]^-$

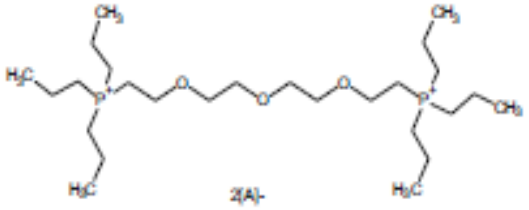
Di-cationic Phosphonium Ionic Liquids

$C_n(P[C_3C_3C_3])_2$



D1 = n = 10 ; $[A]^-$ = $[NTf_2]^-$
D2 = n = 12 ; $[A]^-$ = $[NTf_2]^-$

$PEG3(P[C_3C_3C_3])_2$



E1 = $[A]^-$ = $[NTf_2]^-$

Figure 2.1 Structures of new Phosphonium ILs

Table 2.1 List of all the probe analytes and their solute descriptors

Probe analytes	*E*	*S*	*A*	*B*	*L*
1,2-Dichlorobenzene	0.872	0.78	0	0.04	4.518
Phenol	0.805	0.89	0.6	0.31	3.766
Octylaldehyde	0.16	0.65	0	0.45	4.36
Valeradehyde	0.163	0.65	0	0.45	2.851
o-Xylene	0.663	0.56	0	0.16	3.939
p-Xylene	0.613	0.52	0	0.16	3.839
m-Xylene	0.623	0.52	0	0.16	3.839
Cyclohexanol	0.46	0.54	0.32	0.57	3.758
Nitrobenzene	0.871	1.11	0	0.28	4.511
N,N-Dimethylformamide	0.367	1.31	0	0.74	3.173
2-Pentanone	0.143	0.68	0	0.51	2.755
1-Nitropropane	0.242	0.95	0	0.31	2.894
Toluene	0.601	0.52	0	0.14	3.325
Benzaldehyde	0.82	1	0	0.39	4.008
Pyridine	0.794	0.87	0	0.62	3.003
Aniline	0.955	0.96	0.26	0.53	3.993
Butanol	0.224	0.42	0.37	0.48	2.601
Acetic acid	0.265	0.65	0.61	0.44	1.75
1-Octanol	0.199	0.42	0.37	0.48	4.619
Acetophenone	0.818	1.01	0	0.49	4.501
2-Choloraniline	1.033	0.92	0.25	0.31	4.674
Methyl caproate	0.08	0.6	0	0.45	3.874
Benzene	0.61	0.52	0	0.14	2.786
1-Hexyne	0.166	0.23	0.13	0.1	2.51
Pyrrole	0.613	0.73	0.41	0.29	2.865
2-Propanol	0.212	0.36	0.33	0.56	1.764
Benzonitrile	0.742	1.11	0	0.33	4.039
Propionitrile	0.162	0.9	0.02	0.36	2.082
1-Chlorohexane	0.201	0.4	0	0.1	3.777
Ethyl acetate	0.106	0.62	0	0.45	2.314
p-Cresol	0.82	0.87	0.57	0.31	4.312
Ethylphenyl ether	0.681	0.7	0	0.32	4.242
Naphthalene	1.34	0.92	0	0.2	5.161
Dioxane	0.329	0.75	0	0.64	2.892
Cyclohexanone	0.403	0.86	0	0.56	3.792

purification. The polysiloxane column (RTX-5, 5% phenyl methyl polysiloxane) was purchased from Restek (Columbia, MD, USA). The INNOWAX column (polyethylene glycol) was purchased from Agilent Technologies (Santa Clara, CA, USA).

2.3.2 Synthesis of IL A3

Compound **A3** was obtained through a metathesis reaction between IL **A1** and silver triflate (TfO). Specifically, 1 molar equivalent of IL **A1** was dissolved in acetonitrile, charged with 1 molar equivalent of silver TfO, and stirred overnight. Then, the silver chloride was filtered

from the solution and the solvent was removed by roto-evaporation. Vacuum drying over phosphorous pentoxide resulted in the pure RTIL **A3** (yield >95%).

2.3.3 Synthesis of ILs A4 and A6

Compounds **A4** and **A6** were obtained by performing a metathesis reaction between IL **A1** and lithium bis(trifluoromethane)sulfonamide (NTf_2) or saccharin (SAC) sodium salt. In short, 1 molar equivalent of IL **A1** was dissolved in water. To this solution, 1.2 molar equivalents of lithium NTf_2 or SAC sodium salt was added and the resulting mixture was stirred overnight. After this time, dichloromethane was added to the solution to dissolve the NTf_2 or SAC salt that had phase separated from the water. The lithium or sodium chloride and excess lithium NTf_2 or SAC sodium salt was removed from the dichloromethane phase with successive extractions with water. Removal of dichloromethane through roto-evaporation followed by vacuum drying over phosphorous pentoxide resulted in the pure RTILs **A4** and **A6** (yield >95%).

2.3.4 Synthesis of ILs D1 and D2

Compounds **D1** and **D2**, (1,10-di(tripropyl-phosphonium)decane bis(trifluoromethane)sulfonamide and 1,12-di(tripropyl-phosphonium)dodecane bis(trifluoromethane)sulfonamide, respectively), were synthesized in an identical manner. In a round bottom flask equipped with condenser, 1 molar equivalent (2.00g) of the appropriate dibromo-alkane was dissolved in isopropyl alcohol (50mL). To this solution, 2.5 molar equivalents (**D1**:2.67g, **D2**:2.44g) of tripropylphosphine was added. The resulting mixture was stirred and heated to reflux for 48 hrs.

After this time, the reaction was cooled to room temperature and the solvent was removed by roto-evaporation. The crude product was then dissolved in deionized water (50mL) and washed several times with ethyl acetate (6 x 75mL), allowing for any residual starting material to be removed. Removal of water by roto-evaporation, followed by overnight drying in vacuum over phosphorous pentoxide, yielded pure di-phosphonium bromide salts (yield > 95%).

Next, through a metathesis reaction of the bromide salts and lithium NTf_2^-, compounds **D1** and **D2** can easily be prepared. Specifically, 1 molar equivalent (1.00g) of the bromide salt was dissolved in water (50 mL) and treated with 2.2 molar equivalents (**D1**:1.02g, **D2**:0.97g) of the lithium NTf_2^-. The resulting solution was stirred at room temperature for 24 hrs. After this time, dichloromethane (75 mL) was added to the solution to dissolve the NTf_2^- salt that had phase separated from the water. The lithium chloride and excess lithium NTf_2^- was removed from the dichloromethane phase with successive extractions with water (5 x 75 mL). Removal of dichloromethane through roto-evaporation followed by vacuum drying over phosphorous pentoxide resulted in the pure RTILs **D1** and **D2** (yield >85%).

2.3.5 Synthesis of IL E1

The synthesis of compound **E1** (1,11-di(tripropyl-phosphonium)- 3,6,9-trioxaundecane bis(trifluoromethane)sulfonamide) first required the preparation of a di-bromopolyethylene glycol. The procedure for this has been previously described by Jin et.al.[97] In short tetra(ethylene glycol) was dissolved in ether, cooled in an ice bath, and reacted with 1.1 molar equivalents of phosphorus tribromide. The reaction was then refluxed for 2 hrs. Next, the reaction mixture was poured over ice to react any excess PBr_3. Then the aqueous layer was discarded and the organic layer was washed four times with an aqueous sodium bicarbonate solution. The organic layer was then dried with sodium sulfate and filtered. Next, ether was removed by roto-evaporation and the resulting di-bromopolyethylene glycol was placed under vacuum over night to ensure complete dryness.

The corresponding bromide salt for compound **E1** can then be obtained by reacting 1 molar equivalent (2.00g) of the di-bromopolyethylene glycol with 2.5 molar equivalents (2.50g) of tripropylphosphine in an exact manner as outlined for the synthesis of compounds **D1** and **D2.** Product purification and recovery was also performed in an identical manner as outlined for compounds **D1** and **D2.**

2.3.6 Preparation of IL columns

All IL columns was prepared by the static coating method at 40℃ using a .25% (w/v) coating solution of the IL in dichloromethane. The untreated fused silica capillary (5 m x .25 mm for solvation studies and 30 m x .25 mm for separation studies) used for this coating was obtained from Supelco (Belefonta, PA, USA). Before the static coating was performed, the capillary was pretreated with sodium chloride.[36,98]

Following the coating process, the columns were dried with helium flow while being conditioned to 120℃ overnight. After this time, the efficiency of the column was tested using naphthalene at 100℃. The resulting columns had efficiencies ranging from 3200 to 3800 plates per meter with a .15μm film thickness.

Preparation of the imidazolium based $(MIM)_2PEG_3$-$2NTf_2$ IL column is described elsewhere.[40]

2.3.7 Method of analysis

The kinematic viscosity measurements were made in triplicate using a Cannon-Manning semi-micro viscometer at 30℃. The density measurements were performed with a 10 mL Kimble specific gravity pycnometer at 22℃. Hexane was used an immiscible solvent for these measurements. To obtain the refractive indices, a Bausch & Lomb Abbe-3L refractometer was used at 22℃. Melting point ranges determinations were made using a freezer (-22℃) and a dry ice/acetone bath (-76℃). Lastly, the thermal stability tests were performed on the phosphonium IL columns by ramping the oven temperature from 70-450℃ by 3℃/min. For these analyses, a 1 mL/min He flow was used and the injector and FID temperatures were set to 250℃ and 350℃, respectively. The rise in baseline for the obtained chromatograms represented the volatilization/thermal decomposition of the stationary phase.

For the inverse GC analyses, all probe molecules were dissolved in dichloromethane and were individually gas chromatographed in triplicate, at three different temperatures (50, 70, and 100℃). In between each probe analyte injections at 100℃, the efficiency of the IL

stationary phase was tested with naphthalene to ensure that the integrity of the IL was not compromised after the solvation of a given solute.

All GLC measurements were made using an Agilent 6890N GC, equipped with a flame ionization detector (FID), and computer interfaced with Agilent ChemStation Rev.B.01.03.[204] data analysis software. The injector and detector temperatures were 250℃, the split ratio was 100:1, and the helium mobile phase flow rate was 1.0 mL/min. The retention factors for the probe molecules and their solute descriptors were subjected to MLRA using the statistical computer program Analyse-it® (England, UK).

Separation conditions for the separation of flavors and fragrances, alcohols and alkanes, and Grob compounds were: 40℃ for 3 min, 10℃/min to 150℃; 30℃ for 3 min, 10℃/min to 160℃; and 40-190℃, 6℃/min, respectiv ely. All using 1 mL/min He carrier gas and FID detection.

2.4 Results and Discussion

2.4.1 Physiochemical properties and thermal stabilities

Table 2.2 lists physiochemical properties for the 11 phosphonium based RTILs used in this study, along with the three bromide salt intermediates used in the synthesis of the di-cationic phosphonium ILs. Also, Table 2.2 provides (for comparative purposes) the physiochemical properties of some analogous imidazolium based ILs.[20,73,99]

When comparing the refractive indices of phosphonium ILs **A1, A2, A3, A4,** and **D2** to their analogous imidazolium ILs, it was found that the phosphonium based ILs have a slightly greater refractive index by .02 to .03. This finding indicates that the phosphonium cation plays a role in the refractive index of an IL. The greatest refractive index was found for the phosphonium SAC IL **A6** (1.5047), proving that the anion also has a great effect on the refractive index and that aromatic anions will increase this value.

Comparing the viscosities of the phosphonium ILs to the imidazolium based ILs revealed that phosphonium based ILs are anywhere from three to eight times more viscous,

Table 2.2 Physiochemical properties for the ILs in this study, plus the bromide intermediates for the newly synthesized phosphonium dications, and data for some selected imidazolium ILs

Ionic liquids[a]		Refractive	Viscosity	Density	Solid/liquid transformation	Approximate thermal stability
		Index[b]	(cSt)[c]	(g cm^{-3})[d]	(°C)[e]	(°C)
Monocations						
A1	$P[C_6C_6C_6C_{14}]$ Cl	1.4841	2,077.91	0.895	>−76, <−22	335
	BMIM Cl[f]	Solid	Solid	1.100	65	145
A2	$P[C_6C_6C_6C_{14}]$ BF_4	1.4564	1,021.30	0.941	23	370
	BMIM BF_4[f]	1.429	199.15[g]	1.170	−82	290
A3	$P[C_6C_6C_6C_{14}]$ TfO	1.4585	561.85	0.994	>−76, <−22	405
	BMIM TfO[f]	1.438	69.77[g]	1.290	16	340
A4	$P[C_6C_6C_6C_{14}]$ NTf_2	1.4507	218.60	1.065	>−76, <−22	380
	BMIM NT_2[f]	1.427	36.36[g]	1.430	−4	185
A5	$P[C_6C_6C_6C_{14}]$ NCNCN	1.4840	268.63	0.903	>−76, <−22	360
A6	$P[C_6C_6C_6C_{14}]$ SAC	1.5047	585.32	0.980	>−76, <−22	365
B1	$P[C_8C_8C_8C_8]$ NTf_2	1.4511	185.11	1.067	>−76, <−22	385
C1	$P[C_4C_4C_4C_2]$ DEP	1.4698	360.60	1.013	>−76, <−22	350
Dications						
D1	$C_{10}(P[C_3C_3C_3])_2$ NTf_2	Solid	Solid	1.293	50	425
D2	$C_{12}(P[C_3C_3C_3])_2$ NTf_2	1.4514	1,265.83	1.267	25	425
	$C_{12}(MIM)_2NTf_2$[f]	1.443	432.86[g]	1.400	−26	350[h]
E1	$PEG_3(P[C_3C_3C_3])_2$ NTf_2	1.4469	460.85	1.331	26	410
D1 Br[j]	$C_{10}(P[C_3C_3C_3])_2$ Br	Solid	Solid	1.113	93	>300
D2 Br[j]	$C_{12}(P[C_3C_3C_3])_2$ Br	Solid	Solid	1.141	87	>300
E1 Br[i]	$PEG_3(P[C_3C_3C_3])_2$ Br	Solid	Solid	1.193	66	>250

which is an advantage in the coating of GLC capillary columns. The finding that IL **A4** has a greater viscosity than IL **B1** shows that the length of the alkyl chain on the phosphonium cation effects the IL viscosity. Also, polyethylene glycol linkage chains produce less viscous di-cationic ILs than do hydrocarbon linkage chains (see IL **D2** vs. **E1**). The potent effect of the anion on viscosity can be seen by comparing ILs **A1-6**. For example, by simply changing the anion from Cl to NTf_2, the kinematic viscosity drastically decreased from 2077.91 to 218.60 cSt. It should be noted that the trends in anion effects on viscosity are similar for both phosphonium and imidazolium based ILs, such that Cl > BF_4 > TfO > NTf_2.

The results of a comparison of the densities of phosphonium ILs to imidazolium ILs proved that phosphonium based ILs are always less dense than analogous imidazolium ILs. In fact, all the mono-cationic phosphonium based ILs had densities less than one, with the exception of those which had NTf_2 anions, which had densities just slightly greater than one.

However, it was determined that di-cationic phosphonium ILs have densities that are more similar to, but still slightly less than, di-cationic imidazolium based ILs.

The solid to liquid transformation temperature results were a bit less predictable than the refractive index, viscocity, and density. With the exception of the BF_4 ILs, the mono-cationic phosphonium ILs had solid to liquid transformation temperatures that were less than their imidazolium analogs. However, the di-cationic imidazolium IL had a lower melting point than the di-cationic phosphonium IL with the same dodecane linkage chain. Since many of the phosphonium based ILs preferred to solidify in a glassy state, it was difficult to obtain their melting points and the range given in Table 2.2 indicates the range in which a glass transition was observed. In general, it appears that phosphonium cations with longer alkyl substitutions produce lower melting RTILs. For the di-cationic phosphonium ILs synthesized, it was determined that either an alkyl linkage chain of at least 12 carbons or a poly-ether chain was needed to produce a liquid product at or below room temperature.

Table 2.2 lists the thermal stabilities for all RTILs used in this study. Comparing the mono-cationic phosphonium ILs to mono-cationic nitrogen based ILs, it is clear that the phosphonium ILs are more thermally stable. In general, mono-cationic phosphonium ILs can be about twice as stable as mono-cationic imidazolium ILs. For example, the phosphonium Cl and NTf_2 ILs **A1** and **A4** were stable to 335℃ and 380℃, repectively, where as, their imidazolium based analogs BMIM Cl and BMIM NTf_2 are only stable to approximately 145℃ and 185℃, respectively. Also, the di-cationic phosphonium ILs are more thermally stable than di-cationic imidazolium IL. With these results in mind, it is easy to believe that researchers may choose to use the more robust phosphonium IL columns instead of nitrogen based ILs when high temperatures are needed. Figure 2.2 shows four representative thermal stability curves for a variety of phosphonium-based ILs. The first three curves represent the thermal stability of mono-cationic phosphonium ILs, were as, the last curve represents a di-cationic phosphonium IL. It is important to note that these curves simply represent the loss of the IL stationary phase

and may represent either volatilization or chemical decomposition of the IL. Also, it should be noted that these tests were simply short-term studies; however examples of long term phosphonium column stability can be found in the literature.[100]

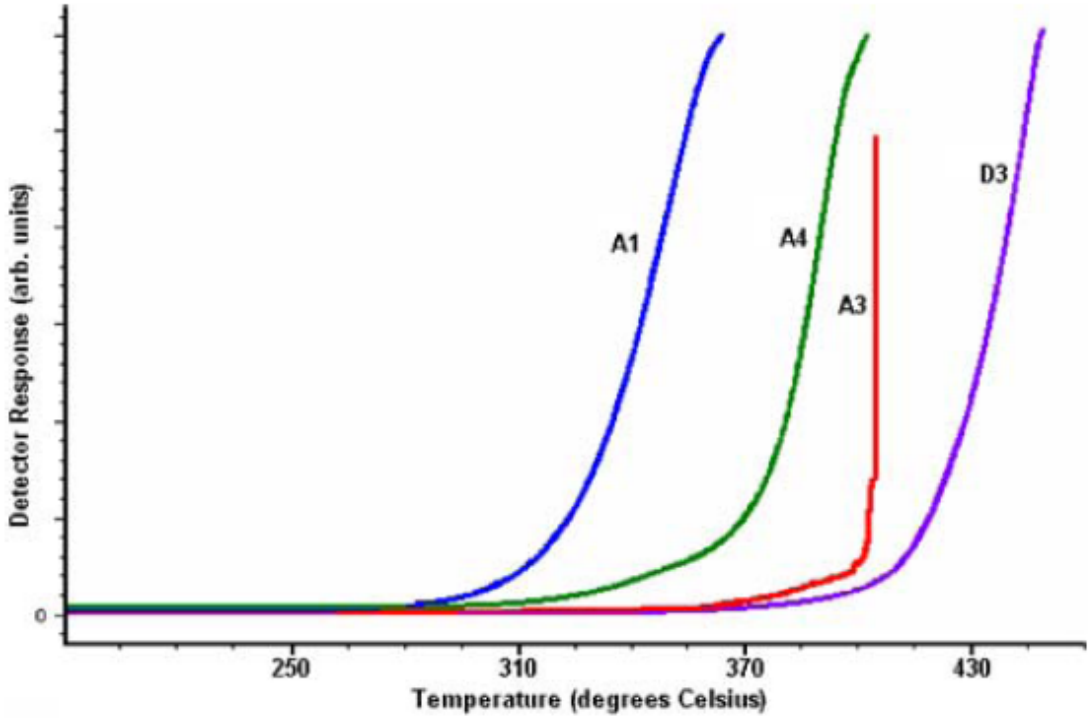


Figure 2.2 Thermal stability diagram for four of the ILs tested in this analysis.

The lowest thermal stability was observed for the chloride IL **A1**. The most thermally stable mono-cationic phosphonium IL was **A3**, with an approximate decomposition temperature of 405℃. This was interesting because typically the IL with the NTf_2^- anion (**A4** in this case) is the most stable due to its weak nucleophilic properties. However, this result does support previous reports which state that phosphonium salts undergo a different decomposition mechanism than ammonium salts.[35] The di-cationic phosphonium ILs showed increased thermal stability possessing decomposition temps up to 425℃ and liquid ranges well over

400℃. These RTILs have not been reported in the literature before and will prove to be very useful as high thermal stability solvents and stationary phases.

2.4.2 Solvation properties of phosphonium RTILs

In this work, we have used an inverse GC approach to obtain data for a linear solvation energy relationship from which specific solvation interactions for phosphonium RTILs can be obtained. Figure 2.1 shows the structures for eight mono-cationic and three di-cationic phosphonium based RTILs that were subjected to this analysis. Table 2.3 shows the derived system constants that describe each phosphonium RTIL at 70℃. The results obtained from interrogating the IL stationary phase at 70℃ proved to be the most useful given the nature of the probe analytes chosen. That is to say, at 100°C some of the more volatile probe analytes eluted with, or too closely to, the dead volume and could not be included in the final data set. In contrast, at 50℃, some of the probe molecules (particularly the H-bond donors) retained too long and suffered from high peak asymmetry factors and were rejected from the data set. Usually, measurements made at 70℃ yielded the maximum number of probe molecules ("n") that could be used for the MLRA. As listed in Table 2.1, "n" ranged from 26 to 34 probe analytes, with the phosphonium chloride **A1** giving the lowest "n" and those ILs with NTf_2^- anions allowing for greater "n"s. As true with most statistical analyses, the ILs that had greater "n"s gave the best regression. Those ILs with $n \leq 28$ resulted in correlation coefficients (R^2) lower than .99, whereas, all other ILs tested had R^2 values of .99. In parallel, the greater the "n" and R^2, the greater the Fisher coefficient (F).

For all ILs tested, the hydrogen bond basicity ("a" term) was one of the more dominant system constants. Hydrogen bond basicity interaction parameters ranged from 1.55 to 6.94. Since IL **A1-6** all posses the same cation, any differences in these system constants can be attributed to the anion. For example, the very large "a" term for the chloride IL **A1** must be due to the chloride anion. The high electron density and small size to charge ratio of the chloride anion makes it an extremely strong hydrogen bond acceptor. This reasoning, along with the

Table 2.3 Interaction parameters for all the ILs studied in this analysis

IL	*e*	*s*	*a*	*b*	*l*	*c*	*n*	R^2	*F*
A1: $P[C_6C_6C_6C_{14}]$ Cl	−0.15	1.51	6.60	−0.58	0.83	−3.63	26	0.97	152.36
(error)	(0.18)	(0.19)	(0.31)	(0.24)	(0.06)	(0.23)			
BMIM Cl[a]	0.291	2.01	5.23	−0.32	0.45	−2.84	22	0.98	
A2: $P[C_6C_6C_6C_{14}]$ BF_4[a]	−0.26	1.66	2.86	−0.50	0.76	−3.28	32	0.99	723.07
(error)	(0.08)	(0.08)	(0.08)	(0.11)	(0.02)	(0.09)			
BMIM BF_4[a]	0	1.46	1.97	−0.13	0.57	−2.74	33	0.98	
A3: $P[C_6C_6C_6C_{14}]$ TfO	−0.24	1.55	2.85	−0.36	0.76	−3.28	33	0.99	358.46
(error)	(0.1)	(0.12)	(0.12)	(0.15)	(0.03)	(0.11)			
BMIM TfO[a]	0	1.73	2.71	0	0.52	−2.64	31	0.99	
A4: $P[C_6C_6C_6C_{14}]$ NTf_2	−0.28	1.55	1.55	−0.15	0.75	−3.29	34	0.99	536.68
(error)	(0.08)	(0.09)	(0.08)	(0.11)	(0.02)	(0.09)			
BMIM NTf_2[a]	0	1.67	1.75	0.38	0.56	−3.03	35	0.99	
A5: $P[C_6C_6C_6C_{14}]$ NCNCN	−0.16	1.39	3.67	−0.15	0.75	−3.24	29	0.99	519.95
(error)	(0.08)	(0.09)	(0.13)	(0.12)	(0.02)	(0.09)			
A6: $P[C_6C_6C_6C_{14}]$ SAC	−0.22	1.66	4.03	−0.49	0.73	−3.28	30	0.99	378.72
(error)	(0.09)	(0.11)	(0.13)	(0.14)	(0.03)	(0.11)			
B1: $P[C_8C_8C_8C_8]$ NTf_2	−0.33	1.58	1.67	−0.25	0.75	−3.26	33	0.99	646.13
(error)	(0.07)	(0.08)	(0.07)	(0.10)	(0.02)	(0.08)			
C1: $P[C_4C_4C_4C_2]$ DEP	−0.16	2.01	6.94	−0.59	0.71	−3.64	28	0.98	227.77
(error)	(0.16)	(0.19)	(0.26)	(0.23)	(0.05)	(0.20)			
D1: $C_{10}(P[C_3C_3C_3])_2$ NTf_2	−0.06	1.76	1.88	−0.10	0.64	−3.30	31	0.99	656.66
(error)	(0.07)	(0.08)	(0.08)	(0.10)	(0.02)	(0.09)			
$C_9(MIM)_2NTf_2$[a]	0.11	1.76	1.75	0.20	0.51	−2.95	33	0.99	644.20
(error)	(0.07)	(0.08)	(0.07)	(0.10)	(0.02)	(0.09)			
D2: $C_{12}(P[C_3C_3C_3])_2$ NTf_2	−0.10	1.71	1.86	−0.02	0.65	−3.31	31	0.99	775.84
(error)	(0.07)	(0.07)	(0.07)	(0.09)	(0.02)	(0.08)			
E1: $PEG_3(P[C_3C_3C_3])_2$ NTf_2	0.05	1.64	1.97	−0.18	0.58	−3.00	30	0.99	744.59
(error)	(0.06)	(0.07)	(0.06)	(0.09)	(0.02)	(0.08)			
$(MIM)_2PEG_3$ NTf_2[a]	0.12	1.80	1.94	0.07	0.50	−3.07	29	0.98	
(error)	(0.09)	(0.10)	(0.09)	(0.13)	(0.03)	(0.11)			

multiple hydrogen bond basic sites on the diethylphosphate IL **C1**, explains why it produced the highest "a" term in this study or any other literature reports for ILs. The ILs with the lowest hydrogen bond basicity were those that had NTf_2^- anions. This is because the NTf_2^- anion has a large size to charge ratio and the negative charge is extremely delocalized. The determination that the anion is main contributor to the hydrogen bond basicity is in line with previous conclusions drawn from nitrogen based ILs.[9,20,79-80] However, it should be noted that there must be some effect of the cation on the hydrogen bond basicity. This is evident in the increase in the "a" term from mono-cationic IL **A4** to di-cationic ILs **D1** and **D2**. The greatest "a" term for an IL with the NTf_2^- anion was found with the polyethylene linked di-cation. Here the ether linkage

is acting as a hydrogen bond base, which slightly increases the overall hydrogen bond basicity of the IL. This phenomena has recently been explored and reported in the literature.[40]

Along with the interaction parameters for phosphonium based ILs determined in this analysis, Table 2.3 also list the interaction parameters deduced for imidzolium ILs in previously reported studies, allowing for an easy comparison of the solvation properties of phosphonium ILs to imidazolium ILs. In comparison to the nitrogen based IL stationary phases,[9,20,79-80] the "a" terms for phosphonium ILs are greater (with the exception of IL **A4** and BMIM NTf_2). Due to these results, it is anticipated that phosphonium ILs will make valuable replacements for nitrogen based ILs in processes that require solvent with strong hydrogen bond basicity.[101]

Despite the overall statistical success of the analysis, an interesting outcome can be seen in the hydrogen bond acidity ("b" term) of the ILs. All phosphonium ILs have unrealistic negative system constants for their "b" terms. By examining the phosphonium IL structures, one can see that there are no acidic hydrogens that can act as hydrogen bond donors, therefore it is sensible that none of the "b" terms are positive. Intuition might lead one to expect zero terms in these cases. Nonetheless, negative "b" terms were also reported for some of the imidazolium ILs, despite the fact that the imidazolium cation posses the ability to act as a hydrogen bond acid. Interestingly, there seems to be a trend followed where the greater the "a" term for these ILs, the more negative the "b" term will be, yet the proper interpretation of this phenomenon is uncertain. In order to investigate the effect of setting the "b" term to zero, the regression was also performed using only the e, s, a, and l terms. The result of doing this was quite interesting in that the correlation coefficient, standard error, and system constants remained virtually unchanged. Since this technique did not greatly improve the analysis, we opted to include the "b" term in all the regressions.

The system constants for the ability for the IL stationary phase to interact with a solute through π and non-bonding electrons ("e" term) are essentially zero or slightly negative for all but one phosphonium IL. The negative values for this system constant are reasonable and

relate to the way the solute descriptor "E" is determined.[41-42] IL **A6** was specially synthesized to examine the effect of having a π electron rich anion. There was no significant increase in the "e" term for this particular IL, thus it is determined that anion has little effect on this interaction parameter. The most interesting aspect of the "e" terms is the positive system constant for the ether linked di-cation IL **E1**. This result implies that the IL stationary phase does interact with solutes though the lone pair electrons possessed by the oxygen atoms in the ether chain, a finding that was briefly noted before in the literature.[40] In comparing "e" terms for phosphonium IL versus imidazolium ILs, it appears that the ability to interact with solutes through π and non-bonding electrons is lower for the phosphonium based ILs tested here. This is because of the π electron rich imidazolium cation, meaning that the "e" term is dominated by the cation.

The "s" term in Table 2.3 represents the ability for the stationary phase to interact with solutes via dipolar interactions. Nearly all of the phosphonium ILs tested had "s" values that were substantial and similar (i.e. statistically overlapped). For ILs **A1-6**, the anion changes drastically, whilst the cation is held constant. Since there is no major change in the "s" term, it can be determined that the anion effects here are very minimal. When the cation is changed, (i.e. mono- to di-cation) there is still no major change in the "s" term. Given these observations, it is difficult to determine the exact effect of the cation and anion on the "s" term. However, it can be concluded that all phosphonium ILs will interact through dipolar interactions. Compared to the imidazolium ILs, the phosphonium ILs have either similar or slightly lower "s" terms. Indeed all ILs seem to interact similarly with solutes through dipolar type interactions.

A substantial system constant was found for the ILs ability to interact through dispersion forces ("l" term). The "l" term is a combination of a negative contribution caused by the endoergic process of cavity formation and breaking solvent-solvent interactions, and a positive contribution from the exoergic process of forming solvent-solute interactions.[41-42] All phosphonium ILs posses net positive "l" terms, thus the dispersive solute-solvent interactions are greater than the solvent-solvent interaction of the stationary phases. The duality embedded

in the “l” system constant makes it a more difficult interaction parameter to understand and use comparatively. For example, just because IL **A1** has the greater “l” term than IL **E1,** does not necessarily mean that IL **A1** interacts with solutes through dispersive interactions more than IL **E1**. Rather, it should be interpreted that the difference in the ease of cavity formation and the strength of the dispersive forces between IL and analyte is greater for IL **A1** than for **E1**. This combinative feature of the “l” term must be understood when comparing the ILs in Table 2.2. Interestingly, the chloride phosphonium IL **A1** has the largest “l” term, whereas, the imidazolium chloride (BMIM Cl) gave the lowest “l” term. An overall comparison of the phosphonium and imidazolium ILs’ “l” terms, shows that phosphonium based ILs have a much greater ability to include and interact through dispersion forces with solutes. The phosphonium ILs with the lowest “l” terms were the di-cationic ILs. Going from mono- to di-cationic phosphonium IL significantly lowered the “l” term. This trend was not observed for the imidazolium ILs.

Lastly, Table 2.4 shows the resulting system constants obtained for IL **D2** at three different temperatures (100, 70, and 50°C). Typically in GLC, when temperature is decreased, retention is increased, which should be reflected in an increase in the interaction parameters (with exception to the “e” term).[42] In short, the results show that when temperature is decreased, interactions through dipolarity, hydrogen bonding, and dispersion all increase. This trend was observed for all phosphonium ILs tested in this analysis and showed that the study was successful.

2.4.3 Separation of complex mixtures

Figure 2.3 illustrates the separation of a complex mixture of 24 flavor and fragrance homologous ester compounds on a phosphonium based IL column, an imidazolium based IL column, a polyethylene glycol column, and a polysiloxane column. The results of the separation on the phosphonium IL column show

Table 2.4 Interaction parameters for IL D2 at three different temperatures

T (°C)	e	s	a	b	l	c	n	R^2	F
100	−0.02	1.53	1.57	−0.02	0.53	−3.24	30	0.99	893.50
(error)	(0.05)	(0.06)	(0.05)	(0.07)	(0.02)	(0.07)			
70	−0.10	1.71	1.86	−0.02	0.65	−3.31	31	0.99	775.84
(error)	(0.07)	(0.07)	(0.07)	(0.09)	(0.02)	(0.08)			
50	−0.13	1.78	2.09	0.01	0.73	−3.26	31	0.99	910.85
(error)	(0.06)	(0.07)	(0.07)	(0.09)	(0.02)	(0.08)			

that phosphonium RTILs can, indeed, be used as highly efficient and selective GLC stationary phases, as it was able to efficiently separate 23 of 24 flavor and fragrance analytes.

Despite the fact that the imidazolium IL column was able to baseline separate a few more of the test mixture components, the phosphonium IL column still possesses some merits of interest. Comparing the chromatograms in Figure 2.3 indicates that the two IL columns can behave differently in terms of selectivity. This can be seen in the many changes in elution order and was expected due to their differing system constants. In comparison to the two commercial columns, the phosphonium IL stationary phase again shows unique selectivity with many differences in elution order. Overall, it appears that the both IL columns tend to act more similar to the polar INNOWAX column than the Rtx-5 column. However, upon close examination, it seems that the phosphonium IL column is less similar to the INNOWAX than the imidazolium column, thus the phosphonium IL stationary phase is less polar than the compared imidazolium IL column. The intermediate polarity of the phosphonium IL column may be part of the reason that it is well suited to separate this mixture of intermediate polarity compounds.

Figure 2.4 shows the separation of a homologous mixture of alkanes and alcohols on the same columns as above. Figure 2.4 also illustrates the classical differences in the polarity of commercially available stationary phases. The very polar INNOWAX phase strongly retains alcohols, but lacks good selectivity for the alkanes. In contrast, the non-polar Rtx-5 column retains alkanes well, yet the alcohols eluted swiftly. Both the phosphonium and imidazolium IL columns proved to have polarities lying between the two commercial columns. This is yet

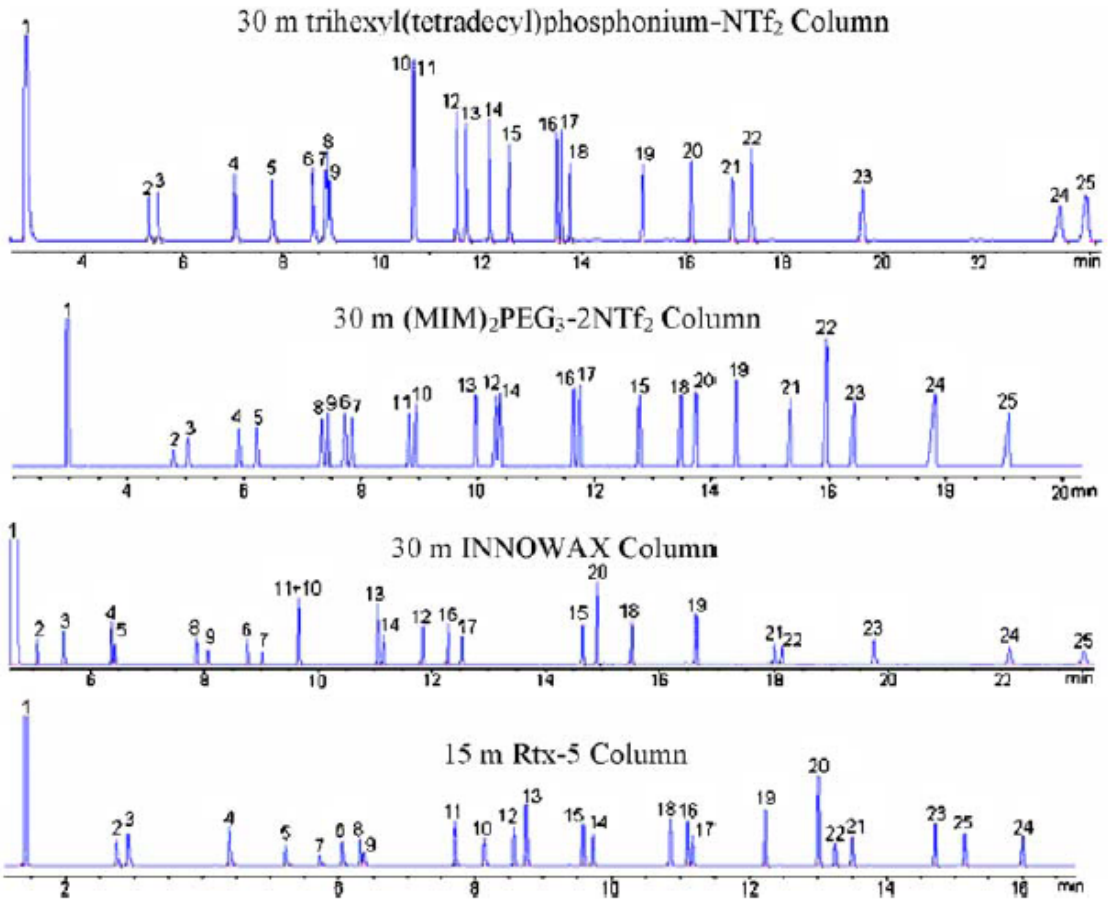


Figure 2.3 Separation of flavor and fragrance mixture

1 dichloromethane, 2 ethyl propionate, 3 methyl butyrate, 4 ethyl butyrate, 5 isopropyl butyrate, 6 allyl butyrate, 7 methyl tiglate, 8 propyl butyrate, 9 ethyl valerate, 10 ethyl hexanoate, 11 isopropyl tiglate, 12 allyl tiglate, 13 propyl tiglate, 14 ethyl heptanoate, 15 furfuryl propionate, 16 hexyl butyrate, 17 ethyl octanoate, 18 furfuryl butyrate, 19 furfuryl pentanoate, 20 hexyl tiglate, 21 furfuryl hexanoate, 22 benzyl butyrate, 23 furfuryl heptanoate, 24 furfuryl octanoate, 25 benzyl tiglate.

another example of the previously mentioned “dual nature” of IL polarity. Also, the two IL columns with NTf_2 anions share the problem of high peak asymmetry factors for alcohols. This is known to be mainly anion dependent and these peak shapes improve dramatically when the triflate anion is used. This kinetic effect depends on the pKa of the protonated anion, and has been reported previously.[79]

The most interesting results that can be seen in Figure 2.4 are the switches in elution between the phosphonium and imidazolium IL columns for the compounds ethanol, heptane, propanol, and octane. For these less retained compounds, it appears that the phosphonium IL column has better retention for the alkanes than the imidizolium column. This again implies that the phosphoium IL phase is somewhat less polar than the imidazolium IL, thus phosphonium IL GLC stationary phases may be better intermediate polarity columns. Overall, it is a good example of the unique selectivity of phosphonium IL stationary phases.

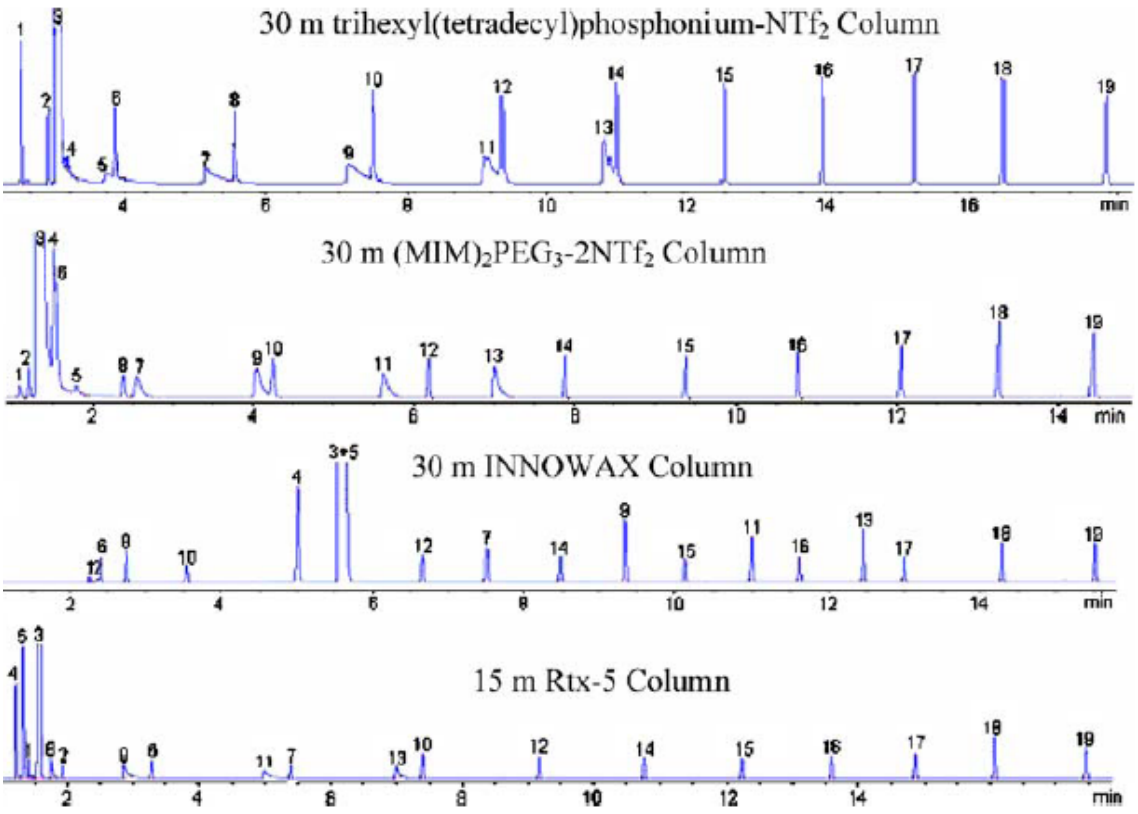


Figure 2.4 Separation of homologous alkane and alcohol mixture.

1 pentane, 2 hexane, 3 dichloromethane, 4 methanol, 5 ethanol, 6 heptane, 7 1-propanol, 8 octane, 9 1-butanol, 10 nonane, 11 1-pentanol, 12 decane, 13 1-hexanol, 14 undecane, 15 dodecane, 16 tridecane, 17 tetradecane, 18 pentadecane, 19 hexadecane.

Figure 2.5 illustrates the separation of the Grob mixture on three different phosphonium IL stationary phases. It is important to note that the Grob test was not performed herein, rather the Grob mixture was simply chosen as a complex analyte mixture containing a diverse selection of solutes. To compare the effect of changing the anion, ILs A4 and A3 can be compared (Fig. 2.5). When changing from the NTf_2^- to the TfO^- drastic changes in elution order were observed. For example, the very polar compound, 2-ethylhexanoic acid (peak 7), retains much longer on the triflate column, whereas the non-polar esters are retained more strongly on the NTf_2^- column. These results indicate that phosphonium ILs with triflate anions are more polar phases than those with NTf_2^- anions. This is in line with the system constants obtained through the solvation parameter evaluation.

To examine the effect of changing the cation, IL phases A4 and E1 can be compared (Fig. 2.5). At first glance, it is easy to see that all compounds retained less on the di-cationic IL stationary phase than the mono-cationic phase. This interesting result may be due to the lower system constant for dispersive interactions for the ether linked di-cation. Also, there are many elution order switches, which shows that not only do phosphonium IL phases have unique selectivity from imidazolium IL phases, but they can also have unique selectivity from one phosphonium IL stationary phase to another.

Lastly, Figure 2.5 clearly illustrates the improvement of mass transfer for hydrogen bonding analytes on the triflate column versus the NTf_2 columns. Examples of solutes that suffer from poor mass transfer on the NTf_2 columns are from 2,3-butanediol, octanol, and 2-ethylhexanoic acid (peaks 3, 4, and 7, respectively). It is clear to see that on the triflate column this problem is alleviated.

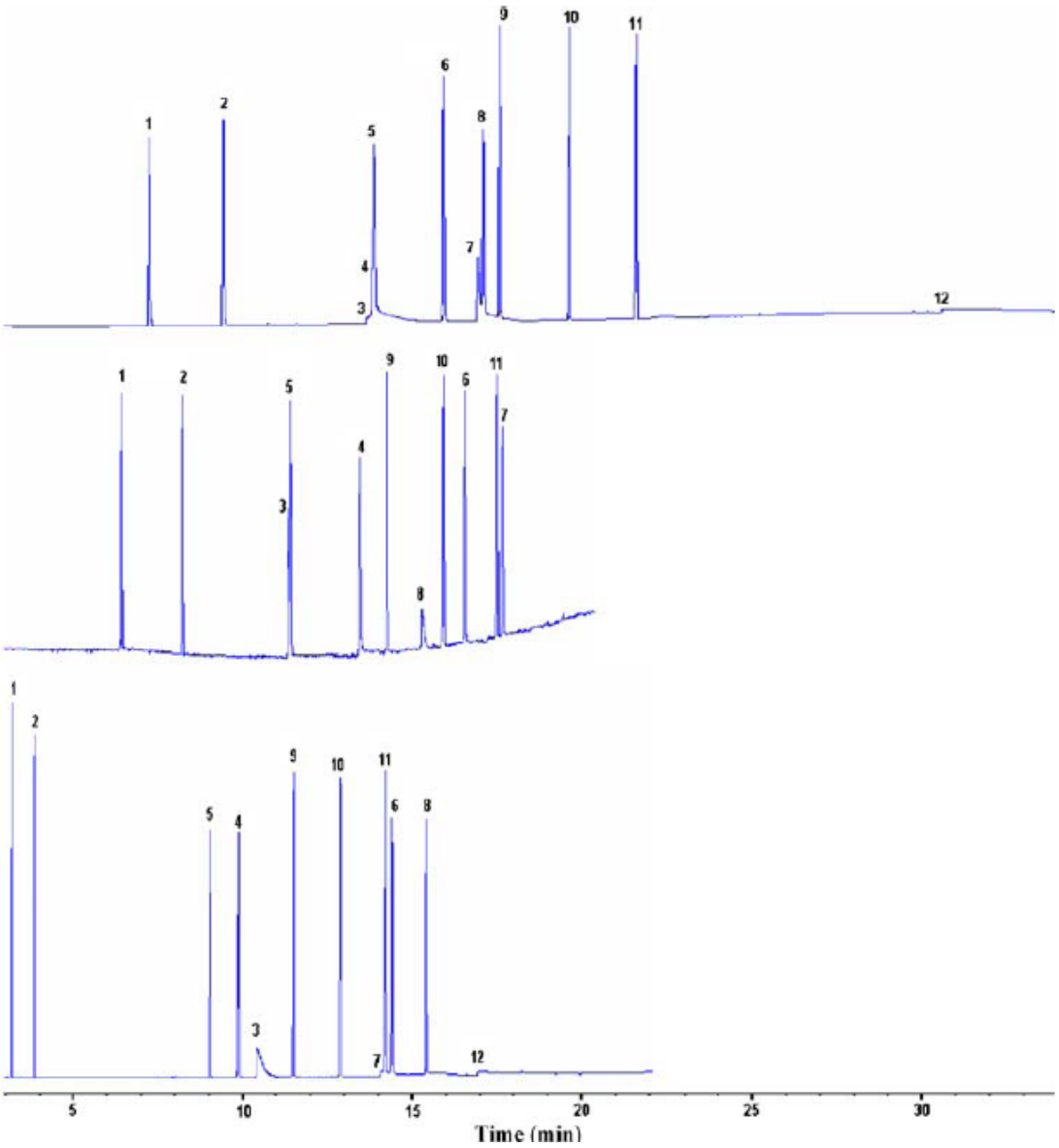


Figure 2.5 Separation of Grob mixture compounds.

The chromatograms obtained using GLC columns coated with phosphonium ILs A4, A3, and E1, respectively from top to bottom. ILs A4, A3, and E1 represent trihexyl(tetradecyl) NTf2, trihexyl (tetradecyl) TfO, and 1,11-di(tripropylphosphonium)-3,6,9-trioxaundecane bis(trifluoromethane) sulfonamide, respectively. 1 Decane, 2 undecane, 3 2,3-butanediol, 4 octanol, 5 nonanal, 6 2,6-dimethylphenol, 7 2-ethylhexanoic acid, 8 2,6- dimethylaniline, 9 methyl decanoate, 10 methyl undecanoate, 11 methyl dodecanoate, 12 dicyclohexylamine (did not elute in b).

2.5 Conclusions

Physiochemical analyses showed that phosphonium based RTILs have greater refractive indices and viscosities than imidazolium based RTILs. Conversely, the densities of phosphonium ILs are less than the densities for imidzaolium ILs, and often times, the density of phosphonium ILs will be less than one. Melting point determinations showed that it is difficult to predict whether a phosphonium or an imidazolium based IL will have a lower melting point. However, it was found that the long alkyl chains on the phosphonium cations almost always result in a RTIL, regardless of the nature of the anion. Also, it was observed that RTILs preferred to solidify in a glass state in a temperature range of -22 to -76℃. Thermal stability tests indicated a significant advantage for the phosphonium-based ILs. Mono-cationic phosphonium ILs are approximately 200℃ more thermally stable than imidazolium based ILs. Newly synthesized di-cationic phosphonium ILs also offered increased thermal stability and resulted in RTILs with liquid ranges greater than 400℃.

The results of the solvation energy relationship analysis for phosphonium RTILs showed that the principal solvating properties of these ILs stems from interactions through hydrogen bond basicity, dipolarity, and dispersive forces, a trend that has also been seen for nitrogen based ILs. The anion controls most of the hydrogen bond basicity, but in comparison to nitrogen based ILs, phosphonium ILs seem to allow for greater hydrogen bond basic interactions. It was determined that interaction through π and non-bonding electrons is controlled by the phosphonium cation and that introducing an ether chain into the cation can increase these types of interactions. Compared to imidazolium ILs, the phosphonium ILs have less of an ability to interact with solutes through π and non-bonding electrons. Interactions through dipolarity can not be solely attributed to either cation or anion, and these interactions do not seem to change greatly when changing the ion pair of the phosphonium or the imidazolium ILs. Lastly, interesting results were found for the phosphonium ILs ability to interact with solutes through dispersion forces. The “l” term for phosphonium ILs was always greater than

imidazolium ILs. Also, going from mono- to di-cationic phosphonium ILs decreased this system constant.

As stationary phases, the phosphonium IL columns displayed a “dual nature” as they were able to separate polar compounds like polar phases, yet, separate apolar analytes like non-polar phases. When compared to an imidazolium IL phase, the phosphonium displayed the ability to perform with unique selectivity and good efficiency. The tetra-alkyl phosphonium IL column used in this study was slightly less polar than the polyethylene glycol linked di-imidazolium IL column used for comparison. The thermal stability and robustness of the phosphonium IL columns dominates nitrogen based IL columns in every aspect. It is expected that the preceding will persuade and attract many researchers to phosphonium RTILs, especially as novel GLC stationary phases.

CHAPTER 3

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY USING A HIGH-TEMPERATURE PHOSPHONIUM IONIC LIQUID COLUMN

3.1 Abstract

A high-temperature ionic liquid, trihexyl(tetradecyl) phosphonium bis(trifluoromethane)sulfonamide, was used as the primary column stationary phase for comprehensive two-dimensional gas chromatography (GC × GC). The ionic liquid (IL) column was coupled to a 5% diphenyl/95% dimethyl polysiloxane (HP 5) secondary column. The retention characteristics of the IL column were compared to polyethylene glycol (DB-Wax) and 50% phenyl/50% methyl polysiloxane (HP-50+). A series of homologous compounds that included hydrocarbons, oxygenated organics, and halogenated alkanes were analyzed with each column combination. This comparison showed that the ionic liquid is less polar than DB-Wax but more polar than HP-50+. The most unique feature of the IL × HP-5 column combination is that alkanes, cyclic alkanes, and alkenes eluted in a narrow band in the GC × GC chromatogram; whereas, these compounds occupied a much larger portion of the DB-Wax × HP-5 and the HP-50+ × HP-5 chromatograms. Each column combination was used to analyze diesel fuel. The IL × HP-5 chromatogram displayed narrow bands for three major compound classes in diesel fuel: saturates, monoaromatics, and diaromatics. The IL column was used at temperatures as high as 290 ℃ for several months without any noticeable changes in column performance.

3.2 Introduction

Numerous stationary phases are now available for gas chromatography (GC). The vast majority of these phases are either substituted siloxanes or polyether phases (such as polyethylene glycol). The selectivities of these stationary phases have been thoroughly

characterized.[102] Ionic liquids (ILs) have been used to produce gas chromatography stationary phases with unique properties.[9,20,32,36,79-80,103] Ionic liquid stationary phases display a high affinity toward dipolar solutes and solutes that can serve as hydrogen bond acids.[79] Such behavior is similar to polar stationary phases such as polyethylene glycol or cyanopropyl-substituted siloxanes. However, several studies[9,20,36,79-70] have shown that ILs retain nonpolar solutes (e.g., alkanes and alkenes) in a manner that is similar to stationary phases with low polarity such as dimethylsiloxane. This unique selectivity has been described as a result of a "dual-nature" retention mechanism. Many IL stationary phases also have the advantageous property of being stable at high temperatures. Most conventional polar stationary phases cannot be used at temperatures greater than 280℃. Anderson and Armstrong recently reported that partially crosslinked ILs can be used at separation temperatures as high as 350 ℃. [80]

Comprehensive two-dimensional gas chromatography (GC × GC) is a high resolution technique that couples a standard gas chromatography separation in a primary column with a series of repetitive, fast separations in a secondary column. The retention order along the primary axis is determined by the selectivity of the primary column, whereas the retention order along the secondary axis is determined by the difference in the selectivities of the secondary column versus the primary column. Numerous stationary phase combinations have been employed for GC × GC separations. To date, there have been no published studies utilizing ionic liquid stationary phases in GC × GC. Ionic liquids are a potentially valuable addition to GC × GC owing to their unique selectivities and hightemperature capabilities.

This article describes a series of GC × GC separations generated with an ionic liquid primary stationary phase and a conventional nonpolar secondary stationary phase. The high-temperature ionic liquid trihexyl(tetradecyl)phosphonium bis(trifluoromethane)sulfonamide was chosen because preliminary single-column studies indicated that it was stable over a wide range of temperatures and capable of producing high chromatographic efficiency. In addition, the single-column studies indicated that the IL had an overall polarity that would be useful in GC

× GC separations. Standard mixtures of organic compounds with a wide range of functional groups were analyzed to characterize the selectivity of the column combination. The GC × GC system was then used to generate a group-type separation of diesel fuel. The selectivity of the IL stationary phase is compared to the selectivities of polyethylene glycol and 50% phenyl/50% methyl polysiloxane.

3.3 Experimental

3.3.1 Chromatography conditions

The basic design and operating principles of flow-switching GC × GC have been described previously.[104-105] A schematic of the setup used for these studies is shown in Fig. 3.1. A Perkin-Elmer Autosystem XL gas chromatograph equipped with electronic pneumatics and dual flame-ionization detectors (FIDs) was used as the experimental platform. Neat mixtures of organic compounds were injected through a split inlet (50:1 split ratio, 250 ℃) in sub-microliter quantities. These compounds then entered a 30 m× 0.25 mm primary column that was coated with a 0.15-μm film of the trihexyl(tetradecyl)phosphonium bis(trifluoromethane) sulfonamide ionic liquid. The process used to produce this column is described in Production of the ionic liquid column. The primary column flow was 1.0 mL min−1. The components entered an in-line differential flow modulator [11] upon exiting the primary column. The modulator was operated at a modulation period of 1.5 s and an auxiliary flow of 18 mL min−1. Ultra-high-purity hydrogen was used for both the primary flow and auxiliary flow. The component pulses exiting the modulator were split evenly between a 5.0 m×0.25 mm HP-5 secondary column (Agilent Technologies, 5% diphenyl/95% dimethyl siloxane, 0.25-μm film thickness) and a 5.0 m×0.25 mm transfer line. The effluent of the secondary column was monitored with a flame ionization detector (FID) held at 290 ℃. The oven temperature was ramped with the following program: 50 ℃ for 2 min; 25 ℃ min−1 to 65 ℃; 18.5 ℃ min−1 t o 90 ℃; 17 ℃ min−1 to 290 ℃; hold at 290 ℃ for 5 min.

GC × GC separations were also performed using a DBWax primary column (Agilent Technologies, polyethylene glycol, 30 m×0.25 mm, 0.25-μm film thickness) or an HP- 50+ primary column (Agilent Technologies, 50% phenyl/ 50% methyl polysiloxane, 30 m×0.25 mm, 0.50-μm film thickness) in place of the IL column. Temperature programs were adjusted for both of these configurations to generate the maximum separations of the homologous sets of standard compounds along the secondary dimension while not producing peak wrap-around. The temperature program used for the DB-Wax × HP-5 column combination was 40 ℃ for 2.5 min; 35 ℃ min−1 to 80 ℃; 20.0 ℃ min−1 to 105 ℃; 15 ℃ min−1 to 260 ℃; hold at 260 ℃ for 5 min. The temperature program used for the HP-50+ × HP-5 column combination was 40 ℃ for 3.0 min; 8 ℃ min−1 to 120 ℃; 7.5 ℃ min−1 to 260 ℃; hold at 260 ℃ for 3 min. All other parameters (e.g., primary and auxiliary flow, modulation timing, etc.) were the same as those employed for the IL × HP-5 separations. In a separate set of experiments, the selectivity of the HP-5 stationary phase was independently characterized by performing single-column, temperature-programmed separations with a 25 m×0.25 mm HP-5 column (0.25-μm film thickness). A 1.0 mL min−1 carrier flow was used in conjunction with the following temperature program: 40 ℃ for 2 min; 10 ℃ min−1 to 240 ℃.

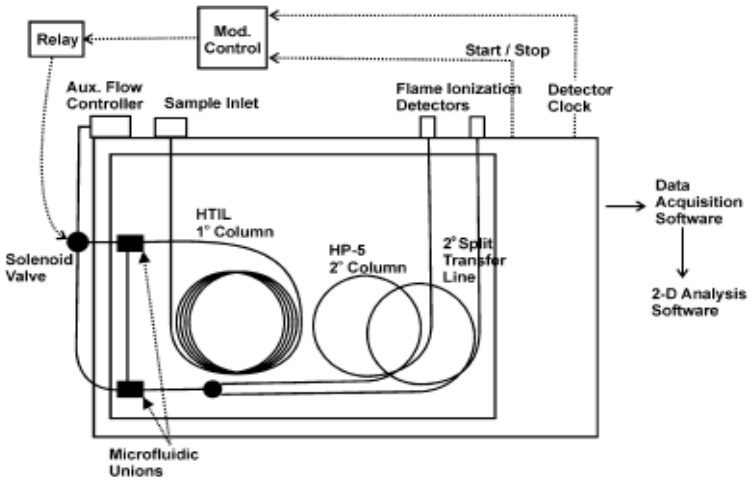


Figure 3.1 Schematic of the GC x GC instrument

3.3.2 Materials for the production of the IL column

Trihexylphosphine can be obtained from Cytec Industries Inc. (West Paterson, NJ). Lithium bis(trifluoromethane) sulfonamide and 1-chlorotetradecane were obtained from Aldrich (Milwaukee, WI).

Trihexyl(tetradecyl)phosphonium chloride was prepared according to the following procedure. In a round-bottomed flask equipped with condenser, trihexylphosphine (4.0 g) was heated to 140 ℃. To this, 1-chlorotetradecane (3.9 g) was added and the resulting mixture was stirred at 140 ℃ overnight.[87] After 24 h, the reaction was cooled to room temperature and the crude product was dissolved in water. Several washings with ethyl acetate allowed for the removal of any excess starting materials. Removal of water by rotoevaporation followed by overnight drying in vacuum over phosphorous pentoxide yielded the pure room-temperature ionic liquid (yield >95%).

Trihexyl(tetradecyl)phosphonium bis(trifluoromethane) sulfonamide was synthesized through a metathesis reaction of the trihexyl(tetradecyl)phosphonium chloride salt with lithium bis(trifluoromethane)sulfonamide (NTf_2^-). Specifically, 1.0 g of the chloride salt was dissolved in water (50 mL) and treated with 0.66 g of the Li+ NTf_2^-. The resulting solution was stirred at room temperature for 24 h. After this time, dichloromethane (75 mL) was added to the solution to dissolve the NTf_2^- salt that had phase separated from the water. The lithium chloride and excess lithium NTf_2^- were removed from the dichloromethane phase with successive extractions with water (5×75 mL). Removal of dichloromethane through roto-evaporation followed by vacuum drying over phosphorous pentoxide resulted in the pure room-temperature ionic liquid (yield >85%).

3.3.3 Column preparation

The (tetradecyl)phosphonium bis(trifluoromethane)sulfonamide IL column was prepared by the static coating method at 40 ℃ using a 0.25% (w/v) coating solution of the IL in dichloromethane. The untreated fused silica capillary (30 m×0.25 mm) used for this coating was

obtained from Supelco (Bellefonte, PA). Before the static coating was performed, the capillary was pretreated with sodium chloride.[36,98] Following the coating process, the column was dried with helium flow while being conditioned to 120 °C overnight. After this time, the efficiency of the column was tested using naphthalene at 100 °C. The resulting column had an efficiency of 3,200 plates per meter.

3.3.4 Characterization of the IL physicochemical parameters

Several fundamental physicochemical parameters of the (tetradecyl)phosphonium bis(trifluoromethane) sulfonamide IL stationary phase were determined. The kinematic viscosity of the IL was found to be 218.60 cSt after triplicate measurements using a Cannon–Manning semimicro viscometer at 30 °C. A density of 1.065 g mL−1 was determined with a 10-mL Kimble specific gravity pycnometer at 22 °C. Hexane was used an immiscible solvent for this measurement. A refractive index of 1.4507 was determined using a Bausch & Lomb Abbe-3L refractometer at 22 °C. The melting point was within a range of −78 to −22 °C based on observations of the IL in a dry ice/acetone bath (−78 °C) and a freezer (−22 °C). Lastly, a thermal stability test was performed on the IL column by ramping the oven temperature from 70 to 450 °C by 3 °C min−1. For this analysis, a 1 mL min−1 He flow was used and the injector and FID temperatures were set to 250 °C and 350 °C, respectively. The baseline of the FID signal was essentially flat until a temperature of 300 °C was reached. The FID signal slowly increased as the temperature increased from 300 to 380 °C. The FID signal increased dramatically at temperatures above 380 °C. Based on these observations a decomposition temperature of 380 °C was assigned to the IL.

3.4 Results and Discussion

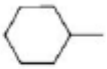
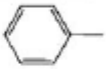
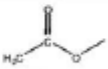
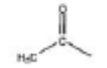
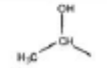
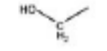
3.4.1 Definition of functional groups

The GC × GC retention times of ten homologous sets of compounds were analyzed with the IL × HP-5, DBWax × HP-5, and HP-50+ × HP-5 column combinations. Each compound had a single functional group joined to a linear alkyl chain; that is, a structure given by

$$Z - (CH_2)_n - H$$

where Z is an organic functional group and n is the number of methylene groups in the alkyl chain. The structure of each functional group Z is shown in Table 3.1. The functional groups were selected to provide a diverse range of intermolecular interactions.

Table 3.1 Homologous sets $Z - (CH_2)_n - H$

Group Name	Structure of Z-	Values of n	Group Symbol
Alkanes		6 - 13	◆
1-Alkenes		6 - 13	◇
Cycloalkanes		0 - 4	◆
Aromatics		0 - 5	×
1-Fluoroalkanes		5 - 8, 10	●
1-Chloroalkanes		4 - 7	○
Acetates		1 - 6, 8	□
2-Ketones		3 - 6	■
2-Alcohols		1 - 7	△
1-Alcohols		0 - 8	▲

3.4.2 GC × GC chromatograms of standard mixtures using an IL primary column

Figure 3.2 shows a GC × GC chromatogram of a mixture of n-alkanes, 1-alkenes, 1-fluoroalkanes, aromatics, 2-alcohols, and 1-alcohols obtained using the ionic liquid primary column and the HP-5 secondary column. The alkanes and 1-alkenes had very similar primary

and secondary retention times and thus are overlapping in the chromatogram. In general, the peaks along the primary axis had widths at half maximum that were approximately 3 s. The widths at half maximum along the secondary axis depended upon the secondary retention time. For example, weakly retained compounds such as 1-alcohols had widths at half maximum of 85 ms, whereas strongly retained compounds such as n-alkanes had widths at half maximum of 100 ms.

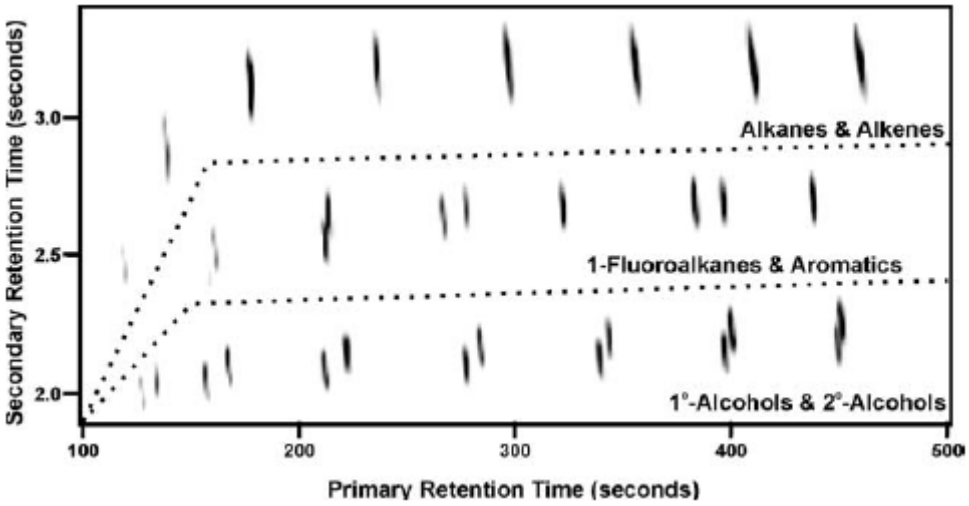


Figure 3.2 GC × GC chromatogram of six homologous sets of compounds obtained with an ionic liquid primary column and an HP-5 secondary column.

Figure 3.3a shows the retention positions of the standard compounds. The compounds within each homologous set had primary retention times that increased with increasing alkyl chain length, and secondary retention times that were essentially constant and determined by the functional group Z. These retention characteristics are typical of GC × GC chromatograms with optimized temperature programs.[104-105] The secondary retention times of the homologous sets had the following retention order: 1-alcohols ≈ 2-ketones < 2-alcohols < acetates < aromatics < fluoroalkanes < chloroalkanes < (alkenes ≈cyclohexanes ≈alkanes).

The IL × HP-5 separations were conducted with a temperature program that reached 290 ℃. Very little column bleed was observed at th is elevated final temperature. Furthermore,

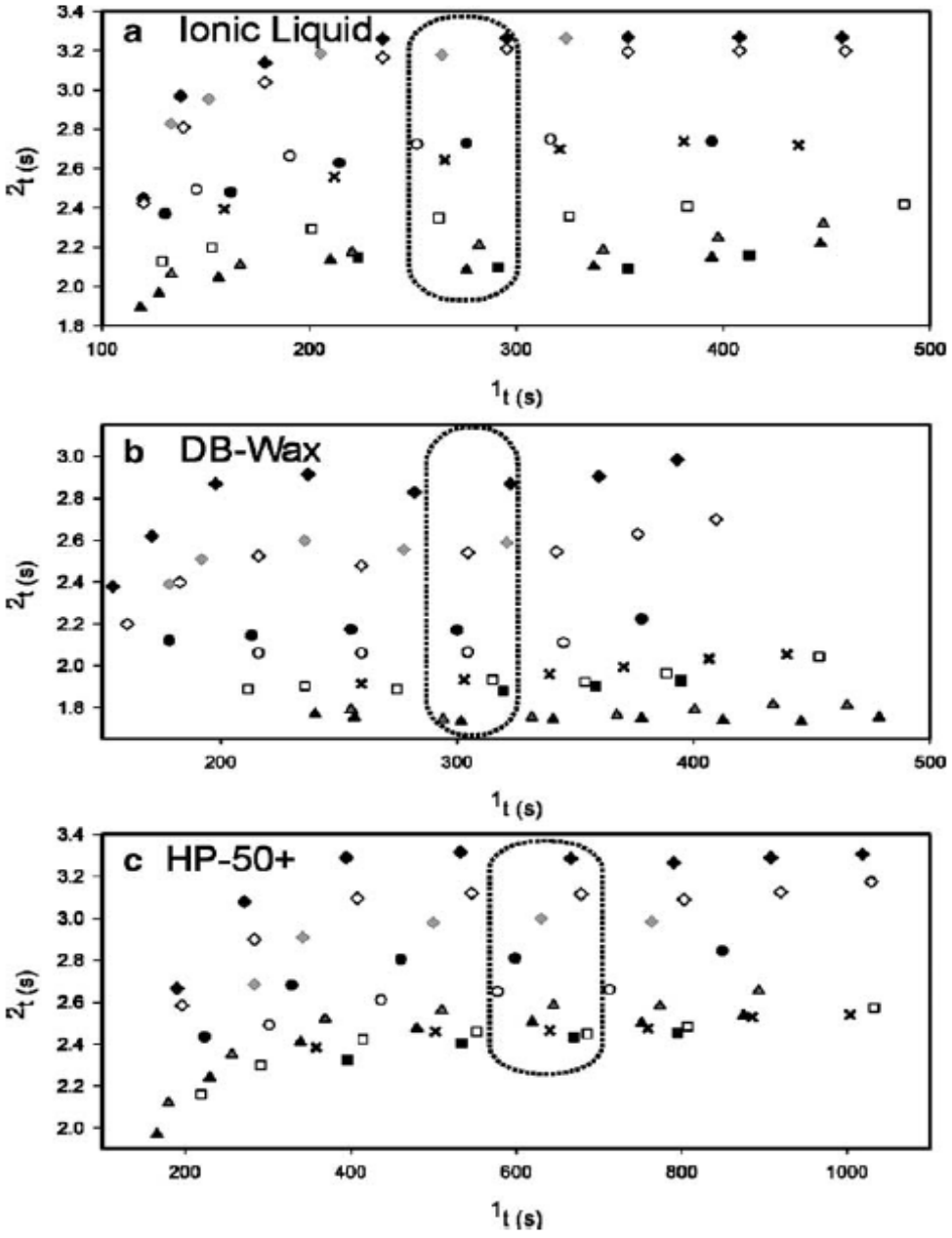


Figure 3.3 Retention times of the homologous compounds

The IL × HP-5 column combination, b the DBWax × HP-5 column combination, and c the HP-50+ × HP-5 column combination. The legend for the graph symbols is shown in Table 3.1. The secondary retention times of the circled compounds are plotted in Fig. 3.5

the quality of the separations on the ionic liquid column did not deteriorate with frequent use over the course of 4 months. This indicates that the IL stationary phase is capable of conducting separations at temperatures higher than those suitable for conventional polar stationary phases such as polyethylene glycol, siloxanes with high levels of cyanopropyl substitution, or trifluoropropyl siloxanes. However, it should be noted that phenyl-substituted siloxanes can operate at temperatures greater than 290 ℃ and that new formulations are being introduced (such as sol-gel phases) that extend the temperature ranges of polar stationary phases.

3.4.3 GC × GC retention comparison

The selectivity generated by the IL primary column was compared to that generated by a polyethylene glycol stationary phase (DB-Wax) and by a 50% phenyl/50% methyl polysiloxane stationary phase (HP-50+). The comparison was based on the relative retention of the homologous compounds along the secondary dimension. The efficiencies of the separations along the primary dimension were not compared, as significantly different temperature programs were required to cause the homologous sets to fully occupy the secondary dimension. This is due to the different film thicknesses and polarities of the IL, DB-Wax, and HP-50+ columns used in this study.

Polyethylene glycol is a highly polar stationary phase that is used frequently for GC × GC separations.[106] The retention times of the homologous sets observed for the DB-Wax × HP-5 column combination are plotted in Fig. 3.3b. When compared with the IL × HP-5 chromatogram, the DB-Wax × HP-5 chromatogram shows a greater primary retention of the 1-alcohols and 2-alcohols relative to the n-alkanes. This is presumably due to strong interactions of the DB-Wax stationary phase with hydrogen bond acid functional groups. The increased primary retention time for these functional groups leads to decreased secondary retention on the HP-5 column. Another major difference is that hydrocarbons such as 1-alkenes,

cyclohexanes, and aromatics also display greater primary retention on the DB-Wax column and therefore have lower secondary retention times.

Siloxanes with high levels of phenyl substitution like the HP-50+ stationary phase have intermediate polarity. Such phases are commonly used in GC × GC separations owing to their ability to operate at high temperatures.[106] The retention times for the homologous sets observed for the HP-50+ × HP-5 column combination are plotted in Fig. 3.3c. The biggest difference as compared to the IL × HP-5 chromatogram is that the hydrocarbons such as 1-alkenes, cyclohexanes, and aromatics display greater levels of retention on the HP-50+ column and therefore have lower secondary retention times. In contrast, oxygenated aromatics such as acetates, 2-ketones, 1-alcohols, and 2- alcohols all display lower levels of retention on the HP-50+ and hence have greater secondary retention times.

A quantitative retention analysis was conducted to clearly highlight the similarities and differences of the selectivity of the IL, DB-Wax, HP-50+, and HP-5 stationary phases. In addition, this analysis leads to the generation of parameters that can be used to understand how the selectivity of the primary column influences the retention along the secondary axis. A graphical alignment procedure was used to measure the affinity of the functional groups of the homologous sets for each stationary phase. This process is illustrated in Fig. 3.4 for the ionic liquid stationary phase. The primary retention time of each solute was plotted as a function of its methylene group number (n) (see Table 3.1 for the definitions of Z and n). Each homologous set was then horizontally shifted by a factor cZ in an iterative fashion to obtain maximum overlap of all of the homologous sets. Only the n-alkanes were held at a fixed position (i.e., cZ=0 for n-alkanes by definition). The aligned data for the ionic liquid is shown in the bottom portion of Fig. 3.4. The values of cZ for each homologous set are shown in Table 3.2. The cZ values represent the retention due to the functional group Z in units of effective methylene number.

The alignment procedure was also done with the DBWax primary retention data, the HP-50+ primary retention data, and the HP-5 single-column retention data. The cZ values

obtained for each stationary phase are shown in Table 3.2. The selectivity of a particular polar phase is most easily assessed from the cZ values when they are expressed relative to the

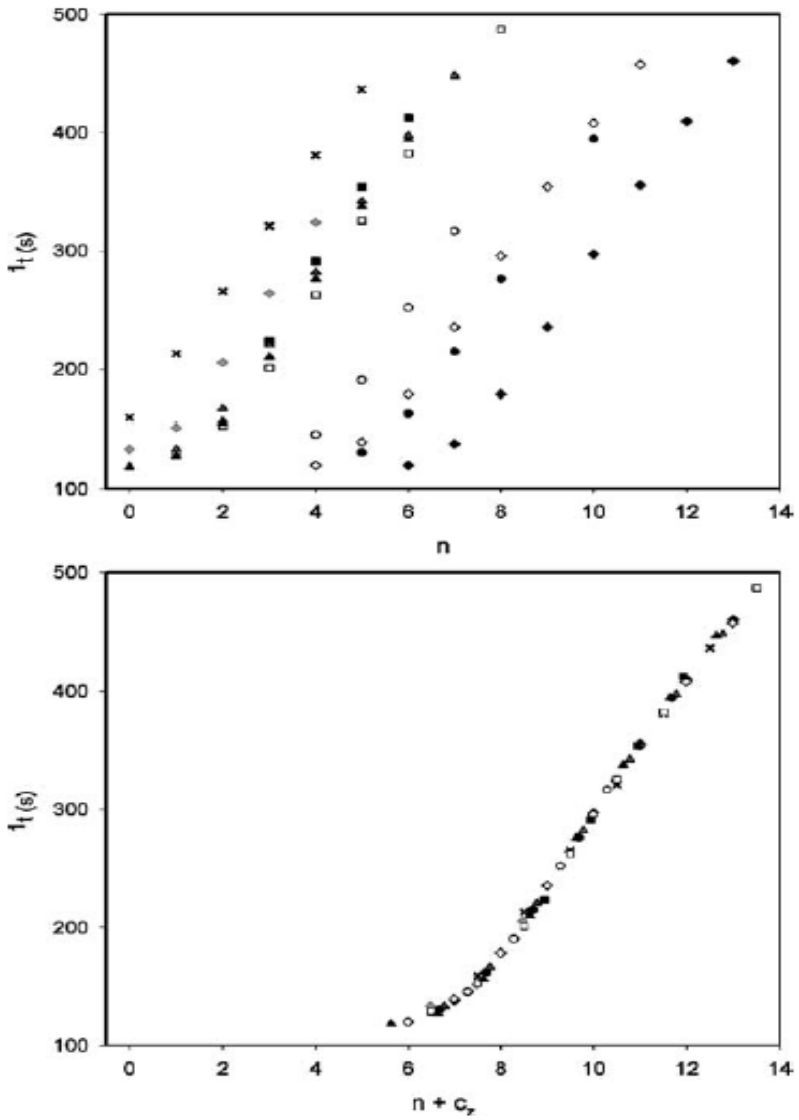


Figure 3.4 Alignment analysis of ionic liquid primary retention time data to determine the cZ factors for the ionic liquid stationary phase.

The upper graph shows the primary retention time of the homologous compounds plotted as a function of the appropriate values of methylene number, n. See Table 3.1 for the definitions of the homologous compounds and the graph symbols. Each homologous data set is shifted by a factor cZ in the bottom graph to obtain the greatest level of alignment among the data sets. The cZ values are shown in Table 3.2

values for a nonpolar phase. The lower portion of Table 3.2 shows ΔcZ values that are defined relative to the cZ values for the HP-5 stationary phase (i.e., ΔcZ =cZ −cZ,HP-5). The magnitude of the ΔcZ values indicates the strength of the interaction of a stationary phase with a particular functional group relative to the strength of the interaction on the HP-5 stationary phase.

The overall relative polarity of the stationary phases can be estimated by the average values of ΔcZ listed at the bottom of Table 3.2. The highly polar stationary phase DBWax has an average ΔcZ value of 2.21, whereas the moderately polar HP-50+ phase has a much lower average of 0.68. The IL stationary phase has an average ΔcZ value of 0.94, which is 40% larger than the HP-50+ value but significantly smaller than the DB-Wax value. Thus, we concluded that this particular IL stationary phase is best categorized as being moderately polar.

Table 3.2 cZ values for the homologous sets on four different stationary phases and the difference of these values (ΔcZ) from the HP-5 values

	HP-5	IL	DB-Wax	HP-50+
c_Z values				
Alkanes	0.00	0.00	0.00	0.00
Alkenes	1.92	1.99	2.47	2.09
Cyclohexanes	6.38	6.47	6.92	6.72
Aromatics	6.68	7.50	9.41	7.79
1- Fluoroalkanes	0.95	1.68	2.40	1.49
1-Chloroalkanes	2.58	3.28	4.53	3.33
Acetates	4.15	5.50	6.92	5.19
2-Ketones	3.96	5.93	7.00	5.02
2-Alcohols	4.01	5.77	8.26	4.83
1-Alcohols	3.70	5.63	8.55	4.63
Δc_Z values[a]				
Alkanes	0.00	0.00	0.00	0.00
Alkenes	0.00	0.07	0.55	0.17
Cyclohexanes	0.00	0.09	0.54	0.34
Aromatics	0.00	0.82	2.73	1.11
Fluoros	0.00	0.73	1.45	0.54
Chloros	0.00	0.70	1.95	0.75
Acetates	0.00	1.34	2.76	1.04
2-Ketones	0.00	1.98	3.05	1.07
2-Alcohols	0.00	1.76	4.25	0.82
1-Alcohols	0.00	1.93	4.85	0.93
Average	0.00	0.94	2.21	0.68

The selectivity of a particular stationary phase is reflected in its distribution of ΔcZ values. The ionic liquid stationary phase shows its largest ΔcZ values for the oxygenated compounds (acetates, ketones, 2-alcohols, and 1-alcohols); moderate values for the aromatics and halogens (1-fluoroalkanes and 1-chloroalkanes); and values near zero for the alkenes and cyclohexanes. DB-Wax shows its highest ΔcZ values for the hydrogen bonding oxygenates (1-alcohols and 2-alcohols); moderate values for aromatics, ketones, and acetates; slightly lower values for halogens; and low values for the 1-alkenes and cyclohexanes. The HP-50+ data show highest ΔcZ values for the aromatics, ketones, 2-alcohols, and 1 alcohols; moderate values for the halogens and cyclohexanes; and a low value for the 1-alkenes.

The most unique aspect of the selectivity of the IL stationary phase is that it displays much stronger interactions with oxygenated organics than those of the nonpolar HP-5 stationary phase, but similar interaction strengths as the HP-5 stationary phase for 1-alkenes and cycloalkanes. This unique selectivity is also observed when the commercial polar stationary phases are compared to the IL stationary phase. The DB-Wax stationary phase displays stronger interactions with oxygenated organics (especially hydrogen bond acids) than the IL stationary phase. The HP-50+ displays weaker interactions with oxygenated organics than the IL stationary phase. However, both the DB-Wax and HP-50+ stationary phases display stronger interactions than the IL stationary phase for 1-alkenes, cyclohexanes, aromatics, and 1-chloroalkanes. These observations are consistent with the prior characterization of similar IL stationary phases and demonstrate the “dualnature” retention mechanism[81], i.e., ionic liquids interact with highly polar compounds in a manner similar to polar polymeric stationary phases and ionic liquids interact with less polar compounds in a manner similar to nonpolar polymeric stationary phases (in this case HP-5). These results are also in agreement with previous characterizations of similar ionic liquids with a solvation parameter model.[81-82] These studies found that many ionic liquids (but not all) interact strongly with hydrogen bond acids and compounds capable of interacting through dipole-type interactions, but do not interact strongly

with solute n- or π-electrons. In contrast, polyethylene glycol and 50% phenyl/50% methyl polysiloxane are known to interact strongly with solute n- and π-electrons.[102] This would explain why the relative retention of the homologous sets that have been classified as interacting significantly through this mechanism (e.g., 1-alkenes, aromatics, cyclohexanes, and 1-chloroalkanes) display relatively low ΔcZ values on the ionic liquid stationary phase.[107]

In addition to providing a quantitative measure of the primary column selectivity, the ΔcZ values are also directly related to the observed secondary retention times. A previous study has shown that the secondary retention time of a solute is exponentially dependent upon the difference in the secondary column retention index and the primary column retention index. Each cZ value represents the contribution of the functional group Z to the retention index. In this case, retention indices are defined as increasing by a value of 1.0 with the addition of a methylene group as opposed to an increase of 100 employed by the Kovats retention index system. The value of ΔcZ for each homologous set represents the difference in the primary retention index and the nonpolar HP-5 secondary retention index. Figure 3.5 depicts the secondary retention times of the solutes circled in Fig. 3.3 as a function of the appropriate ΔcZ values. The secondary retention times are fit with the exponential function $t^2 = a \exp(-b\Delta cz) + d$, where a, b, and d are fitting coefficients. These data demonstrate that the ΔcZ values can be used directly to understand and predict the relative retention of compounds on the secondary column. For example, the values of $\Delta cZ<0.1$ observed for alkanes, 1-alkenes, and cyclohexanes on the IL stationary phase lead to the correct prediction that these compounds should elute in the GC × GC chromatogram as a narrow band with high secondary retention.

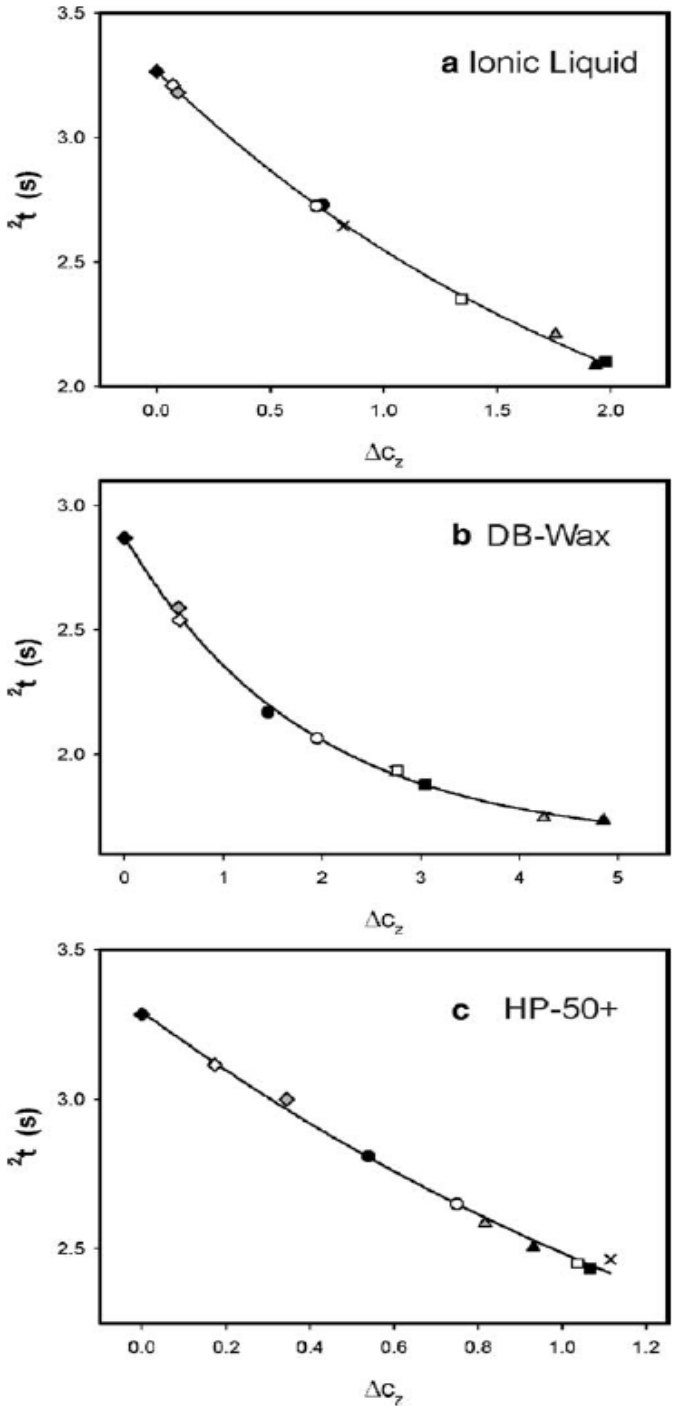


Figure 3.5 Secondary retention times of selected solutes as a function of ΔcZ.

The IL × HP-5 column combination, b the DB-Wax × HP-5 column combination, and c the HP-50+ × HP-5 column combination.

3.4.4 GC × GC analysis of diesel fuel

GC × GC has been used to characterize the composition of petroleum-based fuels since its inception [18–21]. The primary goal of many of these analyses is to separate complex fuel mixtures into compound classes. Under ideal circumstances, a GC × GC separation would generate distinct regions for each compound class. Petroleum fuels have been most commonly analyzed using a nonpolar primary column and a polar secondary column.[108-111] Under such conditions, the secondary retention order is acyclic alkanes < cyclic alkanes < monoaromatics < polyaromatics. Unfortunately, the class regions converge as the carbon number of the compounds is increased. In many cases, the regions are no longer baseline resolved at carbon numbers greater than approximately 14.[112] Reversing the stationary phase order reverses the secondary retention order of the compound classes, but the regions are still not fully resolved.[112] Thus, conventional columns are unable to completely separate complex petroleum mixtures into the four main compound classes.

The results described in the previous section showed that the IL × HP-5 column combination has a unique selectivity towards hydrocarbons. The saturated hydrocarbons (n-alkanes and cyclohexanes) occupied a very narrow region of the GC × GC chromatogram that was well separated from the n-alkylaromatics. Thus, the IL × HP-5 column combination could potentially be effective for the separation of complex fuels into well-defined saturated hydrocarbon and aromatic hydrocarbon classes. However, it is unlikely that the column combination would be effective at separating the saturated hydrocarbons into acyclic and cyclic classes.

Diesel fuel was analyzed by GC × GC with the IL × HP-5 column combination. The resulting chromatogram is shown in Fig. 3.6a. Three distinct groups of peaks are present. Saturated hydrocarbons (acyclic and cyclic alkanes) elute in a long, narrow band at the top of the chromatogram. Monoaromatics elute in a band situated directly beneath the saturated hydrocarbon band. These two regions merge slightly at high primary retention times. Diaromatic

compounds elute in a narrow band with low secondary retention times that is fully separated from the monoaromatic band.

For comparison, GC × GC chromatograms of diesel fuel were obtained with the DB-Wax × HP-5 column combination and the HP-50+ × HP-5 column combination. The DBWax × HP-5 chromatogram (Fig. 3.6b) displays distinct regions for the saturated hydrocarbons, monoaromatics, and diaromatics. However, the separation between the monoaromatic and diaromatic classes is much smaller in the DBWax × HP-5 chromatogram than in the IL × HP-5 chromatogram. Figure 3.6b clearly shows the difference in the saturated hydrocarbon selectivity between the ionic liquid and DB-Wax stationary phases. The saturated hydrocarbons in the DB-Wax chromatogram show a wide range of secondary retention times and occupy approximately 65% of the retention plane. In contrast, the saturated hydrocarbons in the ionic liquid chromatogram elute in a narrow band that occupies less than 25% of the retention plane. The HP-50+ × HP-5 chromatogram (Fig. 3.6c) displays distinct regions for the saturated hydrocarbons and the aromatic compound class; however, the aromatics are not clearly separated into monoaromatic and diaromatic regions. In addition, the saturated hydrocarbons occupy the majority of the retention plane in a fashion that is very similar to that observed for the DB-Wax × HP-5 chromatogram.

All three column combinations show a similar ability to separate saturated hydrocarbons from aromatic hydrocarbons. However, only the IL × HP-5 and DB-Wax × HP-5 are capable of separating monoaromatics from diaromatics. This separation is much more pronounced with the IL × HP-5 column combination. The IL × HP-5 column combination should be particularly effective at inter-class separation of petrochemical hydrocarbons because it confines the saturates, monoaromatics, and diaromatics into narrow bands. However, analyses that seek to isolate individual saturated hydrocarbons would be more effectively conducted with either the DB-Wax × HP-5 or the HP-50+ × HP-5 column combinations.

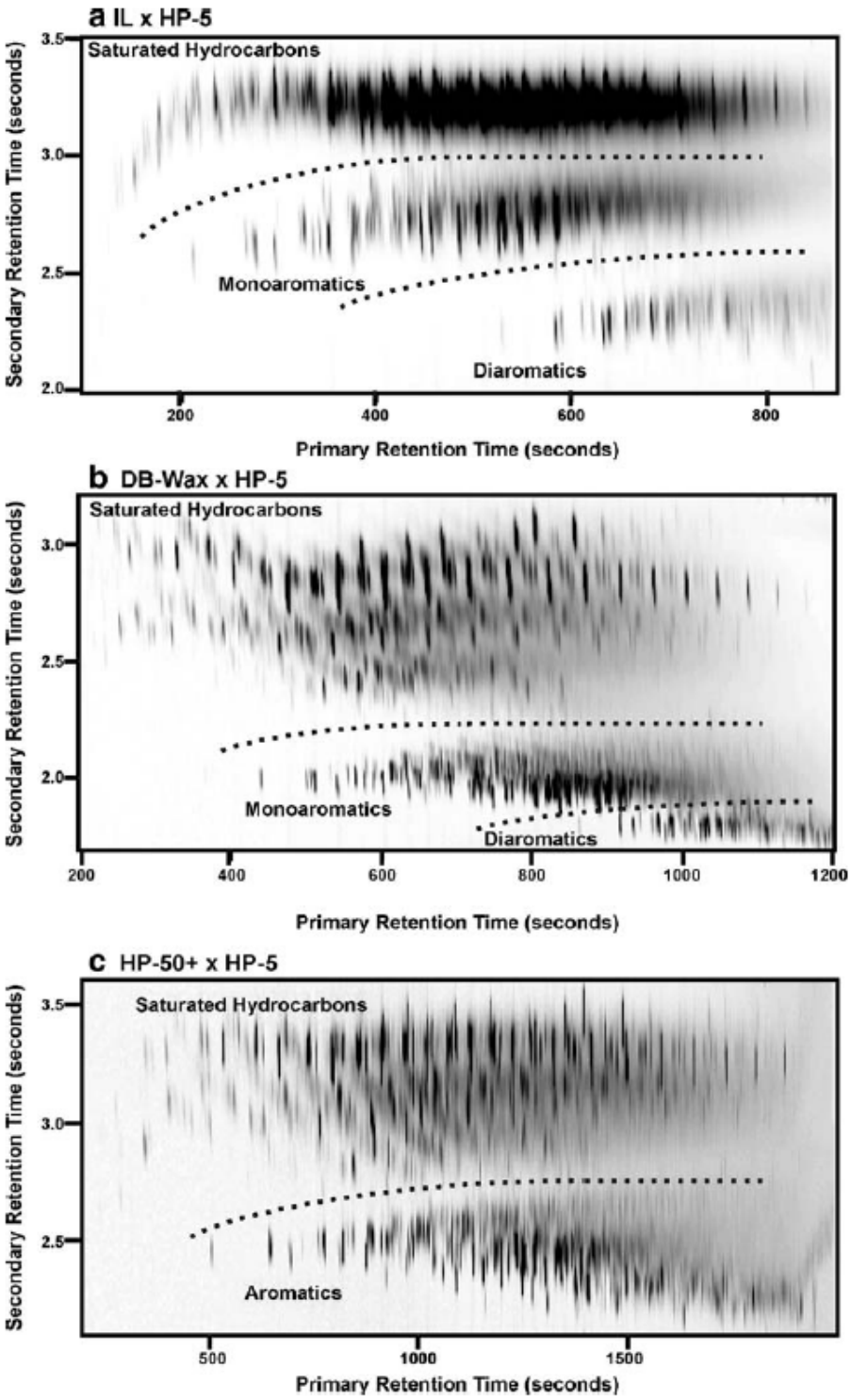


Figure 3.6 GC × GC separation of diesel fuel

3.5 Conclusions

This study demonstrates that ionic liquid stationary phases can be effectively used for GC × GC separations. The phosphonium IL stationary phase that was tested displayed strong interactions with hydrogen bonding and dipolar compounds. In addition, the IL phase is stable at temperatures well above those available to polymeric stationary phases that have similar selectivity toward hydrogen bonding and dipolar compounds (e.g., polyethylene glycol or cyanoproyl-substitued siloxanes). The IL stationary phase also displayed a unique selectivity toward low polarity hydrocarbons such as acyclic and cyclic alkanes and monounsaturated alkenes. These compounds were retained in a manner similar to nonpolar stationary phases. This characteristic resulted in GC × GC chromatograms where the low polarity hydrocarbons all occupied a narrow region within the chromatogram. The IL stationary phase when coupled with a nonpolar secondary column can be used to analyze complex petrochemical samples. Narrow bands were generated for saturated hydrocarbons, monoaromatics, and diaromatics with the diaromatics being fully resolved.

CHAPTER 4

TRIGONAL TRICATIONIC IONIC LIQUIDS: A NEW GENERATION OF GAS CHROMATOGRAPHIC STATIONARY PHASES

4.1 Abstract

Trigonal tricationic ionic liquids (ILs) are a new class of ILs that appear to be unique when used as gas chromatographic stationary phases. They consist of four core structures; 1. **A =** mesitylene core, 2. **B =** benzene core, 3. **C =** triethylamine core, and 4. **D =** tri(2-hexanamido)ethylamine core; to which three identical imidazolium or phosphonium cationic moieties were attached. These were coated on fused silica capillaries and their gas chromatographic properties were evaluated. They were characterized using a linear solvation parameter model and a number of test mixtures. Based on the literature, it is known that both monocationic and dicationic ionic liquids possess almost identical polarities, solvation characteristics, and chromatographic selectivities. However some of the trigonal tricationic ILs were quite different. The different solvation parameters and higher apparent polarities appear to generate from the more rigid trigonal geometry of these ILs as well as their ability to retain the positive charges in relatively close proximity to one another in some cases. Their unique selectivities, retention behaviors and separation efficiencies were demonstrated using the Grob mixture, a flavor and fragrance test mixture, alcohols/alkanes test, and FAME isomer separations. Two ionic liquids **C1** (methylimidazolium substitution) and **C4** (2-hydroxyethylimidazolium substitution) had higher apparent polarities than any know ionic liquid (mono, di and tricationic ILs) or commercial stationary phases. The tri(2-hexanamido)ethylamine core IL series proved to be very interesting in that it not only showed the highest separation efficiency for all test mixtures, but it also is the first IL stationary phase (containing Ntf_2^- anions) that eliminates peak tailing for alcohols and other H–bonding analytes. The thermal stabilities

were investigated using 3 methods: thermogravimetric analysis (TGA) method, temperature programmed gas chromatographic method (TPGC) and isothermal gas chromatographic method. The **D** core series had a high working temperature range, exceptional selectivities and higher separation efficiencies than comparable polarity commercial columns. It appears that this specific type of multifunctional ILs may have the most promising future as a new generation of gas chromatographic stationary phases.

4.2 Introduction

New types of stationary phases are explored constantly in order to come up with entities that have better physico-chemical properties in order to provide better stabilities, selectivities, resolutions and separation efficiencies for qualitative and quantitative determination of increasingly complex analyte systems. Ionic liquids have attracted much attention recently as stationary phases in gas-liquid chromatography (GLC) due to the unique properties these compounds seem to possess. These characteristics include negligible vapor pressure at room temperature, a wide liquid phase temperature range, good thermal stability, non-flammability, resistance to decomposition, ability to undergo multiple solvation interactions, ionic conductivity (>10^{-4} S/cm), and large electrochemical windows (>2V). These properties are highly desirable for many applications in areas of chemistry, physics, and engineering. Some of these applications include replacement for volatile organic solvents in organic synthesis,[2-3,64-65,68,71,113-118] solvents for high temperature reactions,[70] solvents for enzyme catalyzed reactions,[72,119-121] electrochemical applications in photovoltaic cells and fuel cells,[122-126] high temperature lubricants,[127-128] liquid – liquid extractions,[26,73-74,129-130] and mass spectrometric applications.[44,75,131-132] The thermal stability, ability to form multiple solvation interactions, and low volatility makes them ideal candidates for use in gas chromatography as stationary phases. In the recent past, ultra stable stationary phases based on ionic liquids were the focus of many important publications. [9,20,32-33,78-81,133-136] Based on the literature it is evident that multifunctional

ionic liquids can show greater thermal stability than most common monocationic ionic liquids in GC applications but have almost identical solvent propeties.[20]

However, when multiple cationic moieties are present the ionic liquid tends to be a solid at room temperature. For the best performance as a gas chromatographic stationary phase, it is necessary that the ionic liquid be a room temperature ionic liquid (RTIL). These are defined as ionic liquids that are liquids at ambient temperatures.[3] Literature indicates that the highest probability for a multi-functional ionic liquid to be a room temperature ionic liquid is by incorporating the bis(trifluoromethanesulphonyl)imide (NTf_2^-) anion.[20,22,40,137-183] This anion not only gives low melting ionic liquids but also shows high thermal stability.[22,138-139] These two characteristics make the NTf_2^- anion ideal for ILs in GC applications. However, there is a distinct disadvantage of using this anion. That is, NTf_2^- produces peak tailing for alcohols and sometimes for other H-bonding analytes. Many different types of cation combinations have been tested in order to come up with a solution for this peak tailing and so far these attempts have failed. In this work we introduce another class of ionic liquids that not only solves this problem but also provides unique properties and selectivities not found in previously reported ILs. These are trigonal tricationic ionic liquids and they are comprised of three positively charged moieties linked to a central core.

Since trigonal tricationic ILs are a new class of ionic liquids, it is necessary to characterize them based on their solvation properties and relative polarity compared to the general monocationic, dicationic ionic liquids and other common organic solvents. Many methods have been developed over the years for the characterization of ionic liquid solvation properties.[93-94] Some of the earliest developments include an empirical solvent polarity scale derived from either by a solvent dependent reaction rate constant or by the shift in maxima of an absorption or emission band of a solvatochromic dye or a fluorescent probe dissolved in a particular solvent.[37-38,93-94,139-140] However, these methods have failed to provide a comprehensive picture of the polarity of ionic liquids due to the fact that these are single

parameter polarity scales and therefore specific solvent-solute interactions are not taken into account. Ionic liquids can undergo multiple solvation interactions simultaneously such as ionic, dispersive, dipole-dipole, dipole-induced dipole, H-bond donating, H-bond accepting, π-π interactions and π-nonbonding interactions. Furthermore, ionic liquids may have complex extended three-dimensional liquid structures and possibly a supramolecular structure depicted by hydrogen bonding.[37] Hence, single parameter polarity does not correlate with the actual chemical environment of the ionic liquid. The next major development in this field comes with the inverse GLC application of Rohrschneider-McReynolds process.[36] In this method, specific solute-solvent interactions are evaluated by utilizing 5 probe molecules which are assumed to undergo only specific types of interactions.[36,142-143] However, due to the use of only one probe molecule per interaction, statistically the reliability of the values obtained is low.

The method used for the evaluation trigonal tricationic ILs is the Abraham solvation parameter model and it is the most comprehensive method available today. [41-42,107,144-147] This is based on a linear free energy relationship. The principle is similar to Rohrschneider-McReynolds method in that different types of solvent-solute interactions are evaluated separately. However, instead of one probe molecule, several probe molecules are used to characterize one interaction parameter increasing the reliability of the parameter coefficients obtained. The linear solvation energy relationship is given by equation 1:

$$\log k = c + eE + sS + aA + bB + lL$$

Here, the higher case letters *E, S, A, B* and *L* are solute descriptors. *E* represents excess molar refraction of the solute at 20 ^{0}C, *S* is solute dipolarity and polarizability, *A* is Hydrogen bond acidity, *B* is Hydrogen bond basicity and *L* is gas-hexadecane partition coefficient at 25 ºC. Solute descriptor values are evaluated and published in the literature for a number of solutes.[107] The lower case letters are assigned to characterize different types of interactions between the solutes and the solvent. In this case the solvent is the ionic liquid acting as the stationary phase. The value of these coefficients depicts the strength of the

interaction. Here *e* is π and non-bonding electron interactions, *s* is the ability of the phase to interact with dipolar/polarizable solutes, *a* is H–bond donating (H–bond basicity) interactions, *b* is H–bond accepting (H–bond acidity) interactions, *l* coefficient is composed of dispersion forces (positive contribution) and cavity term (negative contribution) and *c* is the system constant. For all the solutes the retention factor *k* can be calculated chromatographically. These values can then be subjected to multiple linear regression analysis (MLRA) to find the five coefficients and system constant.

4.3 Experimental

4.3.1 Materials

Figure 4.1 gives the structures of all the trigonal tricationic ionic liquids synthesized. The reagents 1-methylimidazole, 1-butylimidazole, 1-benzylimidazole, 1-(2-hydroxyethyl)imidazole, tripropylphosphine, 2,4,6-tris(bromomethyl)mesitylene, 1,3,5-tris(bromomethyl)benzene, tris(2-chloroethyl)amine hydrochloride, tris(2-aminoethyl)amine, 6-bromohexanoyl chloride and all the probe molecules in Table 4.1 were purchased from Sigma Aldrich. Detailed synthesis procedure is given else where.[22] Grob test mixture, flavor and fragrance mixture and alcohols and alkanes mixture were also purchased from Sigma Aldrich. The GC capillaries (250 μm internal diameter) were purchased from Supelco. The FAME isomer mixture and the commercial columns Equity-1701, SUPELCOWAX, and SP-2331 were graciously donated by Supelco.

4.3.2 Methods

For the determination of solvation parameter the ionic liquids were coated using static coating technique on salt treated fused silica capillary (5m x .25mm). In this method, the IL was dissolved in dichloromethane to obtain 0.25% (w/v) coating solution and this was injected from one end of the capillary. The capillary tube was kept inside a water bath at 40 ºC. After that, one end of the capillary was sealed while the solvent was evaporated from the other end under high vacuum conditions. Finally the coated columns were flushed with helium gas and conditioned overnight from 30 ºC to 120 ºC at 3 ºC/min. Efficiencies of the 12 IL columns were determined

by using naphthalene at 100 ºC and were higher than 2000 plates m^{-1}.For the determination of solvation parameters, 30 probe molecules were used. The solute descriptors for the 30 probe molecules are listed in Table 4.1. For the determination of each parameter, more than 4 probe molecules having significant range of solute descriptor values were used in order to meet the statistical requirement to obtain meaningful results for the parameters.[42] The solvation parameters were determined using inverse GC method at 2 different temperatures, 70 ºC and 100 ºC. Probe molecules were injected and retention times were measured in triplicate. Methane was used to measure the column hold-up time. For the trigonal tricationic IL columns the three retention factors calculated for each probe molecule were identical within the experimental error. The log of average retention factor from triplicate measurement (log *k*) and solute descriptors (*E, S, A, B, L*) were then subjected to a multi parameter linear least squares fit on Analyse-it for Microsoft Excel software to determine the coefficients. Helium carrier gas flow rate was set at 1 mL min^{-1} for all the analysis with split ratio 100:1. Inlet and detector temperatures were kept at 250 ºC. The values obtained for the solvation coefficients using inverse GC approach are listed in Table 4.4**.** The value *n* represents the maximum number of probe molecules that could be used for MLRA. The value is less than 30 because some compounds co-eluted with the solvent peak especially at the higher temperature. Also, some of the data points had to be removed in order to obtain higher correlation coefficients ($R^2 \geq 0.98$). It was noted that highly peak tailing analytes such as acetic acid and N,N-dimethylformamide were common among the analytes that were removed from the data set.

Separations of Grob test mixture, flavor and fragrance mixture, alcohols and alkanes mixture, and FAME isomers were carried out in Agilent Technologies 6890N Network GC System equipped with Agilent Technologies 5975 inert Mass Selective Detector. Colum dimensions: 30 m x .25 mm x 0.20 µm. Separation conditions for Grob test mixture: 40 – 190 ºC at 6 ºC/min. Flavor and fragrance mixture: 40 ºC for 3 min, 10 ºC/min to 150 ºC. Alcohols and alkanes mixture: 30 ºC for 3 min, 10 ºC/min to 160 ºC. FAME isomers: 165 ºC isothermal.

Table 4.1 List of solute descriptors

probe molecule	*E*	*S*	*A*	*B*	*L*
1,2-dichlorobenzene	0.872	0.78	0	0.04	4.518
phenol	0.805	0.89	0.6	0.31	3.766
octylaldehyde	0.16	0.65	0	0.45	4.36
valeraldehyde	0.163	0.65	0	0.45	2.851
o-xylene	0.663	0.56	0	0.16	3.939
p-xylene	0.613	0.52	0	0.16	3.839
m-xylene	0.623	0.52	0	0.16	3.839
cyclohexanol	0.46	0.54	0.32	0.57	3.758
nitrobenzene	0.871	1.11	0	0.28	4.511
N,*N*-dimethylformamide	0.367	1.31	0	0.74	3.173
2-Pentanone	0.143	0.68	0	0.51	2.755
1-nitropropane	0.242	0.95	0	0.31	2.894
toluene	0.601	0.52	0	0.14	3.325
benzaldehyde	0.82	1	0	0.39	4.008
pyridine	0.794	0.87	0	0.62	3.003
aniline	0.955	0.96	0.26	0.53	3.993
butanol	0.224	0.42	0.37	0.48	2.601
acetic acid	0.265	0.65	0.61	0.44	1.75
1-octanol	0.199	0.42	0.37	0.48	4.619
acetophenone	0.818	1.01	0	0.49	4.501
2-choloraniline	1.033	0.92	0.25	0.31	4.674
pyrrole	0.613	0.73	0.41	0.29	2.865
benzonitrile	0.742	1.11	0	0.33	4.039
propionitrile	0.162	0.9	0.02	0.36	2.082
1-chlorohexane	0.201	0.4	0	0.1	3.777
p-cresol	0.82	0.87	0.57	0.31	4.312
ethylphenyl ether	0.681	0.7	0	0.32	4.242
naphthalene	1.34	0.92	0	0.2	5.161
2-propanol	0.212	0.36	0.3	0.14	2.786
cyclohexanone	0.403	0.86	0	0.56	3.792

Thermal stability of ionic liquids was evaluated using three methods. The first method involves thermogravimetric analysis (TGA) of the pure ionic liquid at 10 ºC/min heating rate. The decomposition temperature of 5% weight loss of the sample is reported in Table 4.2. The second approach is a temperature programmed GC (TPGC) method where the ionic liquid is coated in a 5 m x .25mm capillary and a temperature ramp of 3 ºC/min was applied from 100 ºC until decomposition is observed (see Figure 4.2). Third method is carried out for **D** core ionic liquids only. In this method, the ionic liquid is coated on a NaCl treated fused silica capillary of 5m x .25mm x .15 µm film thickness. and the retention factor of naphthalene was determined at 100 ºC isothermally. It was then subjected to conditioning at higher temperatures for 12 hours

(see Table 4.3) and the naphthalene retention factor was determined again at 100 ºC after each conditioning step. This method yields the most relevant thermal decomposition temperature of the IL stationary phases.

4.4 Results and Discussion

4.4.1 Structures of the trications

Four types of central cores were investigated in this study (see Figure 4.1) and in the order of increasing flexibility they are; A mesitylene core, B benzene core , C triethylamine core, and D tri(2-hexanamido)ethylamine core. These core structures also vary in their ability to form hydrogen bonds with the NTf_2^- anion and other solutes. The A and B cores do not have any intrinsic H–bonding capabilities. In the case of C core, the central nitrogen can be H–bond basic and in D core, the central nitrogen and amide oxygen both can be H–bond basic and the amide hydrogen can be H–bond acidic. It is important to note that to the best of our knowledge this is the first time an amide group is introduced in to the cationic fragment of an ionic liquid. Detailed information of synthesis and impurities present in these ionic liquids were discussed elsewhere. [67] In summary, the purities of these ILs were checked using NMR methods, Mass spectroscopy and elemental analysis. The only detectable contamination found was H_2O which can be removed by drying in vacuum oven at 80 ^{0}C under P_2O_5.

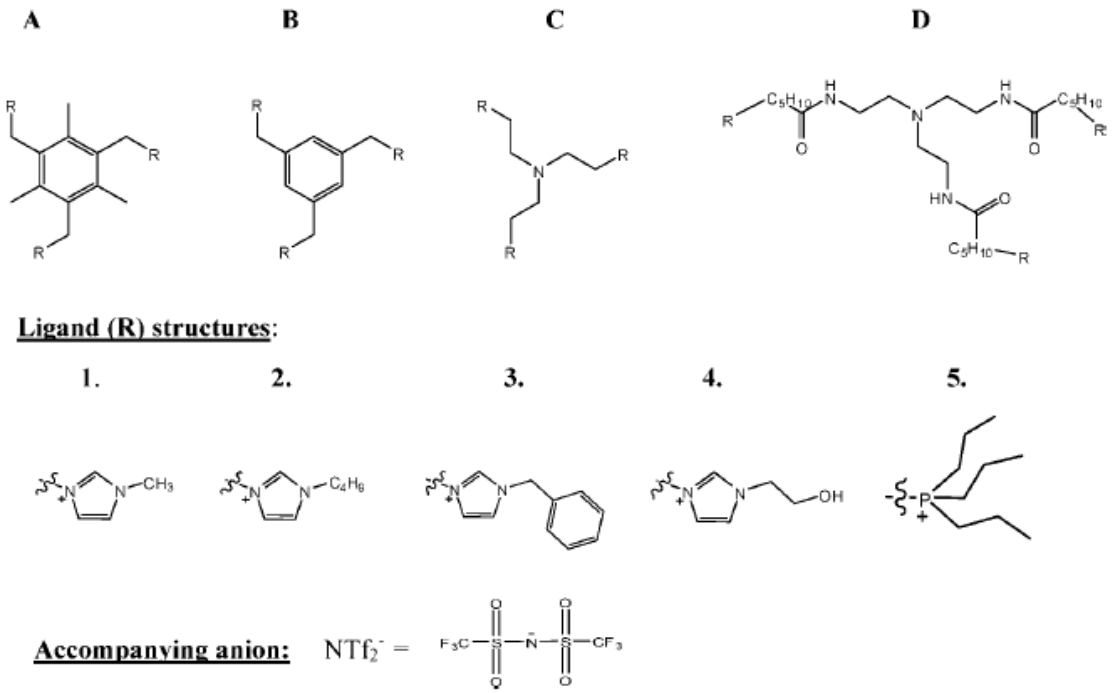


Figure 4.1 Structure of trigonal tricationic ILs

4.4.2 Physical properties of trigonal tricationic ionic liquids

The physical properties of this series of multifunctional ILs are summarized in Table 4.2. Ionic liquid A3 and C1 were solids at room temperature with melting points 62 and 37 ºC respectively. The remaining 10 ionic liquids were room temperature ILs with melting points below 20 ºC. The RTILs were viscous liquids that did not show any air or moisture sensitivity that leads to decomposition at laboratory atmosphere. Densities of these tricationic ILs lie in the range observed for common monocationic and dicationic ILs[138] and are between 1.41 g/cm^3 and 1.64 g/cm^3. Refractive indices range from 1.451 to 1.588. However viscosities of trigonal tricationic ILs are at least one or two orders of magnitude higher than those observed for typical monocationic ILs and dicationic ILs. In fact these are the highest viscosities reported for any ionic liquids. Ionic liquids D3 and D5 have the highest viscosities which range between 40,000-

45,000 cSt and 35,000-40,000 cSt respectively. High viscosities are preferable for ILs that are to be used as stationary phases in GC. [9,79] Furthermore when the benzyl imidazolium moiety is present the viscosities are markedly higher than other imidazolium cationic moieties. This trend was observed for symmetrical and unsymmetrical dicationic ILs as well.[138] This may be attributed to the added π-π stacking of the aromatic ring. Thermal stabilities of the trigonal tricationic ILs were measured using three methods (see Experimental Section) and a detailed discussion follows.

Table 4.2 Physical Properties of the Trigonal Tricationic ILs Used As Stationary Phases.

IL	M.W.	melting point (°C)	density[b] (g/cm^3)	refractive index	viscosity[c] (cSt)	thermal stability[d] (°C)
A3	1474.3	60−2	1.59			365
B1	1203.9	−38.6[a]	1.56	1.467	1280	414
B2	1330.2	−24.6[a]	1.53	1.467	2320	401
B3	1432.2	−87.4[a]	1.55	1.588	20,000−25,000	361
C1	1184.9	36−7	1.56			393
C2	1311.2	−47.5[a]	1.41	1.451	1580	363
C3	1413.2	−6.7[a]	1.51	1.493	25,000−30,000	381
C4	1275.0	−38.5[a]	1.64	1.460	7980	392
D1	1524.4	−16.4[a]	1.59	1.465	7760	351
D2	1650.6	−54.1[a]	1.49	1.466	10,200	335
D3	1752.7	−15.6[a]	1.54	1.495	40,000−45,000	337
D5	1758.8	−31.4[a]	1.48	1.466	35,000−40,000	388
BMIM-Cl[e]	174.7	65	1.10			145[f]
BMIM-TfO[e]	322.3	27	1.30	1.438	69.8	175[f]
BMIM-NTf_2[e]	419.4	−4	1.43	1.427	36.4	185[f]

4.4.3 Thermal stability

The thermal stability of ionic liquids is important as it defines the upper limit of the temperature range where the column can be used as a separation medium. Three methods were used to evaluate thermal stability: First two methods, TGA method and temperature programmed GC method were used to obtain a general idea of the thermal stability. Using the TGA method, the temperatures of 5% thermal degradation of the trigonal tricationic IL samples are reported as the decomposition temperature in Table 4.2. All ionic liquids were thermally stable to at least 335 ºC in the TGA method. In the TPGC method, the baseline rise at the

beginning of the decomposition event can be due to two reasons (see Figure 4.2). It can be due to the volatilization of the ionic liquid or it can be due to the actual decomposition. Either way, this region cannot be used for chromatographic separations due to the increasing baseline from column bleed. At the end of Table 4.2, the thermal stability of some common monocationic ionic liquids (determined by the TPGC method) is listed for comparison purposes.[9] It is important to note that the thermal stability values reported in Table 4.2 for monocationic ionic liquids can be directly compared to the values obtained for trigonal tricationic ionic liquids by the TPGC method in Figure 4.2 as both experiments employed identical procedures. It is evident that the tricationic ionic liquids are much more thermally stable than the common monocationic ionic liquids. All 3 monocationic ILs start to decompose or volatilize before 185 ºC while for the trigonal tricationic ILs, no appreciable bleed is observed before 280 ºC. There is at least a 90 degree advantage of workable temperature range for tricationic ILs over the common monocationic IL stationary phases in gas chromatography.

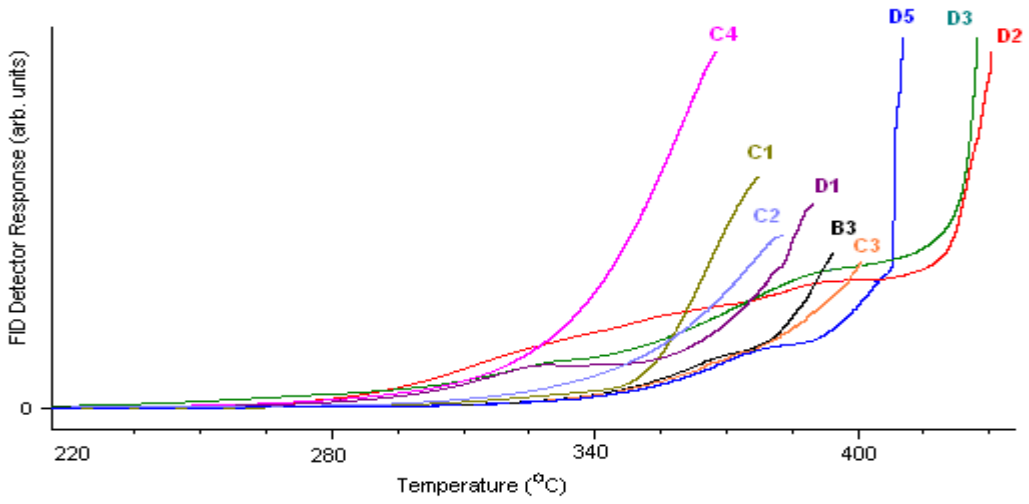


Figure 4.2 Temperature profile for column bleeding in gas chromatography due to thermal decomposition or volatilization of trigonal tricationic ionic liquids

Within the tricationic series since all the ionic liquids have the same anion, the thermal stability variation are solely due to variations in the cationic fragment. IL C4 with hydroxytheylimidazolium cationic moiety and nitrogen core seems to decompose at somewhat lower temperature whereas IL D5 with propylphosphonium cationic moiety and amide linkage shows the highest onset temperature of decomposition. In fact it appears that for D5 the base line does not start to rise appreciably until the temperature exceeds 315 ºC. The above mentioned two methods were used to present general thermal stability comparison between the trigonal tricationic ILs and other ionic liquid based stationary phases since these are the most common methods used in the literature. The third method, an isothermal GC method, is probably the most relevant method for the determination of actual upper limit of temperature for the use of ionic liquids as stationary phases. The retention factor of naphthalene after each thermal treatment is listed in Table 4.3. For all the D core ILs listed, symmetrical sharp peaks were obtained up to 290 ºC conditioning. At 300 ºC thermal treatment the columns show reasonable retention for naphthalene. However the peaks show some tailing. After 310 ºC, there was no retention which indicates column bleed of the ionic liquid stationary phase. These results confirm that the D core IL series is thermally stable up to 300 ºC as a GC stationary phase. In previous work it was shown that the phosphonium cationic moiety is more resistant to thermal degradation than most other N-based cations such as imidazolium and ammonium.[42] This implies that from this set of tricationic ionic liquids, D5 has the largest workable temperature range as a stationary phase in gas chromatography. It exists as a liquid for a range of 331 ºC from – 31.4 ºC to at least 300 ºC. This in it self is quite impressive compared to the monocationic ionic liquids which generally have a liquid temperature range of about 200 ºC or less. It is important to note that the commercial stationary phase SP-2331 which has similar polarity to D5 ionic liquid has an upper temperature limit of 275 ºC. Therefore, IL D5 has at least 25 ºC advantage over the comparable commercial stationary phase which in gas

chromatographic terms leads to better separation efficiencies for heavy and highly polar compounds.

Table 4.3 Variation of Retention Factors (k_{naph}) with Thermal Treatment of D Core Trigonal Tricationic IL Columns[a]

thermal treatment[b] (°C)	k_{naph} in **D1**	k_{naph} in **D3**	k_{naph} in **D5**
100	3.20	8.11	7.21
130	3.11	6.93	7.02
150	3.02	6.50	6.75
200	2.91	5.20	6.51
230	2.86	4.60	6.40
250	2.82	4.28	6.33
270	2.46	3.20	6.19
290	2.12	2.31	6.00
300	3.04[c]	2.34[c]	6.74[c]
310	[d]	[d]	[d]

[a]Measured isothermally for naphthalene, column temperature 100 ℃, He flow rate 1 mL/min. [b]Thermally treated for 12 h under He 1 mL/min. [c] Peak tailing was observed. [d]No retention was observed for naphthalene.

4.4.4 Ionic liquid solvation parameters

The solvation parameters obtained for the trigonal tricationic ionic liquids are listed in Table 4.4. In Table 4.5 these values are compared with the same parameters found for common monocationic and dicationic ionic liquids.[20,79] By comparison, nearly all interaction parameters obtained for monocationic and dicationic ILs are similar to each other whereas those obtained for some of the new tricationic ILs are quite unique. These unique parameters give rise to different behaviors in terms of retention, selectivity and separation efficiency.

Three of the five interaction parameter coefficients i.e., *s* = dipole–type interactions, *a* = H–bond donating interactions, and *l* = dispersion and cavity formation interactions have the greatest magnitude. This implies that solute retention is mainly due to these three types of interactions. Similar observations were made for monocationic and dicationic IL stationary phases.[39,40,43] Ionic liquid C4 (with hydroxyethylimidazole charge carrying moieties and a “N” core) had the lowest *s* and *a* terms ever reported for any ionic liquid stationary phases carrying

Table 4.4 Interaction Parameters Obtained for Trigonal Tricationic IL Stationary Phases

temperature (°C)	*c*	*e*	*s*	*a*	*b*	*l*	*n*	R^2
A3 (BzIM)Mst								
70(std. err.)	−3.34(0.10)	0.07(0.08)	2.02(0.09)	1.86(0.08)	0.72(0.11)	0.47(0.02)	29	0.99
100(std. err.)	−3.37(0.09)	0.10(0.07)	1.87(0.08)	1.61(0.07)	0.58(0.10)	0.39(0.02)	29	0.99
B1 (MIM)Ph								
70(std. err.)	−2.94(0.12)	0.14(0.10)	1.67(0.11)	1.68(0.10)	0.05(0.14)	0.50(0.03)	29	0.98
100(std. err.)	−3.00(0.11)	0.18(0.08)	1.51(0.09)	1.42(0.08)	0.02(0.12)	0.42(0.03)	29	0.99
B2 (BuIM)Ph								
70(std. err.)	−0.18(0.08)	0.07(0.07)	1.72(0.08)	1.80(0.07)	0.23(0.10)	0.56(0.02)	29	0.99
100(std. err.)	3.26(0.08)	0.09(0.07)	1.56(0.08)	1.57(0.07)	0.15(0.10)	0.48(0.02)	29	0.99
B3 (BzIM)Ph								
70(std. err.)	−3.49(0.10)	0.04(0.07)	2.11(0.08)	2.09(0.08)	0.46(0.10)	0.51(0.03)	28	0.99
100(std. err.)	−3.55(0.09)	0.06(0.07)	1.97(0.07)	1.78(0.07)	0.39(0.10)	0.43(0.02)	28	0.99
C1 (MIM)N								
70(std. err.)	−3.53(0.11)	0.05(0.08)	1.55(0.10)	1.81(0.08)	0.35(0.11)	0.53(0.03)	28	0.99
100(std. err.)	−3.70(0.12)	0.04(0.08)	1.58(0.08)	1.51(0.08)	0.31(0.11)	0.45(0.03)	25	0.98
C2 (BuIM)N								
70(std. err.)	−2.92(0.09)	0.05(0.07)	1.57(0.08)	1.55(0.07)	0.14(0.10)	0.55(0.02)	27	0.99
100(std. err.)	−2.98(0.09)	0.06(0.07)	1.43(0.08)	1.29(0.06)	0.16(0.10)	0.46(0.02)	27	0.99
C3 (BzIM)N								
70(std. err.)	−3.23(0.09)	−0.03(0.07)	1.85(0.08)	1.62(0.07)	0.37(0.10)	0.54(0.02)	28	0.99
100(std. err.)	−3.29(0.08)	−0.02(0.06)	1.10(0.07)	1.37(0.07)	0.30(0.09)	0.46(0.02)	28	0.99
C4 (HyIM)N								
70(std. err.)	−3.18(0.10)	0.22(0.09)	0.66(0.10)	0.95(0.09)	0.09(0.12)	0.67(0.03)	29	0.99
100(std. err.)	−3.05(0.09)	0.22(0.06)	0.45(0.07)	0.70(0.06)	0.03(0.08)	0.57(0.02)	25	0.99
D1 (MIM)NAmid								
70(std. err.)	−3.42(0.12)	0.23(0.09)	2.15(0.11)	2.82(0.11)	0.31(0.14)	0.43(0.03)	28	0.99
100(std. err.)	−3.58(0.12)	0.16(0.09)	2.10(0.10)	2.50(0.10)	0.17(0.14)	0.37(0.03)	26	0.99
D2 (BuIM)NAmid								
70(std. err.)	−2.89(0.13)	0.11(0.11)	1.59(0.12)	2.23(0.10)	0.05(0.15)	0.52(0.03)	28	0.99
100(std. err.)	−2.94(0.11)	0.10(0.09)	1.45(0.10)	1.84(0.09)	0.01(0.13)	0.45(0.03)	28	0.98
D3 (BzIM)NAmid								
70(std. err.)	−3.10(0.12)	0.07(0.10)	1.85(0.11)	2.29(0.10)	0.15(0.14)	0.50(0.03)	29	0.99
100(std. err.)	−3.16(0.10)	0.08(0.08)	1.69(0.09)	1.93(0.08)	0.10(0.11)	0.42(0.02)	29	0.99
D5 (PrP)NAmid								
70(std. err.)	−3.30(0.10)	0.13(0.08)	1.91(0.09)	2.72(0.08)	0.03(0.11)	0.52(0.03)	29	0.99
100(std. err.)	−3.34(0.10)	0.14(0.08)	1.72(0.09)	2.17(0.08)	0.07(0.11)	0.44(0.02)	29	0.99

NTf_2^- anion. Hence the C4 IL column exhibited the lowest retention for all the test mixtures investigated (Grob test, flavor and fragrance mixture, alcohols and alkanes, see Figures 4.4, 4.5 and 4.7.) The *e* term which corresponds to π- and nonbonding electron interactions is essentially negligible in this series of trigonal tricationic ionic liquids.

Table 4.5 Comparison of the Interaction Parameters of Monocationic and Dicationic RTILs with Trigonal Tricationic ILs

temperature (°C)	*c*	*e*	*s*	*a*	*b*	*l*	*n*	R^2
BMIM-NTf_2[a]								
70(std. err.)	−3.03(0.09)	0(0.08)	1.67(0.09)	1.75(0.09)	0.38(0.11)	0.56(0.02)	35	0.99
100(std. err.)	−3.13(0.12)	0(0.09)	1.60(0.10)	1.55(0.10)	0.24(0.12)	0.49(0.03)	32	0.98
C_9(mim)$_2$-NTf_2 [b]								
70(std. err.)	−2.95(0.14)	0.11(0.07)	1.76(0.08)	1.75(0.07)	0.20((0.10)	0.51(0.02)	33	0.99
100(std. err.)	−3.06(0.08)	0.11(0.06)	1.64(0.07)	1.50(0.06)	0.15(0.09)	0.43(0.02)	32	0.99
D1 (MIM)NAmid								
70(std. err.)	−3.42(0.12)	0.23(0.09)	2.15(0.11)	2.82(0.11)	0.31(0.14)	0.43(0.03)	28	0.99
100(std. err.)	−3.58(0.12)	0.16(0.09)	2.10(0.10)	2.50(0.10)	0.17(0.14)	0.37(0.03)	26	0.99

It was observed that the *s, a, b,* and *l* coefficients decrease with increasing temperature. The most substantial decrease in magnitude is observed for the *s* and *a* values (dipole–type interactions and H–bond basicity). This is mainly due to the fact that these interactions result from directional bonds and therefore depend largely on the orientation of the solute molecules and the interacting stationary phase molecules. As the temperature increases, translational and rotational energy of the molecules increase. This disrupts the intermolecular interactions between the solute and stationary phase and leads to lower retention and coefficients.[42] When benzylimidazole is the ionic liquid's charge carrying moiety, the H–bond acidity term (*b*) is increased compared to the other cationic moieties (with the exception of the D core ionic liquids in which the methylimidazole cationic moiety shows a higher *b* value). This is observed in symmetrical dicationic ionic liquids as well.[20] This may be due to the increased H–bond acidity of the bridging methylene hydrogens of the benzyl group. This contention is supported by previously published results (illustrated in Figure 4.3 which is a packing diagram of a symmetrical dicationic ionic liquid, C_3(methylimidazolium)$_2$ $2Br^-$). This crystal structure shows H–bonding between the acidic hydrogens and the bromide anion.[20] The hydrogens that are marked with a red arrow are from the methyl group substituted at the 3 position of the imidazole ring. If the methyl group is substituted by a benzyl group, these would be the bridging methylene hydrogens. In that case due to the presence of the benzene ring in the adjacent carbon these hydrogens would be even more acidic giving rise to a larger *b* term.

The D core series of tricationic ILs has the highest *a* values (i.e., hydrogen bond basicities). This is due to the three amide groups present in each ionic liquid. Both amide nitrogen and carbonyl oxygen can participate in H–bonding. The central core nitrogen may be sterically hindered for effective hydrogen bonding.

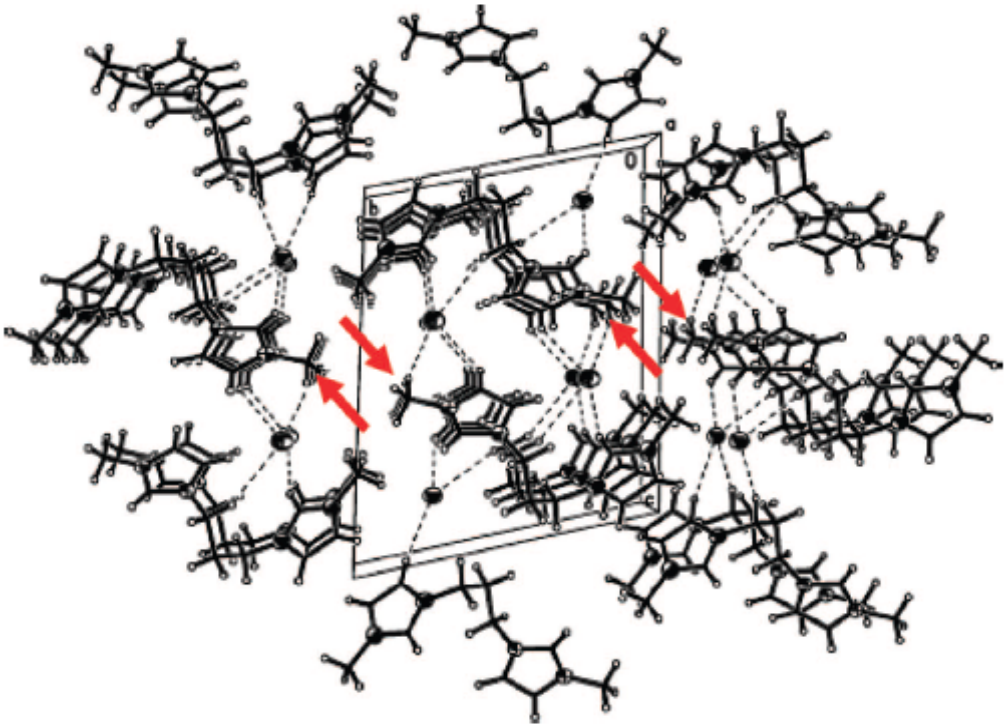


Figure 4.3 X-ray crystallographic data of $C_3(mim)_2 \cdot 2Br$ - showing stacks along short *a*-axis and H-bonding.

The *e* term is probably the least significant coefficient for most of the tricationic ILs investigated. It implies that the interaction between solute and IL stationary phase through π-π and non-bonding electrons is minimal compared to other types of interactions. One would expect the benzylimidazole cationic moiety to introduce some π – bonding interactions but that is not what is observed. The only statistically significant *e* terms are observed for the C4 ionic liquid, with the hydroxyethylimidazole charge carrying moiety; and for the D1 ionic liquid with methylimidazole charge carrying moiety. It is expected that C–core series has the lowest *e* term, as there are no core π – bonding electrons and only one relatively inaccessible non-bonding electron pair on the central nitrogen. However, C4 with hydroxyethylimidazole as the cationic moiety shows the highest π-π and n-π interactions among the trigonal tricationic IL series. Furthermore this ionic liquid has a very low H–bond acidity (*b* coefficient). This implies that the

hydroxyl group interacts with solutes through the non-bonding electrons of oxygen and not as much through H–bonding. Dispersion forces are one of the prominent type of interaction that contributes to analyte retention in these IL stationary phases. However, interaction of tricationic ILs through dispersion forces (*I* coefficient) seems to be similar since there is not much variation in the *I* value from one trigonal tricationic ionic liquid to another. The magnitude of *I* ranges from 0.43 to 0.67 and this falls within the range observed for symmetrical dicationic and monocationic ionic liquids.[9,20,79]

4.4.5 Grob test mixture

This is a single test mixture that is used to evaluate a capillary column chromatographically.[149-150] This mixture can be used to evaluate separation efficiency, acid/base characteristics, adsorptive activity and relative polarity of the column. The mixture contains 12 components and each peak gives information about the column. 1. n-decane and 2. n-undecane represents 100% recovery marker. Symmetrical sharp peaks are expected for properly produced and installed columns. 3. 1-nonanal is used to identify adsorption unique to aldehydes independent of H-bonding. 4. 1-octanol and 5. 2,3-butanediol peak shapes indicate presence of H-bonding sites. Reduced peak heights and unsymmetrical peak shapes for 6. 2-ethylhexanoic acid and 7. 2,6-dimethylphenol indicate H-bonding or basic sites. 8. methyl decanoate, 9. methyl undecanoate and 10. methyl dodecanoate are a homologous series of fatty acid methyl esters and is used to determine the separation efficiency of the column. 11. 2,6-dimethylaniline and 12. dicyclohexylamine peak shapes give information of the acidic nature of the column.

According to Figure 4.4, in trigonal tricationic ionic liquids with B and C core structures (see Figure 4.1), n-decane and n-undecane elute with or near the methylene chloride solvent peak and could not be separated with the given temperature program. Also, 2,3-butanediol, 2-ethylhexanoic acid, and dicyclohexylamine were either retained on the column or eluted with high peak tailing so that they were indistinguishable from the baseline. All of these 3

compounds are polar, have H–bonding capabilities and lack any aromatic substituents. Furthermore, 1-nonanal and 1-octanol show peak tailing and reduced peak heights. These results imply that the B and C core structures can produce IL stationary phases that are highly polar with H–bond accepting capabilities. The C4 IL shows the least retention for Grob test mixture compounds which is in accordance with the previously discussed solvation parameter coefficient results. The homologous series of fatty acid methyl esters were well separated with both B and C core IL stationary phases and showed good separation efficiencies. The acid 2,6-dimethylphenol and the base 2,6-dimethylaniline were the latest eluting detectable peaks. Despite the presence of H–bonding sites, these did not show much peak tailing. The elution orders on the B1 and C4 ILs were similar to highly polar commercial stationary phase SP-2331, whereas the elution order on the C1 IL stationary phase was more comparable with the polar SUPELCOWAX column.

All 12 test compounds were eluted from stationary phases with D core ionic liquids. In D3 and D5 the alkanes are better separated from the solvent peak compared to the B and C core ILs. All D-type columns separated the homologous series of fatty acid methyl esters with exceptionally good separation efficiencies. In both D1 and D3, the two bases elute after the acids which indicate that D1 and D3 are more acidic stationary phases than D5. This also is in agreement with the solvation parameter coefficients obtained. The H–bond acidity term (*b*) for D5 is significantly smaller than that of D1 and D3. In D5, the two acids elute last indicating a more basic, less acidic stationary phase. The D core IL stationary phases show elution orders similar to the highly polar SP-2331 column. This leads to the conclusion that the D series ionic liquids are highly polar and the polarity is comparable to that of SP-2331 (100% cyanopropyl polysiloxane) stationary phase.

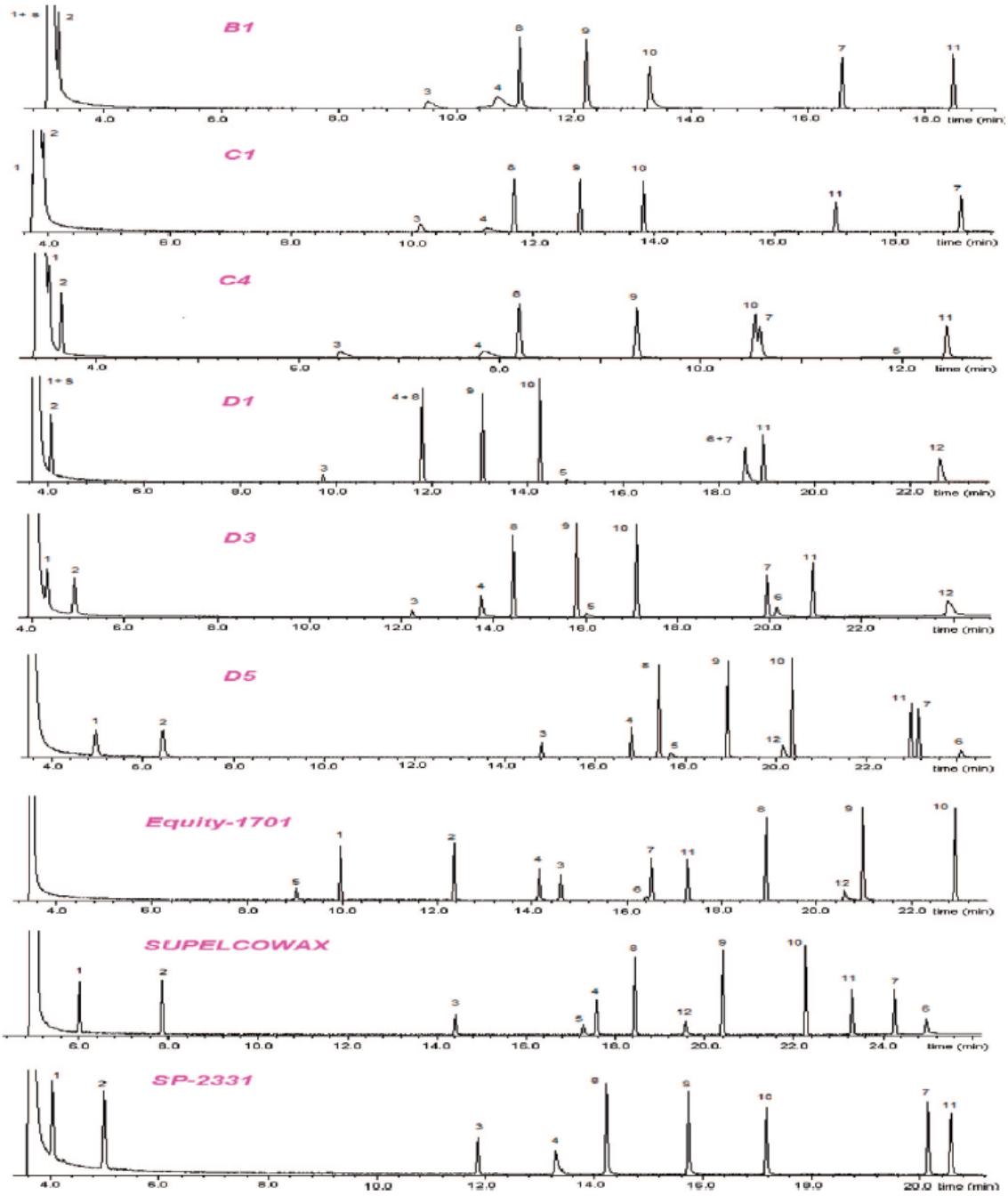


Figure 4.4 Separation of Grob test mixture in trigonal tricationic IL columns and commercial columns with varying degrees of polarity.

(1) *n*-decane, (2) *n*-undecane, (3) 1-nonanal, (4) 1-octanol, (5) 2,3- butanediol, (6) 2-ethylhexanoic acid, (7) 2,6-dimethylphenol, (8) methyl decanoate, (9) methyl undecanoate, (10) methyl dodecanoate, (11) 2,6-dimethylaniline, (12) dicyclohexylamine, s dichloromethane.

4.4.6 Alcohol and alkane mixture

Figure 4.5 illustrates the separation of a mixture of alcohols and alkanes by the six trigonal tricationic ionic liquid columns and commercial columns of diverse polarity. Except for C4, the other IL columns show reasonable retention for both polar alcohols and nonpolar alkanes. This is due to the dual nature of ionic liquids[45] which suggests that ionic liquids act like a polar medium to retain polar compounds and like a nonpolar medium to retain nonpolar compounds simultaneously. One of the most interesting observations of this separation is the relative retention of alcohols and alkanes by these IL columns. In all of the six tricationic liquid stationary phases, the relative retention of alcohols compared to alkanes is much larger than that observed for common monocationic; (Benzyl(methyl)imidazolium-TfO),[9] phosphonium monocationic; (Trihexyl(tetradecyl)phosphonium-NTf_2),[148] or polyethylene glycol linked dicationic; (MIM_2PEG_3-$2NTf_2$)[148] ionic liquid stationary phases (see Figure 4.6). For the B1 and C1 stationary phases, 1- hexanol elutes after hexadecane which is unusual. In fact, to the best of our knowledge, the C1 ionic liquid has the distinction of having the largest relative retention for 1-hexanol with respect to hexadecane ever reported for any commercial stationary phase or any ionic liquid stationary phase. The lowest overall retention for all alcohol and alkane components was again observed for the C4 column. All 18 compounds were eluted before 9.5 minutes. The shorter chain alkanes(pentane – nonane) seems to have almost no interaction with this stationary phase and comes out with the solvent peak under these conditions. It appears that the C1 and C4 ILs may be the most polar GC stationary phases yet reported. This high polarity is unique to trigonal tricationic ionic liquids. All other monocationic, dicationic and linear tricationic ionic liquids (unpublished work) show almost identical solvation characteristics and intermediate polarities. The higher apparent polarity and different solvation properties of trigonal tricationic ILs can be attributed to its more rigid trigonal geometry and the existence of three positive charges in close proximity compared to other forms of ILs.

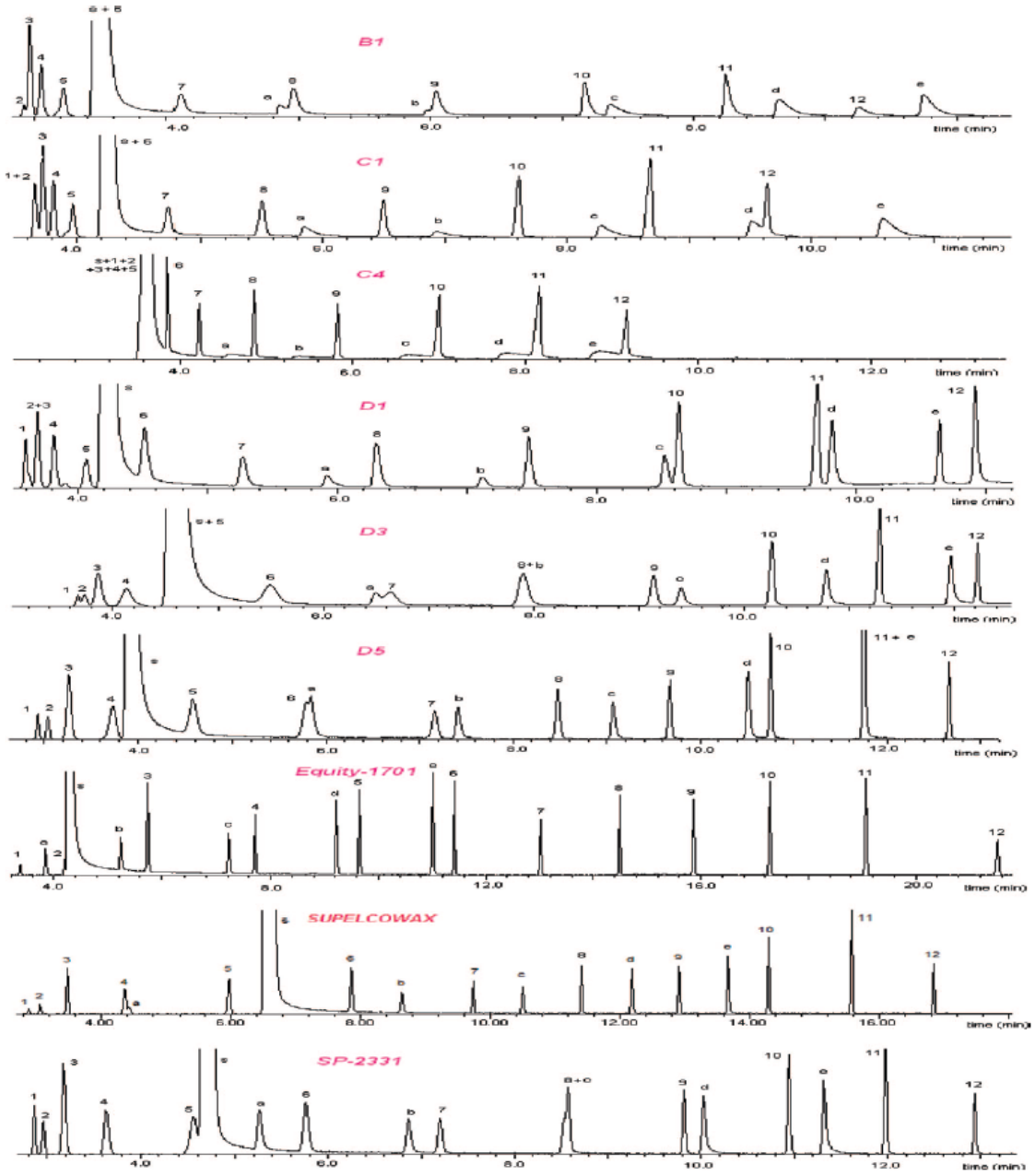


Figure 4.5 Separation of homologous alkane and alcohol mixture

(1) pentane, (2) hexane, (3) heptane, (4) octane, (5) nonane, (6) decane, (7) undecane, (8) dodecane, (9) tridecane, (10) tetradecane, (11) pentadecane, (12) hexadecane, (**a**) ethanol, (**b**) 1-propanol, (**c**) 1-butanol, (**d**) 1-pentanol, (**e**) 1-hexanol, (**s**) dichloromethane.

The B and C core ionic liquid stationary phases produce tailing peaks for the alcohols. This is a common phenomenon observed for ionic liquid stationary phases with the NTf_2^- (bis(trifluoromethane)sulfonimide) counter anion. Both monocationic and dicationic ionic liquids have shown peak tailing for alcohols and this has been one drawback of these types of ionic liquids as stationary phases. To overcome this problem NTf_2^- anion has been replaced by the triflate (TfO^-) anion.[40] However, with multifunctional ionic liquids the NTf_2^- anion is used more frequently in order to obtain lower melting point ILs.

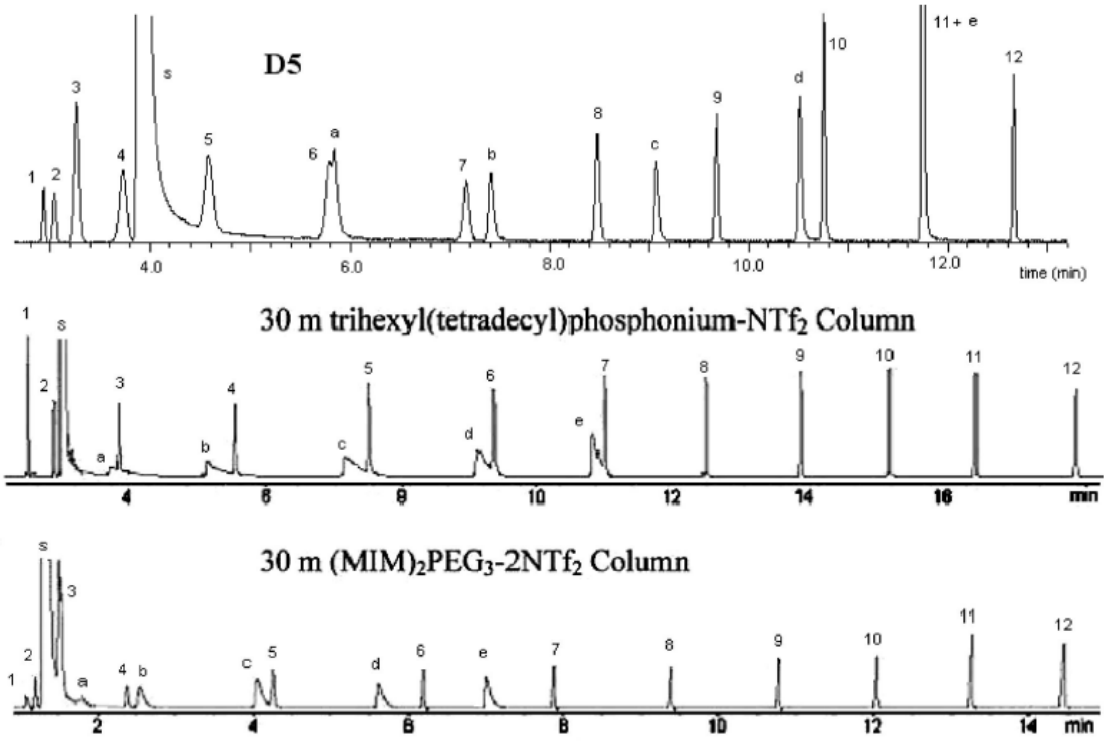


Figure 4.6 Comparison of separation of homologous alkane and alcohol mixture

(1) pentane, (2) hexane, (3) heptane, (4) octane, (5) nonane, (6) decane, (7) undecane, (8) dodecane, (9) tridecane, (10) tetradecane, (11) pentadecane, (12) hexadecane, (**a**) ethanol, (**b**) 1-propanol, (**c**) 1-butanol, (**d**) 1 pentanol, (**e**) 1-hexanol, (**s**) dichloromethane.

One of the unique and probably the more important property of the trigonal tricationic ionic liquid stationary phases is that the D core ILs have reduced and in the some cases almost

eliminated the peak tailing of alcohols even though the NTf_2^- anion is present. As discussed previously, the polarity of the D5 IL stationary phase is comparable to the highly polar SP-2331 commercial phase. However, as shown in Figure 4.5, peak asymmetry for alcohols is less for the D5 stationary phase than for the commercial SP-2331 phase at higher temperatures. Also nonane (5) and dichloromethane (s) were barely separated with SP-2331 phase whereas these compounds are much better separated with the D5 IL stationary phase. Similarly dodecane (8) and 1-butanol (c) co-elute in SP-2331 while these two are completely separated with the D5 IL column. Columns D1 and D5 seem to be complementary to one another in that one column always separates peaks that co-elute on the other (see Figure 4.5). For example hexane (2) and heptane (3) co-elute on D1 and are baseline separated on D5. Decane (6) and ethanol (a) peaks overlap on D5 and separate on D1. Pentadecane (11) and 1-hexanol (e) co-elute in D5 but are well separated on D1. Furthermore, the D-core ILs shows the greatest retention for alkenes among the trigonal tricationic ILs evaluated. The retention times of alkanes in D1, D3 and D5 columns are directly proportional to the solvation parameter coefficient of interaction through dispersion forces (coefficient *l*). Within the D series, D1 has the lowest *l* term, followed by D3, and D5. Accordingly, D1 has the lowest retention for alkanes within the D series followed by D3 and D5 which shows the highest retention. The dual nature of ionic liquids is evident from these separations as both the alkane series and alcohol series are easily separated. Finally it was observed that the retention of alkanes by the tricationic ionic liquids is generally lower than their retention on monocationic and dicationic ionic liquids.[9,80] Therefore other than the high separation efficiency and low peak tailing for alcohols, the D series of ILs have the distinction of being stationary phases that produce good separations for variety of analytes, but with less retention times than conventional columns. This might render trigonal tricationic ILs as desirable stationary phases in two dimensional GC analysis.

4.4.7 Flavor and fragrance mixture

The flavor and fragrance mixture contains structurally related esters (including two homologous series) and has 24 compounds. The separation of this series provides another indication of the selectivity and separation efficiency of the trigonal tricationic ionic liquids compared to commercial columns. According to the Grob test mixture and the alcohol/alkane test results, the commercial column SP-2331 has comparable polarity to the trigonal tricationic ionic liquids, especially the D core series. The flavor and fragrance mixture is used to determine the separation efficiencies of columns with comparable polarities (see Figure 4.7). Column C1 separated 21 compounds and co-eluted 3. The furfuryl homologous series elutes separately from the other esters. Only the last compound of the series furfuryl octanoate overlapped with benzyl butyrate. Again the C4 column showed the least retention for the mixture and all the compound were eluted before 14 minutes. Five compounds were not baseline separated with the C4 column. However, with the D series columns the compounds are much better separated. Especially with the D5 column, the retention and selectivity were comparable to a commercial (SP-2331) column, but the separation was much better. In D5 column, all the 24 compounds are nearly base line separated whereas in the commercial column three esters co-elute and two are only partially separated (isopropyl tiglate and ethyl hexanoate). This confirms the fact that D5 is a highly efficient and selective stationary phase for gas chromatography.

4.4.8 FAME isomer separation

Determination of fatty acid content in edible oils has been an area of high interest due to its importance in dietary, nutritional and therapeutic fields.[153] Generally these isomeric unsaturated carboxylic acids are converted to their methyl esters and the fatty acid methyl esters (FAME) are separated using highly polar stationary phases. [154-156] In this test, a mixture of methyl oleate (9*cis* 18:1) and methyl elaidate (9*trans* 18:1) were separated using the highly polar trigonal tricationic IL stationary phases D1, D3, D5 and the commercial SP-2331 stationary phase (see Figure 4.8). The t*rans*- isomer is twice the concentration (10 mg ml^{-1}) of

the *cis*- isomer (5 mg ml^{-1}) for ease of identification. In the D core trigonal tricationic ionic liquid columns and the commercial SP-2331 column, the *trans* FAME elutes before the *cis* analogue. It is characteristic for cyanopropyl based stationary phases to elute the *trans*- isomer first. With

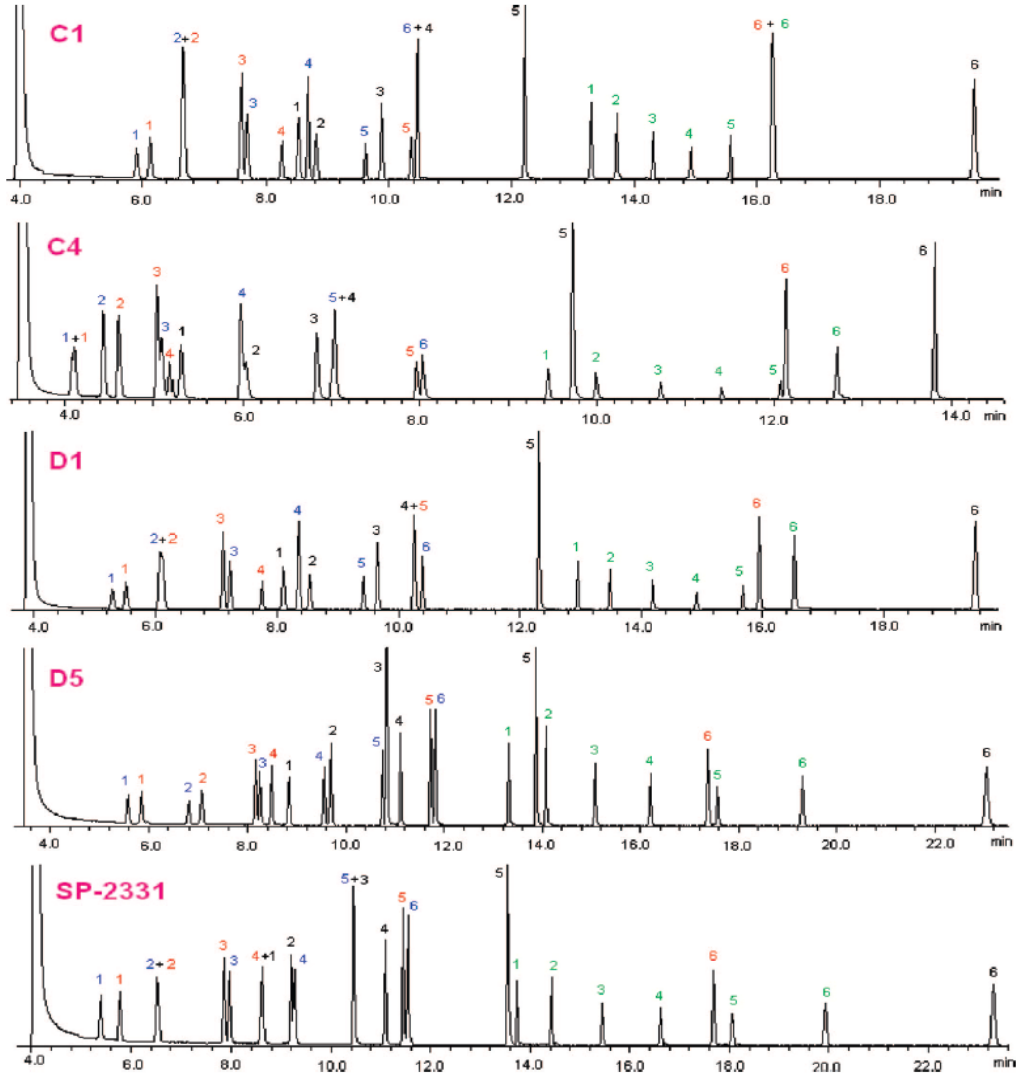


Figure 4.7 Separation of flavor and fragrance mixture.

(blue, 1) Ethyl propionate, (blue, 2) ethyl butyrate, (blue, 3) ethyl valerate, (blue, 4) ethyl hexanoate, (blue, 5) ethyl heptanoate, (blue, 6) ethyl octanoate, (red,1) methyl butyrate, (red, 2) isopropyl butyrate, (red, 3) propyl butyrate, (red, 4) allyl butyrate, (red, 5) hexyl butyrate, (red, 6) benzyl butyrate, (black, 1) methyl tiglate, (black, 2) isopropyl tiglate, (black, 3) propyl tiglate, (black, 4) allyl tiglate, (black, 5) hexyl tiglate, (black, 6) benzyl triglate, (green, 1) furfuryl propionate, (green, 2) furfuryl butyrate, (green, 3) furfuryl pentanoate, (green, 4) furfuryl hexanoate, (green, 5) furfuryl heptanoate, (green, 6) furfuryl octanoate.

polyethylene glycol-based stationary phases, the *cis*- isomer elutes first. This indicates that the D core ionic liquids when used as stationary phases are more similar to the highly polar cyanopropyl stationary phase, but with greater thermal stability. Under similar separation conditions, D5 shows the best separation for the *cis* and *trans* isomers by the D core series of ILs, followed by D3 and D1 respectively.

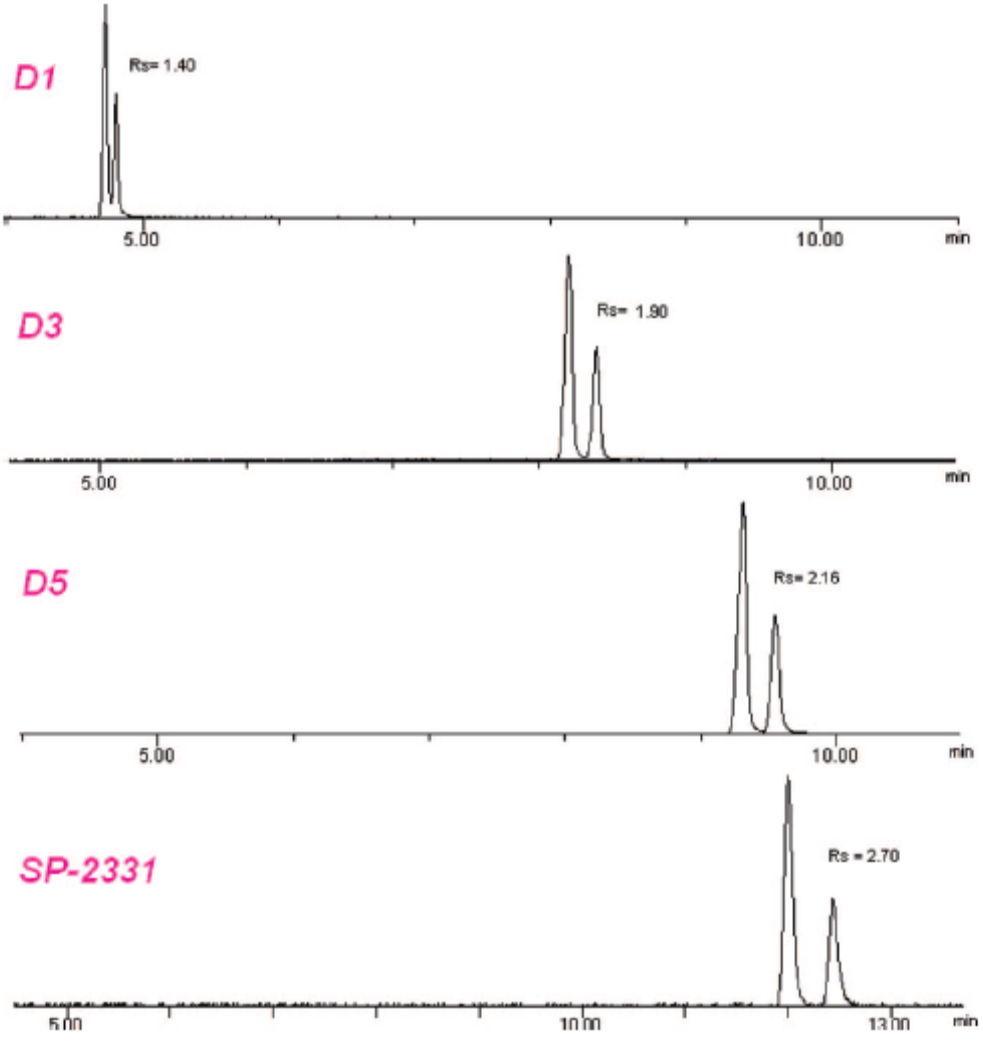


Figure 4.8 Separation of a mixture of methyl oleate and methyl elaidate

4.5 Conclusions

Use of multifunctional ionic liquids as stationary phases for GC can be limited as the most common counter anion for many ionic liquids (i.e., bis(trifluoromethanesulfonyl)imide) results in peak tailing for alcohols and other H-bond forming analytes. Specific D-core trigonal tricationic ILs were shown to overcome this problem. According to the solvation parameter study, all monocationic, dicationic and long chain linear tricationic ionic liquids have almost identical apparent polarities and interaction parameters. However, those were quite different for the some trigonal tricationic ILs, namely the C4, and D-core series, and resulted in unique selectivities and retention behaviors. This uniqueness appears to be the result of two main factors. First is the rigid trigonal geometry which forces the three positive charges to reside in close proximity for ILs with short linkage chains. The second is the contribution from the Amide group. The prominent interaction types of trigonal tricationic ILs were dipole–type interactions, H–bonding interactions and dispersive interactions. Alcohol/alkane mixture and Grob test mixtures indicated that these ionic liquids are far more polar than either the monocationic or dicationic ionic liquids reported thus far. Nitrogen core ionic liquids C1 and C4 were the most polar stationary phases and displayed very low retention for alkanes. Grob test, FAME isomer separation, and elution order of C18:1 *cis-trans* FAME isomers indicated that D core ionic liquids, especially D5 have polarities comparable to SP-2331, a 100% cyanopropylpolysiloxane commercial stationary phase. Ionic liquid D5 stands out as it shows minimum peak tailing for alcohols and other H–bonding analytes. According to the flavor and fragrance test, D1 and D5 are complementary to each other and show higher selectivity and superior separation efficiencies than the commercial SP-2331 stationary phase which has roughly comparable polarity. Furthermore, D5 is more thermally stable than the SP-2331. It was observed that benzylimidazolium cationic moiety introduces much higher viscosities to the ionic liquid systems. IL D3 has the highest viscosity among ionic liquids ever to be reported. All these

trigonal tricationic ILs were highly thermally stable and had a minimum liquid temperature range of about 300 ºC. These values far exceed those observed for traditional monocationic ionic liquids. According to these results, trigonal tricationic ionic liquid D5 is very promising as a highly polar stationary phase that has high thermal stability and yields symmetrical peaks for H-bonding analytes.

CHAPTER 5

EVALUATION OF DICATIONIC REAGENTS FOR THEIR USE IN DETECTION OF ANIONS USING POSITIVE ION MODE

5.1 Abstract

Twenty-three different dications were investigated for their effectiveness in pairing with singly charged anions, thereby allowing the electrospray ionization mass spectrometry (ESI-MS) detection of anions as positively charged complexes. Nitrate, iodide, cyanate, monochloroacetate, benzenesulfonate, and perfluoro-octanoate were chosen as representative test anions as they differ in mass, size-to-charge ratio, chaotropic nature, and overall complexity. Detection limits were found using direct injection of the anion into a carrier liquid containing the dication. Detection limits are given for all six anions with each of the 23 dications. Each anion was easily detected at the ppb (µg/L) and often the ppt (ng/L) levels using certain dicationic reagents. The ability of dicationic reagents to pair with anions and produce ESI-MS signals varied tremendously. Indeed, only a few dications can be considered broadly useful and able to produce sensitive results. Liquid chromatography (LC)-ESI-MS also was investigated and used to show how varying the dicationic reagent produced significantly different peak intensities. Also, the use of tandem mass spectrometry can lead to even greater sensitivity when using imidazolium based dications.

5.2 Introduction

Detection and quantitation of anions is important in a wide variety of scientific fields. Scientists in environmental chemistry, biochemistry, and the food and drug industries all routinely use analytical techniques to study anions. The most common methods for anion analysis include ion-selective electrodes,[157-159] ion chromatography (IC),[159-160] flow injection analysis (FIA),[161-12] and a variety of other spectroscopic and electroanalytical approaches. Mass

spectrometry is an obvious choice for detection of anions since they are charged species. The advent of electrospray ionization allowed routine analysis of the ionic components in a liquid sample.[163] By coupling ESI-MS with a separation method (i.e., liquid chromatography), a means to separate and detect most compounds is easily accomplished. However, while ESI-MS is widely used in both the positive and negative ion modes, the positive ion mode often is preferred as it can have lower detection limits and higher stability.[46,164-165] For positive mode analysis, an acidic additive is commonly employed to facilitate protonation of the analyte and to provide a stable electrospray. However, the addition of a basic compound to a water/methanol solvent system does not seem to provide a stable spray for negative mode analysis, resulting in fluctuations of the ion current.[46] It is known that corona discharge is more prevalent in the negative ion mode as opposed to the positive ion mode, which can produce a significant rise in background peaks and can also lead to reduced stability for the ion current.[166] Also, undesirable arcing is more prevalent in the negative ion mode. It has been suggested that halogenated solvents such as chloroform[167], hexafluoroisopropanol[168] and 2,2,2,-trifluororethanol[46] be used as opposed to more common solvents. These halogenated solvents produce an abundance of halogen ions at the capillary tip, resulting in a more stable spray formation. To reduce the occurrence of corona discharge, both electron-scavenging gases[56] and halogenated solvents[169] have proven useful. While carefully choosing amongst the aforementioned solvents may lead to better signals in the negative ion mode, it must be noted that these are not common solvents for use in LC, IC, or FIA. Ideally, one would like to be able to use common solvents such as methanol and water and also take advantage of using the positive ion mode, so less optimization is necessary and the problems with negative mode can be avoided.

Recently a method was developed to detect singly charged anions in the positive ion mode, thus eliminating the necessity of using negative ion mode and also eliminating any need for unconventional solvents. This method entails the addition of a small amount of a relatively large, chaotropic, organic dication to the carrier flow solvent, which can pair with a single anion

to give a positively charged complex of a higher *m/z*. This approach was first used for the trace analysis of perchlorate.[59-62] Most recently, it was shown to be advantageous for the analysis of over 30 different anions, proving its broad applicability and effectiveness.[63] There are several advantages to this method, among the more important of which are its ease of use and its sensitivity. Indeed, this single method provided the best reported limits of detection (LOD) for a variety of anions, proving to be more sensitive than negative ion mode ESI-MS methods as well as other analytical techniques.[63] Only a small concentration of a dication reagent is needed (tens of µM) and it can be added post-column if a separation method is employed so there is no effect on the separation. Finally, there is a key advantage to this method when it is employed with certain quadrupole instruments. By pairing the anion with a large dication, one can eliminate any problems with detection of an anion either below or near the low mass cutoff (LMCO). That is, whereas the anion previously may either have fallen below the LMCO or so close to it that detection was severely hindered, it can be paired with a dication, thereby moving the detected mass-to-charge ratio several hundred units higher, to a region of low background interference noise and few interfering peaks.

Apart from the original perchlorate study[59], there has not been any substantial amount of research done on what types of dications provide good or poor results. The dication that was found to provide the best results in the original study (1,1=-(nonane-1,9-diyl) bis(3-methylimidazolium), dication **VIII** in Table 5.1 was consequently used for multi-anion study of reference.[63] Obviously, differences in the structure and nature of the dication could cause a significant difference in its affinity for different anions, as well as its stability and overall efficacy. The purpose of this study is to examine the effects of using a variety of different types and structures of dication reagents, and to determine whether or not their selectivity, sensitivity, and efficacy vary for different anions. Our previous efforts included extensive research in developing dicationic compounds.[20,40,138,170] Originally synthesized as ultra-stable ionic liquids20, this research has led to the development of a wide array of dicationic compounds, including

imidazolium and phosphonium based dications, as well as those with differing linkage chains and even unsymmetrical species.[40,138]

Table 5.1 Structures and Masses of the Dications Used in this Study.

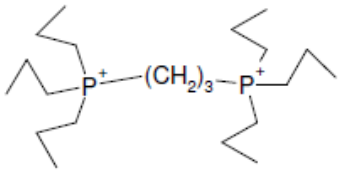
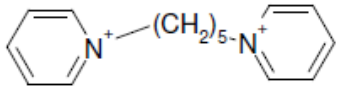
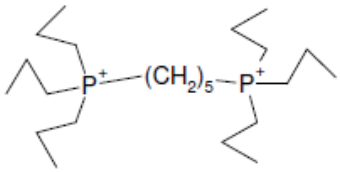
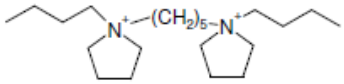
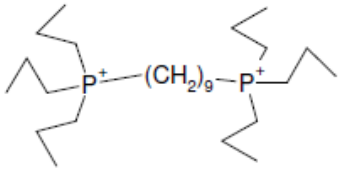
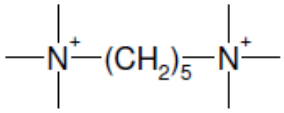
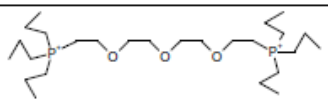
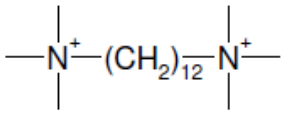
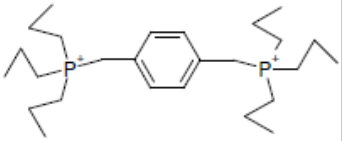
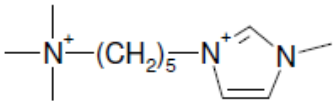
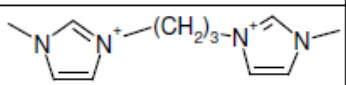
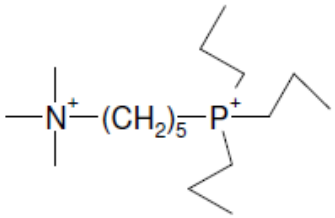
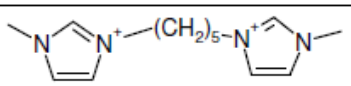
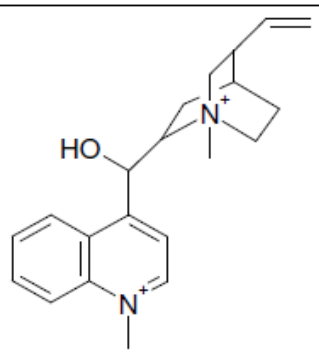
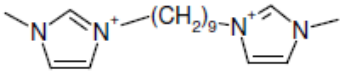
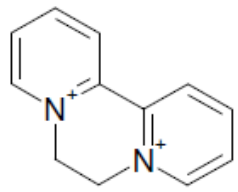
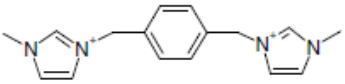
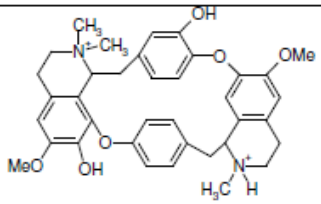
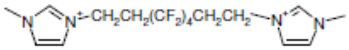
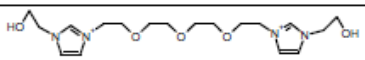
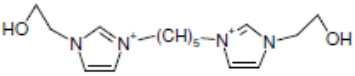
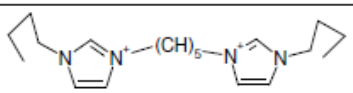
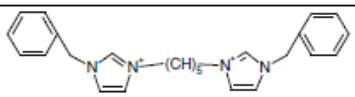
No.	Mass	Structure	No.	Mass	Structure
I	362.6		XV	228.1	
II	390.6		XVI	324.6	
III	446.6		XVII	188.4	
IV	480.6		XVIII	286.6	
V	424.8		XIX	211.2	
VI	206.3		XX	289.4	

Table 5.1 *continued*

VII	234.3		XXI	324.4	
VIII	290.3		XXII	184.1	
IX	268.3		XXIII	610.6	
X	420.4				
XI	384.4				
XII	294.3				
XIII	318.4				
XIV	386.3				

In this work, 23 dication salts are studied for their ability to form a complex with several different anions to be detected by ESI-MS. The salts encompass a wide range of cationic moieties (including imidazolium, pyrrolidinium, pyridinium and phosphonium-based cations), and

structures (differing chain lengths, aromaticity, symmetrical and unsymmetrical dications, etc.). Detection limits via direct injection are used to determine efficacy for the complex formed between the dication (dissolved in the carrier stream) and the anion of interest. The results are evaluated to discern which reagents provide the highest selectivity and sensitivity, as well as the structural features that make an effective or ineffective pairing agent. Finally, representative LCESI- MS analyses are done to illustrate the effect of using different dicationic reagents for anion analysis in the positive ion mode.

5.3 Experimental

Methanol and water were of HPLC grade and obtained from Burdick and Jackson (Morristown, NJ). Reagent grade sodium hydroxide and sodium fluoride were from Fisher Scientific (Pittsburgh, PA). Anions used were purchased as either the sodium/potassium salt or as the free acid from Sigma-Aldrich (St. Louis, MO). Stock solutions of each anion were made weekly. Chemicals used for the syntheses of the dicationic compounds were also obtained from Sigma-Aldrich.

Dication **I** from Table 5.1 was synthesized by dissolving one molar equivalent of 1,5-dibromopropane in isopropanol. To this solution, 3 M equivalents of tripropylphosphine were added. The resulting mixture was stirred and heated to reflux for 48 h. The solution was then cooled to room temperature and the solvent was removed by rotoevaporation. The crude product was then dissolved in deionized water and washed several times with ethyl acetate to remove any residual starting material. The water was then removed through rotoevaporation, followed by overnight drying in vacuum over phosphorous pentoxide. Dications **II**, **III**, **V–X**, and **XII–XVIII** were made in an analogous manner. Dications **XIX** and **XX** were synthesized by refluxing 1 M equivalent of (5-bromopentyl)-trimethylammonium bromide in isopropyl alcohol with 3 M equivalents of 1 methylimidazole and tripropylphosphine, respectively. The resulting product was then purified as described above. To produce dications **IV** and **XI**, synthesis of the dibromopolyethylene glycol linker chain was first needed. This was accomplished by dissolving

tetra-(ethylene glycol) in ether, which was then cooled in an ice bath and reacted with 1.1 M equivalents of phosphorus tribromide. The reaction was then refluxed for 2 h. Next, the reaction mixture was poured over ice to react the excess PBr3. The aqueous layer was discarded and the organic layer was washed four times with an aqueous sodium bicarbonate solution. The organic layer was then dried with sodium sulfate and filtered. Next, the ether was removed by rotary evaporator and the resulting linker was placed under vacuum overnight to ensure complete dryness. This linker was then reacted with the appropriate end groups to produce the dication. Dication **XXI** was synthesized by first dissolving one molar equivalent of cinchonidine in *N,N*-dimethylformamide at 80 ℃. Four molar equivalents of methyl iodide were then added to the mixture and allowed to react for 48 h. After the solvent was removed by rotary evaporation, the residue was dissolved in methanol. Upon addition of diethyl ether, the product precipitated out of solution, and was collected by filtration and then washed with cold ether. Dications **XXII** and **XXIII** are commercially available compounds (Sigma-Aldrich). All dicationic compounds were anion exchanged to their fluoride form to maximize complex formation between the dication and the injected analyte. This exchange procedure is given in reference.[59]

For direct injection analysis, a 40 µM dication-fluoride (DF2) solution was directed into a Y-type mixing tee at 100 µL/min via a Shimadzu LC-6A pump (Shimadzu, Columbia, MD). Also directed into the mixing tee was a carrier flow consisting of a 2:1 ratio of methanol to water at 300 µL/min from a Surveyor MS pump (Thermo Fisher Scientific, San Jose, CA). After the mixing tee, the final conditions were then 50/50 water/methanol with 10 µM DF2 at a flow rate of 400 µL/min. Sample introduction was done with the six port injection valve on the mass spectrometer using a 2 µL sample loop. A linear ion trap mass spectrometer (LXQ; Thermo Fisher Scientific, San Jose, CA) was used for this study. The ESI-MS settings were spray voltage, 3 kV; capillary temperature, 350 ℃; capil lary voltage, 11 V; tube lens voltage, 105 V; sheath gas, 37 arbitrary units (AU); and auxiliary gas, 6 AU. For the negative ion mode analysis, voltage polarities were reversed, while all other parameter settings were kept. ESI-MS settings

for the optimized MCA detection are as follows; spray voltage: 4.5kV, capillary temperature: 350℃, capillary voltage: 35 V, tube lens voltage: 80 V, sheath gas: 25 AU, and auxiliary gas: 16 AU. The ion trap was operated using single ion monitoring (SIM).

For the chromatographic experiments, sample introduction was done by a Thermo Fisher Surveyor autosampler (10 µL injections). The stationary phase used was a 10 cm C-18 (3 µm particle size) obtained from Advanced Separations Technology (Whippany, NJ). In the chromatograph of the multi-anion sample used for Figure 5.1, the column was equilibrated with 100% water at 300 µL/min. At 1 min, a linear gradient to 100% methanol began and was completed at 3 min. The addition of the DF2 solution was done post-column at 100 µL/min via the mixing tee. For the chromatographs of the benzenesulfonate samples, the mobile phase consisted of 100% water at 300 µL/min for the entire analysis. To help with spray formation, the DF2 was prepared as a methanol solution and again added post-column. For the negative ion mode runs, pure methanol was introduced into the mixing tee as opposed to the DF2 in methanol solution. The MS was again operated in SIM mode, monitoring the mass-to-charge ratio of each analyte for the entire run. Where single reaction monitoring was used, the normalized collision energy was set at 25 while the activation time was for 30 ms. Xcalibur and Tune Plus software (Thermo Fisher Scientific, San Jose, CA) was used for data collection and analysis.

The experimental parameters described above were adopted from reference.[63] The authors strongly recommend further optimization when using a specific dicationic reagent for use in the detection of (a) specific anion(s). It is believed that these detection limits may be lowered when considerable time is given to optimization or when using a more sensitive mass spectrometer.

5.4 Results and Discussion

Table 5.1 provides the structure and mass of the wide variety of dications used in this study. Dications **I-V** are phosphonium based while **VI-XIV** contain imidazolium structures (**X** also

contains a fluorocarbon linkage chain). Compounds **XV-XXIII** contain other charged moieties including trimethylammonium, pyridinium, and pyrrolidinium. In addition, some “mixed” and non-symmetrical dicationic entities are included (**XIX**, **XX**, **XXI** and **XXIII**). Table 5.2 lists the limits of detection (LOD) for each of the six representative anions (benzenesulfonate, cyanate, pefluorooctanoic acid, iodide, nitrate, monochloroacetic acid) when successfully paired with the 23 different dicationic reagents. These values were determined by direct injection ESI-MS (see Experimental) and are listed (from top to bottom) in order of sensitivity. Consequently, identifying the dicationic reagents that produce the best results (lowest LOD) as well as those which are ineffective is straight-forward (Table 5.2). The test anions were selected from to provide a cross-section of ions having different sizes and functionalities. Iodide, cyanate, and nitrate are relatively common and simple anions, but vary in size and number of constituent oxygen moieties. Benzenesulfonate (BZSN) was chosen as it is a somewhat larger organic anion and the only test analyte containing a sulfonate group. Monochloroacetic acid is a representative small haloorganic anion with environmental significance. Perfluorooctanoic acid (PFOA), a large, anionic fluorocarbon, is unlike any of the other anions. This, along with recent research interest in this as an environmental contaminant make it a good choice for inclusion in this study.

It was expected that using different types of positively charged end groups would lead to differing performance. To show this effect, ten different dicationic reagents that each contain the same pentane linkage can be compared. These ten include dications **II**, **VII**, **XII-XVII**, and **XIX-XX**. Of these, four outperformed the rest. Both dications **XIV** and **XVI** produced good results (low LODs) even when compared to all other dications, while **II** and **VII** did almost as well. While both **VII** and **XIV** are imidazolium based compounds, **II** and **XVI** contain vastly different charged groups (phosphonium and pyrrolidinium). It must also be noted that **XII** produced the worst results of these ten dications. Since **XII** is very close in structure and mass to **XIII**, it seems like the hydroxyl group leads to poorer detection limits. This is possibly due to its increased polarity

which would then lead to incomplete desolvation in the gas phase. It is of no surprise that BZSN paired better with the aromatic dications (other than **XII**) which points to pi-pi interactions playing a prominent role in gas phase association. Interestingly, both iodide and cyanate do not seem to pair well with the imidazolium based dications.

The length of the "chain" connecting the cationic moieties is another parameter to consider. There are several analogous dications in this study that differ only by the length of the hydrocarbon linkage chain. Namely, **I-III** consist of phosphonium based dications, **VI-VIII** are all methyl-imidazolium based, and X**VII** and X**VIII** are alkylammonium based. Looking at the phosphonium reagents, it can be seen that the C5 linked (**II**) and the C9 linked (**III**) behave similarly. However, the C3 linked (**I**) outperforms these with most of the anions tested, and by a wide margin. The only anions that are not improved upon are MCA (which have similar values) and cyanate. The opposite trend seems to be true for the methyl-imidazolium based reagents (**VIVIII**), in that the larger C9 linked dication **VIII** produces superior results compared to all of the shorter linked imidazoliums for all anions. The two alkyl-ammonium dications behaved similarly, apart from PFOA and cyanate. For both of these anions, the C12 linked dication (**XVIII**) produced significantly lower detections limits. However, both the C3 linked phosphonium and the C9 linked imidazolium dications produced lower detection limits than did **XVIII** for all anions except for cyanate.

The effect of using different types of linkage chains was also studied. Three different chain types were studied. A p-xylene linker was used for dications **V** and **IX**, tetraethylene glycol was used for **IV** and **XI**, and a fluorocarbon chain is present on **X**. In general, these more "exotic" linkage chain types were no better and generally worse than their corresponding optimal chain length hydrocarbon counterparts. Since the synthesis of these compounds is generally more complicated, there seems to be no advantage in using these linkage chain types.

A few dications studied did not fit into the categories above and thus, could not be compared in a systematic fashion. These compounds (**XXI-XXIII**) differ significantly from the others in that they do not contain two distinct charged moieties connected by a linkage chain. Some of these are naturally occurring compounds (**XXI**, **XXIII**) while one is a commercially available “diquat” (**XXII**). These types of compounds were not found to be useful for this method. Most of the anions could not even be detected as a complex with these particular dications. While it is unknown exactly how the dication interacts with the anion, it seems like an appropriate linkage chain that provides some flexibility is very important to ion association. This empirical observation may explain the poor performance of **XXI-XXIII** as well as why dications **V** and **IX** did not perform as well. The p-xylene linked dications (**V, IX**) are the most rigid amongst the symmetrical dications having a linkage chain. Clearly the flexibility of the dication is one factor that is important for complex formation. Ion mobility studies could provide insight into these dication-anion interactions and perhaps indicate how exactly the dication conforms to the anion.

From the results described above, a few reagents stand out above the rest. The first is dication **VIII**. This dication performs well for all anions apart from cyanate. The best dication to analyze cyanate was found to be dication **XVI**, which also performs well for the other anions, especially iodide and nitrate. Dication **I** is also a reagent that should be among the first to be evaluated when using this method for any other anion, as it was the top performer for both benzenesulfonate and iodide. Finally, while dication **XIV** was not the best for any particular anion, but it generally was in the top quartile for all of the tested anions, and thus also is considered to be among the most useful dicationic reagents. These four dications (**I**, **VIII**, **XIV**, and **XVI**) encompass a phosphonium based dication, a pyrrolidinium based dication, and two imidazolium based dications. Each of these has a different optimum hydrocarbon linkage chain length. It is recommended that these four dications should be evaluated first when analyzing an anion that has not been previously studied with this gas-phase ion association method.

Table 5.2 Limits of Detection for Each Anion as Detected as a Dication-Anion Complex.

NCO^- LOD		$PFOA^-$ LOD		NO_3^- LOD	
Dication	Mass Inj (ng)	Dication	Mass Inj (ng)	Dication	Mass Inj (ng)
XVI	6.00E-02	VIII	1.22E-04	VIII	1.84E-03
XVIII	8.00E-02	I	2.50E-04	I	5.00E-03
XXI	2.00E-01	XI	5.00E-04	VII	6.00E-03
III	3.00E-01	IV	2.00E-03	XVI	1.60E-02
IV	6.00E-01	II	3.00E-03	XIII	2.00E-02
II	6.00E-01	V	4.00E-03	XVIII	2.00E-02
XX	8.00E-01	XX	4.00E-03	XIV	2.00E-02
XVII	1.20E+00	XIV	4.50E-03	XVII	2.50E-02
XV	3.00E+00	XVI	6.00E-03	XII	3.00E-02
IX	4.00E+00	XIX	8.00E-03	XIX	4.00E-02
VIII	6.42E+00	VII	8.00E-03	IX	4.00E-02
X	8.00E+00	XVIII	1.00E-02	III	5.00E-02
XXIII	8.00E+00	III	1.00E-02	X	6.00E-02
I	1.50E+01	X	1.01E-02	II	6.50E-02
XIX	2.00E+01	VI	1.40E-02	IV	8.00E-02
V	2.00E+01	IX	1.41E-02	XX	8.00E-02
VI	2.00E+01	XIII	2.02E-02	XI	1.20E-01
VII	2.00E+01	XV	2.01E-02	V	2.00E-01
XIV	1.50E+02	XVII	5.00E-02	XV	2.00E-01
XII	ND	XII	6.06E-02	VI	6.00E-01
XIII	ND	XXI	1.60E+00	XXIII	ND
XI	ND	XXIII	ND	XXI	ND
XXII	ND	XXII	ND	XXII	ND

ND = Not Detected (150 ng highest amount injected)

Table 5.2 *Continued*

$BZSN^-$ LOD		MCA^- LOD		I^- LOD	
Dication	Mass Inj (ng)	Dication	Mass Inj (ng)	Dication	Mass Inj (ng)
I	1.03E-03	XVI	6.00E-03	I	1.08E-03
XIV	2.00E-03	II	6.18E-03	V	1.62E-03
V	2.06E-03	IV	6.18E-03	XVI	2.00E-03
VIII	2.06E-03	XIV	1.00E-02	IV	2.16E-03
X	4.04E-03	III	1.17E-02	XIV	4.00E-03
VII	5.00E-03	X	1.24E-02	XVIII	4.04E-03
XIII	5.00E-03	VIII	1.50E-02	II	4.32E-03
IV	6.18E-03	I	1.65E-02	VIII	6.00E-03
IX	7.00E-03	VII	1.80E-02	XX	6.48E-03
VI	8.00E-03	XVII	2.00E-02	III	6.48E-03
XV	8.08E-03	XIII	2.00E-02	VII	8.00E-03
XIX	1.00E-02	XX	2.06E-02	IX	8.08E-03
III	1.55E-02	XVIII	3.00E-02	VI	1.00E-02
XX	1.55E-02	IX	3.00E-02	XIII	1.21E-02
XII	2.00E-02	XV	6.36E-02	XVII	2.00E-02
XVI	2.00E-02	XIX	1.20E-01	X	2.00E-02
II	2.06E-02	XI	3.00E-01	XI	2.00E-02
XVII	4.00E-02	XII	5.00E-01	XII	3.04E-02
XI	5.00E-02	VI	2.00E+01	XIX	5.00E-02
XVIII	1.00E-01	XXI	2.06E+01	XV	1.50E-01
XXI	4.00E+00	XXIII	4.12E+01	XXIII	4.32E+01
XXII	ND	V	5.16E+01	XXII	ND
XXIII	ND	XXII	ND	XXI	ND

ND = Not Detected (150 ng highest amount injected)

It should be stated that the interpretation of the empirical results stated thus far has been primarily explained as a consequence of differing binding affinities between the dicationic reagent and the anion. However, it is essential to consider instrumental factors and the role they play in the sensitivity of these measurements. This is particularly true since only a single set of instrumental parameters was used for all dication-anion complex experiments. To demonstrate how instrumental response can significantly alter sensitivity, a complete optimization of instrumental parameters was done for the determination of monochloroacetate (MCA) using

dication **XVI**. After optimization of both the electrospray and mass spectrometer parameters (see Experimental), the limit of detection was reduced by a factor of three (from 6.00 pg to 2.00 pg, results not shown). It can clearly be seen that individual optimization will produce increased sensitivity for most of the anions in this study, and that instrument settings/configurations are important.

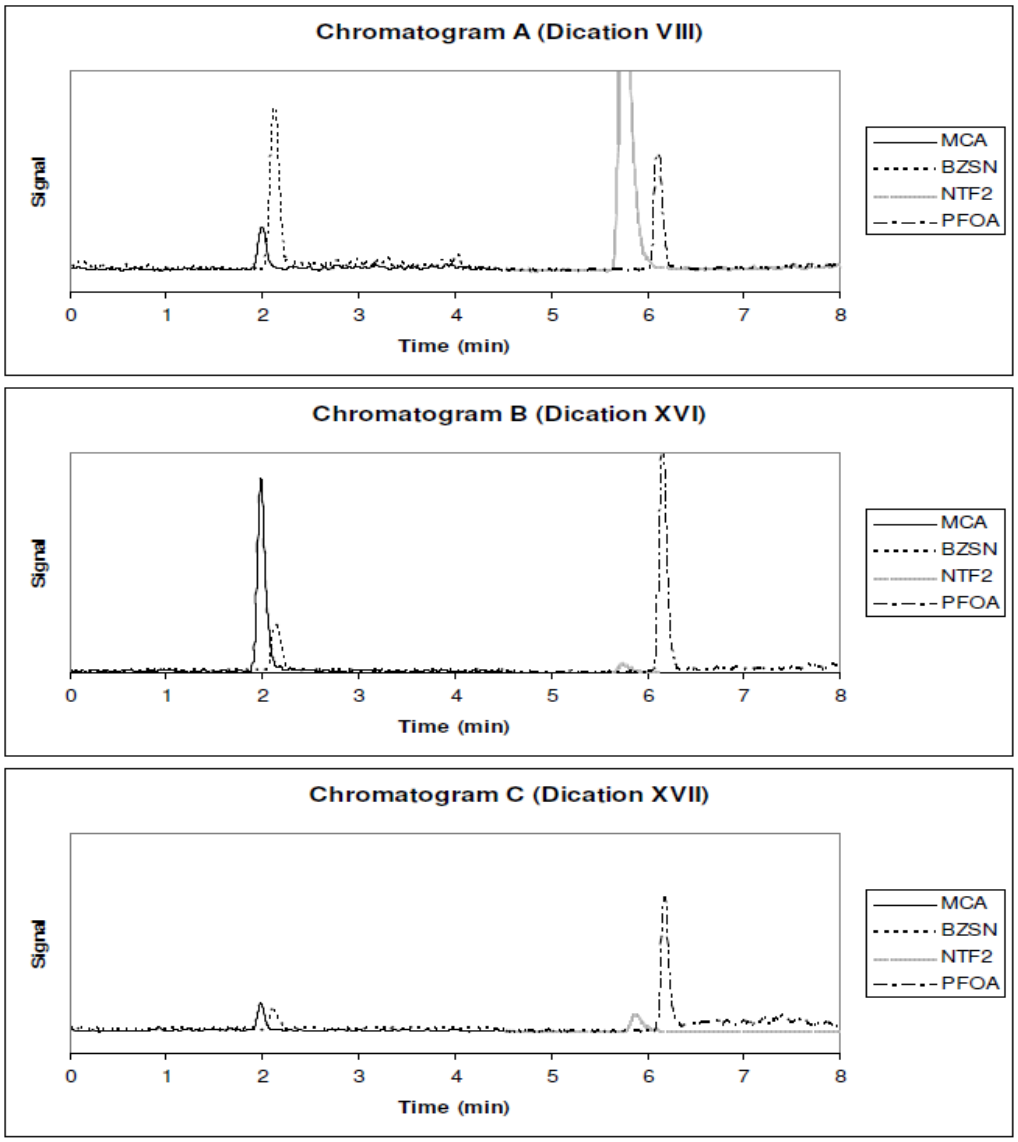


Figure 5.1 Chromatograms showing the separation of a sample containing four anions.

As an illustration of the pronounced effect of different dicationic reagents on the positive ion ESI-MS signal of anions, three analogous LC-ESI-MS analyses were compared (see Figure 5.1).Two of the recommended dications are used (**VIII,** Figure 5.1A and **XVI,** Figure 5.1B), as well as a moderately successful but not recommended dication **XVII** (Figure 5.1C). Each cation/anion complex was monitored at its appropriate m/z (i.e., the sum of the mass of the dication and the mass of the anion). As can be seen, significant changes in peak area occur for each anion in successive chromatograms. As expected, the recommended dications (chromatograms A and B) outperform dication **XVII**. It should be noted that the worst performing dications (those in the bottom quartile of Table 5.2) would produce peaks that could not be discerned under the conditions of Figure 5.1. Also apparent in Figure 5.1 is that there are great differences even between the two recommended dications. So while the recommended dications generally perform well across the board, one should always be sure to test at least three or four of the reagents to obtain optimal signal intensity.

Often, this method can achieve significantly lower limits of detection by using tandem MS capabilities. Since this method takes place in the positive mode, the daughter fragment formed after excitation also must be a positive ion, which is a fragment of the dication used. This is another key advantage of using this approach when determining the concentration of structurally-simpler anions (e.g. iodide) that cannot undergo fragmentation under MS/MS analysis in the negative ion mode. In a previous study, it was found that when a dication anion complex was excited, it lost the anion and either a proton or a methylimidazolium group, resulting in a singly charged fragment that was left for detection. In many cases this reduced the LOD for a variety of anions. This is one distict advantage of using the imidazolium-based dicationic reagents, as they lend themselves to MS/MS fragmentation more easily than other dications. A typical mass spectrum of the mobile phase under operating conditions (see Experimental) is shown in Fig. 5.2. The dication used in this instance is compound **XIV**. Several discernable fragments can be seen in the background even without excitation. The main

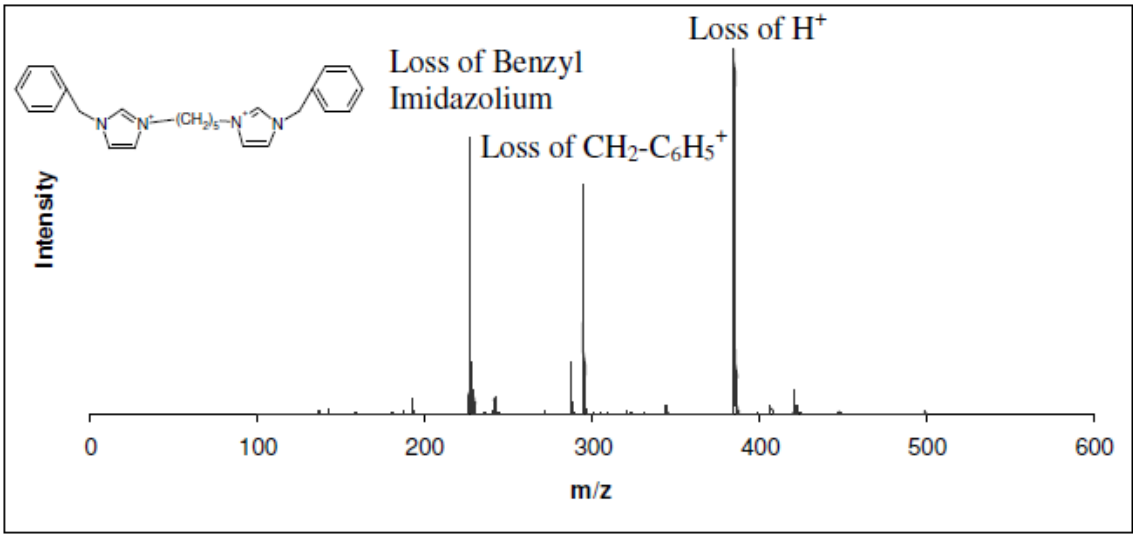


Figure 5.2 Mass spectrum of the mobile phase containing the dicationic reagent.

fragments include the peaks at 227.3 (loss of benzyl imidazolium), 295.3 (loss of [CH2-C6H5]+), and 385.3 (loss of the acidic proton in the 2- position of imidazolium). Any of these peaks can be monitored after the excitation of the dication-anion complex, usually resulting in a significant increase in sensitivity. This increased sensitivity is illustrated in Figure 5.3, which shows three separate chromatographic runs of 100 ng/mL of benzenesulfonate. While operating under negative ion mode with the addition of methanol post column, a peak can be seen which gives a moderate S/N of 14. By simply using 40 µM of dication **XIV** in methanol and changing to the positive ion mode, an instant increase in the S/N of almost 10 fold (to 128) is seen. It can easily be seen why this approach is advantageous. This peak can even further be increased by the application of single reaction monitoring (SRM). When the transition of the complex mass (m/z =543.3) to the fragment observed at 227.3 (loss of both the anion and benzyl imidazolium group) is monitored, the S/N increases to 510. This is a 36-fold increase over using the "traditional" negative ion mode to monitor an anion. Since ESI is a "soft" ionization source, the relative abundance of fragments is surprisingly high. The amount of fragmentation seems to be dependent of the capillary temperature. A lower capillary temperature, while decreasing the

amount of fragments, did not lead to an increase in sensitivity (possibly due to incomplete desolvation), while a higher capillary temperature (> 400° C) actually led to decreased sensitivity. Interestingly, these types of fragments are only readily seen when using the imidazolium based dications, as other dications (such as phosphonium or pyrrolidinium types) did not lend themselves to fragmentation as well and consequently, no increases in sensitivity were seen when using MS/MS. So while phosphonium or pyrrolidinium based dicationic reagents produce excellent results when using SIM, imidazolium based reagents should be evaluated if MS/MS capabilities are available. The four dications recommended above include two imidazolium based dications that can be used in MS/MS analysis.

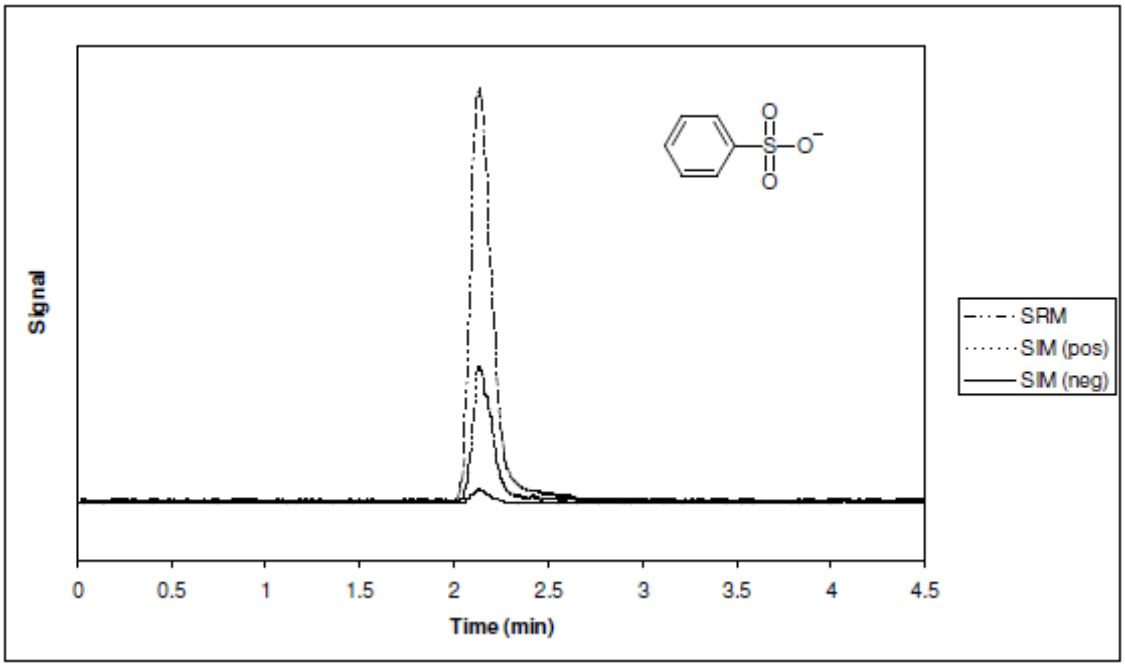


Figure 5.3 Chromatograms of benzenesulfonate in negative, positive, and SRM.

5.5 Conclusions

The use of dicationic reagents to detect singly charged anions via gas phase ion association has been shown to be a highly sensitive method and offers several significant improvements over using the negative ion mode when using tradition solvents. In this work, twenty-three different dications were evaluated to give insights as to the significant differences in dicationic reagents and which ones were most broadly useful. Four specific dicationic reagents (out of 23) stood out as far as producing superior performance and these are recommended when analyzing other anions. It was shown how this approach can be easily coupled to chromatography to study multiple anions. Also, the importance of choosing the correct dication in order to get significant signals for the anions of interest is demonstrated. Finally, the advantage of using the imidazolium based dications is shown through the application of MS/MS. Further work is needed to determine exactly how the dications interact with anions before any predictive capabilities are possible. Future work will include using this method to lower detection limits of methods that employ the negative ion mode and the possibility of studying doubly charged anions using tricationic species.

CHAPTER 6

EVALUATING THE USE OF TRICATIONIC REAGENTS FOR THE DETECTION OF DIVALENT ANIONS IN THE POSITIVE MODE BY ESI-MS

6.1 Abstract

The analysis of anions remains an important task for many areas of science and new sensitive analysis methods continue to be of great interest. In this study we discuss the use of seventeen tricationic reagents for use as gas phase ion pairing agents for divalent anions. When the anion pairs with the tricationic reagent, an overall positive charge is retained and enables detection by ESI-MS in the positive mode. The seventeen tricationic reagents were made from one of four core structures and seven terminal charge groups. The effect of these structural elements on the detection sensitivity of the complex is examined empirically. A comparison of signal to noise ratios achieved in positive and negative modes also is presented.

6.2 Introduction

The analysis of anions remains an important task for many areas of science including environmental analysis, the pharmaceutical industry, and the food industry. Flow injection analysis and separation techniques such as ion chromatography have employed ion-selective electrodes[171-178] and spectrophotometric techniques[176-177] to detect anions. However, these detection methods are generally not considered to be universal detectors. Conductivity detection can be used as a universal detector for anions, but the lack of specificity can be a problem for complex samples, even when combined with a separation technique.[183] Mass spectrometry is growing in popularity as a universal detector for anions and it can be used alone[178-179] or in combination with a separation method.[180-184]

The negative ion mode is the most common way of detecting anions using ESIMS. However, operating in negative ion mode with standard solvents found in chromatography

(primarily water and methanol) can lead to corona discharge, poor spray stability, and a propensity for arcing.[46,166] These effects can be suppressed by using electron scavenging gases[56] or halogenated solvents.[166-169] The substitution of isopropanol or butanol[164] for methanol has also been recommended for operation in negative ion mode. However, these solvents are less commonly used in LC methods involving water and result in higher operating pressures.

Recently, we have successfully used dicationic reagents to detect singly charged in the positive mode by ESI-MS.[59,63] The dicationic reagent paired with the anion in the gas phase and enabled detection in the positive mode using common LC solvents. Additional benefits include (a) moving anions to a higher mass range out of the low mass region dominated by chemical noise (b) increasing sensitivity for anions with masses near the low mass cutoff of quadrupole instruments (e.g. traps), and (c) helping to discriminate against interferences with the same mass to charge ratio. This approach has also been used with ion chromatography to determine the levels of perchlorate and two other anions in human urine, milk, and seawater.[60-62] The success of dicationic reagents to detect singly charged anions in the positive mode has encouraged us to use a similar approach for the detection of doubly charged anions. When various dicationic reagents were used to detect singly charged anions in the positive mode using ESI-MS, it became clear that some dications were better suited for this type of application than others.[185] Thus, the goal of this note is twofold: (1) to serve as a proof of concept that doubly charged anions can be detected in the positive mode in ESI-MS using tricationic reagents and (2) to begin identification of the structural elements of the tricationic reagents that will enable sensitive detection. Here, we report our findings to these ends.

6.3 Experimental

6.3.1 Tricationic reagents

Figure 6.1 gives the structure of the seventeen cationic reagents used in this study. After purification, the tricationic salts were exchanged to the fluoride form using the procedure

reported previously with some modifications.[59,63] The same amount (4 mLs) of anion exchange resin was packed into a disposable 10 mL syringe and put into the fluoride form by washing the column with ten column volumes of 1 M NaOH followed by ten column volumes of water, seven volumes of 0.5 M NaF, and ten volumes of water. The tricationic reagents were dissolved in either water or methanol at a concentration of 0.05M and one milliliter of this solution was passed through the at a concentration of 0.05M and one milliliter of this solution was passed through the resin and eluted by water into a volumetric flask. This stock solution was diluted with water to make the working tricationic reagent solution at concentration so that when it was mixed with the carrier solvent the concentration of the reagent was 10 μM.

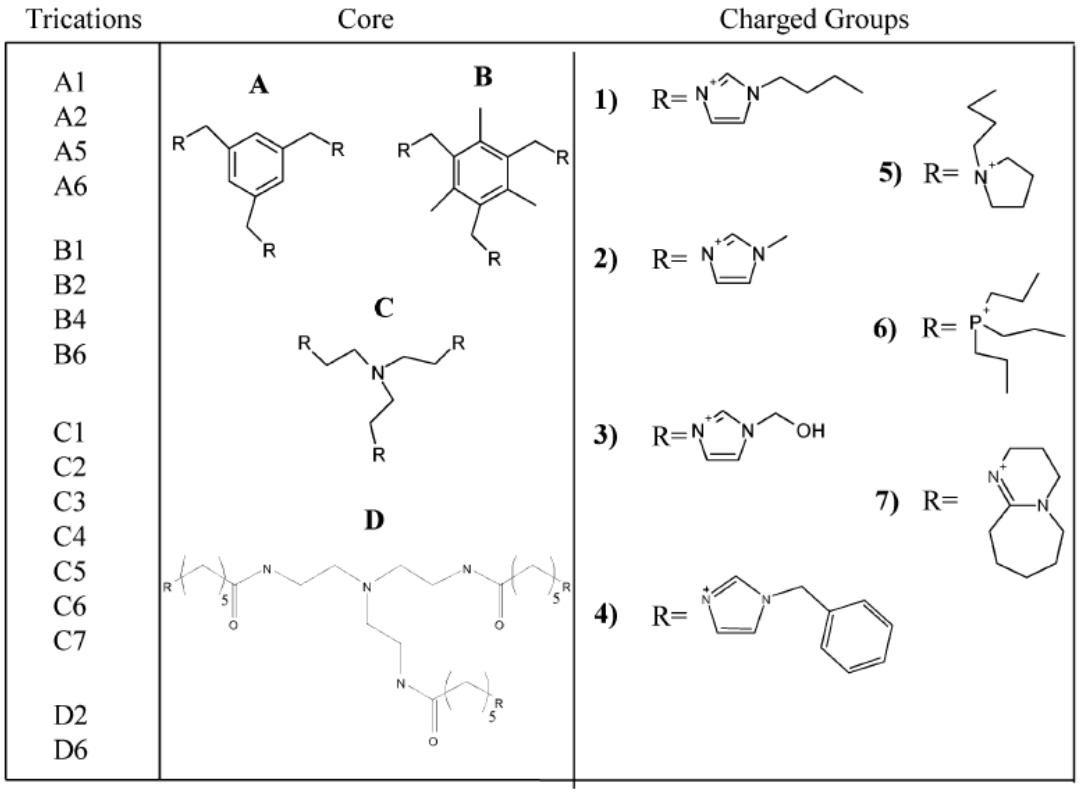


Figure 6.1 Structures of trigonal tricationic ion-paring reagents.

6.3.2 ESI-MS

ESI-MS analysis was carried out on a LXQ (Thermo Fisher Scientific San Jose, CA, USA) linear ion trap. A Surveyor MS pump (Thermo Fisher Scientific) with a vacuum degasser provided the carrier flow (67% MeOH/ 33%Water) at 300 µL/min. The tricationic reagent was introduced to carrier flow using a Y-type tee and a Shimadzu 6A LC pump operated at 100 µL/min was used for this purpose. For analysis in the negative ion mode, water replaced the aqueous tricationic reagent solution. The test anions were introduced into the carrier solvent using a six-port injection valve located between the Surveyor MS pump and the Y-type tee. ESI ionization conditions for positive mode were as follows: spray voltage: 3 kV; capillary temperature: 350ºC; capillary voltage: 11 V; tube lens: 105 V; Sheath gas 37 arbitrary units (AU); Auxiliary gas: 6 AU. Optimized conditions for detecting fluorophosphate with cation **D6** were spray voltage: 5 kV; capillary temperature: 250ºC; capillary voltage: 28 V; tube lens: 95 V; Sheath gas 37 arbitrary units (AU); Auxiliary gas: 6 AU. In negative mode the conditions were: spray voltage: -4.7 kV; capillary temperature: 350ºC; capillary voltage: -21 V; tube lens: -96 V; Sheath gas 37 arbitrary units (AU); Auxiliary gas: 6 AU. Detection limits (defined as S/N=3) for the eleven anions were determined by five replicate injections. The mass spectrometer was operated in single ion monitoring mode for the determination of all limits of detection (LODs). Data analysis was performed in Xcalibur 3.1 software.

Table 6.1 ESI-MS parameters.

MS parameters	general positive mode	general negative mode	optimized for FPO_3
spray voltage (kV)	3	−5	4.7
capillary temp (°C)	350	250	350
capillary voltage (V)	11	28	−21
tube lens (V)	105	95	−96
sheath gas (AU[a])	37	37	37
auxiliary gas (AU[a])	6	6	6

[a] AU, arbitrary units.

6.4 Results and Discussion

Eleven divalent anions were used to evaluate seventeen different tricationic reagents (see Table 6.2). The anions included both inorganic and organic types and were structurally diverse. Metal-based anions such as dichromate, nitroprusside, and hexachloroplatinate were among the inorganic anions included. Some of the anions were chosen based on the behavior of singly charged anions with dicationic reagents. Singly charged anions with halogen atoms paired very well with dicationic reagents and so representative divalent anions with bromine or fluorine atoms (bromosuccinate, dibromosuccinate, fluorophosphate) also were included in this study.

The trications synthesized for this study had one of four different "core" structures (Fig. 6.1). **A** and **B** have a benzene core while the nitrogen at the middle of core **C** is less hydrophobic. **D** is by far the most flexible of four core structures. Seven different charge carrying groups were used to create the seventeen tricationic reagents. Trications are named by the core used (A, B, or C) and the type of charged group (1-7). For example, trication **A1** has the benzene core and butyl imidazolium charged groups.

The detection limits for the anions in the positive mode by ESI-MS are given in Table 6.1. Except for dichromate, detection limits for most of the anions were in the hundreds of picograms to nanogram range with the tricationic reagents. The trications are arranged from lowest to highest according to the determined LODs. Using this arrangement, there are a few trends that emerge. From Table 6.2 it becomes obvious that trications **A6** and **B1** provide good sensitivity for a broad range of the representative divalent anions. **A6** (1,3,5-tris (tripropylphosphonium) methylbenzene trifluoride) performs the best overall since it ranks as one of the top three trication reagents for all of the anions except sulfate and oxalate. Even then, it ranks as the fifth best tricationic reagent for detecting oxalate. Trication **B1** (1,3,5-tris-(1-(3-butylimidazolium))

Table 6.2 LODs of doubly charged anions with trigonal tricationic reagents.

sulfate		dichromate		oxalate		thiosulfate	
trication	LOD (ng)	trication	LOD (ng)	trication	LOD (ng)	trication	LOD (ng)
B1	1.00×10^{-1}	B1	4.58×10^{-1}	C6	1.50×10^{-2}	A6	1.25×10^{-1}
B4	1.00×10^{-1}	B4	2.00	A1	4.00×10^{-2}	C1	1.25×10^{-1}
A5	1.00×10^{-1}	A6	1.00×10^{1}	B1	4.00×10^{-2}	B2	1.50×10^{-1}
C3	1.25×10^{-1}	C4	1.00×10^{1}	B6	2.34×10^{-1}	C5	1.53×10^{-1}
D6	1.50×10^{-1}	B2	1.00×10^{1}	A6	2.50×10^{-1}	B4	1.61×10^{-1}
C4	2.50×10^{-1}	A1	1.02×10^{1}	C1	3.43×10^{-1}	C4	2.00×10^{-1}
B2	2.50×10^{-1}	A2	1.25×10^{1}	C3	3.75×10^{-1}	B1	2.41×10^{-1}
A1	5.00×10^{-1}	B6	1.49×10^{1}	C4	4.35×10^{-1}	B6	2.60×10^{-1}
A6	5.00×10^{-1}	C2	1.73×10^{1}	A2	4.99×10^{-1}	C6	4.50×10^{-1}
A2	6.25×10^{-1}	C1	1.75×10^{1}	A5	5.00×10^{-1}	C3	4.99×10^{-1}
D2	7.00×10^{-1}	C3	2.00×10^{1}	B2	5.00×10^{-1}	A2	7.50×10^{-1}
C2	7.50×10^{-1}	C5	2.50×10^{1}	C2	7.18×10^{-1}	C2	7.80×10^{-1}
C1	8.75×10^{-1}	C6	4.50×10^{1}	B4	7.50×10^{-1}	A1	1.00
B6	1.50	D6	4.88×10^{1}	D6	8.75×10^{-1}	D2	1.38
C5	1.88	C7	4.96×10^{1}	C5	1.00	A5	2.14
C6	2.38	A5	1.75×10^{2}	D2	1.50	C7	5.20
C7	2.75	D2	2.50×10^{2}	C7	4.28	D6	1.50×10^{1}

nitroprusside		bromosuccinate		*o*-benzenedisulfonate		hexachloroplatinate	
trication	LOD (ng)	trication	LOD (ng)	trication	LOD (ng)	trication	LOD (ng)
B4	3.22×10^{-3}	A6	7.50×10^{-2}	A6	1.50×10^{-2}	B4	2.60×10^{-2}
A6	7.50×10^{-3}	B6	4.99×10^{-1}	C1	2.25×10^{-2}	B1	3.90×10^{-2}
B1	8.55×10^{-3}	C3	5.00×10^{-1}	B1	2.50×10^{-2}	A6	7.50×10^{-2}
B6	1.38×10^{-2}	D6	5.00×10^{-1}	B4	2.50×10^{-2}	C1	1.00×10^{-1}
C4	2.00×10^{-2}	C6	7.50×10^{-1}	C4	3.00×10^{-2}	A1	1.30×10^{-1}
C1	2.73×10^{-2}	A5	1.50	C6	3.75×10^{-2}	B6	1.58×10^{-1}
C5	2.73×10^{-2}	C5	1.63	A1	5.00×10^{-2}	B2	2.00×10^{-1}
C3	4.25×10^{-2}	A2	4.99	C2	5.00×10^{-2}	C4	2.50×10^{-1}
A1	4.29×10^{-2}	C1	5.00	B6	5.00×10^{-2}	D6	5.00×10^{-1}
C2	4.42×10^{-2}	B2	5.00	C5	5.00×10^{-2}	C5	8.75×10^{-1}
A2	4.86×10^{-2}	C2	7.00	A2	7.50×10^{-2}	C3	1.00
C7	6.00×10^{-2}	A1	7.50	C3	1.25×10^{-1}	C2	1.05
B2	1.00×10^{-1}	C4	8.75	D6	1.50×10^{-1}	A2	1.58
D6	1.25×10^{-1}	B4	1.00×10^{1}	A5	2.00×10^{-1}	C7	1.58
C6	2.00×10^{-1}	D2	1.25×10^{1}	C7	3.75×10^{-1}	C6	2.00
A5	3.15×10^{-1}	B1	1.75×10^{1}	B2	1.13	D2	2.13
D2	8.75×10^{-1}	C7	4.50×10^{1}	D2	1.75	A5	2.25

dibromosuccinate		fluorophosphate		selenate	
trication	LOD (ng)	trication	LOD (ng)	trication	LOD (ng)
B6	1.50×10^{-1}	A6	3.75×10^{-2}	A6	7.50×10^{-2}
A6	1.79×10^{-1}	D6	7.50×10^{-2}	B4	2.25×10^{-1}
C4	3.75×10^{-1}	B6	1.25×10^{-1}	B1	2.80×10^{-1}
C2	5.00×10^{-1}	D2	1.25×10^{-1}	C4	3.50×10^{-1}
C1	5.00×10^{-1}	B2	1.50×10^{-1}	C6	3.75×10^{-1}
C3	5.00×10^{-1}	C3	2.00×10^{-1}	B6	3.92×10^{-1}
C6	5.00×10^{-1}	B1	2.49×10^{-1}	D6	4.25×10^{-1}
C5	1.13	B4	2.49×10^{-1}	C1	5.00×10^{-1}
B1	1.25	C6	2.50×10^{-1}	C7	5.60×10^{-1}
B4	1.50	C4	2.75×10^{-1}	C3	7.50×10^{-1}
C7	1.50	C7	2.82×10^{-1}	D2	7.50×10^{-1}
D6	1.50	C1	4.25×10^{-1}	C2	1.12
A1	3.00	C5	5.00×10^{-1}	A2	1.13
A5	5.00	A5	7.50×10^{-1}	B2	1.38
A2	5.00	C2	1.00	A5	2.50
B2	5.00	A1	4.99×10^{1}	A1	4.00
D2	5.00	A2	5.00×10^{1}	C5	1.75×10^{1}

methyl-2,4,6-trimethylbenzene trifluoride) also does well, but is in the top three less consistently than **A6**. Table 6.2 also clearly shows that trication **C7** does not pair well with any anion, making it the most ineffective additive tested. **A5** also ranked in the lower half of the trication list for many of the anions. These two tricationic reagents would be poor choices for developing a sensitive method for the detection of divalent anions by positive ion mode ESI-MS.

When the terminal cationic moieties of the trication are the same, it is possible to compare the effect of the core structure on the performance of the tricationic reagent. While there are exceptions, cores **A** and **B** tend to pair more effectively with the doubly charged anions than those based on core **C** (Fig. 6.1). For these eleven anions, a tricationic reagent with a **C** core performs in the top three only four times. Thus, a tricationic reagent with a more rigid aromatic core seems to produce better results. However, the decision whether or not to include methyl groups as substituents on the benzene core is less straightforward. When the charged group is phosphorus-based, the plain benzene core (**A1**) provided lower detection limits compared to the trimethylbenzene core (**B6**). However, the opposite trend was seen in comparing **A1** and **B1**. **A1** seemed to be more susceptible to the loss of one of the butyl imidazole groups under MS conditions (data not shown) than **B1**, which appears to be stabilized by the methyl groups on the benzene ring. It should also be noted that these cores may have limited flexibility due to the repulsion among their identically charged moieties. Flexibility of the pairing agent was found to be an important factor in the pairing of singly charged anions with dicationic reagents.[185] Trications **D2** and **D6** are more flexible due to their longer chains. However, these trications do not provide good sensitivity for any divalent anions except fluorophosphate. This core structure has several heteroatoms and carbonyl groups which could compromise its effectiveness as a gas phase ion pairing agent that can provide good detection limits. It seems that a more ideal tricationic core would use longer (perhaps solely) hydrocarbon chains to attach the charged groups to a hydrophobic core. This would reduce charge repulsion and increase flexibility.

The nature of the terminal charged groups also influenced the detection limits observed for the anions. For example, the phosphonium based tricationic reagents (**A6**, **B6**, **and C6**) generally paired well with all of the anions. Benzyl imidazolium groups provided the lowest detection limits for nitroprusside and hexachloroplatinate and decent detection limits for o-benzenedisulfonate. This seems to indicate that pi-pi and n-pi interactions play a role in the association of certain specific anions with tricationic reagents. Analogous trends were seen with dicationic reagents.[185] However, two of the charged groups that did well with the dicationic reagents gave lower than expected sensitivities for the representative anions in this study. Reagents with methyl imidazolium and pyrrolidinium groups consistently placed in the middle to lower half of the trications tested regardless of the core structure. Instead, butyl imidazolium groups on the trimethyl benzene core (**B1**) performed better than expected.

It should be noted that the empirical data presented here are the result of several factors in addition to the binding affinity of the anions to the tricationic reagents. A single set of instrumental settings was used for the evaluation of the tricationic reagents. Some variance in instrumental performance between the different complexes is to be expected. The detection limit for oxalate was lowered from 250 pg to 75 pg when conditions were completely optimized (see Experimental) for the oxalate/**A6** complex. Increasing the spray voltage and decreasing the capillary temperature had the biggest impact on the signal intensity.

Figure 6.2 shows is a comparison of signal to noise ratios in the positive and negative ion modes for the two anions hexachloroplatinate and o benzenedisulfonate. In both cases, using a tricationic reagent in the positive mode produced superior signal to noise ratios even though ten times less sample was injected. By detecting divalent anions in the positive mode as a complex, the sensitivity for the two anions increases by almost two orders of magnitude. This demonstrates the ability of tricationic reagents to improve the sensitivity of mass spectrometry for divalent anions.

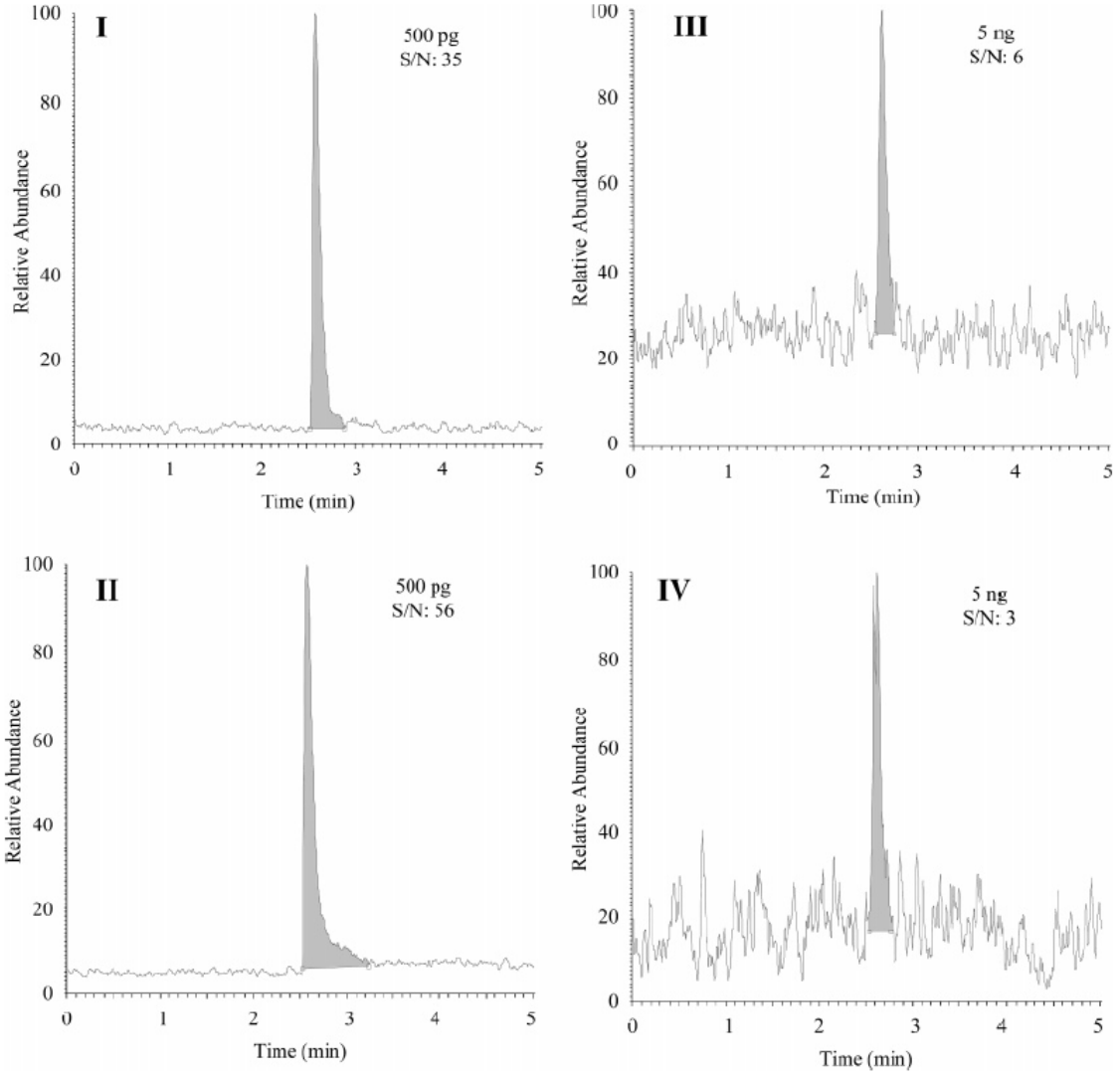


Figure 6.2 Comparison of positive (I, II) and negative modes (III, IV) for hexachloroplatinate (I, III) and o benzenedisulfonate (II, IV).

Tricationic reagents A6 (I) and B1 (II) in water were introduced into the carrier flow after anion injection in positive ion mode while only water was used in negative ion mode (III, IV).

6.5 Conclusions

Seventeen tricationic reagents have been evaluated as pairing agents for detecting eleven doubly charged anions in the positive mode by ESI-MS. Structural features of the tricationic reagents including the terminal charged groups and the core structure influenced the

detection limits for the doubly charged anions. The nature of the optimal charged groups for the tricationic reagents were often different from that found in a previous study for dicationic reagents. The use of tricationic reagents in the positive ion mode increased the S/N ratios of hexachloroplatinate and obenzenedisulfonate compared to negative mode even though ten times more sample was injected in the negative ion mode.

CHAPTER 7

EVALUATION OF FLEXIBLE LINEAR TRICATIONIC SALTS AS GAS-PHASE ION-PAIRING REAGENTS FOR THE DETECTION OF DIVALENT ANIONS IN POSITIVE MODE ESI-MS

7.1 Abstract

Anion analysis is of great importance to many scientific areas of interest. Problems with the negative mode ESI-MS prevent researchers from achieving sensitive detection for anions. Recently, we have shown that cationic reagents can be paired with anions, such that detection can be done in the positive mode, allowing for low limits of detections for anions using ESI-MS. In this analysis, we present the use of 16 newly synthesized flexible linear tricationic ion-paring reagents for the detection of 11 divalent anions. These reagents greatly differ in structure from previously reported trigonal tricationic ion-pairing agents, such that they are far more flexible. Here we present the structural features of these linear trications that make for good ion-pairing agents, as well as, show the advantage of using these more flexible ion-pairing reagents. In fact, the limit of detection for sulfate using the best linear trication was found to be 25 times lower than when the best rigid trication was used. Also, MS/MS experiments were performed on the trication/di-anion complex to significantly reduce the detection limit for many di-anions. Limits of detection in this analysis were as low as 50 femtograms.

7.2 Introduction

Anion analysis is of great importance to environmental researchers, biochemists, food and drug researchers, and the pharmaceutical industry; all of which are continually in need of facile, sensitive analytical techniques that can be used to both detect and quantitate trace anions.[186-202] Often, the anions of interest exist in complex matrices such as blood, water, and urine.[61,178,190,195-197] For this reason, separation techniques are routinely coupled with anion detection. Currently, some of these techniques utilize flow injection analysis or ion

chromatography,[200-205] with detection frequently obtained through the use of ion selective electrodes, conductivity, or spectroscopic techniques.[53-54,175,206-207] Yet, these detection methodologies lack either universality or specificity.[207]

For many analytes, ESI-MS has provided broad specificity and lower detection limits. Given the anion's inherent charge, it is not surprising that negative ion electrospray ionization mass spectrometry (ESI-MS) has come to the forefront as a general analytical approach that can be directly coupled with liquid chromatography (LC) if desired. Unfortunately, for most types of analytes, the negative ion mode often results in poorer limits of detection (LOD) than the preferred positive ion mode.[46,164-166] Due to high negative voltages, the negative ion mode is more prone to corona discharge than the positive mode.[56,166] This causes the negative mode to have an increased chance for arcing events and ultimately more noise resulting in unsatisfactory LODs.[46] Corona discharge in the negative mode can be controlled by using halogenated solvents and substituting more alkylated alcohols (i.e., butanol or IPA) for methanol.[164-167] Ideally, LC-ESI-MS methodologies would use more common solvents, such as, methanol, water, and acetonitrile. Furthermore, it would be more practical to do all ion detection in the more stable and sensitive positive ion mode.

Recently, we have developed a method for the detection of singly charged anions in positive mode ESI-MS using only water/methanol solvents.[63] This technique involves the addition of a low concentration of a dicationic ion pairing reagent to the mobile phase. The dication pairs with the singly charged anion, resulting in a complex possessing an overall plus one charge, which can be detected in the positive ion mode. Benefits of this technique include: (a) the use more practical solvents, (b) substantial increases in the sensitivity, (c) ease of use, (d) the ability to detect anions that fall below a trapping mass spectrometer's low mass cutoff region, and (e) detection of the complex at a much higher mass-to-charge region were there is far less chemical noise. To fully take advantage of factor (e) alone, it is best to choose a

relatively high molecular weight pairing agent that will result in a complex of a single positive charge.

Subsequently, the dicationic ion-pairing agent was used to determine the LODs for over 30 singly charged anions.[63] Also in this work, it was shown for the first time that MS/MS can often be used to further lower the LODs of these anions. Overall, this analysis showed the true ultra-sensitivity of ion-pairing by producing the lowest reported LODs for several anions by any know technique.[63] The effectiveness of over 20 dicationic ion-pairing agents was evaluated in order to determine the structural properties that allow for low LODs.[185] A major finding in this study was that flexibility of the dication seemed essential for good sensitivity. Therefore, the best dicationic ion-pairing reagents cited were those which possessed a flexible alkyl chain that linked the two cationic moieties. Recently, the ion-paring technique was extended to the use of tri-cationic reagents for the detection of divalent anions.[208] The essential tricationic reagents were found to bind divalent anions, and monitoring the complex in the positive ion mode was a more sensitive detection method than monitoring the naked doubly charged anions in the negative mode. However, the tricationic reagents used had a somewhat rigid trigonal structure (for a representative structure see the bottom of Fig. 7.1), which may be an undesirable feature of an ion-pairing agent from a sensitivity standpoint.

Recently, we devised a synthetic method to produce more flexible linear trications. In this work, we present the use of 16 newly synthesized linear tricationic ion-pairing reagents to determine the LOD for 11 divalent anions. Herein, we describe the differences and advantages of using the more flexible linear trications versus the more rigid trigonal trications. Also, we show that MS/MS experiments can be performed on the linear trication/di-anion complex, and that by monitoring a fragment of the complex, the LOD often can be dramatically lowered. This is the first ever report of using this type of an MS/MS experiment to detect doubly charged anions in the positive ion mode with any tricationic ion-pairing agent.

7.3 Experimental

7.3.1 Materials

Figure 7.1 gives the structures of all the linear tricationic ion-pairing reagents used in this study. The reagents required for synthesis included sodium imidazole, 1,3-dibromoproapne, 1,6-dibromohexane, 1,10-dibromodecane, 1,12-dibromododecane, methylimidazole, butylimidazole, benzylimidazole, and tripropylphosphine which were purchased from Sigma-Aldrich (Milwaukee, WI, USA). All synthetic reagents were of reagent grade and were used without further purification. The anions that were tested for LOD (listed in Table 7.1) were ordered as either the lithium, sodium, or potassium salt or as the disassociative free acid. They were also obtained from Sigma-Aldrich and were used as the reagent grade without further purification. HPLC grade water and methanol were purchased from Burdick and Jackson (Honeywell Burdick and Jackson, Morristown, NJ, USA).

7.3.2 Synthesis

Linear trications A1-4, B1-4, C1-4, and D1-4 were synthesized in an analogous manner, which included two steps. The first step was to produce a 1,3-(dialkylbromide)imidazolium bromide salt core. In short, this was done by first slowly adding a solution of sodium imidazole (1 molar eq.) in anhydrous DMF with a syringe pump to the corresponding dibromoalkane (5 molar eq.) under a vigorous stream of nitrogen. The reaction mixture was stirred for 12h at room temperature, then heated to 70℃ and allowed to react for an additional 12h. Next, DMF was removed by roto-evaporation and the excess dibromoalkane was extracted with hexane. The resulting viscous liquid was purified by column chromatography (SiO_2, methanol:dichloromethane (1:9)) to obtain the pure 1,3-(dialkylbromide)imidazolium bromide salt core in 65% yield. The next step is to react a solution of the preceding (1 molar eq.) in anhydrous THF (100mL) with the corresponding imidazole or phosphine (2.5 molar eq.) under reflux for 24h. Then the solvent was removed by roto-evaporation and the resulting tricationic bromide salt was dissolved in DI water (10-20 mL) and washed with ethyl acetate (5 x 50 mL) to

remove the excess imidazole or phosphine. Lastly, water was removed by roto-evaporation and the product was dried under vacuum over phosphorous pentoxide resulting in the pure tricationic ionic liquid in 90% yield. A full report of the synthesis of these unique tricationic ionic liquids, including the NMR, MS, and other physiochemical data will be reported in due course. The bromide salt then had to be exchanged to the fluoride form for use as the ion-paring agent in ESI-MS. We have previously reported the procedure for this anion exchange.[63,208]

7.3.3 ESI-MS

The ESI-MS conditions used here were the same as those previously used and optimized for the detection of perchlorate with a dicationic reagent, and were as follows: spray voltage, 3kV; sheath gas flow, 37 arbitrary units (AU); auxiliary gas flow rate, 6 AU; capillary voltage, 11 V; capillary temperature, 350℃; tube lens voltage, 105 V. When detecting the trication/dianion complex in the positive SIM mode, the SIM width was 5. When performing the SRM experiments, the isolation widths were between 1 and 5, the normalized collision energy was 30, and the activation time was 30 ms. All data analysis was performed using the Xcalibur and Tune Plus software.

Throughout this study, a Finnigan LXQ (Thermo Fisher Scientific, San Jose, CA, USA) ESI-MS was used for all of the analyses. The MS was equipped with a six port injector (5 µL loop) and was coupled with a Finnigan Surveyor MS Pump. Between the injector and the ionization source, a Y-type mixing tee allowed for the addition of flow from a Shimadzu LC-6A pump. It was from this pump that the tricationic ion-pairing agent was introduced to the solvent flow. Overall, the total flow to the ESI was 400 µL/min. The MS pump accounted for 300 µL/min (67% MeOH : 33% H_2O), while the LC pump applied the 40 µM trication solution in water at a rate of 100 µL/min. All the anions were dissolved HPLC grade water, such that their initial concentration was 1 mg/mL. Serial dilutions were made from the stock solutions and the anions were directly injected using the six port injector. New stock solutions were prepared weekly and the injector was expected to be the largest cause for possible experimental error ($\pm$ 5%). The

limits of detection were determined to be when an injection at a given concentration resulted in peaks giving a signal-to-noise ratio of three.

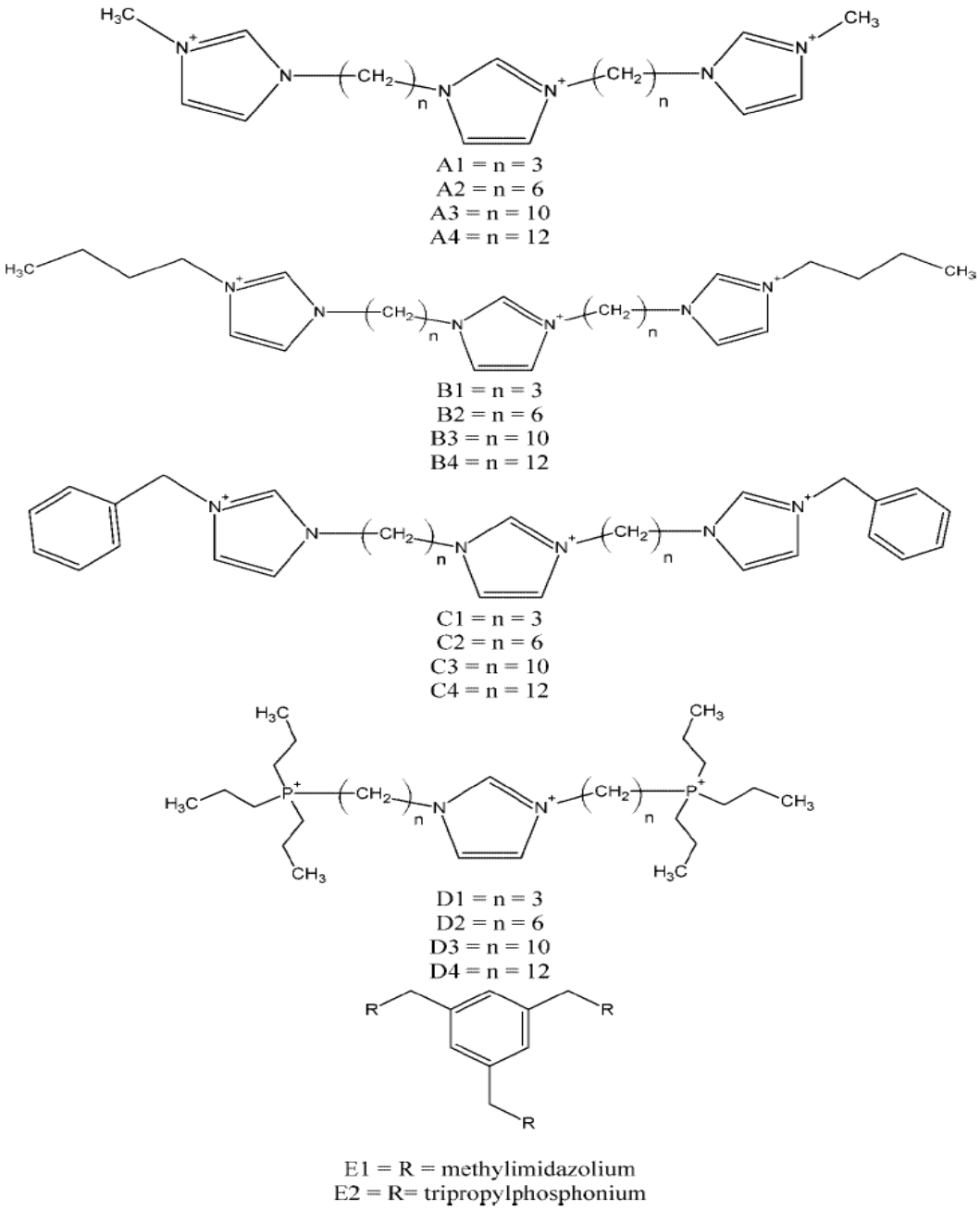


Figure 7.1 Structures of linear tricationic ion-pairing reagents.

7.4 Discussion

In previous reports, we have shown that dicationic ion-pairing reagents can be used to pair with singly charged anions, such that, the positively charged complex can be monitored in the positive mode, resulting in extremely low LODs.[63,208] More recently, we demonstrated that tricationic reagents could also be used to complex doubly charged anions, leading to much lower LODs for those divalent anions when detecting the complex in the positive mode.[63] Since the trications used previously had relatively rigid structures, a series of flexible ion-pairing agents were synthesized and tested to see if they offer greater sensitivity for the detection of anions in positive mode ESI. In addition, MS/MS of the paired ions was examined in hopes of further lowering the LOD in many cases.

Figure 7.1 shows the structures of the 16 linear tractions used in this analysis (A1-4, B1-4, C1-4, and D1-4). All of the 16 linear trications have the same imidazolium core. They differ in the length of the alkyl chain (C_3, C_6, C_{10}, and C_{12}) that tethers the terminal charged moieties to the central imidazolium, as well as, in the nature of the terminal charged moieties (methylimidazolium, butylimidazolium, benzylimidazolium, and tripropylphosphonium). By examining this series of linear trications, we were able to observe possible advantages of varying the chain length (i.e., flexibility), as well as, determining which cationic moieties produce the lowest LOD for the sample anions. Also shown in Figure 7.1, are the structures of two previously reported rigid trications.[208] Of these, the E1 trication was shown to be a moderately successful pairing agent, while trication E2 was found to be the best known trigonal tricationic ion-pairing agent.[208] The results of these two rigid trications allows for a definitive comparison to the new flexible trications developed for this study.

Table 7.1 lists the LODs for the 11 doubly charged anions, when paired with the 16 linear trications and monitored in the positive mode. Overall, the LODs for the divalent anions ranged from the nanogram (ng) to the picogram (pg) level. In order to evaluate the effect of the

Table 7.1 LODs of doubly charged anions with linear tricationic reagents.

sulfate		thiosulfate		oxalate		fluorophosphate	
trication	LOD (pg)	trication	LOD (pg)	trication	LOD (pg)	trication	LOD (pg)
D3	2.00×10^1	**D3**	6.25×10^1	**C2**	1.20×10^1	D4	2.50×10^1
D4	7.50×10^1	**C2**	6.25×10^1	D2	3.50×10^1	**D3**	2.63×10^1
D2	1.25×10^2	B3	6.25×10^1	A2	8.10×10^1	*E2*	3.75×10^1
B3	2.00×10^2	B2	6.25×10^1	D4	1.25×10^2	D2	4.25×10^1
B4	2.60×10^2	D2	7.50×10^1	B4	1.25×10^2	B3	9.00×10^1
C1	3.00×10^2	B4	7.50×10^1	**D3**	2.50×10^2	C3	1.50×10^2
B2	3.25×10^2	C1	8.75×10^1	*E2*	2.50×10^2	A3	2.00×10^2
C4	3.50×10^2	D4	9.00×10^1	A3	3.00×10^2	A2	2.00×10^2
C3	3.75×10^2	D1	1.00×10^2	B1	3.00×10^2	D1	2.00×10^2
C2	4.50×10^2	C4	1.00×10^2	B2	3.25×10^2	C4	2.10×10^2
B1	5.00×10^2	A3	1.00×10^2	C4	4.00×10^2	**C2**	2.25×10^2
E2	5.00×10^2	A4	1.00×10^2	C3	4.40×10^2	B2	2.75×10^2
A2	5.50×10^2	A2	1.25×10^2	C1	5.00×10^2	A4	4.50×10^2
A4	5.75×10^2	B1	1.25×10^2	*E1*	5.00×10^2	B4	5.00×10^2
A3	6.00×10^2	*E2*	1.25×10^2	A4	5.50×10^2	B1	8.75×10^2
D1	6.25×10^2	C3	1.75×10^2	A1	6.50×10^2	C1	1.50×10^3
E1	6.25×10^2	A1	5.00×10^2	D1	8.25×10^2	A1	4.50×10^3
A1	1.75×10^3	*E1*	7.50×10^2	B3	2.08×10^3	*E1*	5.00×10^4

dibromosuccinate		hexachloroplatinate		nitroprusside		dichromate	
trication	LOD (pg)	trication	LOD (pg)	trication	LOD (pg)	trication	LOD (pg)
D3	1.25×10^2	D2	3.50×10^1	**C2**	7.00	C4	3.50×10^3
E2	1.79×10^2	B2	3.50×10^1	D1	7.50	B4	3.75×10^3
D1	2.00×10^2	D1	3.75×10^1	*E2*	7.50	C3	3.88×10^3
C1	2.75×10^2	**D3**	4.00×10^1	C1	1.00×10^1	B3	4.25×10^3
B4	3.25×10^2	**C2**	5.00×10^1	D2	1.25×10^1	A3	5.00×10^3
B1	3.50×10^2	B1	7.00×10^1	B1	1.25×10^1	D4	5.50×10^3
B3	3.75×10^2	B3	7.50×10^1	**D3**	2.00×10^1	**D3**	6.25×10^3
A3	4.50×10^2	B4	7.50×10^1	B2	2.00×10^1	A4	6.25×10^3
C3	5.00×10^2	C1	7.50×10^1	C3	2.25×10^1	B1	6.25×10^3
D4	5.00×10^2	A2	7.50×10^1	B3	2.50×10^1	**C2**	6.38×10^3
A4	5.00×10^2	*E2*	7.50×10^1	A2	2.50×10^1	C1	6.50×10^3
D2	6.25×10^2	C4	8.50×10^1	A3	3.00×10^1	D2	7.50×10^3
C2	7.50×10^2	D4	1.00×10^2	B4	3.25×10^1	B2	7.50×10^3
B2	7.50×10^2	C3	1.25×10^2	D4	3.75×10^1	A2	7.50×10^3
A2	2.50×10^3	A3	1.25×10^2	C4	3.75×10^1	D1	8.75×10^3
A1	3.00×10^3	A4	1.75×10^2	A1	3.75×10^1	*E2*	1.00×10^4
E1	5.00×10^3	A1	5.00×10^2	*E1*	4.86×10^1	*E1*	1.25×10^4
C4	5.00×10^4	*E1*	1.58×10^3	A4	5.00×10^1	A1	1.50×10^4

selenate		*o*-benzenedisulfonate		bromosuccinate	
trication	LOD (pg)	trication	LOD (pg)	trication	LOD (pg)
E2	7.50×10^1	*E2*	1.50×10^1	*E2*	7.50×10^1
B3	2.50×10^2	D1	1.63×10^1	C4	6.25×10^2
C4	2.75×10^2	C1	1.75×10^1	**D3**	7.50×10^2
D3	3.75×10^2	B1	2.00×10^1	D1	7.50×10^2
B1	4.00×10^2	**C2**	3.20×10^1	A4	8.00×10^2
C2	4.25×10^2	B4	4.00×10^1	**C2**	1.00×10^3
C3	4.40×10^2	B2	4.00×10^1	B4	1.00×10^3
D4	5.00×10^2	D2	4.75×10^1	C3	1.50×10^3
D2	5.00×10^2	**D3**	6.50×10^1	D4	2.00×10^3
C1	5.00×10^2	A4	6.50×10^1	D2	2.25×10^3
B2	5.00×10^2	C3	7.50×10^1	A3	3.75×10^3
B4	5.25×10^2	*E1*	7.50×10^1	B3	4.00×10^3
A4	5.50×10^2	D4	1.00×10^2	*E1*	4.99×10^3
A3	7.00×10^2	B3	1.00×10^2	C1	5.00×10^3
D1	7.50×10^2	A3	1.00×10^2	A2	5.00×10^3
A2	7.50×10^2	A2	1.25×10^2	B2	5.50×10^3
E1	1.13×10^3	A1	3.75×10^2	B1	7.50×10^3
A1	3.38×10^3	C4	8.75×10^3	A1	1.25×10^4

[a] The limit of detection was determined to be the amount of analyte that resulted in S/N = 3. Also, the data for E1 and E2 were extracted from ref 40. Note that the bold numbered ion-pairing agents are the two best linear trications, and the italicized ion-pairing agents are the two trigonal trications.

chain length in the linear tricationic ion-pairing reagent, one can compare the trications of the same letter. For example, trications D1-4 differ only in the length of the hydrocarbon chain connecting the charged moieties (Fig. 7.1). In general, it appears that the common trend is that linear trications with hexyl or decyl linkage chains gave the lowest LODs, whereas, trications with propyl or dodecyl linkages resulted in higher LODs. This trend can be easily seen by comparing the LOD for thiosulfate when using the "D" series of linear trications. In this comparison, the order from best to worst ion-pairing agent was found to be D3, D2, D4, and D1. A likely explanation for this observation is that when the alkyl linkage chain is too short, the linear trication is less flexible and not as likely to "bend" around the anion. This finding supports our hypothesis that flexibility is a key feature in a good tricationic ion-pairing reagent. In contrast, when the alkyl chain gets too long, the cationic moieties are too far from each other and can not work as a single unit when binding the anion. However, the effect of the linkage chain being too short is far more unfavorable then it being too long. An example of this can be seen in Table 7.1, were trication A1 with the shortest linkage chain was found to be one of the three worst ion-pairing agents for all anions. Clearly, the results (Table 7.1 and Fig. 7.1) suggest that when using linear tricationic ion-pairing reagents, the alkyl linkage chain should be between six and ten carbons in length.

By evaluating the data for a series of trications that all have the same linkage chain, but different cationic moieties, the best terminal charged groups can be determined. Typically, the linear trications possessing the benzylimidazolium (the "C" moiety) and the tripropylphosphonium (the "D" moiety) terminal charged groups resulted in lower LODs than the methylimidazolium (the "A" moiety) or butylimidazolium (the "B" moiety) cationic groups. This observation is shown by the LODs for oxalate when paired with the linear tricationic "2" series. The order from best to worst ion-pairing agents was found to be C2, D2, A2, and B2. Another example of this can be seen in the LODs for both nitroprusside and dichromate, where (from best to worst) the order was C2, D2, B2, and A2. These results, along with the previously noted

optimum linkage chain lengths, allow for the determination that trications C2 and D3 were the overall best tricationic ion-pairing agents. Trication C2 has hexyl linkage chains and benzylimidazolium terminal charged groups, and trication D3 has decyl linkage chains and tripropylphosphonium cationic moieties. Interestingly, in the three comprehensive studies we have done on ion-pairing agent structures, the tripropylphosphonium cationic moiety is the only one that has always resulted in a recommended ion-pairing agent.[63,208]

The other important comparison to be made with the data in Table 7.1 is the LODs resulting from using the flexible linear trication versus the more rigid trigonal trications (E1 and E2). As can be seen, the best linear trications, C2 and D3, rank very near the top for most of the anions tested. However, the best trigonal trication, E2, also ranks very near the top for many of the tested anions. From this observation, it was determined that the best linear trications and the best trigonal trications both work well when monitoring the same divalent anions. Interestingly, the linear and trigonal ion-pairing reagents seem to be complimentary to one another. Overall, the best linear trication was not found to be a greatly superior ion-pairing agent when compared to the best trigonal trication. Yet, some very useful and somewhat complimentary tricationic ion-pairing reagents were added to our repertoire. However, if you compare trigonal trication E1 (the moderately successful trigonal trication) to the flexible linear trications, it can be seen that trication E1 ranks near the bottom for all the anions tested. It was determined that in general, the more flexible trications are better ion-pairing agents than the rigid trications. Obviously, there are other factors that play a part in finding the optimum ion-pairing agent, which allow trication E2 to work as well as the linear trications. Perhaps the most important factor is that it contains the highly favorable tripropylphosphonium moiety.

Figure 7.2 illustrates the benefits of using a linear trication versus a trigonal trication for the detection of sulfate in the positive mode. In both detection scenarios, the same concentration of sulfate was injected (500 pg). In the upper panel (I), the ion-pairing agent was the best linear trication D3, and in the lower panel (II) the best trigonal trication E2 was used. It

is apparent that the linear trication resulted in superior detection of sulfate, with a signal-to-noise seven times greater that that for the trigonal trication. It should be noted that sulfate itself has a mass-to-charge ratio of -48, thus falling below the low mass cutoff of our MS instrument and rendering itself undetectable in the negative mode.

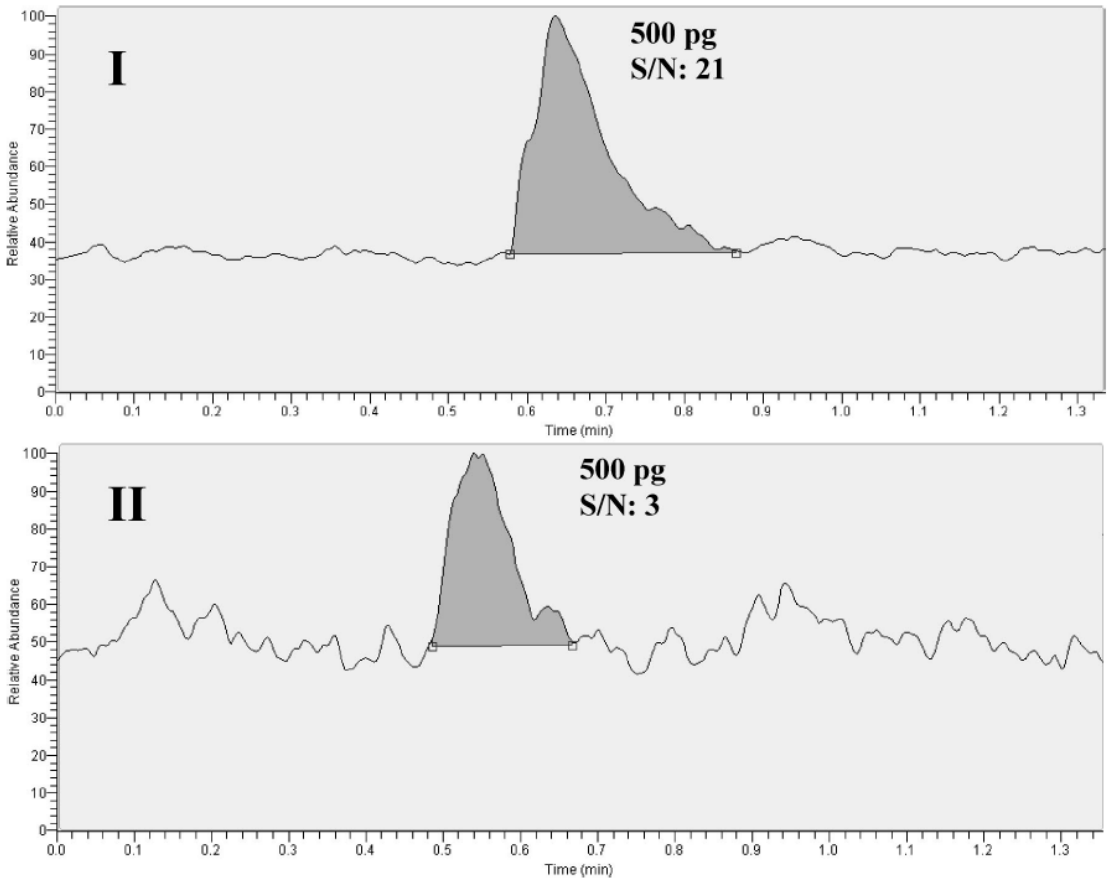


Figure 7.2 Comparison of the detection of sulfate in the positive mode using tricationic ion-paring reagents D3 (I) and E2 (II).

Another facet of this study was to show that SRM experiments could be performed on the trication/anion complex, and that by monitoring a positively charged fragment of the complex, lower LODs for the divalent anions could be achieved. The key part of this type of

experiment is to find the proper fragment to monitor. In many cases the fragmentation was the same, but not always. Figure 7.3 shows a proposed fragmentation pattern for the more commonly observed disassociation of a trication D3/di-anion complex. As is shown by Figure 7.3, collision induced disassociation (CID) typically resulted in a singly charged alkyl linked phosphonium imidazole, which had a mass-to-charge ratio of 367.4. Monitoring this fragment can lead to a decrease in the LOD for the anion that was part of the parent complex.

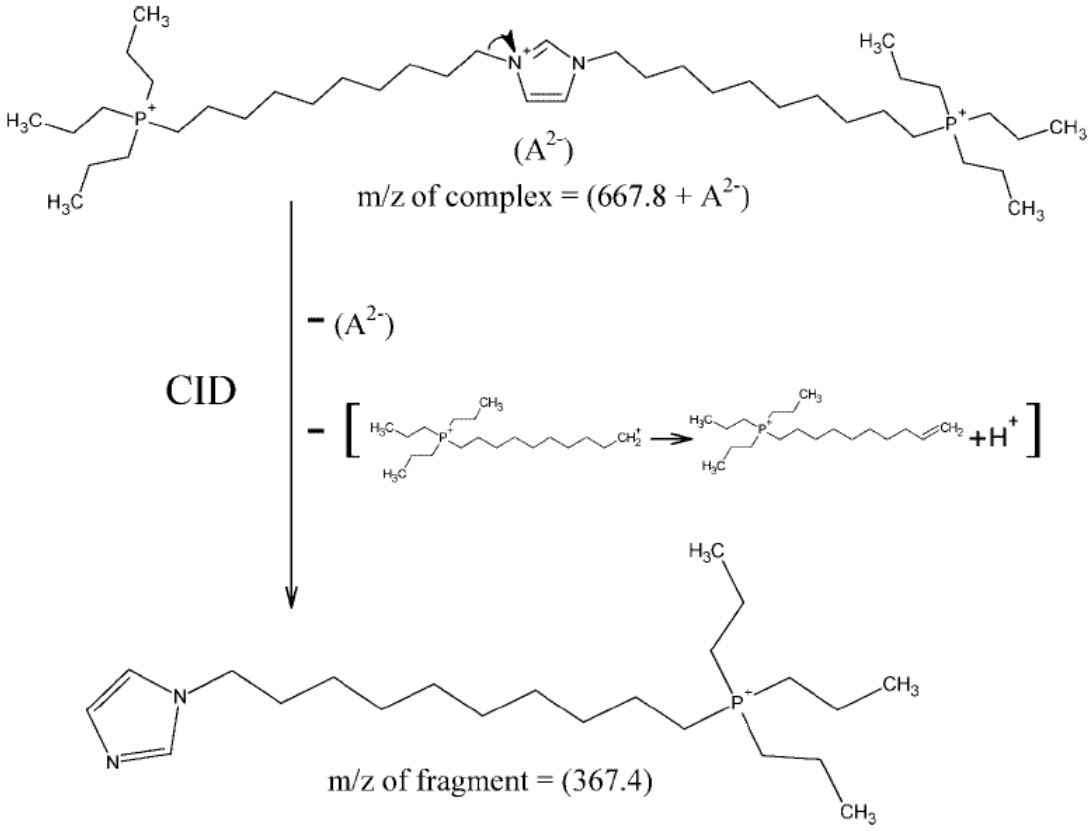


Figure 7.3 Proposed fragmentation pattern for a typical SRM experiment using trication D3.

Table 7.2 lists the results for the SRM experiments that were performed in this analysis. Trications D3 and C2 were paired with 11 divalent anions and tested for their LOD using the

SRM method. For comparison, the SIM results are listed next to the SRM results. As can be seen, the SRM mode often resulted in lower LODs than the SIM mode. There were two analytes (D3/bromosuccinate and C2/oxalate) that showed no improvement, but in general there was nearly an order of magnitude improvement when using the SRM mode. In three cases, the SRM mode resulted in a two order of magnitude decrease in the LOD. One of these cases was the detection of nitroprusside using trication C2 as the ion-pairing agent and employing the SRM mode. For this system, the LOD for nitroprusside was determined to be 50 femtograms (fg), which is the lowest LOD for any mono- or divalent anion that has tested to date. Clearly this is a very facile and sensitive method.

Also, listed in Table 7.2 are the SRM fragment masses that were monitored. As noted previously, many complexes produce the same fragment; 367.4 for trication D3 and 309.2 for trication C2. However, it was observed that there are some trication/di-anions that follow different disassociation pathways. For example, the trication D3/hexachloroplatinate complex produced a fragment with a mass-to-charge ratio of 1003.5. This fragment corresponds to the loss of one chlorine atom from the hexachloroplatinate, while the overall cation-anion complex remained intact. A similar effect was seen with the SRM detection for nitroprusside. Here, nitroprusside loses a nitro group and still stays complexed with the trication. For these cases, it is interesting to see that the non-covalent trication/di-anion complex remains intact, while covalent bonds have been broken. One more example of this type of fragmentation was for bromine containing anions. Here the central imidazolium looses its acidic proton (in the 2 postion of the imidazolium ring) and becomes a dication. This dication then complexes with a bromide anion that was lost from the di-anion. This means that for any bromine containing di-anions, the same fragment could be monitored (m/z: 745/747 for D3 and m/z: 629/631 for C2).

It should be noted that although the LODs for the 11 divalent anions in SIM and SRM are already quite low, they could be lowered further by completely optimizing the conditions for a particular complex. In this analysis, one general set of conditions were used for the entire

study. Previously, we have shown that the LODs can be further decreased by a factor of three to ten with individual optimization.[37-39] Finally, the use of some other types of MS systems (triple quad, etc.) with this technique can further reduce detection limits.

Table 7.2 Comparison of LODs in the SIM positive and SRM positive Modes.

	trication D3			trication C2		
	SIM LOD (pg)	SRM LOD (pg)	SRM mass	SIM LOD (pg)	SRM LOD (pg)	SRM mass
sulfate	2.00×10^{1}	1.50×10^{1}	367.4	4.50×10^{2}	3.00×10^{2}	309.2
thiosulfate	6.25×10^{1}	5.00×10^{-1}	367.4	6.25×10^{1}	3.50×10^{1}	309.2
oxalate	2.50×10^{2}	1.00×10^{2}	367.4	1.20×10^{1}	7.50×10^{1}	549.2
fluorophosphate	2.63×10^{1}	2.05×10^{1}	367.4	2.25×10^{2}	1.00×10^{2}	309.2
dibromosuccinate	1.25×10^{2}	1.25×10^{1}	745/747	7.50×10^{2}	2.00×10^{1}	629/631
hexachloroplatinate	4.00×10^{1}	4.50	1003.5	5.00×10^{1}	2.00×10^{1}	889.4
nitroprusside	2.00×10^{1}	3.50	853.5	7.00	5.00×10^{-2}	737.4
dichromate	6.25×10^{3}	5.75×10^{2}	367.4	6.38×10^{3}	3.00×10^{3}	643.4
selenate	3.75×10^{2}	2.00	367.4	4.25×10^{2}	6.00×10^{1}	309.2
o-benzenedisulfonate	6.50×10^{1}	1.00×10^{1}	367.4	3.20×10^{1}	3.75×10^{1}	309.2
bromosuccinate	7.50×10^{2}	1.00×10^{3}	745/747	1.00×10^{3}	1.00×10^{3}	629/631

7.5 Conclusions

Sixteen newly synthesized linear tricationic ion-pairing agents were evaluated for their ability to detect doubly charged anions in positive mode ESI-MS. It was found that for linear trications, the optimum alkyl chain lengths coupling the cationic moieties should be between six and ten carbons in length. It was determined that the best cationic moieties were tripropylphosphonium and benzylimidazolium. In comparison to previously reported rigid tricationic ion-pairing agents, the flexible linear trications presented here generally make better MS ion-pairing agents. It was shown that when the same amount of sulfate was injected, the signal-to-noise ratio when using the best linear trication was seven times greater than when using the best trigonal trication. However, it was found that trigonal trication E2 remained useful as it was often complimentary to the linear trications. Lastly, one to three orders of magnitude decreases in the LODs were found when using SRM.

CHAPTER 8

THE EVALUATION AND COMPARISON OF TRIGONAL AND LINEAR TRICATIONIC ION-PAIRING REAGENTS FOR THE DETECTION OF ANIONS IN POSITIVE MODE ESI-MS

8.1 Abstract

A general and sensitive method for detecting divalent anions by ESI-MS and LC/ESI-MS as positive ions has been developed. The anions are paired with tricationic reagents to form positively charged complexes. In this study, four tricationic reagents, 2 trigonal and 2 linear, were used to study a wide variety of anions, such as disulfonates, dicarboxylates, and inorganic anions. The limits of detection for many of the anions studied were often improved by tandem mass spectrometry. Tricationic pairing agents can also be used with chromatography to enhance the detection of anions. The tricationic reagents were also used to detect monovalent anions by monitoring the doubly charged positive complex. The limits of detection for some of the divalent anions by this method are substantially lower than by other current analytical techniques.

8.2 Introduction

The analysis of anions is of great necessity and interest in many fields of science. Low levels of organic acids have been determined in a variety of samples such as food, environmental, and biological matrices.[209-217] Some dicarboxylic acids, such as glutaric, fumaric, and adipic acids are marker compounds for certain metabolic disorders and have been determined in urine samples.[218] Aromatic sulfonates are used in many industrial processes and consumer products, such as laundry detergents. Many of these sulfonates end up in wastewater and municipal water supplies and have been determined by various methods.[211,219] Because of the ramifications of low levels of anions in the environment, fast and effective trace methods of analysis are very important.

Complex environmental sample matrices often require a separation technique to isolate the analyte. Common separation methods include ion chromatograph,[60-61,179-180,209] ion pair chromatography,[211,219,220] reverse-phase mode chromatography,[221--223] and capillary electrophoresis (CE).[212-213,224] To enhance the spectroscopic detection of anions that do not contain a UV chromophore, some CE and high performance liquid chromatography (HPLC) methods utilize sample derivatization[225-226] or indirect UV or fluorescence detection methods.[173,227-228] Ions have also been detected by ion selective electrodes and conductivity [26-31]. Mass spectrometry (MS) provides universal detection for anions and is being used more and more, either alone[179] or paired with a separation technique.[211,214,222]

Electrospray ionization (ESI)-MS is a logical choice for ion detection because of the inherent charge state of the analyte. Negative mode ESI-MS is the most common way of detecting anions. Problematically, negative ion mode operation with standard chromatographic solvents, such as methanol and water, can lead to poorer spray stability, corona discharge, and arcing, which ultimately lead to poor detection limits.[166,229] Halogenated solvents[164-168] or electron scavenging gases[56] can be used to suppress these effects.

Operating in positive mode ESI would help to avoid the stability problems of negative mode ESI-MS and the use of unconventional solvents. A method was developed to detect singly charged anions using positive mode ESI-MS by pairing the anion with a dicationic reagent to create a positively charged complex.[59-63,185] There are multiple advantages to this method beyond the use of positive mode ESI-MS. One benefit of monitoring the anion/dication pair is moving the anion to a higher mass region where there is lower background noise. Additonally, anions of low mass are moved well above the low mass cutoff when quadrupole instruments, such as an ion trap, are used. Also, the pairing reagents may be used to differentiate between the analyte of interest and an interference of the same m/z.[59]

Most recently, tricationic reagents were paired with divalent anions, which again could be detected as a singly charged complex.[208,230] The first group of tricationic reagents used as

pairing agents were classified as trigonal trications.[208] These trications have fairly rigid structures and provided detection sensitivity enhancement for many of the anions tested. Past results have indicated that rigid dicationic pairing agents did not work as well as more flexible dications[185], so a second class of tricationic reagents was developed. The second group of tricationic reagents is linear and more flexible.[231] The limit of detection (LOD) for some of the divalent anions tested was lower for the linear trications than the trigonal cations.[230] In the present study, the best two trigonal and two linear tricationic reagents from these previous studies will be used to determine detection sensitivity for a wide variety of divalent anions. LOD trends for a given tricationic reagent or class and analyte type (e.g. dicarboxylate, disulfonate) would aid in future method development. The use of tricationic reagents in MS-MS and possible dissociation mechanisms are discussed as well. Additionally, these tricationic reagents can be used for the detection of monovalent anions as a doubly charged complex, which has not been previously studied with tricationic reagents. This leads to the possibility of detecting both singly and doubly charged anions using a singular tricationic reagent.

8.3 Experimental

The water and methanol used in these experiments were of HPLC grade and obtained from Burdick and Jackson (Morristown, NJ). Reagent grade sodium hydroxide and sodium fluoride were from Fisher Scientific (Pittsburgh, PA). The anions listed in Tables 8.1 and 8.3 were purchased as either the sodium or potassium salt or in the acid form from Sigma-Aldrich, with the exception of butanedisulfonic acid and 1,5-naphthalenedisulfonic acid which were purchased from TCI America (Portland, OR). Stock solutions (1 mg/mL) were made weekly and diluted serially for analysis.

The tricationic reagents evaluated in this study, as shown in Figure 8.1, were synthesized according to previous reports.[208,230-231,22] Before analysis, each trication was anion exchanged to the fluoride form as previously reported.[208]

For direct injection analysis, a 40 μM trication-fluoride solution was pumped into a Y-type mixing tee at 100 μL/min using a Shimadzu LC-6A pump (Shimadzu, Columbia, MD). Also directed into the mixing tee was a 2:1 mixture of methanol:water at a flow rate of 300 μL/min using the Surveyor MS pump (Thermo Fisher Scientific, San Jose, CA). This set up leads to an overall solvent composition of 50/50 water/methanol with 10 μM tricationic reagent and a total flow rate of 400 μL/min. The six-port injection valve on the mass spectrometer (5 μL loop) was used for sample introduction.

A Finnigan LXQ (Thermo Fisher Scientific) ESI-MS instrument was used for the analysis of anions in this study. The ESI-MS conditions used were: spray voltage 3kV; sheath gas flow, 37 arbitrary units (AU); auxiliary gas flow rate, 6 AU; capillary voltage, 11 V; capillary temperature, 350° C; tube lens voltage, 105 V. The trication-anion complex was monitored in SIM mode with a width of 5 m/z units. This range was chosen to include isotope peaks, and LOD determinations were made from extracted ion chromatograms of the cation-anion complex m/z. For SRM experiments, the isolation width was 1-5 units with a normalized collision energy of 30 and an activation time of 30 ms. Data was analyzed using the Xcalibur and Tune Plus software. The limits of detection were determined when multiple injections of a given concentration resulted in a signal-to-noise ratio of three.

For the chromatography experiments, sample introduction was made by a Thermo Fisher Surveyor autosampler (5 μL injections). The stationary phase used was a Cyclobond 1 (25 cm x 2.1 mm) obtained from Advanced Separation Technology (Whippany, NJ). The flow rate was 300 μL/min, and the column was equilibrated with 100% methanol and a step gradient to 100% water was applied at 5 minutes. The tricationic reagent (40 μM) was added to the column effluent at 100 μL/min via the mixing tee. The mass spectrometer was operated in SIM mode, monitoring the mass of each di-anion/trication complex throughout the chromatographic run.

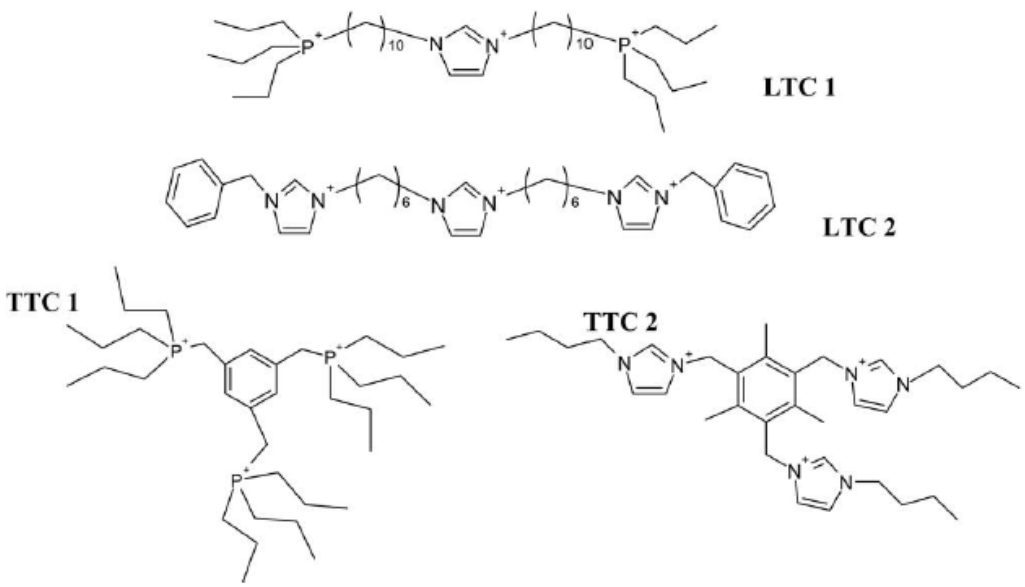


Figure 8.1 Structures of the tricationic ion-pairing reagents.

8.4 Results and Discussion

The tricationic reagents used in this study were chosen to represent the best performing trigonal and linear trications used in previous studies.[208,230] These four tricationic reagents offer a variety of functional groups as well as differences in rigidity. The linear trications contain both an imidazolium core with different chain lengths and terminal charged groups. Linear trication 1 (LTC 1, Fig 8.1) has C_{10} linkages between the central imidazolium and tripropylphosphonium (TPP) terminal charged groups. Linear trication 2 (LTC 2, Fig 8.1) has benzylimidazolium terminal charge groups with a C_6 linkage chain. Trigonal trication 1 (TTC 1, Fig 8.1) has a benzene core with three TPP charged groups. Trigonal trication 2 (TTC 2, Fig 8.1) consists of a mesitylene core with three n-butylimidazolium groups in the 2,4,6 positions.

A variety of divalent anions were chosen to evaluate the ion-pairing performance of the tricationic reagents. The anions can be divided into categories based on their functional groups. The groups are: disulfonates, dicarboxylates, metal containing compounds, other sulfur

containing compounds, and miscellaneous compounds. Within the disulfonate and dicarboxylate categories, an effort was made to include compounds with varying chain lengths and functional groups to investigate any effect these might have on limits of detection.

Table 8.1 shows the 34 divalent anions used in this study and their limits of detection using each of the 4 tricationic reagents. They are arranged into the anion categories with the lower limits of detection at the top of each category. An examination of the LODs with the bold type-face, which indicate the lowest LOD for each anion, in Table 8.1 indicates that about 2/3 of the lowest LODs are for the linear tricationic reagents. Additionally, LTC 1 and TTC 1, which are the phosphonium containing reagents, (Fig. 8.1), account for 26 (of 34) of the lowest LODs. The exceptional overall performance of the TPP reagents for this set of divalent anions is in agreement with previous studies.[230]

Generally, the disulfonates have lower limits of detection than dicarboxylates. The lowest LODs for the disulfonates are for dihydroxynaphthalenedisulfonate and *m*-benzenedisulfonate using TTC 1. The disulfonates with aromatic groups (dihydroxynaphthalenedisulfonate, *m*-benzenedisulfonate, 4-formyl-*m*-benzenedisulfonate, anthraquinone-2,6-disulfonate) usually had lower LODs than the straight chain disulfonates. Methane, ethane, propane, and butane disulfonic acids were evaluated with each tricationic reagent. There does not appear to be a trend in the detection limit based on the increasing chain length for the disulfonic acids except when using TTC 1, where methane disulfonic acid had a higher LOD than for the longer chain disulfonates. For the disulfonate category as a whole, the trigonal trication reagents performed better than the linear ones.

Two of the other sulfur containing compounds, besides the disulfonates, also showed low LODs. In fact, the LOD for tetrathionate, using LTC 1, is the lowest of all the anions tested when operating in SIM mode (50 femtograms). Tetrathionate and peroxidisulfate were very near the lowest LODs for both LTC 1 and 2, but had LODs higher than most of the disulfonates for TTC 1 and 2. There appears to be excellent complexation for these sulfur-oxo compounds with

Table 8.1 LODs for divalent anions using four tricationic pairing reagents in SIM.

	Linear trications		Trigonal trications	
	LTC 1 LOD (ng)	LTC 2 LOD (ng)	TTC 1 LOD (ng)	TTC 2 LOD (ng)
Disulfonates				
Dihydroxynaphthalenedisulfonate	7.50E-02	5.00E-02	**7.50E-03**	1.20E-02
m-Benzenedisulfonate	2.50E-02	5.00E-02	**8.75E-03**	1.00E-02
4-Formyl-m-benzenedisulfonate	1.25E-01	3.75E-02	**1.00E-02**	1.50E-02
Naphthalene-1,5-disulfonate	6.00E-02	**1.25E-02**	2.00E-02	3.00E-02
Butanedisulfonate	1.25E-01	5.00E-02	3.00E-02	**2.00E-02**
Propanedisulfonate	1.00E-01	**2.00E-01**	2.45E-02	7.50E-02
Anthraquinone-2,6-disulfonate	**2.50E-02**	5.00E-02	7.50E-02	5.00E-02
Methanedisulfonate	1.00E-01	**3.00E-02**	6.00E-02	**3.00E-02**
Ethanedisulfonate	**3.50E-02**	2.25E-01	3.60E-02	4.00E-02
Dicarboxylates				
Dipivolyltartarate	1.75E-02	**1.25E-02**	1.50E-02	1.50E-02
Camphorate	**6.00E-02**	1.50E-01	**6.00E-02**	5.00E-01
Phenylsuccinate	1.50E-01	7.50E-02	**5.00E-02**	1.00E-01
Glutarate	**7.00E-02**	2.00E-01	1.00E+00	5.00E-01
Malate	2.60E-01	**5.00E-02**	2.25E-01	5.00E-01
Methylsuccinate	2.00E-01	**7.50E-02**	2.50E-01	1.00E-01
Fumarate	**1.50E-01**	4.00E-01	1.50E+00	7.50E+00
Pimelate	**1.50E-01**	2.00E-01	2.50E+00	7.50E-01
Malonate	2.00E+00	1.38E+00	8.75E-01	**3.00E-01**
Adipate	**5.00E-01**	8.00E-01	2.25E+00	1.50E+00
Dibromomaleate	8.50E-01	1.00E+00	1.00E-01	**1.75E-01**
Chlorosuccinate	3.75E+00	1.88E+00	**2.25E-01**	9.00E-01
Metal containing compounds				
Hexachlororhenate $ReCl_6$	**1.50E-02**	3.00E-02	1.50E-01	2.00E-02
Chromate CrO_4	2.50E-01	7.50E-01	6.25E+00	**7.50E-02**
Molybdate MoO_4	**1.50E-01**	2.50E+00	3.75E-01	7.50E-01
Manganate MnO_4	1.00E+00	—	**3.75E-01**	8.75E-01
Arsenate AsO_4	**7.50E-01**	2.25E+00	2.50E+00	1.00E+00
Other sulfur compounds				
Tetrathionate S_4O_6	**5.00E-04**	2.25E-02	2.50E-02	5.00E-02
Peroxidisulfate S_2O_8	**1.20E-02**	1.65E-02	7.50E-02	2.00E-01
Succinaldehyde bisulfite	1.25E+01	2.50E+01	**1.25E+00**	5.00E+00
Glutaraldehyde bisulfite	3.50E+00	2.50E+00	**1.75E+00**	2.50E+00
Miscellaneous compounds				
Phenylphosphate	**4.00E-02**	1.00E-01	7.50E-02	5.00E-02
Rhodizonate	**1.05E-01**	5.00E-01	3.75E+00	3.75E-01
Hydrogen phosphite	**1.50E-01**	5.00E-01	3.75E-01	2.50E-01
Selenite	1.25E+00	—	**3.50E-01**	3.75E+00

*Limit of detection determined where the amount of analyte used results in S/N = 3.
Bold typeface indicates the lowest limit of detection for each anion.
— Indicates that a dianion/trication complex was not observed.

the linear trications. Two other sulfur containing compounds (i.e., the bisulfites) had nearly the highest LODs for all of the trications (Table 8.1).

Among the dicarboxylates studied, dipivaloyl-tartrate has the best LOD when pairing with all of the trications studied and for LTC 1 has a lower LOD than all of the disulfonates. For the tricationic reagents with benzene/mesitylene cores or charged groups (LTC 2, TTC 1, and

TTC 2), the dicarboxylates with non-halogen chain substitutions (dipivaloyl-tartrate, phenylsuccinate, methylsuccinate, and malate) have lower limits of detection than the straight chain dicarboxylates (Table 8.1). The halogenated dicarboxylates (chlorosuccinate and dibromomaleate) had lower LODs using the trigonal trications (Table 8.1). For the straight chain dicarboxylates studied, glutarate, (C_5), had the lowest limit of detection, followed by pimelate, (C_7), and then adipate, (C_6). With LTC 1, the LOD for adipate is about 7 times higher than for glutarate, though they only differ by one carbon in chain length. For the dicarboxylate category in general, the linear trications outperformed the trigonal ones.

The inorganic compounds studied generally had higher LODs than the organic acids and disulfonates. $ReCl_6$ showed the best results of the inorganic compounds studied and had a limit of detection in the top five for LTC 1, LTC 2, and TTC 2. Two phosphorus containing compounds were also studied. Phenyl phosphate had lower LODs than hydrogen phosphite. This result is in general agreement with earlier work that used dicationic reagents and singly charged anions, which found that more oxidized species had better LODs.[63]

The additional application of the tricationic reagent to enhance detection for chromatography is shown in Figure 8.2. Three dianions (camphorate, phenylsuccinate, and naphthalene-1,5-disulfonate) are separated using a β-cyclodextrin stationary phase. The trication is added post-column. The better peak shape for the late eluting naphthalene-1,5-disulfonate peak is likely due to the step gradient employed. The first two peaks are broadened before the mobile phase is changed, while the third peak is eluted by the strong solvent. Chromatographic retention and separation of dianions could be very useful in cases of complex sample matrixes.

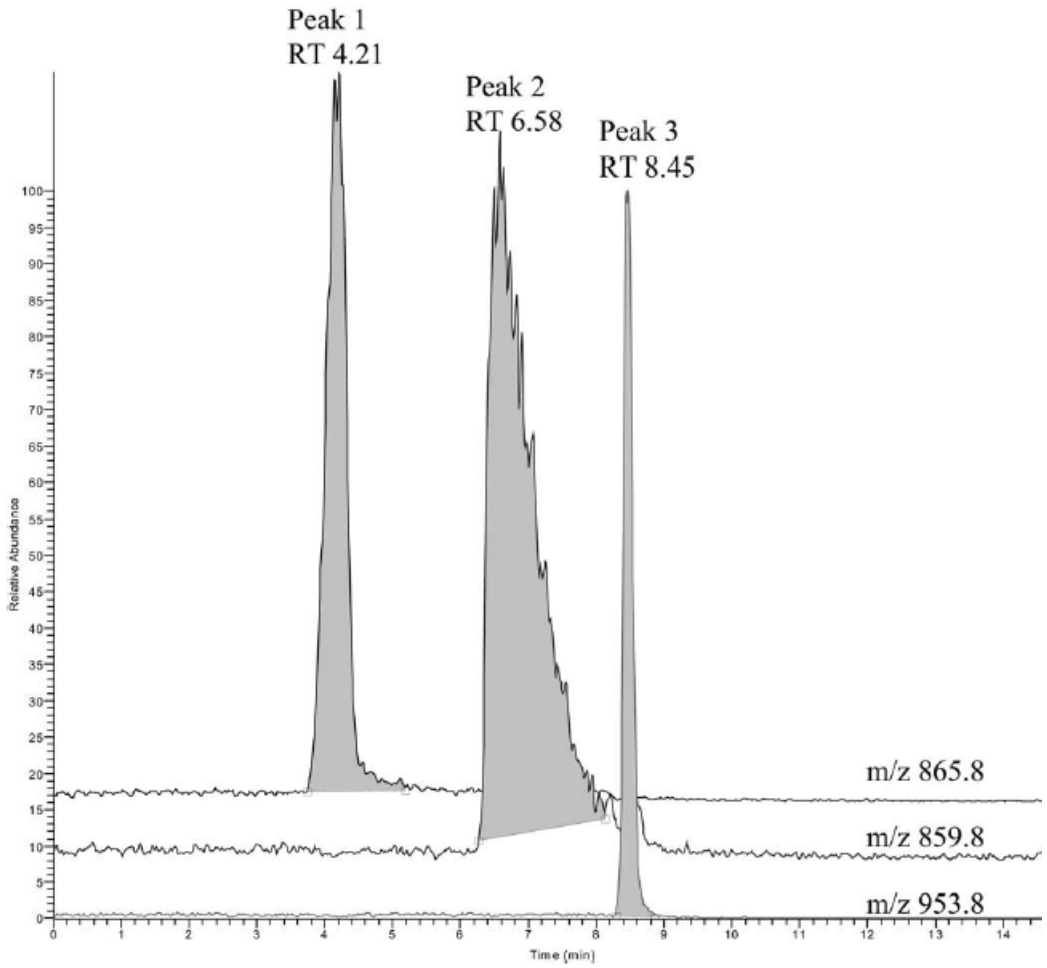


Figure 8.2 An extracted ion chromatogram using tricationic ion-paring agents.

LC separation of camphorate (peak 1), phenylsuccinate (peak 2), and naphthalene-1,5-disulfonate (peak 3) with the retention times (RT) also listed. This separation was performed on a β-cyclodextrin stationary phase (2.1 mm x 25 cm), which was equilibrated with 100% methanol. A step gradient to 100% water was applied at 5 min. The flow rate was 300 µL/min and 40 µM LTC 1 was introduced with a tee-piece to the effluent at a flow rate of 100 µL/min. The three trication–dianion complex masses were monitored simultaneously in SIM mode.

The limits of detection for most of the divalent anions could be reduced by using single-reaction monitoring (SRM). Some advantages of SRM are to improve specificity in analysis, to lower noise in the region being analyzed, and/or to eliminate interference by a background ion in the mass spectrometer. In SRM, the dianion-trication complex is trapped, excited, and the

transition to a resultant fragment is monitored. SRM analysis was peformed for each dianion and the results are shown in Table 8.2.

Table 8.2 LODs for divalent anions using four tricationic pairing reagent in SRM.

	Linear tications		Trigonal tications	
	LTC 1 LOD (ng)	LTC 2 LOD (ng)	TTC 1 LOD (ng)	TTC 2 LOD (ng)
Disulfonates				
Dihydroxynaphthalenedisulfonate	2.75E-03	5.00E-03	7.50E-03	**1.20E-03**
m-Benzenedisulfonate	**5.00E-04**	3.00E-03	6.25E-03	1.25E-03
4-Formyl-m-benzenedisulfonate	5.00E-03	1.00E-02	—	**1.50E-03**
Naphthalene-1,5-disulfonate	**4.61E-04**	4.38E+00	4.50E-03	3.60E-03
Butanedisulfonate	4.50E-03	6.25E-03	**3.50E-03**	**4.50E-03**
Propanedisulfonate	2.00E-02	1.25E-02	7.50E-03	4.50E-03
Anthraquinone-2,6-disulfonate	1.13E-03	**7.50E-04**	3.60E-03	7.90E-03
Methanedisulfonate	3.25E-03	3.15E-03	4.50E-02	**3.00E-03**
Ethanedisulfonate	**1.50E-03**	8.75E-03	1.44E-02	9.80E-03
Dicarboxylates				
Dipivolyltartarate	6.25E-03	**3.75E-03**	1.00E-02	5.50E-03
Camphorate	4.50E-02	4.50E-02	**3.00E-02**	2.00E-01
Phenylsuccinate	1.00E+00	7.50E-02	1.00E-01	**2.50E-02**
Glutarate	**3.75E-02**	6.00E-02	7.50E-01	1.50E-01
Malate	7.00E-02	**1.50E-02**	—	—
Methylsuccinate	**2.40E-02**	3.75E-02	1.05E-01	4.00E-02
Fumarate	**1.00E-02**	2.25E-02	1.50E+00	—
Pimelate	**3.00E-02**	7.50E-02	3.25E+00	7.50E-01
Malonate	**1.00E-01**	1.20E-01	3.00E-01	5.00E-01
Adipate	**1.20E-01**	2.25E-01	2.25E+00	1.50E+00
Dibromomaleate	7.50E-02	3.00E-02	3.50E-02	**2.50E-03**
Chlorosuccinate	1.50E+00	3.75E+00	**4.50E-01**	—
Metal containing compounds				
Hexachlororhenate $ReCl_6$	**2.00E-03**	3.00E-03	1.00E-02	2.00E-02
Chromate CrO_4	7.50E-02	2.25E-01	3.00E-01	**4.00E-02**
Molybdate MoO_4	**2.50E-02**	2.50E+00	5.00E-01	1.58E-01
Manganate MnO_4	3.75E-01	—	**1.25E-01**	7.50E-01
Arsenate AsO_4	**9.00E-02**	2.00E-01	5.75E-01	2.75E-01
Other sulfur compounds				
Tetrathionate S_4O_6	**1.00E-05**	4.00E-04	5.00E-04	5.00E-03
Peroxidisulfate S_2O_8	1.25E-03	**1.15E-03**	6.75E-03	6.00E-03
Succinaldehyde bisulfite	7.50E+00	1.50E+01	**1.50E+00**	5.50E+00
Glutaraldehyde bisulfite	**5.00E-02**	2.50E+00	8.75E-01	3.00E+00
Miscellaneous compounds				
Phenylphosphate	**5.00E-06**	1.00E-03	1.13E-02	1.50E-02
Rhodizonate	**1.05E-01**	5.00E-01	3.75E+00	1.25E-01
Hydrogen phosphite	**3.25E-02**	2.00E-01	1.00E+00	3.50E-02
Selenite	7.50E-02	—	**7.00E-02**	2.63E+00

*Limit of detection determined where the amount of analyte used results in S/N = 3.
Bold typeface indicates the lowest limit of detection for each anion.
— Indicates that a dianion/trication complex was not observed.

For LTC 1, most SRM transitions were to a fragment of the trication. Most of the dianion/trication complexes fragmented to either m/z 665.5 $[LTC1\text{-}2H]^{+1}$ or m/z 367.4 corresponding to the C_{10}TPPImidazole (shown in Fig. 8.3a). The inorganic anions, tetrathionate, peroxidisulfate, fumarate, phenylphosphate, and phenyl succinate did not fragment to m/z 665

or 367.4. For these -2 anions, a portion of the dianion was lost and the +1 complex between the trication and the remainder of the dianion was monitored. An example is tetrathionate where the complex fragment monitored (m/z 811.6) corresponds with the loss of SO_3. The most common fragments for LTC 2, were either the loss of 1 hydrogen each from 2 of the imidazolium rings (m/z 551.3) or the loss of the benzylimidazolium group (Fig 8.3b). For the complexes that lost the benzylimidazolium group, the dianion stayed complexed with the remainder of the trication. This unconventional fragmentation occurred with LTC 2 for the inorganic anions, peroxidisulfate, tetrathionate, rhodizonate, phenylphosphate, and dihydroxynaphthalenedisulfonic acid.

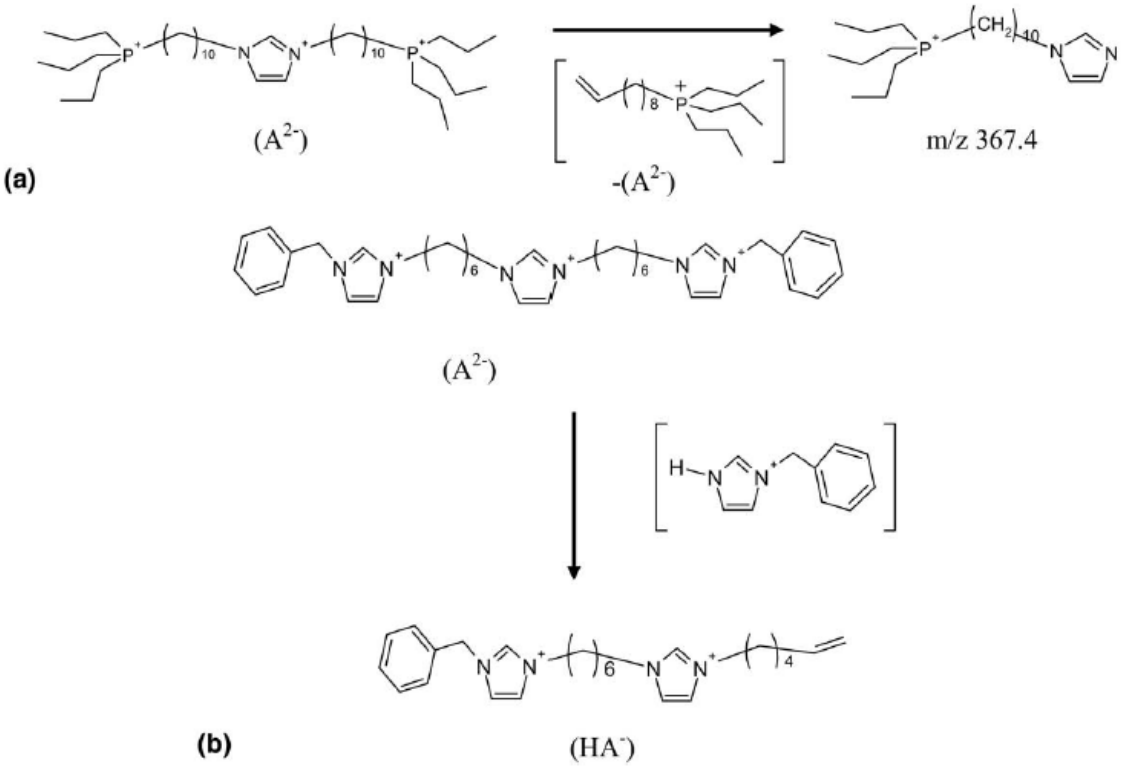


Figure 8.3 Proposed fragmentation pathways for the disulfonates using LTC 1.

The predominant fragment monitored for TTC 1 is the loss of two hydrogens from the methylene carbons between the phosphorus and benzene ring. Only manganate, peroxidisulfate, tetrathionate, hexachlororhenate, and chromate underwent alternate fragmentation. The major fragmentation pathway for TTC 2 is the loss of the butylimidazolium group from the overall complex, so the dianion remains with the rest of the trication. Arsenate, peroxidisulfate, tetrathionate, rhodizonate, hexachlororhenate, glutaraldehyde bisulfate, dihydroxynaphthalenedisulfonic acid, adipate, and pimelate, succinaldehyde bisulfite, and camphorate followed alternate fragmentation patterns with TTC 2.

The group of compounds that had the largest improvements in LOD between SIM and SRM were the disulfonates. With one or more of the trications studied, each disulfonate had its LOD improved by at least an order of magnitude. The disulfonates were the only analytes to follow fragmentation for LTC 1 and LTC 2 as shown in Figure 8.3a and 8.3b, respectively. While the largest change in LOD was seen for the linear trications, the trigonal trications had the lowest LOD for 5 of the 9 disulfonates studied.

Chlorosuccinate and dibromomaleate also had interesting fragmentation patterns. In the case of these analytes, the halogen is lost from the anion and remains paired with the trication (or a portion of it). This was seen in our previous study on the linear trications.[230] Figure 8.4a illustrates a proposed fragmentation pattern for dibromomaleate using TTC 1. The distinct isotopic pattern for bromine (Fig. 8.4c) is evidence of the gas phase association of the bromine with a +2 fragment of the trication. The improvement in LOD between SIM and SRM was larger for the halogenated dicarboxylates using the trigonal trications.

Phenylphosphate showed an improvement of 2-3 orders of magnitude by SRM for both linear trications. The SRM LODs for the dicarboxylates ranged from just slightly better than SIM LODS to about 8 times better, with the exception of fumarate and malonate which showed 18-fold (LTC 2) and 20-fold (LTC 1) improvements, respectively. Arsenate (LTC 2), hexachlororhenate (LTC 2, TTC 1), and glutaraldehyde bisulfite (LTC 1) were the only other

analytes with improvements of an order of magnitude or more. In general, the linear trications had lower LODs for SRM than the trigonal cations.

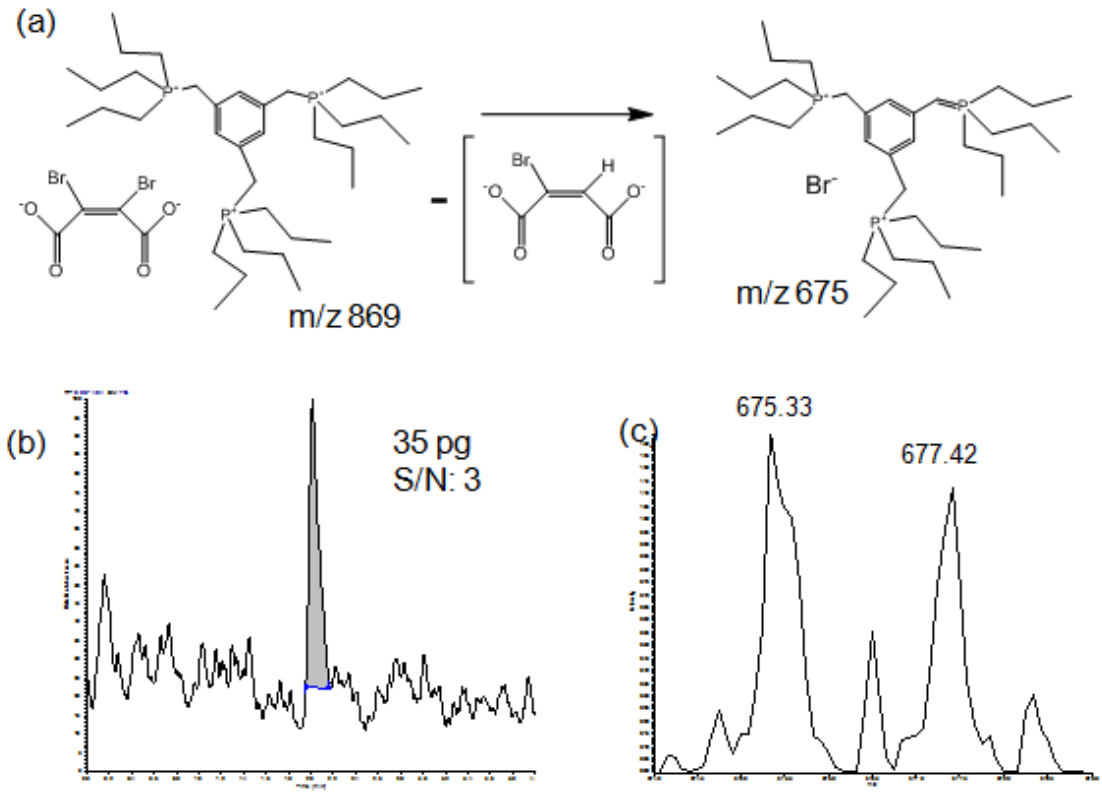


Figure 8.4 Proposed fragmentation pathway of the SRM transition for dibromomaleate.

Panel (**a**) shows the pathway. Panel (**b**) shows an injection monitoring the SRM transition from *m/z* 869à to 675.33 and 677.42. Panel (**c**) is the fragment spectrum observed for the peak shown in (**b**). The main peaks are two mass units apart and nearly the same height, indicative of Br.

The tricationic reagents can pair with doubly charged anions to form complexes with an overall +1 charge, but can also pair with singly charged anions to form +2 complexes. Five "mono-anions" were evaluated using the four tricationic reagents to determine their limits of detection. The data for SIM and SRM for these anions is shown in Table 8.3. The LOD for benzenedisulfonate both by SIM and SRM is the lowest for the five singly charged anions tested. In comparison to the SIM LOD for the dicationic reagents tested in a previous study[185]

the LOD for benzenedisulfonate ranks second using LTC 1 as a pairing reagent. The LODs in this study for perfluoroctanate and monochloroacetate are better than 7-8 (of 23) of the dicationic pairing reagents previously studied.[185] The ability of the tricationic reagents to pair with doubly and singly charged anions shows that the use of a single tricationic pairing reagent could be used to evaluate both monovalent and divalent anions simultaneously.

The LODs in this study compare favorably with those reported for anion analysis by other methods. There are many methods reported for the analysis of biologically releveant organic acids. In our study, the LODs for fumarate and methylsuccinate were 10 and 24 pg, respectively. Lower limits were determined, 0.9 pg fumarate and 0.5 pg methylsuccinate, by an LC method where the analytes were subjected to a long derivatization process to use fluorescence detection.[225] CE analysis with indirect UV detection was used to determine levels of various organic acids. The LODs under the optimized CE conditions for malonic acid, methylsuccinic acid, glutaric acid, and adipic acid reported are 144 pg, 37.3 pg, 34.9 pg, and 72.2 pg respectively. Our SRM tricationic method showed lower LODs for the malonic and methylsuccinic acids (100 pg and 24 pg), similar results for the glutaric acid (37.5 pg), and higher results for adipic acid (120 pg).[173] A number of the analytes in that study had very similar migration times and without a more specific detection method, might be indistinguishable in that analysis.

Larger improvements over previous methods were seen with the disulfonates. An LOD of 200 pg for benzenedisulfonate by LC-UV was reported.[223] Using our method and TTC 1, the LOD for the same analyte is 8.75 pg using SIM detection and 500 fg using LTC 1 and SRM detection. Other aromatic sulfonates were determined in concentration ranges of 0.1-1 ng/ml by solid phase extraction-ion pair chromatography using UV detection[219] and 100-400 ng/ml by CE/MS.[211] The LOD for napthalene-1,5-disulfonic acid was determined by ion interaction chromatography both by the direct injection of a large sample volume (100uL) and preconcentration (sample volume of 50 mL).[220] The LODs were 20 ng for the large sample

volume and 30 ng for the sample preconcentration. Using the tricationic pairing method and no preconcentration, the LOD for this analyte is 12.5 pg in SIM mode and 461 fg in SRM.

The analysis of inorganic ions is also important, though not always as facile as the detection of organic acids or disulfonates. A coated-wire membrane sensor electrode was used to determine chromate levels in solution.[232] The LOD for this method was determined in a solution that was 116 ng/mL. In our analysis of chromate, the lowest solution concentration we analyzed was 8 ng/mL in SRM mode using TTC 2, for an absolute detection limit of 40 pg. Molybdate levels in various water samples were determined by coprecipitation and neutron activation analysis, a very labor intensive technique which can necessitate the use of a reactor.[233] The limit of detection for this method was 1 pg/mL using a 100 mL sample, for an absolute detection of 100 pg of molybdate. Using LTC 1, the LOD for molybdate in SRM is 25 pg. Another precipitation method was used to preconcentrate $ReCl_6$ followed by detection using selective excitation of probe ion luminescence.[234] In this study, 150 pg of $ReCl_6$ was needed to see an observable signal. In our study, $ReCl_6$ was determined well below 150 pg in both SIM (15 pg) and SRM (2 pg) monitoring modes.

Table 8.3 LODs in SIM and SRM modes for monovalent anions using four tricationic reagents.

	Linear trications		Trigonal trications	
SIM mode	LTC 1 LOD (ng)	LTC 2 LOD (ng)	TTC 1 LOD (ng)	TTC 2 LOD (ng)
Benzenesulfonate	**1.50E-03**	9.00E-03	1.50E-02	3.13E-03
Perfluorooctanoate	5.00E-02	2.75E-02	**1.50E-02**	5.00E-02
Trifluoromethanesulfonimide	**1.05E-02**	7.50E-02	3.00E-02	1.00E-01
Monochloroacetate	7.00E+00	1.25E+01	1.00E-01	**2.00E+00**
Benzoate	6.25E+01	8.75E+00	3.75E+00	**9.65E-02**
SRM mode	LOD (ng)	LOD (ng)	LOD (ng)	LOD (ng)
Benzenesulfonate	**9.50E-05**	2.70E-03	3.50E-03	1.38E-03
Perfluorooctanoate	**3.00E-04**	4.13E-03	3.00E-03	1.63E-03
Trifluoromethanesulfonimide	1.05E-02	6.00E-02	2.50E-02	**3.43E-04**
Monochloroacetate	—	1.00E+01	**1.00E-02**	7.50E-02
Benzoate	—	8.75E+00	3.75E-01	**9.65E-02**

*Limit of detection determined where the amount of analyte used results in S/N = 3.
Bold typeface indicates the lowest limit of detection for each anion.
— Indicates that a dianion/trication complex was not observed.

8.5 Conclusions

Four optimal tricationic pairing reagents were used to determine the limits of detection for 34 divalent anions and 5 monovalent anions. The linear and trigonal tricationic reagents performed about equally as a whole, but the two trications with tripropylphosphonium cationic moieties outperformed trications with imidazolium based charge groups. When evaluating tricationic reagents, our results show that the linear trications provide lower limits of detection for most classes of compounds and should be tested first. The exception to this is the determination of disulfonates, where trigonal trications generally perform better. The use of tandem MS on the trication/di-anion complex helps to improve the sensitivity of detection for most of the dianions studied. Those complexes that dissociate into fragments not common to the trication showed the lowest limits of detection. Tricationic ion-pairing agents can also be used to determine monovalent anions by monitoring the +2 complexes. Therefore, mixtures of monovalent and divalent anions could be studied using a single tricationic reagent. Many of the LODs in this study are better or similar to those that have been previously reported, however this method is advantageous as it does not involve intricate sample preparation nor preconcentration and may be accessible to more laboratories.

CHAPTER 9

CE-ESI-MS ANALYSIS OF SINGLY CHARGED INORGANIC AND ORGANIC ANIONS USING A DICATIONIC REAGENT AS A COMPLEXING AGENT

9.1 Abstract

A dicationic ion pairing reagent, N, N'-dibutyl 1,1'-pentylenedipyrrolidinium, was used to form complexes with singly charged anions for their subsequent analysis by CE-ESI-MS in positive ion mode. This methodology offers the advantages of greater versatility and sensitivity relative to direct detection of the anions in negative ion mode, and it can be realized by a number of possible complexation strategies, including pre-column, on-column, and post-column modes. Four model anions, perfluorooctanoate (PFOA), benzenesulfonate (BZSN), monochloroacetate (MCA), and trifluoromethanesulfonimide (NTF_2) were amenable to complexation with the dicationic reagent, yielding singly charged cations with greater m/z ratios. By optimizing various parameters, including the CE separation buffer composition and pH, the concentration of the dicationic reagent, the mode of complexation, the nebulizing gas pressure, and the sheath liquid composition, it was possible to develop a robust CE-ESI-MS method appropriate for the analysis of anions in a tap water sample. By this method, limits of detection were found to be 20.9 and 1.31 ng/mL for MCA and BZSN, respectively.

9.2 Introduction

The diverse fields of environmental, pharmaceutical, and food science, amongst others, share the common need for methods capable of determining anions with great sensitivity. Established methods that permit the direct detection of anions without incorporating any prior separation techniques include mass spectrometry[63,208,235], spectrophotometry[59,186], ion-selective potentiometry and other electrochemical techniques.[174,236]

However, complex sample matrices often necessitate the incorporation of separation methods in conjunction with anion detection. Ion chromatography is the most common separation method used in this context.[60-61,173,237] Gas chromatography[238], reverse-phase liquid chromatography[239], and capillary electrophoresis (CE)[31,240] methods for anion analysis have also been demonstrated, and each of these can be routinely coupled to MS for anion detection. The detection of anions by these coupled techniques can be complicated by the need for derivatization into volatile species (GC-MS) or by the higher background and poorer electrospray stability experienced in negative ion mode.[166,238] Also, very small anionic analytes may fall below the low mass cutoff of the spectrometer and thus be undetectable in their native state, while other (detectable) low mass anionic analytes reside in a region of high chemical noise and may suffer from reduced sensitivity relative to larger mass ions.[59] To overcome these limitations, small quantities of large, chaotropic, organic dicationic or tricationic ion-pairing agents can be added to pair with singly-charged or doubly-charged anions, yielding positively charged complexes of higher m/z.[63,185,208,230,241-242] The trace analysis of perchlorate in various samples first demonstrated the success of this approach.[59] Armstrong and coworkers introduced the use of an imidazolium-based dicationic reagent as a complexing agent for 34 singly-charged anions.[63] This allowed for the detection of these anions in the more stable and sensitive positive-ion mode ESI-MS. The analysis of a tap water sample by this method revealed the presence of five anions (chloride, nitrate, bromide, monochloroacetate (MCA), and benzenesulfonate (BZSN)), which were also quantified. A mixture of five other selected anions (thiocyanate (SCN), triflate (TFO), BZSN, perfluorooctanoate (PFOA), and trifluoromethanesulfonimide (NTF_2)) were separated by HPLC prior to a comparison of detection in both positive and negative selected ion monitoring (SIM) mode. The results proved the ultra-sensitivity of this technique and demonstrated its compatibility with HPLC.

The structure and nature of the dicationic reagent can have significant effects on the reagent's affinity for anionic analytes and stability. A recent study by Remsburg *et al* compared

23 dicationic salts to evaluate their efficacies as pairing agents for anion analysis in positive ion mode.[185] From these, four dications were recommended for general use in subsequent anion analysis by this ion-pairing method. The dication N, N'-dibutyl 1,1'-pentylenedipyrrolidinium, which possesses a pentane linkage and butylpyrrolidinium charged groups (Fig. 9.1), was found to be the best to analyze for cyanate while also performing well for other anions (especially iodide and nitrate).

Capitalizing on this earlier work, and recognizing the inherent advantages of CE as a separation technique (including reduced solvent/reagent consumption, reduced sample requirements, and high efficiencies relative to LC), we have developed a CE-ESI-MS method using the dication N, N'-dibutyl 1,1'-pentylenedipyrrolidinium fluoride as a complexing agent for anion analysis in the positive-ion mode. Review articles have critically examined the performance of CE-ESI-MS relative to LC-ESI-MS[243], and the utility of CE-ESI-MS as a quantitative tool[244], and as a tool for bioanalysis.[245] The sensitivity and efficiency of the new anion analysis method introduced herein depends on the mode by which the dicationic reagent is introduced to the sample solution, and so a comparison of three possible modes is presented. These modes for the introduction of the dicationic reagent include: pre-column (before injection and CE separation); on-column (by incorporation of the dication in the CE separation buffer); and post-column (by introduction of the dication to the separated analytes via a sheath liquid necessary to the ESI process). Finally, a practical application of our CE-ESI-MS method with dication pairing is demonstrated by the detection of two environmental contaminants (BZSN and MCA) in a tap water sample. The rapid and reproducible separation, quantitation, and identification of singly-charged anions permitted by this CE-ESI-MS method with dication pairing is important for future biological and environmental research.

9.3 Experimental

9.3.1 Reagents and solutions

All chemicals and solvents used were of analytical grade or HPLC quality. Perfluoro-octanoic acid (PFOA), sodium benzenesulfonate (BZSN), monochloroacetic acid (MCA), and lithium trifluoromethanesulfonimide (NTF_2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of each anion were prepared in distilled, deionized water (Millipore, Bedford, MA, USA) to the appropriate concentration. N, N'-dibutyl 1,1'-pentylenedipyrrolidinium dication (Fig. 9.1) was synthesized as its bromide salt according to a previously published procedure from Armstrong and coworkers.[185] To maximize complex formation between the dicationic reagent and singly-charged analyte anions, the dication was subjected to ion exchange to produce a stock solution (10.00 mM) of the fluoride salt in water according to the procedure described by Martinelango *et al*.[59] The dicationic reagent solution was either added to the anion sample, to the CE separation buffer, or to the sheath flow liquid in the appropriate concentration according to the method being studied. Ammonium acetate (>99.99%), ammonium formate, ammonium carbonate (ACS reagent), ammonium bicarbonate (ACS reagent), ammonium hydroxide (28 – 30%, ACS reagent) and formic acid (> 99.9%, ACS reagent) were from Sigma-Aldrich, and were used to prepare CE separation buffers. Working buffer solutions were prepared to the required concentrations and their pH values were adjusted by adding ammonium hydroxide or acetic acid as appropriate. Buffers were filtered through 0.2 µm nylon syringe filters (Corning, NY, USA) before use.

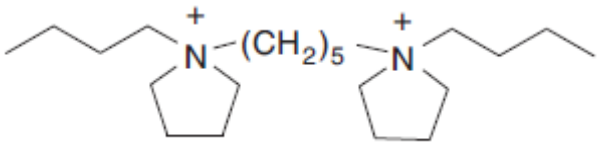


Figure 9.1 Structure of the dicationic paring agent used for CE-ESI-MS.

9.3.2 CE-ESI-MS analysis

A HP^{3D}CE capillary electrophoresis system (Agilent Technologies, Palo Alto, CA, USA) equipped with a UV absorbance diode array detector was used for CE-MS coupling. CE-MS experiments were performed with a 75.0 cm (21.5 cm to the UV absorbance detector), 48 µm i.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ). The capillary temperature was controlled at 25^{o}C inside the CE safety interlock compartment. The capillary was conditioned prior to its first use by consecutively flushing with H_2O for 10 min, 0.1 M NaOH for 10 min, H_2O for 10 min, and the electrophoresis buffer for 20 min. After each sample run, the capillary was rinsed with the electrophoresis buffer for 2 min. The buffer in the inlet reservoir was renewed after every three runs for improved reproducibility. The separation voltage was 20 kV. Analytes were hydrodynamically injected at 50 mbar for 10 s.

The CE system was coupled to an Esquire 3000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an orthogonal electrospray ionization (ESI) source. The CE-ESI-MS coupling was facilitated by a coaxial sheath liquid interface (Agilent Technologies). The ion trap mass spectrometer was used in the positive ion mode, and the capillary voltage was set at 3.5 kV. Dry nitrogen gas was heated to 325^{o}C and delivered at a flow rate of 5 L/min, while the nebulizing gas (N_2) pressure was 15 psi. The lenses and block voltages were fixed using the tuning software, assuming the compound stability to be 100%. The ion trap was operated in ion charge control mode to accumulate 30000 ions, for a maximum accumulation time of 200 ms. The sheath liquid (50:50, v/v; MeOH/H_2O) was delivered at 0.4 mL/min by a pump equipped with a 1:100 splitter (thus providing sheath liquid to the CE-MS interface at 4 µL/min).

9.4 Results and Discussion

9.4.1 Foreword

In order to develop a robust and sensitive method for the analysis of a variety of anionic analytes by CE-ESI-MS, a number of parameters had to first be optimized. Of these, the buffer

composition and pH are of the highest priority, since these affect both the “front end” CE separation and the “back end” MS detection. The sheath liquid, necessary as make-up flow and for grounding the separation voltage, also requires optimization in terms of its composition and flow rate. Uniquely, the sheath liquid in the present work was also able to provide a mechanism for the introduction of the dicationic reagent for complexation of the anionic analytes as they emerged, separated, from the electrophoresis capillary in the post-column complexation mode. Finally, instrumental parameters such as nebulizing and drying gas flow rates and temperatures and MS trap voltages are of importance to the overall method viability, although the optimal values for these generally fell within the recommended instrument default ranges. A thorough examination of parameter optimization follows.

9.4.2 Optimization of CE separation buffer and pH

Optimization of the CE separation buffer with regard to analyte migration behavior, analysis time, peak shape, and resolution is complicated by coupling to MS due to the stringent requirements of the latter for buffer volatility. Commonly used CE-MS buffers are based on the ammonium salts of acetate, formate, carbonate, and bicarbonate. Fortuitously, these typical CE-MS buffer anions are relatively poorly complexed by the dicationic pairing regeant[63], and as such, are well suited for the dicationic reagent approach to anion analysis by CE-ESI-MS. A comparison of the electropherograms obtained using each of the four common CE buffers, prepared with the addition of 20.0 µM of N, N'-dibutyl 1,1'-pentylenedipyrrolidinium dication, for a mixture of four anionic analytes (PFOA, BZSN, NTF_2 and MCA) is shown in Fig. 9.2. Although baseline resolution of the four anions was easily achieved using each of the four buffers and “on-column” complexation with the N, N'-dibutyl 1,1'-pentylenedipyrrolidinium dication, the sensitivity of the method towards MCA was significantly diminished in the carbonate and bicarbonate buffers, especially relative to the acetate buffer. This is likely due to the fact that acetate is not efficiently complexed by the dication and so poses relatively less interference than the other buffer anions. Although the relative sensitivity of the method obtained with the

formate buffer was comparable to that obtained with the acetate buffer (and was even better in the case of BZSN with formate), migration times increased and peak shapes worsened, and so the ammonium acetate buffer was employed in subsequent experiments.

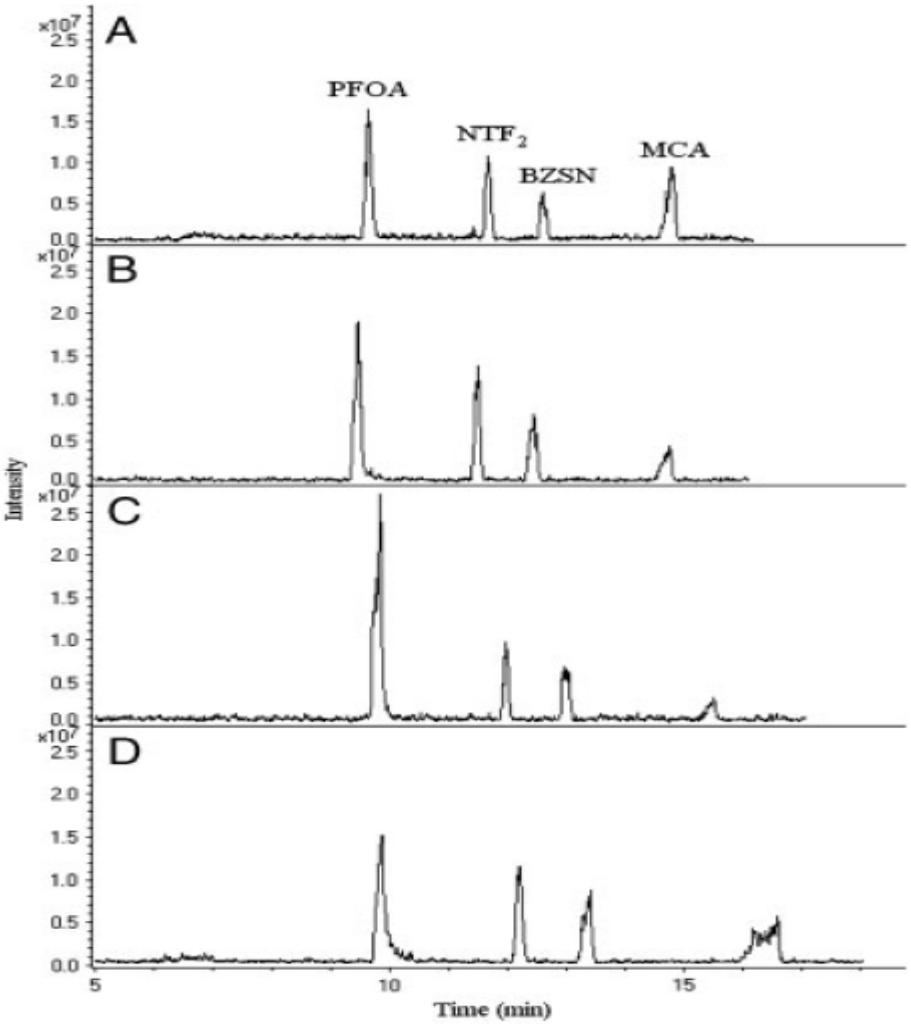


Figure 9.2 Electropherograms showing the effect of electrophoresis buffer composition on the separation of four anions

Anions used: (20 mg/ mL PFOA, 1 mg/mL NTF2, 10mg/mL BZSN, and 100 mg/mL MCA) by CE-ESI-MS. Each buffer contains 20 mM *N,N*0-dibutyl 1,10- pentylenedipyrrolidinium for on-column ion pairing of the analytes. (A) 30mM ammonium acetate, pH 8.93; (B) 30mM ammonium carbonate, pH 8.85; (C) 30mM ammonium bicarbonate, pH 7.79; (D) 30mM ammonium formate, pH 7.50.

The net mobility of the cationic complexes that were formed with the four model anions increased as the pH of the ammonium acetate buffer was increased over the range from 5.00 to 8.93, as seen in Fig. 9.3. Electroosmotic flow increases with increasing pH, and it is likely this effect that dominates the change in net mobility observed in this study. Since baseline resolution was achieved at all pHs studied, it was possible to optimize the pH on the basis of analysis time (eliminating the use of pH 5.00 based on its increased analysis time) and signal intensity (eliminating the use of pH 8.93 based on its reduced sensitivity). Thus, pH 6.68 was determined to be optimal for the acetate buffer in these studies.

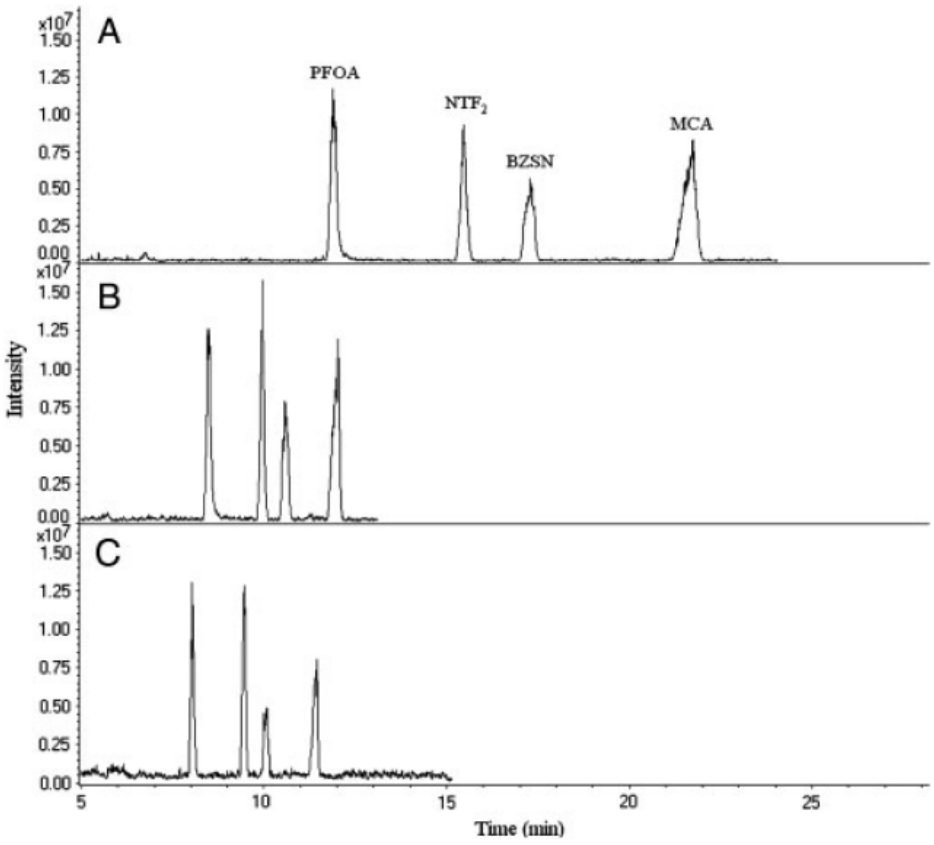


Figure 9.3 Electropherograms showing the effect of electrophoresis buffer pH on the separation of four anions.

9.4.3 Effect of dicationic reagent concentration

It is necessary to use a sufficient quantity of the dicationic reagent to ensure complexation of the analyte anions; however, a large excess of the dicationic reagent could have the effect of degrading overall sensitivities due to carryover (of the reagent to the ion source), space charge effects, trap volume, and/or interference from the excess reagent signal itself. To minimize the latter, base peak electropherograms were recorded by scanning the mass-to-charge ratio from 410 – 740, thus effectively removing possible interfering signals from uncomplexed dicationic reagent. To determine a suitable concentration of the dicationic reagent, electropherograms were recorded for the standard four anion sample mixture in 30.0 mM ammonium acetate buffer (pH 6.68) with either 10.0, 20.0, or 40.0 µM N, N'-dibutyl 1,1'-pentylenedipyrrolidinium added to the buffer for on-column complexation (Fig. 9.4). As can be seen in Fig. 9.4, peak heights increased (increasing sensitivity) with increasing concentration of the dicationic reagent added to the separation buffer up to 20.0 µM of added N, N'-dibutyl 1,1'-pentylenedipyrrolidinium, while a subsequent reduction in signal was observed in the case of 40.0 µM of added N, N'-dibutyl 1,1'-pentylenedipyrrolidinium. Additionally, migration times increased as dicationic reagent concentration increased, presumably due to the reduction of electroosmotic flow resulting from increased buffer ionic strength upon the addition of dicationic reagent. Thus, the optimum concentration for the dicationic reagent in these studies was determined to be 20.0 µM. This reagent concentration was appropriate for the analysis of a range of analyte concentrations (as determined during calibration studies, Section 3.6), but optimization of this parameter would have to be repeated if analyte concentration ranges were orders of magnitude higher than those studied here.

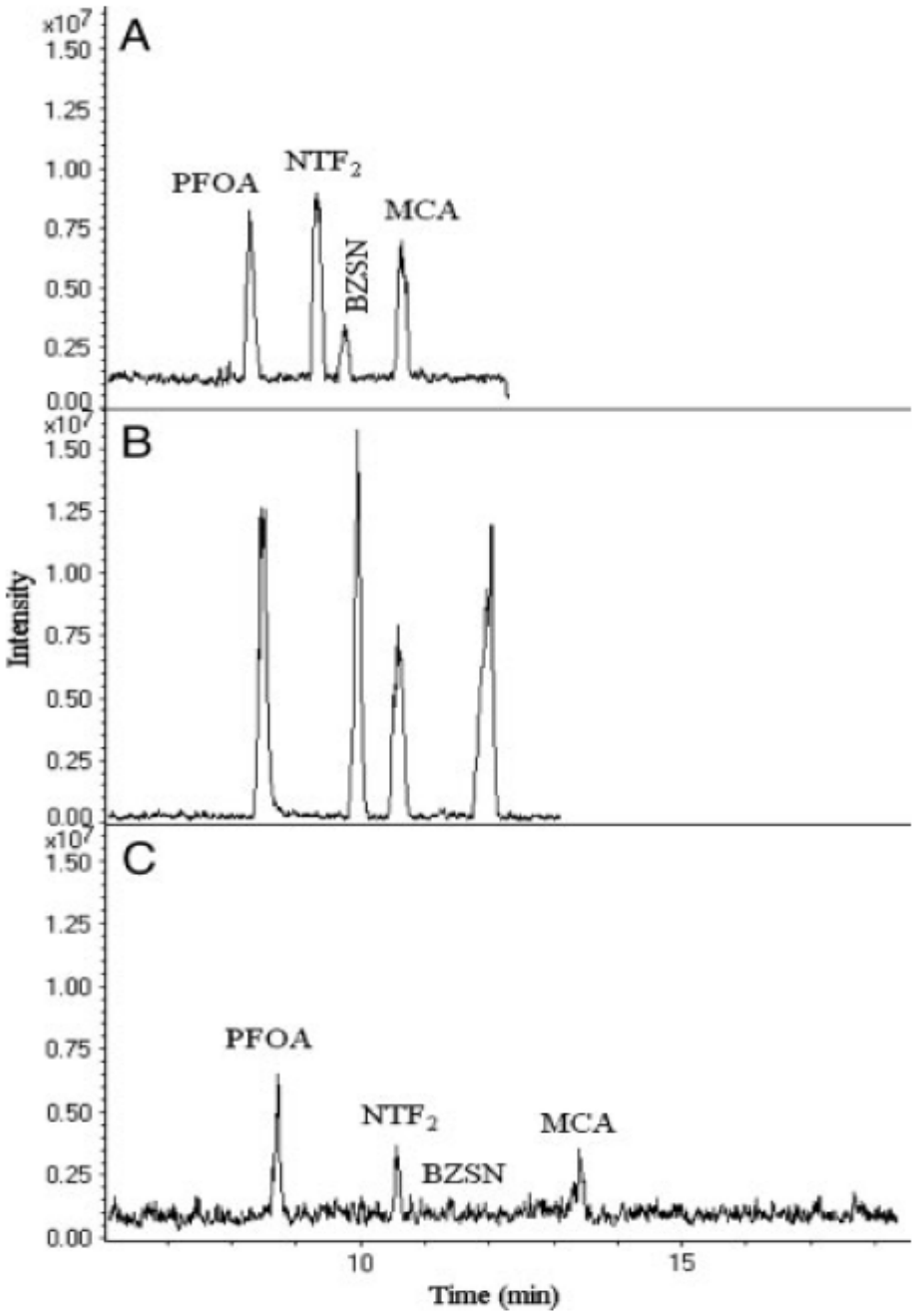


Figure 9.4 Electropherograms resulting from varying the concentration of dicationic reagent.

Agent added to a 30mM ammonium acetate separation buffer (pH 6.68) for on-column complexation and CE-ESI-MS analysis of four anions (20 mg/mL PFOA, 1 mg/mL NTF2, 10mg/mL BZSN, and 100 mg/mL MCA). (A) 10.0 mM *N,N*0- dibutyl 1,10-pentylenedipyrrolidinium; (B) 20.0 mM *N,N*0-dibutyl 1,10-pentylenedipyrrolidinium; (C) 40.0 mM *N,N*0-dibutyl 1,10- pentylenedipyrrolidinium.

9.4.4 Effect of sheath liquid composition

The optimization of the ESI parameters is crucial to achieve MS signal for any analyte, and this is especially true in CE-ESI-MS, where a sheath liquid is typically employed to make up the necessary volume flow from the capillary to the electrospray interface as well as to serve as a place in which to provide a ground for the separation voltage. In all cases, the sheath liquid affects the transfer of analyte from the liquid phase into the gas phase, thus having a significant impact on the resulting MS signal (and sensitivity). It has been reported that small amounts of formic acid, acetic acid, ammonium formate, or ammonium acetate can be added to the sheath liquid to enhance ESI-MS in positive ion mode.[246-247] Furthermore, incorporation of an organic solvent in the sheath liquid can improve the efficiency of ion evaporation.[248] Juan-García *et al.* investigated the effect of methanol and isopropanol as sheath liquids and found that methanol gave the most stable and highest MS signal.[249] Thus, methanol was employed as a component of the sheath liquid in the present studies.

The concentration of methanol in the sheath liquid was optimized by comparing the sensitivity and stability of the MS signal resulting from experiments conducted with three different concentrations of methanol (20, 50, 80% v/v) in water as the sheath liquid. As can be seen in Fig. 9.5, peak areas (sensitivities) for the four analytes (PFOA, NTF_2, BZSN, and MCA) were reduced when 20/80 methanol/water was employed as the sheath liquid. Increasing the methanol content of the sheath liquid up to 50% resulted in markedly improved sensitivities (and peak shapes); however, increasing the methanol content further still (up to 80%) resulted in higher background noise, which degraded overall sensitivities. Thus, a 50/50 (v/v) methanol/water mixture was employed as the sheath liquid in subsequent experiments.

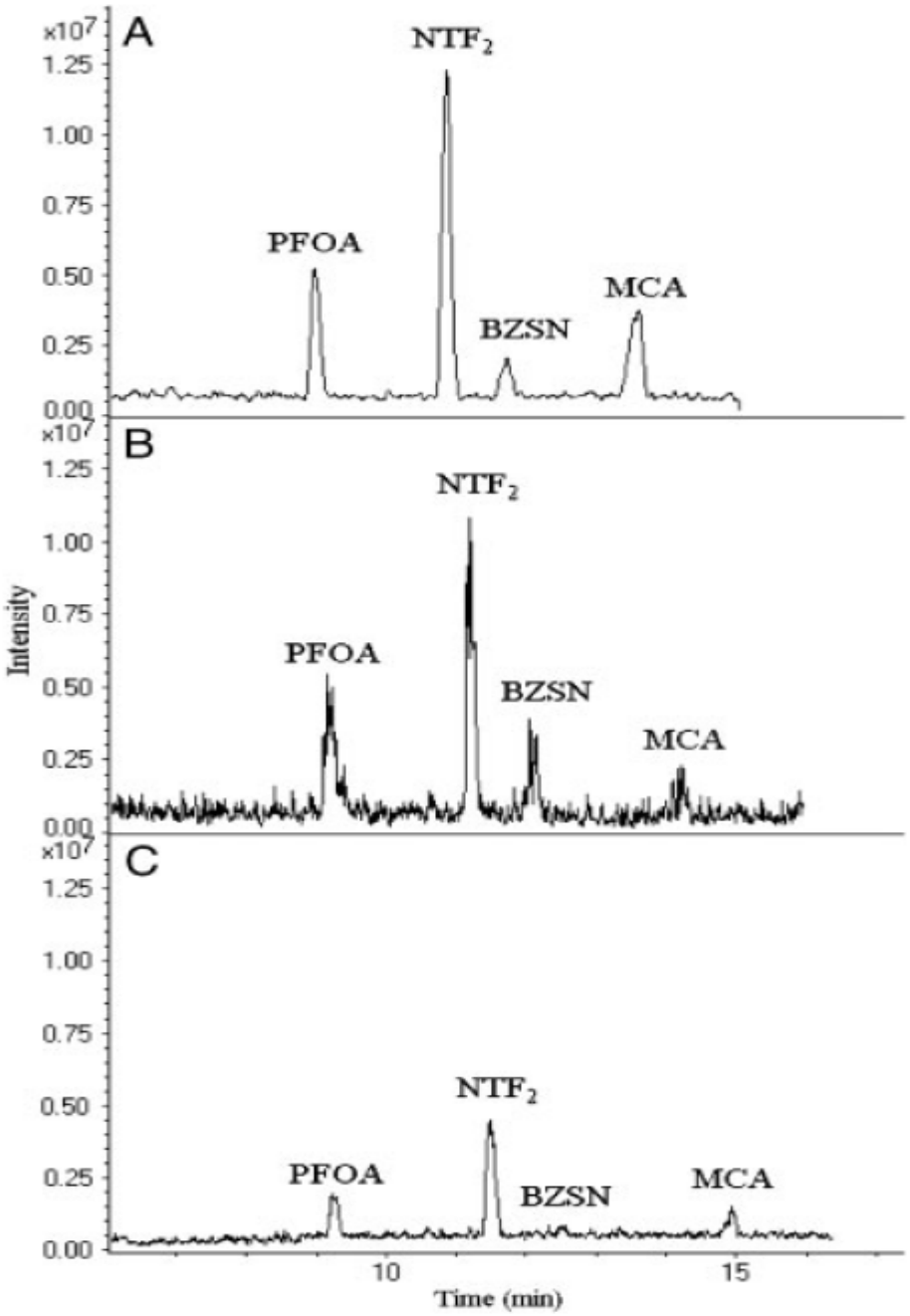


Figure 9.5 The effect of sheath liquid composition on the separation of four anions.

Anions: (20 mg/mL PFOA, 1 mg/mL NTF2, 10 mg/mL BZSN, and 100 mg/mL MCA) by CE-ESI-MS with oncolumn complexation by 5.0 mM *N,N*0-dibutyl 1,10-pentylenedipyrrolidinium in a 30mM ammonium acetate separation buffer (pH 6.68). (A) 50/50 v/v methanol/water; (B) 80/20 v/v methanol/ water; (C) 20/80 v/v methanol/water.

The effect of formic acid concentration (0.0, 0.1%, 0.5%) in the sheath liquid was also investigated. Peak areas (sensitivities) for the four analytes (20 µg/mL PFOA, 1.00 µg/mL NTF_2, 10.00 µg/mL BZSN, and 100.0 µg/mL MCA) were reduced as the formic acid concentration was increased, until BZSN and MCA could no longer be detected with 0.5% formic acid in the sheath liquid (data not shown). Longer migration times were observed when the highest concentration of formic acid (0.5%) was employed in the sheath liquid. This result was unexpected but reproducible, and may be attributed to alteration of the electrophoresis buffer composition by the presence of higher concentrations of acid in the sheath liquid, which, in turn, could reduce electroosmotic flow and hence, increase migration times.[250] Since the greatest sensitivity (and no alteration of migration times) was achieved with no added formic acid, the simple 50/50 (v/v) methanol/water sheath liquid was determined to be optimum.

9.4.5 The effect of instrumental parameters

In order to examine the effect of the flow rate of the sheath liquid, the flow rate was increased from 0.2 to 1.0 mL /min (although it should be recognized that the actual sheath flow at the CE-MS interface was 100-times lower due to the use of a pump with a 1:100 split ratio, as described previously). When the flow rate was increased from 0.2 to 0.4 mL/min, a gradual increase in peak intensity was observed (data not shown). However, when the flow was increased from 0.4 to 0.6 mL/min, no further increase in sensitivity was found. Furthermore, at flow rates greater than 0.6 mL/min, a gradual decrease in peak intensity was observed. The decrease in sensitivity may be a consequence of the sample dilution effect and decreased ionization efficiency at higher flow rates. Thus, 0.4 mL/min was selected as the optimal flow rate of sheath liquid.

The drying gas in ESI is generally used to accelerate desolvation, to increase sensitivity, and to avoid the entry of undesirable ions into the mass spectrometer. Drying gas flow rates in the range of 4 -10 L/min were tested, with no affect on peak height (data not shown). A drying gas flow rate of 5 L/min was used in these experiments.

The effect of nebulizing gas pressure on CE-ESI-MS performance was examined for N_2 pressures of 10, 15, and 20 psi. Sensitivity was found to increase with increasing nebulizing gas pressure, but at the expense of CE resolution (data not shown), presumably because the nebulizing gas can have the effect of siphoning or drawing the contents of the separation capillary more rapidly toward the outlet. To retain sufficient resolution for the separation of many potential analytes in a complex mixture with enhanced sensitivity, a nebulizing gas pressure of 15 psi was selected.

9.4.6 Comparison of modes of complexation

In this work, the purpose of forming ion-pair complexes of singly-charged analyte anions with the N, N'-dibutyl 1,1'-pentylenedipyrrolidinium dicationic reagent was to increase the overall m/z of the species being detected and to render them positively charged, thus permitting their detection in positive ion mode with greater sensitivity than could be achieved by direct detection of the analyte anions in negative ion mode. To demonstrate this, Fig. 9.6 compares the detection of four anions, complexed and uncomplexed, in positive and negative polarity modes, respectively. The peak areas and signal-to-noise ratios of the four anions when complexed with N, N'-dibutyl 1,1'-pentylenedipyrrolidinium and detected in positive ion mode (Fig. 9.6(b)) were significantly larger than those of the uncomplexed ions in negative ion mode (Fig. 9.6(a)). In fact, the anion MCA could not even be detected at a concentration of 100.0 µg/mL in the negative mode. It should be noted that no attempts were made to optimize separation and detection parameters in the negative ion mode for this work, based on previous documentation of poor responses to ESI-MS analysis of acidic and polar species in negative ion mode and the complexity of optimization in this mode.[165] Clearly, complexation with the dicationic reagent led to the enhanced sensitivity and versatility that was sought. In particular, the signals for BZSN and MCA showed the greatest increases when detected in the positive ion mode, while NTF_2 showed the greatest sensitivity overall. This is due to the NTF_2 anion being

quite chaotropic and more surface active, allowing for detection at low levels in both positive and negative ion mode.[46]

However, it should be noted that the mode of complexation (of the anions with N, N'-dibutyl 1,1'-pentylenedipyrrolidinium) can also have an impact on detectability. Ion pairing can take place in the "pre-column" mode, whereby the dicationic reagent is mixed with the sample prior to injection and analysis by CE-ESI-MS; or in the "on-column" mode, whereby the dicationic reagent is added to the electrophoresis buffer so that the sample ions have a chance to undergo ion pairing throughout the duration of the separation and their migration through the capillary towards the detector; or in the "post-column" mode, whereby the dicationic reagent is added to the sheath liquid so that the sample ions undergo separation in their native state prior to the formation of ion-pair complexes and introduction to the MS detector. A comparison of pre-column, on-column and post-column complexation modes is shown in Fig. 9.6 (b), (c) and (d). On-column complexation resulted in greater sensitivities, and was characterized by shorter migration times compared with pre-column. In CE-LIF studies employing noncovalent fluorescent labeling of proteins with dyes, enhanced sensitivities are likewise achieved for on-column labeling relative to pre-column labeling. This can be attributed to the presence of complexing agent (or dye) at a constant concentration throughout the capillary, so that the equilibrium between free and bound complexing agent (or dye) can be readily established and maintained throughout the separation. Also, variations in analyte migration times (observed for pre-column versus on-column labeling or complexation methods) are likely due to variations in electroosmotic flow caused by alteration of the buffer composition upon the addition of complexing agents.

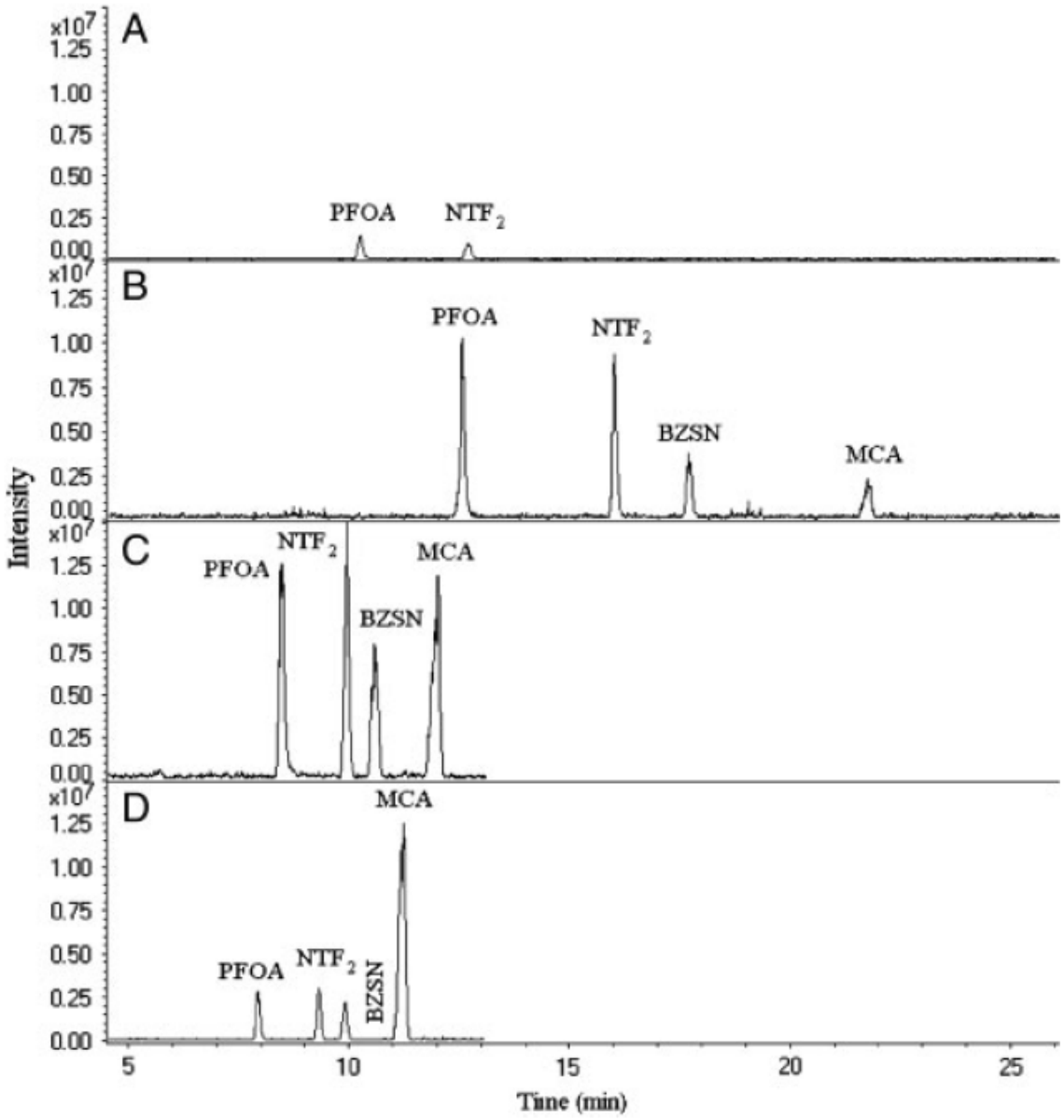


Figure 9.6 Electropherograms showing the effects of separation mode (positive ion *versus* negative ion mode; and precolumn *versus* on-column *versus* postcolumn ion-pairing mode) on the analysis of four anions

Anions: (20 mg/mL PFOA, 1 mg/ mL NTF2, 10mg/mL BZSN, and 100 mg/mL MCA) by CE-ESI-MS employing a 30mM ammonium acetate separation buffer (pH 6.68). (A) Negative ion mode with no ion pairing; (B) positive ion mode with precolumn complexation of the anions by mixing the sample with 20 mM *N,N*0- dibutyl 1,10-pentylenedipyrrolidinium prior to injection; (C) positive ion mode with on-column complexation with 20 mM *N,N*0-dibutyl 1,10-pentylenedipyrrolidinium in the separation buffer; (D) positive ion mode with post-column complexation with 20 mM *N,N*0-dibutyl 1,10-pentylenedipyrrolidinium in the sheath liquid.

A further comparison of the on-column and post-column modes of complexation for the test mixture of four analyte anions with N, N'-dibutyl 1,1'-pentylenedipyrrolidinium is shown in Fig. 9.6 (c) and (d). In these experiments, 20.0 µM N, N'-dibutyl 1,1'-pentylenedipyrrolidinium was either added to the separation buffer (Fig. 9.6(c)) or to the sheath liquid (Fig. 9.6(d)). Post-column complexation resulted in greater sensitivity (greater peak height) only in the case of MCA, while the other peak sizes were diminished relative to on-column complexation. It should be noted that the sensitivity for all analytes gradually decreased with repeated runs using the post-column complexation methodology (due to some as-yet unknown cause), and so it was determined that the on-column complexation method would provide optimal sensitivity for a tap water analysis, as described presently.

9.4.7 Tap water analysis

A tap water sample was collected from the cold-water tap of a laboratory sink at Wake Forest University. The water was allowed to run for 15 min before collection. For the determination of anions in the tap water sample by CE-ESI-MS with dicationic reagent complexation, the optimized experimental conditions, as previously determined, were used (namely: a separation buffer consisting of 30.0 mM ammonium acetate with 20.0 µM N, N'-dibutyl 1,1'-pentylenedipyrrolidinium (pH 6.68) for on-column complexation; a 50/50 (v/v) methanol/water sheath liquid delivered at 0.4 mL/min; and a 5 L/min flow of N_2 nebulizing gas at 15 psi and 325^{o}C). Quantitation of individual anions was performed using the extracted ion electropherogram mode, which allows for the extraction of signal corresponding to a given m/z ratio from the total ion current. The limit of detection for anions BZSN and MCA was calculated based on 3*s*/*m*, where *s* is the standard deviation in the baseline (approximated as 1/5 of the peak-to-peak noise in the blank signal); and *m* is the slope or sensitivity of a five-point calibration curve constructed from peak area versus injected anion standard concentration. Determination of ions in tap water samples by this CE-ESI-MS method with on-column

dicationic reagent complexation, as summarized in Table 9.1, revealed the presence of MCA and BZSN at levels similar to those found in tap water samples analyzed by LC-MS.[63] This new CE-ESI-MS method thus provides sufficient sensitivity to permit the rapid and accurate analysis of anions in water samples, as may be necessitated by environmental, health, industrial, or processing applications.

Table 9.1 Quantifiable anions in Winston–Salem tap water sample.

Anion	Concentration[a)] (ng/mL)	Regression equation	R^2	LOD (ng/mL)
MCA	47.2 (±7.8)	$Y=(2.30\times10^{7})x-(7.864\times10^{4})$	0.9947	20.89
BZSN	5.31 (±8.9)	$Y=(1.05\times10^{8})x+(4.851\times10^{4})$	0.9903	11.31

9.5 Conclusions

In this paper, a CE-ESI-MS method was developed for the separation and identification of four anions in the positive ion mode using a dicationic ion-pairing reagent. Compared to LC-ESI-MS methodologies, this CE-ESI-MS method has several advantages, such as higher separation efficiencies and lower sample and solvent consumption. Method optimization revealed that increasing concentrations of dicationic reagent in the electrophoresis buffer led to increasing sensitivities for singly-charged anionic analytes, but this held true only up to 20.0 μM of added N, N'-dibutyl 1,1'-pentylenedipyrrolidinium. Additionally, a comparison of complexation modes – pre-column, on-column, and post-column – revealed that on-column complexation (achieved by adding the dicationic reagent directly to the electrophoresis buffer) was most effective. Finally, our results showed that CE-ESI-MS could be applied to quantitative water analyses, with limits of detection for MCA and BZSN comparable to those obtained by LC-ESI-MS.

CHAPTER 10

EVALUATION OF TETRACATIONIC SALTS AS GAS-PHASE ION-PAIRING AGENTS FOR THE DETECTION OF TRIVALENT ANIONS IN THE POSITIVE MODE ESI-MS

10.1 Abstract

In previous studies, new ESI-MS approaches were developed for the highly sensitive detection of singly and doubly charged anions in the positive mode ESI-MS by using specially synthesized dicationic and tricationic ion pairing agents respectively. By detecting the positively charged ion complex in the positive mode, LODs for the anions can be lowered by several magnitudes. In this work, we used eighteen newly synthesized tetracationic ion pairing agents, constructed with different geometries, linkages and cation moieties, for the detection of eighteen triply charged anions of different structural motifs. The LODs for these anions were from ten to several thousand times lower in the SIM positive mode than in the negative mode were. These tetracationic agents also were shown to be useful for the detections of -1 and -2 anions. In addition, the LODs for -3 anions can be further lowered by monitoring the daughter fragments of the ion pair complexes in the SRM mode.

10.2 Introduction

New methods of anion analysis are of continual interest provided such methods prove advantageous for analytes of importance in a variety of environmental, biochemical or medicinal applications. Several facile and sensitive methods to detect and quantify anions have been developed to accomplish this task. Currently, ion selective electrodes,[157-158] conductivity,[252-253] atomic spectroscopic techniques coupled with flow injection analysis (FIA) ,[254-255] and ion chromatography [159,256] are widely used for the analysis of anion. However, none of these techniques are completely satisfactory because they are either not universal or lack the ability to provide structural information for complex ions.[183] ICP-MS (inductively coupled plasma mass

spectrometry) is another common method that is known for its high sensitivity and low limits of detection (LOD). It is now widely used in medical, biological, and forensic fields.[257-259] However, ICP-MS is not applicable for all anions nor does it provide structural information for complex ions because they are destroyed before detection.

Electrospray ionization mass spectrometry (ESI-MS) provides an alternative approach for the analysis of anions and in particular complex ions can be detected in their native forms without decomposition. Coupled with separation methods, ESI-MS is capable of detecting most ionic species. However, as powerful as is ESI-MS in the positive mode, it can suffer from lower sensitivity in the negative mode.[46,164-166] One cause for the decrease in sensitivity in the negative mode is the prevalence of corona discharge sometimes leading to arcing events.[56,166] This phenomenon results in an unstable Taylor cone and higher background noise leading to poorer LODs.[46] Studies have shown that corona discharge in the negative mode can be suppressed by using halogenated solvents or alcohols with longer alkyl chains such as propanol, 2-propanol, and butanol.[56,164,167] However, more commonly used solvents such as water, methanol, and acetonitrile are still preferred especially when ESI-MS is coupled with reversed phase liquid chromatography (LC) or ion chromatography. Consequently, it would be highly beneficial to develop methods for sensitive anion detection by ESI-MS using typical LC operating conditions.

Recently, a new approach for anion detection in the positive mode has been developed.[58,63,185,208,230] This technique uses cationic ion pairing agents to form complexes with anions which can in turn be detected in the positive mode. The first use of this method was to detect very low levels of perchlorate anions by allowing them to pair with a dicationic reagent in a carrier flow solvent to form a singly positively charged complex detected in the positive mode. This technique was then extended to the detection of a plethora of singly charged anions.[63,185] This general approach to anion analysis was shown to have many advantages. First, the LODs achieved with this method in the positive mode are much lower than those possible in the

negative mode. Second, only small amounts of the ion pairing agents are needed for any analysis and it can be added pre-column or post-column when LC is employed. Third, common solvents such as water, methanol and acetonitrile can be used. Finally, many anions which fall below the low-mass-cut-off (LMCO) of trapping MS can now be detected since the complexes are brought to a higher mass range by the pairing reagent. Indeed, moving the lower mass detection of any ion away from a region of higher chemical noise into a higher mass region of less noise (upon complexation with the pairing agent) is usually beneficial. For these reasons, detection of anion/cation complexes in the positive mode has proven to be much more sensitive than detection of the native anion in the negative mode. Also, operation in SRM (single reaction monitoring) mode can further lower LODs.[63,185] Recently, dicationic ion pairing agents for the LC-ESI-MS of singly charged anion became available commercially.[260]

Following upon the success of using dicationic ion pairing agents to improve the detection limit of singly charged anions, we extended the use of this technique to the detection of dianions through complexation with tricationic pairing agents.[208,230] It has been determined that benzylimidazolium and tripropylphosphonium are the best cationic moieties and the reagents of flexible linear structure generally work better than rigid trigonal trications.[230] In this work, we move one step further in advancing this technique for anion detection. Eighteen tetracationic reagents constructed with different cationic moieties connected by different linkages were synthesized. These reagents have been evaluated for their ability to complex 18 trivalent anions for detection in positive mode ESI-MS. SRM experimentation was performed in an attempt to further lower LODs. The best results were compared with the LODs obtained in the negative mode. The four best tricationic reagents identified in previous studies also were tested and compared. [230]

10.3 Experimental

10.3.1 Chemicals

Water and methanol were of HPLC grade and were obtained from Burdick and Jackson (Morristown, NJ, USA). Amberlite IRA-400 ion exchange resin, sodium hydroxide (reagent grade) and sodium fluoride (reagent grade) for the ion exchange were obtained from Sigma-Aldrich (St Louis, MO, USA). The anions listed in Table 10.1 were purchased as the sodium/potassium salt or in the acid form from Sigma-Aldrich (St Louis, MO, USA) and all were of reagent grade or better.

10.3.2 Ion-pairing agents

The structures of the tetracationic reagents are in Fig 10.1. The synthesis of the tetracationic reagents is briefly described by the following steps. Step 1: 1-(3-bromopropyl)-3-methyl-imidazolium bromide was first synthesized by reacting methyl imidazole with excess of 1,6-dibromopropane in DMF at 80℃ overnight. It was purified by flash chromatography with 9:1 dichloromethane and methanol. Step 2: 1-(6-(imidazolyl)hexyl)- imidazole was synthesized by reacting excess of sodium imidazole with 1,6-dichlorohexane in DMF at room temperature for 12 hours. It was purified by flash chromatography with 20:1 dichloromethane and methanol. Step 3: Linear tetracation A1 was synthesized by reacting one equivalent of 1-(6-(imidazolyl)hexyl)- imidazole with two equivalent of 1-(3-bromopropyl)-3-methyl-imidazolium bromide in DMF at 80℃ for 24 hours. It was purified by flash chromatography with 4:1 dichloromethane and methanol. All the other linear tetracation salts were synthesized in the same fashion.

The four tricationic reagents (Fig 10.2.) used in this analysis was the same as described in earlier studies.[208,230] All reagents were synthesized in the bromide salt form and exchanged to its fluoride salt form prior to the analysis. The anion exchange was performed with a 10mL syringe filled with 4mL of ion exchange resin in the same manner as described in earlier papers.[63,185,208,230]

Table 10.1 Structures of the trivalent anions studied.

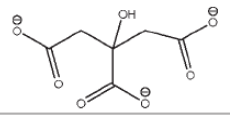
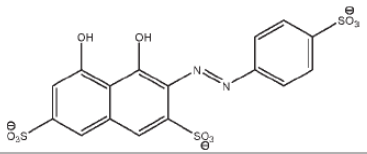
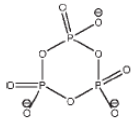
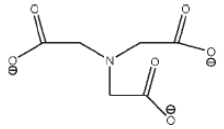
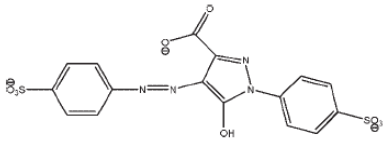
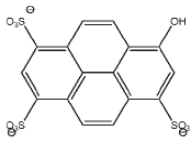
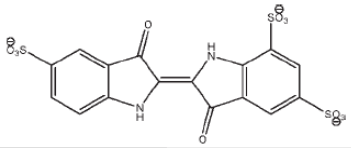
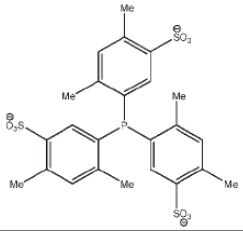
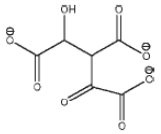
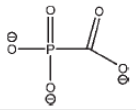
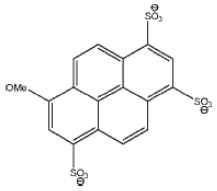
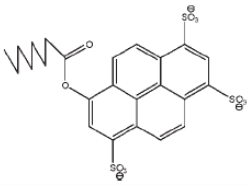
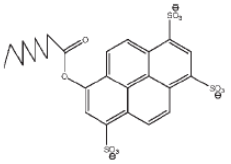
	Name	Mass	Structure
I	citrate	189.0	
II	sulfanilic acid azochromotrop	500.9	
III	trimetaphosphate	236.9	
IV	nitrilotriacetic tricarboxylate	188.0	
V	phosphate	95.0	PO_4^{3-}
VI	tartrazine	465.0	
VII	hexanitrocobaltate	334.9	$Co(NO_2)_6^{3-}$
VIII	pyranine	454.9	
IX	indigotrisulfonate	498.9	
X	hexachlororhodate	315.6	$RhCl_6^{3-}$
XI	tris(2,4-dimethyl-5-sulfophenyl)-phosphine	583.0	
XII	oxalomalic tricarboxylate	203.0	

Table 10.1 *Continued*

	Name	Mass	Structure
XIII	phosphoformate	123.0	
XIV	orthovanadate	114.9	VO_4^{3-}
XV	hexacyanocobaltate	215.0	$Co(CN)_6^{3-}$
XVI	8-methoxypyrene-1,3,6-trisulfonate	468.9	
XVII	8-octanoyloxypyrene-1,3,6-trisulfonate	581.0	
XVIII	8-nonanoyloxypyrene-1,3,6-trisulfonate	595.0	

10.3.3 ESI-MS analysis

A Finnigan LXQ (Thermo Fisher Scientific, San Jose, CA) ESI-MS was used for all of the analyses in this study. An ion pairing reagent aqueous solution (40 μM) was pumped at 100 μL/min using a Shimadzu LC-6A pump (Shimadzu, Columbia, MD) and mixed with 300 μL/min carrier flow (Water/MeOH=2/1, v/v) pumped by a Finnigan Surveyor MS pump. The positive mode ESI-MS conditions were as follows: spray voltage, 3 kV; sheath gas flow, 37 arbitrary units (AU); auxiliary gas flow rate, 6 AU; capillary voltage, 11 V; capillary temperature, 350℃; tube lens voltage, 105 V. When detecting the complex in the positive SIM (Selective Ion Monitoring) mode, the SIM width was set to 5 so as to include the isotope peaks. For the detection in SRM mode, the isolation widths were between 1 and 5, the normalized collision energy was 30, and the activation time was 30 ms. Xcalibur and Tune Plus software were used

to analyze data. The initial concentrations of anion stock solutions were 1 mg/mL. Serial dilutions were made from the stock solutions, and 5 µl of the anions were directly injected using the six port injector. New stock solutions were prepared every week, and the major error source for this experiment was from the injector (±5%). The limits of detection were determined to be when a series of five injections at a given concentration resulted in peaks giving a signal to noise ratio of 3. In order to prevent problems from the possible accumulation of the dilute cationic reagents, all the connecting tubing was rinsed with methanol/water 50/50 at 400µl/min for two hours at the end of each day run. Also, the capillary transfer tube was manually washed with methanol/water 50/50 every week. With this protocol, no problem was ever observed to come from the cationic pairing reagent.

10.4 Results and Discussion

10.4.1 Tested anions

The structures of the 18 trivalent anions used in this study are listed in Table 10.1. Both inorganic and organic species are included. Many of the inorganic trivalent anions are metal complexes, such as hexanitrocobaltate, hexachlororhodate, and hexacyanocobaltate. There are two phosphorous based anions: trimetaphosphate and phosphate. Orthovanadate is a protein-phosphotyrosine phosphatase inhibitor.[261] The organic anions contained either carboxylic and/or sulfonate groups as the anionic moieties. Sodium citrate has three carboxylate groups and is a very common flavor additive in soft drinks. Sulfanilic acid azochromotrop, tartrazine, indigotrisulfonate, 8-methoxypyrene-1,3,6-trisulfonate, 8-octanoyloxypyrene-1,3,6-trisulfonate, pyranine and 8-nonanoyloxypyrene-1,3,6-trisulfonate are dyes. Among them, tartrazine is a commonly used food pigment and also found to be associated with a variety of children's behavioral changes when ingested. [262]

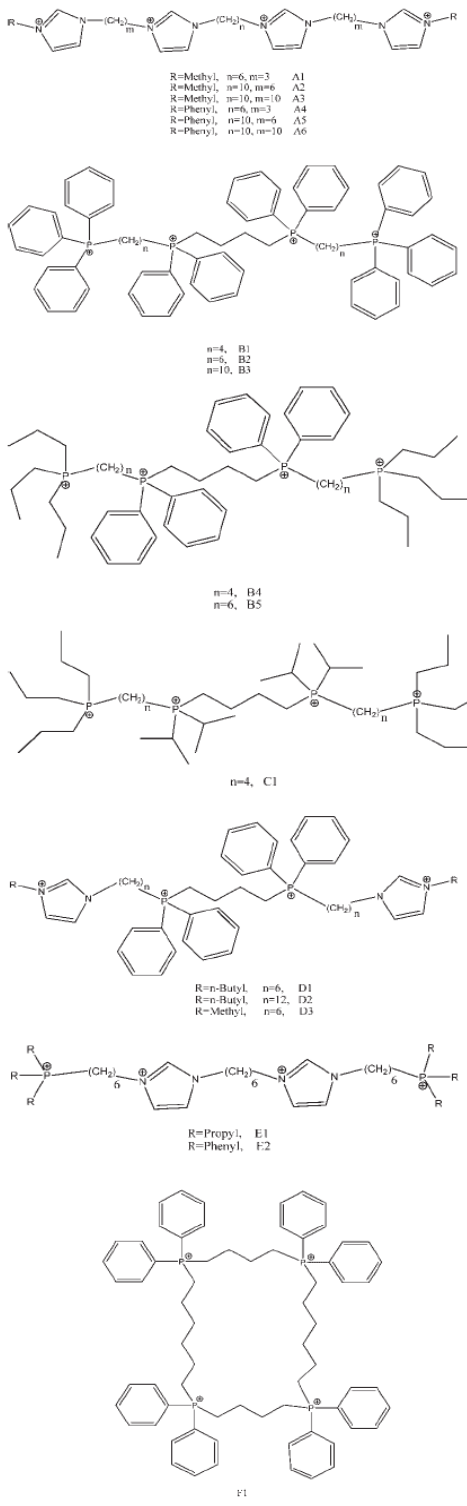
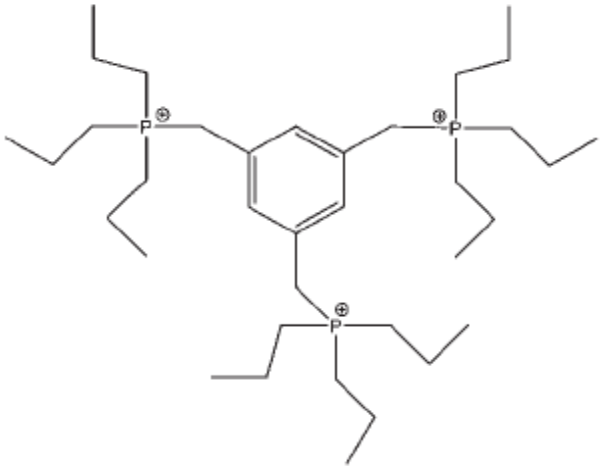
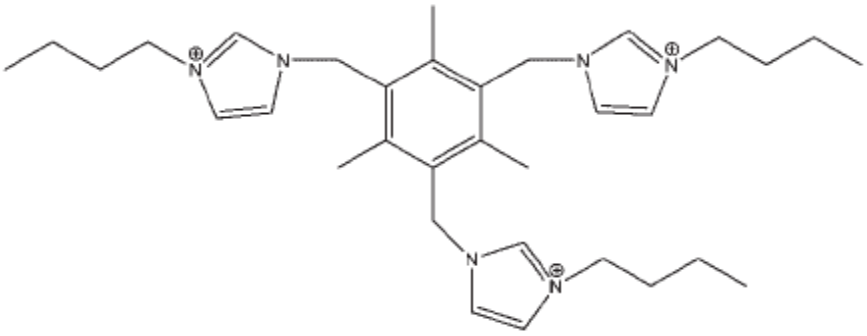


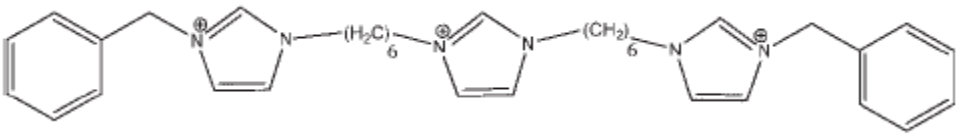
Figure 10.1 Structure of the tetracationic ion-pairing agents.



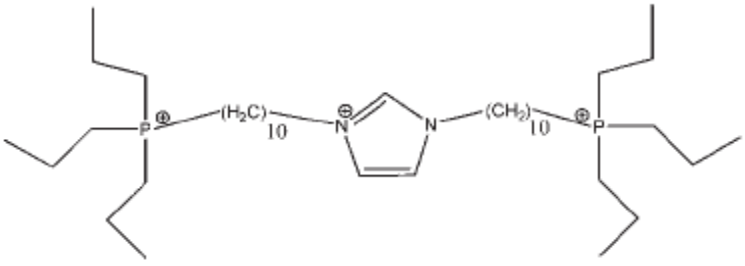
trication 1



trication 2



trication 3



trication 4

Figure 10.2 Structures of the tricationic ion-pairing agents.

10.4.2 Tetracationic ion-pairing reagents

The tetracations synthesized for this study are shown in Figure 10.1. Seventeen of them have the same general linear motif, while one is a cyclic tetracation, which is much more rigid. In previous studies, it was found that linear tricationic ion pairing reagents generally produced better results than trications with a more rigid trigonal geometry.[230] Although it remains interesting to compare the linear tetracations with at least one rigid tetracationic pairing agent in this study, attempts to synthesis more compact rigid tetracations failed due to the repulsion between closely placed cationic moieties. As tetracations have one more charged moiety and one more carbon linkage chain than the linear trications, more variations in the structures can be made, such as the length of the middle and side carbon chain linkages, as well as, the arrangement of different cationic moieties at the middle and end. All these linear tetracations can be divided to three groups: pure imidazolium based, pure phosphonium based, and imidazolium and phosphonium mixed tetracations. They differ in the center carbon chain length (C_4, C_6, and C_{10}), side carbon chain length (C_3, C_6, and C_{10}), center cation moieties (imidazolium, diisopropyl phosphonium, diphenyl phosphonium), and terminal groups (methyl imidazolium, benzyl imidazolium, triphenylphosphonium and tripropylphosphonium).

10.4.3 LODs in the negative mode

The LODs for the trivalent anions in the negative mode were determined and listed in Table 10.2. It should be noted that most trivalent anions do not exist in their -3 charged state in aqueous solution. They can either be singly protonated to become a divalent anion or doubly protonated to a singly charged ion. Therefore, the LODs in the negative mode were determined from the base peak. For example, all the three charged states (-1, -2, -3) of trimetaphosphate can be seen in the negative mode, and the LOD was obtained by monitoring only the -3 peak as it had the best signal to noise ratio. Three anions (X, XIII, XV) are not detectable at a concentration of 10µg/ml (50ng) which was the highest concentration injected. Signal peaks of anions XII, VI, and VII can be seen at concentrations of 10µg/ml (50ng) but the signal-to-noise-

ratios were less than 3, therefore the LODs for these three anions were determined to be greater than 50ng. The poor LODs are the result of the unstable spray conditions in the negative mode leading to the low ionization efficiency and the fact that some -3 anions (V, XIII) have mass to charge ratios which fall below the low-mass-cut off of the ion trap mass spectrometer. The LODs for the rest of the anions range from 250pg (XI) to 20ng (V). Anions containing sulfonate groups generally had lower LODs while metal containing anions had relatively high LODs. The LODs for eight anions (II, VIII, IX, XI, XIV, XVI, XVI, XVIII) were determined based on their singly protonated form (-2), three anions (I, IV, V) were determined as doubly protonated (-1) species, and only one anion (III) from the unprotonated (-3) species.

Table 10.2 LODs for trivalent anions in the negative mode.

Anion	Mass	LOD (ng)	Base peak*
XI	583.0	0.250	291.9/−2
XVI	491.0	1.50	245.9/−2
XVIII	595.0	1.50	297.7/−2
VIII	454.9	2.50	227.9/−2
XVII	581.0	2.50	291.0/−2
II	500.9	5.00	251.0/−2
I	189.0	5.00	191.0/−1
III	236.9	5.00	79.0/−3
IV	188.0	5.00	190.0/−1
IX	498.9	10.0	249.9/−2
XIV	114.9	15.0	117.0/−2
V	95.0	20.0	97.0/−1
XII	203.0	>50	205.0/−1
VI	465.0	>50	155.0/−3
VII	334.9	>50	111.7/−3
X	314.7	>50	not detected
XIII	123.0	>50	not detected
XV	215.0	>50	not detected

* Base peak indicates the detected mass and charge state of the anion.

10.4.4 LODs in the positive mode (selected ion monitoring)

Table 10.3 lists the LODs for the 18 trivalent anions in the positive mode when complexing with the 18 tetracations. The detected complexes and mass to charge ratios are also listed. The best LODs for the trivalent anions ranged from 7.5pg to 18ng. As the anions may exist in three different charge states in solution, it is not surprising that each anion can form three possible complexes (+1, +2, +3) with tetracationic reagents. However, different tetracations pair preferentially with different anionic species. For example, for the detection of phosphate, tetracationic paring reagents C1 and B4 gave the best LODs (380pg and 400pg) when the +3 complex was used for detection. Conversely, with other reagents, +1 or +2 complexes gave better signal-to-noise ratios. For some anions, +1 complexes generally gave the best signal to noise ratio compared to +2 and +3 complexes, such as hexacyanocobalte, 8-octanoyloxypyrene-1,3,6-trisulfonate, 8-nonanoyloxypyrene-1,3,6-trisulfonate, tris(2,4-dimethyl-5-sulfophenyl)-phosphine and pyranine. Anions typically giving the best LODs for +2 charged complexes were phosphate and 8-methoxypyrene-1,3,6-trisulfonate. The prevalence of +2 complexes or +1 complexes can be related to the pKa of the conjugate acid of the trivalent anion. For example, as the pKa of HPO_4^{2-} is 12.76, thus the protonated dianionic form of phosphate will be most abundant in the aqueous solution. This could be the reason why the +2 complexes (tetracation plus HPO_4^{2-}) produce a stronger signal than the +1 complex (tetracation with PO_4^{3-}). However, it should be noted that not all of the complexes are detectable. For example, the complexes of A3, B3, B4, B5 and D2 with hexanitrocobaltate were not observed.

Among the anions studied, tris(2,4-dimethyl-5-sulfophenyl)-phosphine and hexacyanocobaltate gave the lowest LODs overall (7.5pg with A2 or B5). Compared to the LODs in the negative mode, the greatest improvement was achieved with hexacyanocobaltate when pairing with A2 or B5, The sensitivity was more than 6600 times better in positive mode (7.5pg), as it was undetectable in the negative mode (at 50ng). In general, sensitivity improvements in the positive mode were in the range of 10-1000 times those in the negative

mode. Hexachlororhodate had the highest LOD (18ng), which is still more than 10 times better than the negative mode (also undetectable). Except for hexacyanocobaltate, metal containing trivalent anions generally have higher LODs than other anions. On the other hand, sulfonate based anions typically had lower LODs than all the other anions. Although 8-octanoyloxypyrene-1,3,6-trisulfonate and 8-nonanoyloxypyrene-1,3,6-trisulfonate have very similar structures, it is interesting to see that their LODs and best pairing reagents are different. This indicates that even a small change in structure can affect ion pairing. The difference may also be due to the different background noise at the mass to charge ratios of the complexes. For carboxylate based anions, oxalomalic tricarboxylate had the highest LOD (2.5ng) and nitrilotriacetic tricarboxylate gave the lowest LOD (125pg). Among the phosphate based anions, trimetaphosphate had the lowest LOD (37pg) and phosphate (380pg) had the highest.

Among the 18 cationic paring agents tested, phosphonium based tetracations generally produced better results than pure imidazolium based tetracations in the SIM mode. Unsurprisingly, the imidazolium and phosphonium mixed tetracations showed moderate performance. For example, the best eight pairing agents for citrate are all phosphonium based, while the the four worst reagents were imidazolium tetracations. Also, it was found that pairing agents with more aromatic group substitutents worked better for many aromatic anions while alkyl substituted phosphonium agents paired better with alkyl group containing anions. For example, B1 and B4 worked generally better than C1 for anions with aromatic groups. This indicates that π-π interactions can be important for effective ion pairing agents. However, C1 worked better for nonaromatic anions than did B1 and B4. One possible reason is because C1 has relatively less steric bulk about its cationic moieties than do B1 and B4 resulting in stronger cation-anion interactions as the ionic moieties are closer to one another.

Table 10.3 LODs for trivalent anions using tetracationic pairing agents in SIM mode.

I citrate			II sulfanilic acid azochromotrop			III trimetaphosphate		
cation	LOD (ng)	Base peak*	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
C1	0.150	456.4/+2	B1	0.0500	782.3/+2	C1	0.0368	959.6/+1
B1	0.300	626.5/+2	E2	0.0700	706.3/+2	B4	0.0438	1095.5/+1
B4	0.375	524.5/+2	B4	0.0875	680.3/+2	B1	0.0750	1299.4/+1
tri2	0.400	721.3/+1	D2	0.100	1512.0/+1	F1	0.0875	1230.1/+1
D1	0.500	516.5/+2	C1	0.125	612.4/+2	D1	0.125	1080.0/+1
D2	0.750	600.4/+2	B2	0.150	810.3/+2	B2	0.150	1355.3/+1
F1	1.00	591.3/+2	D1	0.175	1344.0/+1	D2	0.250	1248.0/+1
B5	1.25	552.3/+2	F1	0.200	747.6/+2	B5	0.315	1151.5/+1
D3	1.25	474.3/+2	A3	0.220	1219.7/+1	A6	0.625	1107.7/+1
E2	1.25	550.3/+2	E1	0.225	604.4/+2	A3	0.750	955.6/+1
tri4	1.50	857.8/+1	B3	0.250	1732.7/+1	D3	0.750	498.3/+2
A1	2.00	655.4/+1	D3	0.250	1259.6/+1	A1	1.00	703.3/+1
A3	2.00	907.7/+1	tri1	0.250	1099.5/+1	A4	1.00	855.3/+1
B2	2.00	654.5/+2	A4	0.275	560.2/+2	B3	1.00	1468.6/+1
B3	2.50	710.3/+2	A6	0.300	1371.6/+1	A2	1.25	843.4/+1
E1	2.50	448.4/+2	A1	0.375	1169.8/+1	tri2	1.50	769.3/+1
tri3	2.50	741.4/+1	A5	0.425	1259.7/+1	E1	2.50	472.4/+2
A6	3.00	1059.8/+1	B5	0.500	1415.8/+1	E2	2.50	575.5/+2
A2	3.50	398.3/+2	A2	0.575	1107.5/+1	tri3	2.50	789.4/+1
A4	3.75	404.3/+2	tri2	1.00	1033.3/+1	tri1	4.50	835.4/+1
tri1	5.00	787.3/+1	tri3	2.50	1053.4/+1	tri4	5.00	905.6/+1
A5	>50	No complex	tri4	3.75	1169.8/+1	A5	>50	No complex

IV nitrilotriacetic tricarboxylate			V phosphate			VI tartrazine		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
C1	0.125	455.9/+2	C1	0.375	409.4/+2	E2	0.150	688.5/+2
tri2	0.300	720.3/+1	B4	0.400	477.3/+2	B1	0.175	764.3/+2
B4	0.350	524.0/+2	D1	0.560	469.3/+2	A2	0.310	536.3/+2
B1	0.500	625.8/+2	B1	2.00	386.6/+3	A5	0.360	612.4/+2
F1	0.600	590.8/+2	B5	2.00	505.3/+2	D2	0.375	1476.0/+1
B5	0.750	552.8/+2	D3	2.25	427.3/+2	A3	0.500	1183.6/+1
D3	0.750	473.8/+2	F1	2.50	544.3/+2	A4	0.500	542.2/+2
D1	0.875	515.8/+2	tri4	2.50	763.8/+2	tri1	0.500	1063.5/+1
E2	0.875	550.0/+2	D2	3.00	553.4/+2	B4	0.600	662.5/+2
A4	1.00	855.3/+1	E2	3.00	503.3/+2	A1	0.625	931.3/+1
D2	1.00	600.0/+2	A1	3.75	561.3/+1	A6	0.625	1335.8/+1
A1	1.50	654.3/+1	B3	5.00	442.7/+3	B2	0.750	1584.0/+1
A3	1.50	453.8/+2	tri2	5.00	627.3/+1	C1	0.750	1187.7/+1
tri1	1.50	786.3/+1	A2	6.00	351.3/+2	D1	0.750	1308.0/+1
tri4	1.50	856.8/+1	A3	6.00	407.4/+2	D3	0.750	1223.6/+1
tri3	1.75	740.3/+1	A4	6.00	357.2/+2	B3	1.00	1696.5/+1
A6	2.50	529.9/+2	tri1	25.0	693.4/+2	B5	1.00	1380.6/+1
B2	2.50	653.8/+2	E1	35.0	404.4/+2	E1	1.00	586.4/+2
B3	2.50	710.0/+2	A5	50.0	427.4/+2	F1	1.00	1458.2/+1
E1	2.50	447.9/+2	A6	50.0	483.4/+2	tri2	2.50	499.2/+2
A2	3.00	397.8/+2	B2	50.0	405.3/+3	tri3	2.50	590.2/+2
A5	5.00	473.5/+2	tri3	>50	No complex	tri4	5.00	1133.8/+1

VII hexanitrocobaltate			VIII pyranine			IX indigotrisulfonate		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
D3	10.0	365.2/+3	B1	0.0750	1517.5/+1	B1	0.150	781.3/+2
E2	18.0	613.8/+2	D1	0.0750	1297.6/+1	D1	0.250	671.3/+1
B1	20.0	1397.5/+1	D2	0.125	1465.5/+1	D2	0.250	1511.0/+1
tri2	20.0	867.0/+2	tri1	0.125	1053.5/+1	A1	0.500	965.4/+1
tri3	25.0	434.5/+2	B4	0.150	1313.5/+1	A3	0.500	1217.7/+1
B2	37.5	1454.5/+1	D3	0.175	1213.4/+1	B2	0.500	809.3/+2
A5	50.0	1093.7/+1	B2	0.200	1574.5/+1	A6	0.625	1370.7/+1
A6	50.0	603.4/+2	B5	0.200	1369.7/+1	A4	0.700	559.7/+2
C1	50.0	1057.7/+1	A1	0.250	921.3/+1	E2	0.750	706.0/+2
D1	50.0	589.5/+2	A5	0.250	1236.2/+1	F1	0.750	1493.5/+1

Table 10.3 *Continued*

VII hexanitrocobaltate			VIII pyranine			IX indigotrisulfonate		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
F1	50.0	1309.5/+1	C1	0.250	1177.6/+1	A2	1.00	1106.5/+1
A1	>50	401.2/+2	E2	0.250	1366.0/+1	A5	1.00	1258.7/+1
A2	>50	471.3/+2	A2	0.300	531.3/+2	D3	1.00	1258.6/+1
A3	>50	351.9/+3	B3	0.300	1686.5/+1	B4	1.13	680.0/+2
A4	>50	No complex	F1	0.375	1448.5/+1	tri1	1.50	1097.4/+1
B3	>50	No complex	A3	0.500	1173.7/+1	B3	2.50	1730.7/+1
B4	>50	No complex	A4	0.500	1073.4/+1	B5	2.50	1415.0/+1
B5	>50	No complex	A6	0.625	1325.6/+1	C1	2.50	611.5/+2
D2	>50	No complex	E1	0.625	1161.8/+1	E1	2.50	603.9/+1
E1	>50	No complex	tri2	2.50	987.4/+1	tri2	5.00	1031.4/+1
tri1	>50	No complex	tri3	3.50	1007.4/+1	tri3	20.0	1052.4/+1
tri4	>50	No complex	tri4	50.0	1153.8/+1	tri4	50.0	1168.8/+1

X hexachlororhodate			XI tris(2,4-dimethyl-5-sulfophenyl)-phosphine			XII oxalomalic tricarboxylate		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
E2	18.0	613.8/+2	C1	0.00750	1305.8/+1	tri2	2.50	735.0/+1
tri3	25.0	434.5/+2	B4	0.0123	1441.7/+1	A1	5.00	669.2/+1
B1	30.0	688.7/+2	B5	0.0125	1498.8/+1	C1	15.0	925.5/+1
E1	30.0	341.5/+3	B2	0.0150	1701.8/+1	D1	15.0	925.5/+1
A6	42.5	395.9/+3	B1	0.0200	1646.5/+1	tri3	15.0	755.4/+1
A1	50.0	391.5/+2	B3	0.0250	1814.8/+1	F1	20.0	1196.2/+2
B2	50.0	1434.2/+1	F1	0.0250	1576.7/+1	B2	40.0	441.3/+3
C1	50.0	1038.7/+1	D1	0.0300	1425.7/+1	A4	50.0	821.4/+1
D1	50.0	579.8/+2	D2	0.0350	1594.0/+1	A5	50.0	481.3/+2
D3	50.0	537.6/+2	E2	0.0375	747.8/+1	B1	50.0	1266.0/+1
F1	50.0	1309.5/+1	D3	0.0500	1342.5/+1	B3	50.0	717.5/+2
A3	>50	1033.3/+1	A1	0.0750	1049.6/+1	B4	50.0	531.5/+2
B4	>50	1174.6/+1	A2	0.0750	1189.5/+1	B5	50.0	559.5/+3
B3	>50	1546.6/+1	A3	0.0750	1301.7/+1	D3	50.0	481.3/+2
A4	>50	467.5/+2	A4	0.125	1201.4/+1	E2	50.0	557.5/+2
D2	>50	663.8/+2	A6	0.125	1454.3/+1	E1	>50	909.9/+1
tri2	>50	847.9/+1	E1	0.150	645.4/+2	A2	>50	No complex
A2	>50	No complex	tri1	0.150	1181.6/+1	A3	>50	No complex
A5	>50	No complex	A5	0.175	1342.7 /+1	A6	>50	No complex
B5	>50	No complex	tri2	0.250	1115.4/+1	D2	>50	No complex
tri1	>50	No complex	tri3	0.500	1135.9/+1	tri1	>50	No complex
tri4	>50	No complex	tri4	1.50	1049.6/+1	tri4	>50	No complex

XIII phosphoformate			XV orthovanadate			XV hexacyanocobaltate		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
C1	0.250	423.4/+2	F1	0.425	554.0/+2	A2	0.00750	821.5/+1
B4	0.300	491.7/+2	B1	0.500	589.3/+2	B5	0.00750	1329.5/+1
B5	0.500	519.3/+2	B2	1.00	1233.3/+1	D1	0.0125	1057.6/+1
tri2	0.750	655.2/+1	B4	1.25	487.5/+2	B1	0.0175	1278.3/+1
B2	1.00	621.3/+2	B5	2.50	515.6/+2	B4	0.0210	1073.6/+1
tri4	1.00	791.6/+2	C1	2.50	419.4/+2	C1	0.0250	938.0/+1
D1	1.25	966.0/+1	B3	3.00	673.7/+2	D3	0.0250	973.5/+1
B1	1.50	593.3/+2	E2	5.00	1025.3/+1	F1	0.0375	1207.4/+1
D2	1.50	1133.6/+1	tri2	5.00	647.3/+1	A1	0.0500	681.3/+1
E2	2.00	517.5/+2	tri4	7.00	392.4/+2	A6	0.0500	1085.7/+1
A1	2.50	589.3/+1	A4	21.0	245.1/+3	B2	0.0500	1334.5/+1
B3	2.50	1354.0/+1	tri1	30.0	713.3/+1	D2	0.0500	1226.5/+1
tri3	3.00	675.4/+1	A6	50.0	329.3/+3	E1	0.0625	921.7/+1
D3	3.20	441.3/+2	D1	50.0	479.5/+2	A3	0.0750	933.5/+1
A4	4.00	371.2/+2	A1	>50	No complex	A4	0.0750	833.7/+1
F1	5.25	558.6/+2	A2	>50	No complex	E2	0.0750	1126.3/+1
A6	6.25	497.4/+2	A3	>50	No complex	B3	0.100	1445.5/+1
A3	10.0	841.7/+1	A5	>50	No complex	A5	0.125	973.6/+1
E1	10.0	415.4/+2	D2	>50	No complex	tri4	0.500	1159.8/+1
A5	10.5	441.3/+2	D3	>50	No complex	tri1	1.25	813.3/+1
tri1	25.0	721.3/+1	E1	>50	No complex	tri3	2.00	767.2/+1
A2	30.0	365.3/+2	tri3	>50	No complex	tri2	5.00	747.3/+1

Table 10.3 *Continued*

XVI 8-methoxypyrene-1,3,6-trisulfonate			XVII 8-octanoyloxypyrene-1,3,6-trisulfonate			XVIII 8-nonanoyloxypyrene-1,3,6-trisulfonate		
cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak	cation	LOD (ng)	Base peak
B5	0.0500	777.3/+2	B1	0.0500	1496.6/+1	B5	0.0350	821.5/+1
B3	0.0875	675.3/+2	B4	0.0875	1812.7/+1	D2	0.0500	1329.5/+1
B1	0.100	667.3/+2	D1	0.100	1644.5/+1	D3	0.0500	1057.6/+1
A4	0.185	625.3/+2	A5	0.185	1199.9/+1	A5	0.0750	1278.3/+1
B2	0.250	549.3/+2	A2	0.250	1700.7/+1	B1	0.0750	1073.6/+1
C1	0.250	703.3/+2	B5	0.250	1303.8/+1	B4	0.0750	938.0/+1
D1	0.250	701.5/+2	E2	0.250	1424.7/+1	A2	0.100	973.5/+1
E2	0.250	1023.4/+1	tri2	0.250	746.8/+1	D1	0.100	1207.4/+1
B4	0.275	625.2/+2	D3	0.275	1440.7/+1	F1	0.105	681.3/+1
D2	0.375	805.3/+2	B2	0.375	1592.5/+1	A1	0.125	1085.7/+1
D3	0.500	751.7/+2	D2	0.500	1339.6/+1	A6	0.125	1334.5/+1
A1	0.500	1089.5/+1	tri1	0.500	1047.5/+1	C1	0.125	1226.5/+1
A6	0.650	555.2/+2	A4	0.650	1452.3/+1	A3	0.150	921.7/+1
F1	0.750	607.4/+2	C1	0.750	1574.5/+1	B2	0.150	933.5/+1
E1	0.750	742.5/+2	F1	0.750	644.4/+2	E2	0.150	833.7/+1
A5	1.00	861.3/+2	B3	1.00	1342.7/+1	A4	0.250	1126.3/+1
A3	1.25	957.3/+1	A1	1.25	1299.7/+1	B3	0.250	1445.5/+1
A2	1.25	605.4/+2	A3	1.25	1188.0/+1	tri1	0.300	973.6/+1
tri1	1.25	681.4/+2	A6	1.25	1180.5/+1	E1	0.500	1159.8/+1
tri3	2.50	1197.8/+1	E1	2.50	1133.9/+1	tri2	2.50	813.3/+1
tri2	2.75	1133.9/+1	tri3	2.75	1113.5/+1	tri3	2.50	767.2/+1
tri4	5.00	1250.3/+1	tri4	5.00	1250.3/+1	tri4	5.00	747.3/+1

We selected B1, B4 and C1, which are all phosphonium based tetracations, as the ion pairing reagents that outperformed all others for the detection of trivalent anions. These are the first recommended cations to use for the detection of -3 anions. The cyclic phosphonium tetracation worked fairly well, but not as good as the best linear tetracations. This indicates that flexibility also may be an important feature for tetracationic pairing agents. This is analogous to what was found for trication ESI-MS pairing agents.[230]

In addition, since some of the analytes exist mainly in the -2 charge state in solution, it should be possible for them to form +1 complexes with trivalent cations. Consequently, we also used the four best tricationic agents previously found for the detection of -2 anions (Fig. 10.2). The results are also listed in Table 10.3. It is obvious that tetracationic reagents are typically superior to tricationic ones in detecting these anions (except for oxalomalic tricarboxylate, for which trication 2 worked best). In many cases, the tricationic reagents performed worst, thus these agents should not be among the first tested for the detection of -3 anion. Therefore, we

recommend the tetracationic ion pairing agents outlined in this study for detecting trivalent anions in ESI-MS.

Tetracationic pairing agents not only complex trivalent anions but also form cation-anion complexes when paired with singly charged and doubly charged anions. Therefore, we also tested two tetracations (B1 and C1) for the detection of four singly charged and four doubly charged anions. The results are shown in Table 10.4. For monoanions, the results (i.e. sensitivities) found when using tetracations B1 and C1 were not as good as those that found for the less charged pairing agents studied in earlier papers.[63] For example, the best LOD for perfluorooctanate obtained by using a dicationic agent was 0.12pg while B1 and C1 only gave 150pg and 90pg LODs, respectively. For trifluoromethanesulfonimide, the LOD achieved by B1 (4.5pg) was close to the best LOD achieved by a dicationic ion pairing agent (2.3pg).[63] Interestingly, B1 and C1 performed well for the detection of dianions. For example, the LODs of *m*-benzenedisulfonate obtained by B1 (12.5pg) and C1 (12.5pg) are two times lower than the best LODs obtained with tricationic agents (32pg).[185] Although further study on the complexation of tetracationic agents with mono- and dianions needs to be done, it has been shown that tetracations can be possibly used as universal ion pairing agents for detecting mono-, di-, and trivalent anions.

Table 10.4 LODs for -1 and -2 anions using tetracationic pairing agents.

		B1		C1	
Anions	Mass	LOD (pg)	Base peak	LOD (pg)	Base peak
perfluorooctanate (−1)	413.1	150	491.8/+3	90.0	424.0/+3
benzenesulfonate (−1)	157.2	15.0	406.6/+3	15.0	338.6/+3
monochloroacetate (−1)	93.5	1000	625.0/+2	2500	523.0/+2
trifluoromethanesulfonimide (−1)	280.0	4.50	447.5/+3	15.0	379.8/+3
hexachloroplatinate (−2)	407.9	25.0	735.2/+2	50.0	633.5/+2
m-benzenedisulfonate (−2)	236.2	12.5	649.4/+2	12.5	547.4/+2
fluorophosphate (−2)	98.0	500	387.3/+3	20.0	477.3/+2
nitroprusside (−2)	216.0	10.0	639.3/+2	25.0	537.6/+2

10.4.5 LODs in the positive mode (Single Reaction Monitoring)

It has been demonstrated in previous papers that using ion pairing agents in the SRM (single reaction monitoring) mode can further reduce the detection limits of anions.[63,185,208,230] In the SRM mode, anion-cation complexes are first selected, and then disassociated into fragments. The LODs were obtained by monitoring the strongest fragment peak. In this study, we tested the three best tetracations (B1, B4 and C1) for the detection of trianions in the positive SRM mode. The SRM results are listed in Table 10.5. Typically 3 to 10 times better (lower) LODs were achieved. However, the metal containing anions did not show immense improvements in the SRM mode. For example, the LOD of hexachlororhodate in the SRM mode (10ng) was only slightly better than the LOD in the SIM mode (18ng). The LODs of some anions were lowered to the 100 fg range, for example hexacyanocobalte, 8-methoxypyrene-1,3,6-trisulfonate and tris(2,4-dimethyl-5-sulfophenyl)-phosphine. When compared to the LODs in the negative mode, the biggest improvements found by using the tetracationic agents are more than four orders of magnitude (as for hexacyanocobaltate).

10.5 Conclusions

Eighteen newly synthesized tetracationic ion pairing agents with diverse structures have been evaluated for the detection of trivalent anions in both positive SIM and SRM modes of ESI-MS. The best LODs obtained in the positive mode were compared with the LODs for the negative mode. Improvements from 10 to greater than 6600 times were found in the SIM positive mode. It has been determined that the phosphonium based reagents generally gave lower LODs than the imidazolium based tetracations. The pairing agents overall geometry plays as an important role as the nature of the cationic moieties in its effectiveness. The three best tetravalent reagents were selected for SRM mode experiments. Furthermore, the utility of these tetracations was demonstrated by also using them to successfully complex mono- and dianions. The LODs of most anions were lower in the SRM mode and up to four orders of magnitude of improvement was seen for the SRM mode as compared to the negative mode. Finally, it needs

to be noted that fluorescence spectroscopy also would be highly sensitive for the pyranine type trivalent anions. However, ESI-MS maintains certain other distinct advantages in that it is universal (i.e., also does nonfluorescent samples), can analyze several ions simultaneously and provide structural information.

Table 10.5 LODs for trivalent anions in the SRM positive mode.

	Tetracation B1		
Anion	Precursor ion	Base fragment peak	LOD (ng)[b]
I	626.5/+2	530.3/+2	**0.0625**[a]
II	782.3/+2	773.3/+2	**0.0150**
III	1299.4/+1	1037.4/+1	0.0150
IV	625.8/+2	530.3/+2	0.150
V	386.3/+3	353.9/+3	50.0
VI	764.3/+2	742.3/+2	0.0188
VII	1397.5/+1	1185.3/+1	10.0
VIII	1518.5/+1	1255.4/+1	**0.0125**
IX	781.5/+2	819.2/+1	**0.500**
X	688.7/+2	652.3/+2	250
XI	1646.5/+1	1385.3/+1	0.00250
XII	1266.0/+1	1133.5/+1	75.0
XIII	593.3/+2	571.3/+2	0.50
XIV	589.3/+2	500.3/+2	0.0150
XV	1278.3/+1	503.3/+1	0.0125
XVI	777.3/+2	769.8/+2	**0.00113**
XVII	1644.0/+1	1381.5+1	**0.0125**
XVIII	1658.5/+1	1395.5+1	**0.0200**
	Tetracation B4		
Anion	Precursor ion	Base fragment peak	LOD (ng)
I	524.5/+2	428.3/+2	**0.0625**
II	782.3/+2	655.8/+2	**0.0150**
III	1095.5/+1	401.3/+1	0.0200
IV	524/+2	215.2/+1	0.0650
V	477.33/+2	428.3/+2	**0.250**
VI	662.5/+2	640.3/+2	**0.0150**
VII	N[a]	N	N
VIII	1313.5/+1	401.3/+1	0.125
IX	680.0/+2	241.1/+1	1.00
X	N	N	N
XI	1441.7/+1	1281.5/+1	0.0250
XII	531.5/+2	295.2/+1	**0.750**
XII	491.7/+2	469.3/+2	**0.0175**
XIV	487.5/+2	448.3/+2	**0.0125**
XV	1073.6/+1	401.3/+1	**0.00200**
XVI	675.3/+2	595.3/+2	0.0750
XVII	1440.7/+1	1279.6/+1	0.125
XVIII	1454.5/+1	1293.6/+1	0.200
	Tetracation C1		
Anion	Precursor ion	Base fragment peak	LOD (ng)
I	456.4/+2	425.4/+2	0.0250
II	612.4/+2	485.8/+2	0.0250
III	959.6/+1	917.6/+1	**0.00375**
IV	455.9/+2	215.3/+2	**0.0500**
V	409.4/+2	280.3/+2	**0.250**
VI	1187.0/+1	1143.8/+1	0.125
VII	1057.7/+1	845.6/+1	**5.00**
VIII	1177.6/+1	1135.6/+1	**0.0125**
IX	611.5/+2	590.3/+2	**0.500**
X	1037.8/+1	541.4/+2	**25.0**
XI	1305.8/+1	1263.8/+1	**0.000600**
XII	925.5/+1	792.6/+2	25.0
XIII	423.4/+2	397.3/+2	0.25
XIV	419.4/+2	621.4/+2	0.0188
XV	938.0/+1	333.3/+2	0.00220
XVI	607.4/+2	546.3/+2	0.00375
XVII	1303.8/+1	1261.7/+1	0.0250
XIII	1317.8/+1	1275.8/+1	0.0250

[a] Complex is not detected. [b] Numbers in bold indicate the best LODs.

CHAPTER 11

DICATIONIC ION-PARING AGENTS USED FOR THE DETECTION OF ANIONS IN POSITIVE ION MODE ESI: MECHANISTIC DETERMINATIONS AND CONSIDERATIONS

11.1 Introduction

Throughout the preceding chapters (Chapters 5-10) a new technique for the detection of anions in the positive ion mode of electrospray ionization mass spectrometry (ESI-MS) has been described. This has been achieved through complexation of the anions with multiply charged cationic ion-paring reagents. In each of these experiments, many limits of detection (LOD) have been reported. Nearly all the LODs determined were far lower than those found using the negative ion mode with no cationic ion-pairing reagent. Furthermore, several of these LODs were found to be lower than any reported by any analytical technique.[63]

All of the LODs were determined empirically with little disscusion about the mechanism by which these ion-paring reagents produce such sensitive results. In fact, it is very interesting that complexation can produce such high sensitivity considering the formation of complexes is generally regarded as a source for ionization suppression. For example, it has been documented that the addition of trifluoracetate to a spray solution will suppress the signal of positively charged analytes (such as peptides).[263-265] Nonetheless, the complexation described herein has proven to enhance the signal for most anionic species.

In previous studies, many empirical observations were made concerning the structural motifs of both the anions and the ion-pairing reagents which seemed to enhance detection. First, it was generally observed that more chaotropic anions yielded lower LODs. Soukup-Hein et. al. showed that the relative order of sensitivity for some anions loosely followed the Hofmeister series (a series that describes the ability for an anion to change the ordering of water molecules).[63] Also, it was observed that anions containing halogen atoms were more

sensitive than non-halogen containing analogues. This again was attributed to the fact that these anions were more chaotropic. Lastly, it was observed that anions of a higher oxidation state had lower detection limits than those of a lower oxidation state.

It was observed that flexible dicationic agents performed better than rigid reagents. This was also found to be true for tricationic and tetracationic ion-paring reagents. Other trends that were observed are as follows: i) reagents having hydroxyl groups worked more poorly than those that did not; ii) the presence of aromatic groups in the ion-paring agents was found to be advantageous when detecting aromatic anions, indicating that π-π interactions are important; iii) it was found that reagents containing alkyl linkage chains between the cationic moieties performed as well as polyethylene or perfluoro-alkyl linked dications (thus the extra synthetic work required for the later could be avoided); iv) phosphonium based tetracationic ion-pairing reagents outperformed imidazolium based agents. From these observations, four dicationic, four tricationic, and three tetracationic ion-pairing agents have been identified as most successful. Their success has lead to the commercialization of one dicationic and one tricationic ion-pairing reagent.[260]

Though these empirical observations have been made, there is still a lack of understanding as to the exact mechanism(s) that allow this ion-pairing method to be so successful. It appears there are at least three factors. First, there must be some consideration as to the role of the solution phase binding between the ion-pairing agent and the anion. The second factor is the ionization efficiency of the complexes. A third point to be considered is the potential for the anion (in its complexed form) to undergo oxidation during the electrospray process. The later is one source for the observation that anions in higher oxidation states were detected more sensitively. This factor will not be examined in this study.

In this work three dicationic ion-pairing reagents and four mono-valent anions are studied. The solution phase binding constants, as determined by Nuclear Magnetic Resonance spectroscopy (NMR) are evaluated. For comparison, an online dynamic titration technique is

used to determine the binding constants by ESI-MS.[266] The ionization efficiency of these systems are correlated to their surface activity and measured using surface tensiometry. A comparison of the LODs produced by these systems to the results found herein will be made. From these results, a clearer understanding of the factors that effect these systems will be revealed and additional mechanistic considerations will be presented.

11.2 Experimental

11.2.1 Materials

Sodium bromide (NaBr), sodium iodide (NaI), sodium benzoate (NaBzO), and sodium benzenesulfonate (NaBZSN) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The deuterated methanol and deuterated water used in the NMR titrations, as well as, the sodium hydroxide, sodium fluoride, and Amberlite IRA-400 (chloride form) used for the ion exchange, were also purchased from Sigma-Aldrich. The deionized water used for the surface tension experiments was obtained from our in-house Milli-Q (Millipore Corporation, Billerica, MA, USA) system. The water and methanol used in the ESI studies were purchased from Burdick and Jackson (Morristown, NJ, USA). The dicationic reagents were synthesized in their bromide form as outlined in Chapter 5 and were subsequently ion exchanged to their fluoride form following a previously outlined procedure.[63]

11.2.2 ESI-MS analyses

The MS used in this study was a Thermo Finnigan LXQ (Thermo Electron Corporation, San Jose, CA, USA). The general set-up for both the LOD determinations and the dynamic titrations was the same. In short, a Thermo Finnigan Surveyor MS pump (Thermo Electron Corporation, San Jose, CA, USA) was used to provide a 300 µL/min solvent stream (67/33 methanol/water) which was mixed (via a low volume mixing tee) with a 100 µL/min solvent stream containing 8 µM dicationic ion-pairing reagent. The second solvent stream was applied using a Shimadzu LC-6A pump. The resulting solution was a 2 µM dicationic ion-pairing reagent solution in 50% water 50% methanol. This solution was pumped directly into the ESI

interface at 400 µL/min without any flow manipulation. The flow from the Surveyor pump provided the solvent for which the anions were added. Red PEEK tubing (i.d. 0.005 in) and Blue PEEK tubing (i.d. 0.010 in) were used for all the solvent streams in the LOD studies and dynamic titration experiments, respectively. The anions were added using direct injection via a 6-port injector equipped with 5 and 10 µL injection loops for the LOD measurements and dynamic titrations, respectively. The MS conditions were as follows: spray voltage, 3 kV and 5 kV (for the LOD and dynamic titration studies, respectively); capillary temperature, 350 ℃ and 275 ℃ (for the LOD and dynamic titration studies, respectively); capillary voltage, 11 V; tube lens voltage, 105 V; sheath gas flow, 37 arbitrary units (AU); auxillary gas flow, 6 AU.

For the LOD measurements, stock solutions of the anions were made at a concentration of 1 mg/mL. From the stock solutions, serial dilutions were made to minimize error. To find the LODs, the solutions were injected five times and successively diluted until a set of five injections at a given concentration resulted in a signal-to-noise (S/N) ratio of 3. Selected Ion Monitoring (SIM) was applied to find the LODs.

For the dynamic titration measurements, solutions containing either 10 µg/mL (for NaI and NaBZSN) or 20 µg/mL (for NaBr and NaBzO) were injected. The only difference in the general set-up was the addition of a long length of tubing (≈6 m) after the mixing tee and before the ESI interface. This tubing is essential for the dynamic titration technique, such that the complex will diffuse longitudinally and create a Gaussian distribution.[266] The titration for each system was repeated six times while spectra was taken from a m/z range of 100 to 1000. The data was treated using an in-house software tool that was written in Microsoft Visual C# 2005 Express Edition. [266] To run this program, the relative response ratio of complex to free host was assumed to be 1. The software automatically generated dissociation/binding constants for the systems.

11.2.3 NMR analyses

The instrument for the NMR binding determinations was a JEOL ECX 300 MHz (Tokyo, Japan). The solvent used was 50% deuterated methanol 50% deuterated water. Methanol was used as the lock solvent. All experiments were completed at 25 ℃. The shifts were determined in reference to a tetrametylsilane (TMS) standard.

The dicationic ion-pairing reagent solutions were prepared by weighing 10 mg of the dicationic salt and dissolving them in 0.8 mL of 50% deuterated methanol 50% deuterated water. The anion stock solutions were prepared in deuterated water such that 2 µL of the stock contained 0.1 molar equivalent of the ion-pairing reagent that was being titrated. A zero measurement was first made for just the free dicationic solution. Next, successive additions of the anions and measurements of the chemical shift changes were recorded. From 0.1 to 1.0 molar equivalents, measurements were made at every 0.1 equivalents. From 1.0 to 2.0 molar equivalents, measurements were made at every 0.2 equivalents. Finally, 2.5 and 3.0 molar equivalents were measured. In each case, protons alpha to the positive charge in the dications were monitored as the titration was performed. The change in the chemical shifts and the concentration of the titrant were then used to determine the binding constants through non-linear least squares regression using the WinEQNMR2 (Galway, Ireland) computer software.[267] The error was estimated using a biased linear regression estimate treating the partial derivatives as constant coefficients of the parameters.

It is important to note, that the systems were also titrated with sodium fluoride to observe in changes in chemical shift from the change in ionic strength. In doing so, it was determined that changes in ionic strength did not cause measurable changes in chemical shifts.

11.2.4 Surface tension analyses

The instrument used for the surface tension measurements was a Fisher Model 20 Surface Tensiometer. The platinum ring used had a mean circumference of 5.94 cm and a ring/wire radius ratio of 53.2113942. The surface tensiometer was calibrated using an object of

known mass, such that the readings obtained were directly in Dynes/cm. All measurements were made at 25 ℃.

The surface tension based titrations were performed by the successive addition of anions to a 0.1 M dication bulk solution. The increments for the addition of titrant (anions) were 0.1 molar equivalents up to 2.0 molar equivalents, then 0.5 molar equivalents up to 5.0 molar equivalents. Measurements were made in triplicate. Also, pure water was titrated with the anions an identical fashion to serve as a blank.

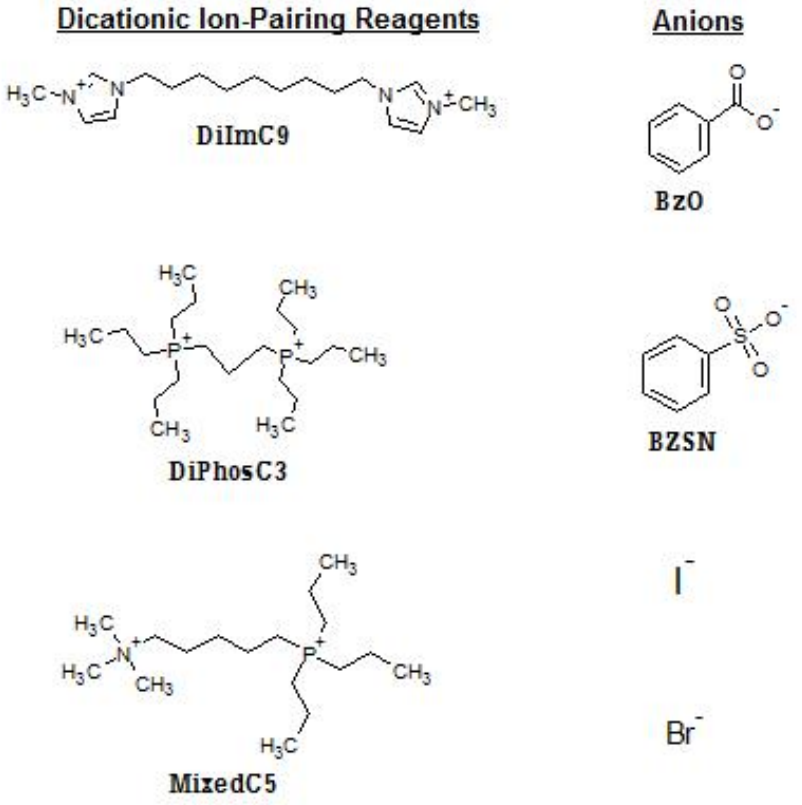


Figure 11.1 Structures of the pairing agents and anions.

11.3 Results and Discussion

11.3.1 LODs in the SIM positive mode

Many of the ion-pairing systems studied herein have been reported previously[63], while others have not. All anions used in this study were in their sodium salt form, which in some cases (sodium benzoate) differed from the original analysis. For this reason, it was first necessary to determine the limits of detection (LODs) for all the systems used in this study. Table 11.1 lists the LODs found for four anions (Figure 11.1) in conjuction with three dicationic ion-pairing agents (Figure 11.1).

As can be seen in Table 11.1, the LODs spanned a range of approximately two orders of magnitude. The DiPhosC3/iodide complex was detected at 1.08 x 10^{-3} ng, while the MixedC5/benzoate complex was only detected at 1.68 x 10^{-1} ng. The data was consistent with previous studies[63,185], with the exception of the detection of benzoate (BzO). Here the detection limits for BzO are lower. This is because the initial studies used benzoic acid as the source for BzO, whereas, sodium benzoate was used in this study. Due to the weak acidity of benzoic acid, it is sensible that the use of the sodium salt would yield lowed LODs. However, the overall trends in sensitivity, as reported earlier, have been preserved. In short, benzenesulfonate (BZSN) and iodide (I) produced lower LODs than bromide (Br) and BzO. Also, DiPhosC3 and DiImC9 generally produce lower LODs than MixedC5.

Table 11.1 LODs for the dication/anion complexes.

Dication	$BZSN^-$	BzO^-	I^-	Br^-
DiImC9	2.06×10^{-3}	1.68×10^{-2}	6.00×10^{-3}	6.00×10^{-2}
DiPhosC3	1.03×10^{-3}	1.26×10^{-1}	1.08×10^{-3}	1.17×10^{-1}
MixedC5	1.55×10^{-2}	1.68×10^{-1}	3.88×10^{-2}	7.77×10^{-2}

LODs reported as the abosolute mass in ng.

11.3.2 Solution phase binding constants determined by NMR

The first factor that may lead to the low LODs (and the differences from complex to complex) obtained through this method is the ability for the dicationic ion-pairing reagent to associate with the anions of interest in solution. It important to note that this association must take part in the solution phase. If it did not take part in solution, large dication/fluoride complex signals should be obsereved. This is because the dicationic ion-pairing agent is added in its fluoride form, and if the binding were occurring exclusively in the gas phase, fluoride complexes would dominate, due the small size-to-charge ratio of fluoride and its higher concentrations. These complexes were never observed in the background. Knowing this, it is of up-most importance to determine the solution phase binding constants for these systems.

Our first attempt to determine the association constants for the complexes was done using CE. Binding constants for some monocationic and monoanionic salts, as determined by CE, have been reported previously.[268-270] However, severe wall interaction of the dicationic ion-pairing agents, among other reasons, hindered the success of this approach. Binding constant determinations using NMR also is a well established method. In fact, NMR based evaluations of cation-anion associations have already been reported.[271] In these reports, NMR titrations were performed and the binding constants were determined through a non-linear regression fitting program WinEQNMR2. Association constants for all of the dication-anion pairs in this study were obtained by this approach (see Experimental). Figure 11.2 shows an example of a non-linear NMR titration performed for this study. As can be seen, the titration data exhibits a good fit to the curve and thus could be used to obtain the association constant.

Figure 11.3 illustrates the solution phase binding constants as determined by NMR. The determined binding values ranged from 53 M^{-1} to 128 M^{-1} for the DiImC9/BzO complex and the DiPhosC3/I complex, respectively. Literature values for ion interactions is aqueous media were just slightly less than this range, while other reports for ion interactions in organic media (ACN) were much higher (due to the absence of waters of hydration).[271] Since the solvent used

in these experiments was 50% methanol and 50% water (by volume), the magnitude of these constants are quite reasonable.

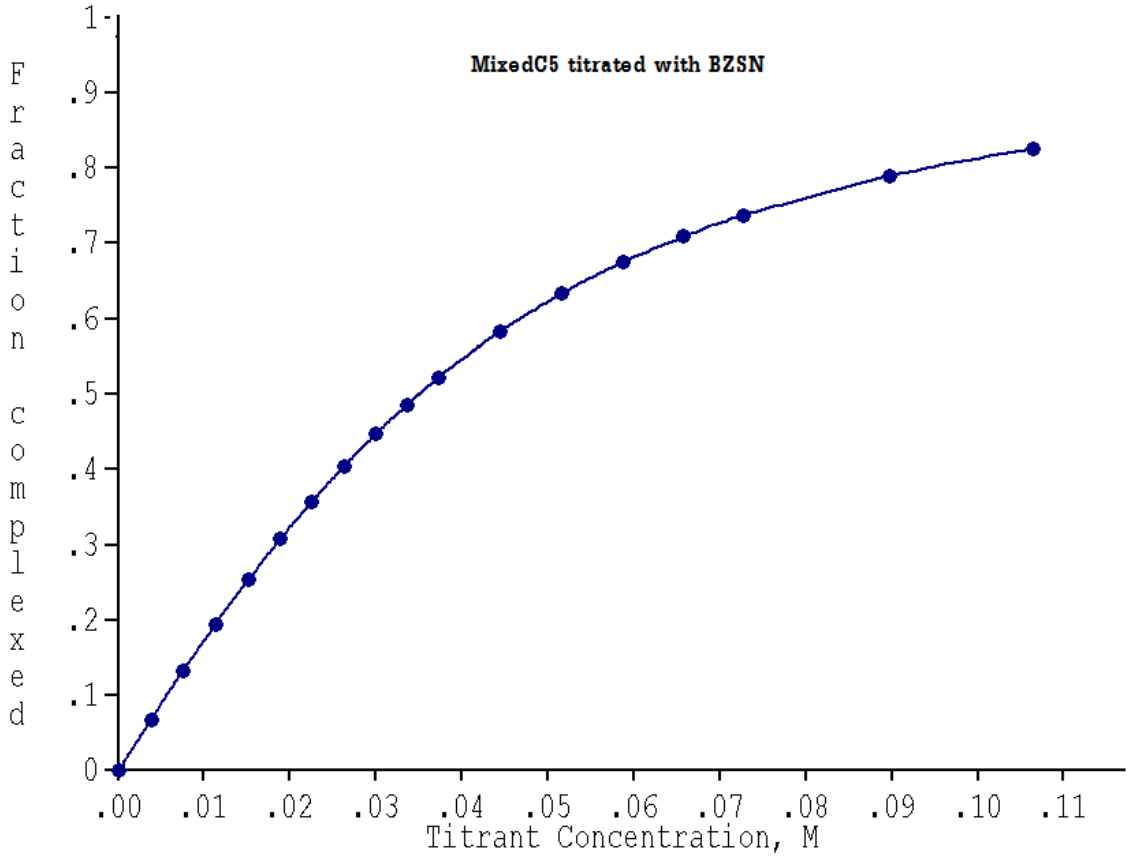


Figure 11.2 Non-linear curve used to determine binding constants by NMR.
For the conditions used to obtain this curve, see the Experimental section

Though the binding between dication and anion is fairly weak (compared to gas phase interactions), some notable differences were observed. The most interseting observation was that DiPhosC3 typically produced larger association constants. As reported previously, phosphonium based ion-pairing agents tend to work quite well for the ESI-MS detection of

anions. It seems that at least one of the reasons for their success is their ability to bind anions in solution more strongly than other pairing agents.

Next, it was observed that iodide associates most strongly to the non-aromatic dications (DiPhosC3 and MixedC5), while the aromatic DiImC9 dication preferred to bind benzenesulfonate (BZSN). This indicates that π-π interactions are important for the association of the DiImC9 dication to pair with anions. Lastly, it was observed that in all cases, bromide had smaller association constants than iodide. This result indicates that binding takes part in the solution phase, as it is known that bromide will be more hydrated than iodide, and thus bind cations less strongly.

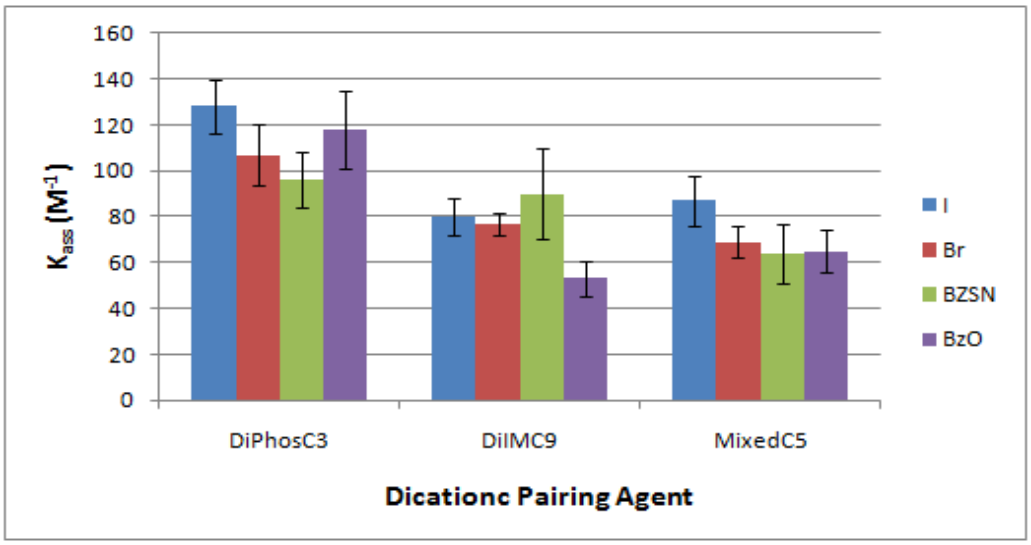


Figure 11.3 Solution phase binding constants determined by NMR.

For the experimental parameters used and the estimates of the errors, see the Experimental section.

However, a direct correlation between the solution phase binding constants and the LODs in ESI-MS was not always found. However, some trends can be explained. First, the binding constants for iodide are always higher than those for bromide. This directly follows the

LOD trend. Second, iodide and BZSN typically have larger accociation constants than bromide or BzO. This again is reflected in the LODs. In contrast, the large range in LODs (two orders of magnitude or more) cannot be fully explained by looking at the solution binding alone. For example, MixedC5 always generated LODs about an order of magnitude less than DiImC9. This is not reflected in the binding data which suggests these two paring agents have similar binding efficacies for these anions.

11.3.3 Association constants determined by ESI-MS

Considering that these LODs are obtained using ESI, a measure of the association behavior using ESI would be useful. To accomplish this, on-line dynamic titrations were performed. This technique is explained in detail elsewhere.[266] In short, a constant amount of host (dication) is introduced to the ESI-MS, while and injection of guest (anion) is made. The host-guest complex is then allowed to diffuse in a length of tubing to yield a Gaussian distribution of the complex. By treating each scan of the MS as an individual titration step, a large number of steps can be recorded. Plotting the ratio of complex intensity over free dication intensity versus the time, and using a Gaussian peak fitting program, a binding constant can be generated. An example of the Gaussian peak fit is shown in Figure 11.4 and the resulting binding constants are shown in Figure 11.5.

As can be seen in Figure 11.5, most of the trends observed in the NMR experiments were not maintained. One observation was that iodide and BZSN yielded larger association constants than did bromide or BzO. This followed the LOD trends. The NMR results indicated that DiPhosC3 generally yieled the largest binding constants, whereas the ESI-MS results indicate that MixedC5 binds anions best. This is not in correlation with the LOD results, where MixedC5 was the least effective ion-pairing agent. It should be noted that in the determination of these binding constants, the ratio of the response factors for the complex and the dication was assumed to be unity. This introduces a large potential error in these measurements. Ways to determine the actual response factor of the complex continue to be investigated.

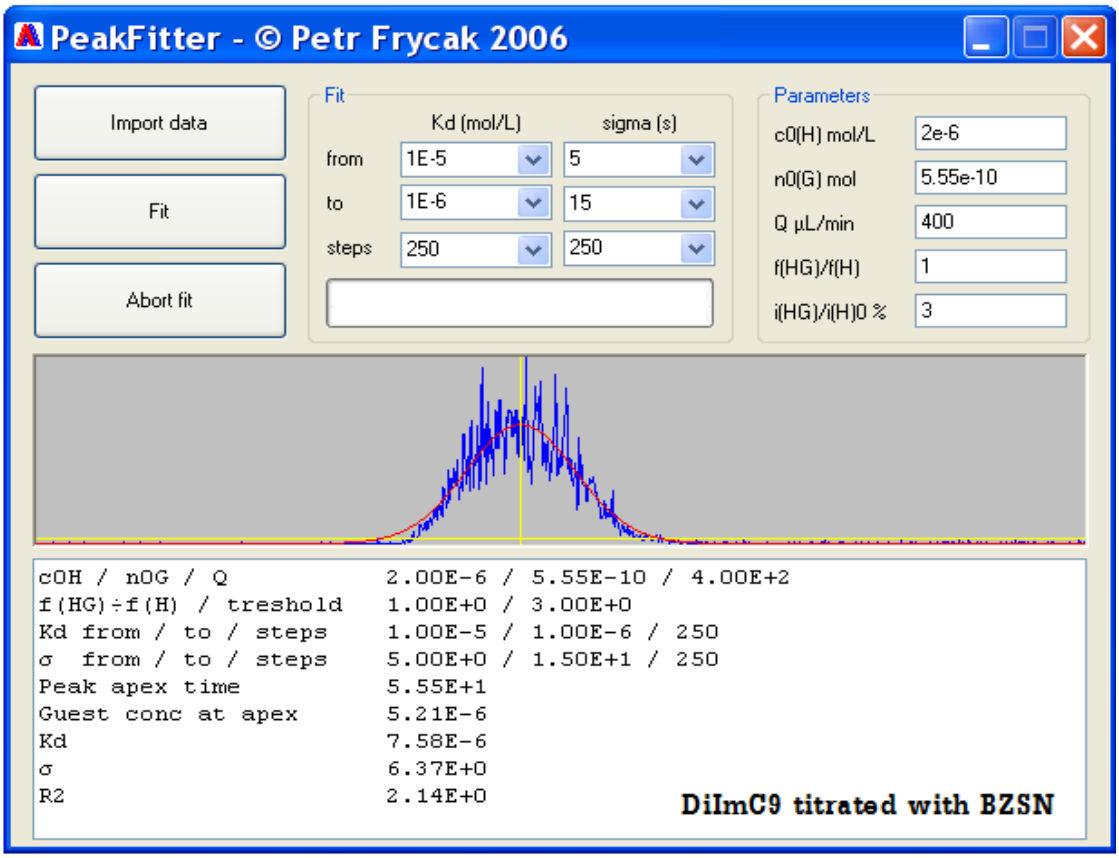


Figure 11.4 Example of Gaussion peak fitting.

This is the output file from the in-house peak fitting program. The system used to produce this figure was DiImC9/BZSN. For ther parameters, see the Experimental section.

Perhaps the most profound discrepancy between the binding constants determined by ESI and those found using NMR is simply the amount (i.e. orders of magnitude) by which they differ. All the solution phase binding constants were found to be approximately 10^2 M^{-1}, whereas the values obtained by ESI were approximately 10^3-10^5. This means their values differ by about 2 orders of magnitude. This indicates that a considerable enhancement in the binding occurs when these complexes are desolvated. Though the desolvation process is complicated, there have been reports of enhancement of host-guest binding systems when they undergo electrospray ionization.[272-276]

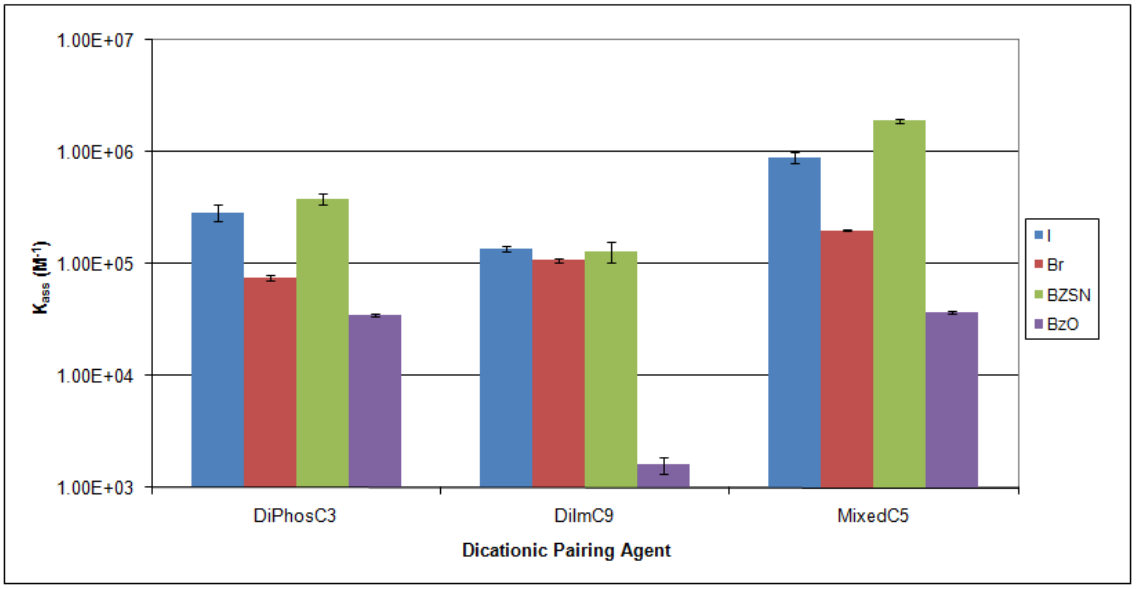


Figure 11.5 Solution phase binding constants determined by ESI-MS.

One such study showed that the "shrinking of the droplet" and consequent increase in concentration can lead to enhanced complex formation.[274] Since the dication/anion complexes observed in the ESI-MS are 1:1 binding systems (1:2 would be neutral and not observed), their rate constant would be second order. If this rate (the rate at which the dication, anion, and complex equilibrate) can occur faster than the desolvation process, the concentration of the complex will increase exponentially.

A second model which further describes the enhancement of binding systems during the ESI process is referred to as the equilibrium partitioning model (EPM).[275-276] Other similar models have been reported earlier for the enhancement of analyte signals in charged droplets containing surfactants.[277-278] This model was referred to as modified aerosol ionic redistribution. In short, the EPM describes the ESI droplets as biphasic, in which the outer phase is hydrophobic and the inner phase is hydrophilic. Figure 11.6 represents this model. If there are two phases, there will be a partitioning of the host, guest, and complex between the inner phase

to the outer phase. Thus, there are far more equilibria (3 partitioning and 2 binding) to be considered than just that of the complex formation in the bulk solution. These different equilibria are also represented in Figure 11.6. Thus, this model suggests that the ion intensities observed in the mass spectra are not only a result of the ion binding, but also the partitioning equilibria of the species. The following section will investigate the affinity for the anions, dications, and the complexes for the more "hydrophobic" outer phase.

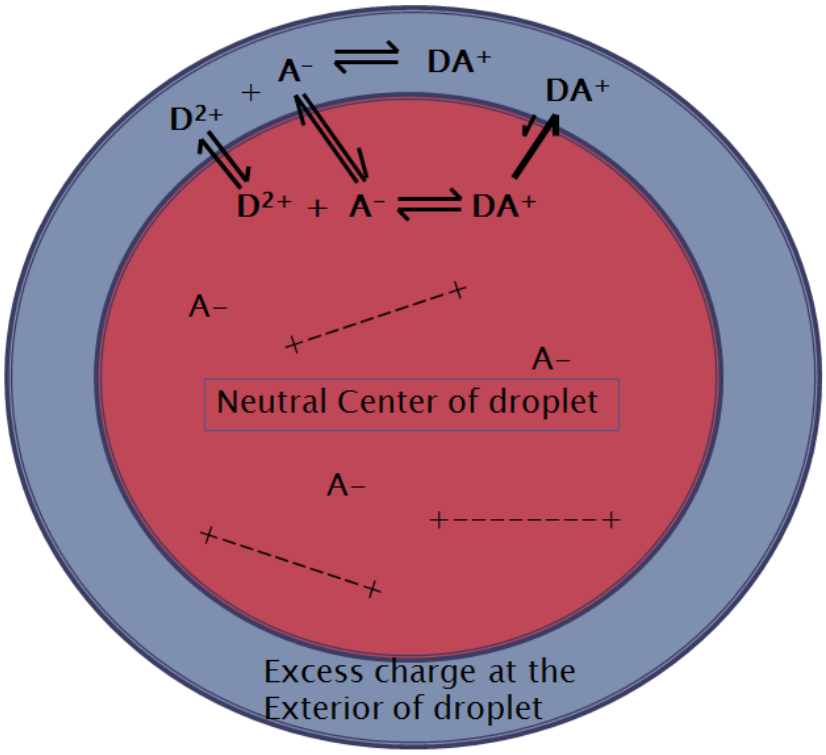


Figure 11.6 Equilibrium partitioning model.

11.3.4 Surface tension measurements

To better understand the relative ability for the dications, the anions, and the complexes to partition to the outer phase of the electrospray droplet, surface tension experiments were performed. These experiments will help show that the 1:1 complexes have a much greater surface activity than the free dication or the anion.

For a compound to have high surface activity it should posses a lipophilic portion which will prefer to reside in or near the droplet-air interface, which is more hydrophobic. The hypothesis for this experiment is that the dicationic ion-pairing reagents themselves are not very surface active, as they possess two symmetrically spaced cationic moieties. However, when the complex forms, one of the charged moieties is neutralized, thus creating a surfactant-like compound.

Figure 11.7 shows the results of titrating 0.1 M dication (DiImC9) bulk solution with an anion (BZSN) and taking several surface tension measurements over the course of the titration. Also, provided in Figure 11.7 is result of adding the anion just to water rather than the dication bulk solution. What should first be discussed is the surface activity of the anion itself. As can be seen by Figure 11.7, the addition of BZSN only slightly decreases the surface tension of water, meaning it is not very surface active. It should be noted that when NaBr and NaI were used, the surface tension of water increased. This is a well documented phenomenon.[279,280] Thus, when the droplets first form, the anions will surely reside in the center of the droplet.

The next observation that was made from these experiments is that the dications alone are just slightly more surface active than the anions, lowering the surface tension of water by no more than 7 dynes/cm. All three dications had similar surface activity as determined by surface tensiometry. For reference, the surface tensions of 0.1 M DiPhosC3, DiImC9, and MixedC5 were 64.8, 66.3, and 67.2 dynes/cm, respectively. This supports the hypothesis that the dicationic reagents themselves are not tremendously surface active.

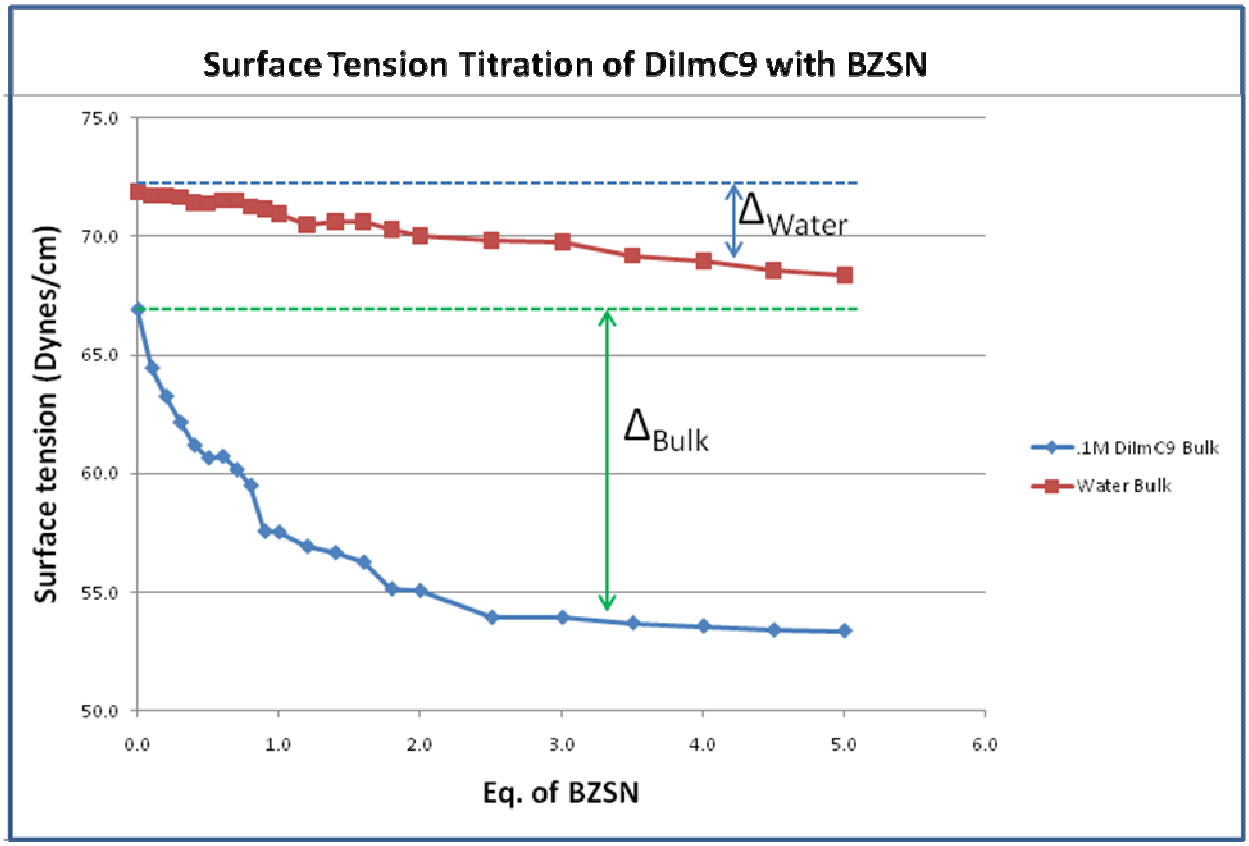


Figure 11.7 Surface tension measurements for titrating DiImC9 with BZSN.

This plot represents surface tension vs. benzenesulfonate (BZSN) concentration in neat water and a 0.1M aq. solution of the dication DiImC9. For more details on the procedure used to obtain this plot, see the Experimental section.

The most intersting result that was observed throughout this experiment was the dramatic decrease in the surface tension of dication bulk solutions when titrated with anions. This effect is illustrated in Figure 11.7. At two molar equivalents of BZSN added, the surface tension of the dication bulk solution was lowered by 12 dynes/cm, whereas when an equivalent amount of BZSN was added to water, the surface tension only decreased by 2 dynes/cm. As a result, the existence of the DiImC9/BZSN complex lowered the surface tension of water by nearly 20 dynes/cm. To make the data comparison for each dication/anion system simpler, the

$\Delta\Delta\gamma_{x\ eq}$ values will be compared (where $_{x\ eq}$ denotes the number of molar equivalents of anion that was added). $\Delta\Delta\gamma_{x\ eq}$ was determined through the following relationship:

$$\Delta\Delta\gamma = \Delta_{bulk} - \Delta_{water}$$

where Δ_{bulk} and Δ_{water} are the differences in surface tension from the point where no anion was added to the point where the described number of molar equivalents ($_{x\ eq}$) of anion is added. These values also are indicated in Figure 11.7. Subtraction of Δ_{water} serves as a normalization factor and was a slightly negative number for the inorganic salts which increased the surface tension of water and a slightly positive number for the organic salts (as shown in Fig. 11.7).

Table 11.2 lists the $\Delta\Delta\gamma$ values for all the systems in this study. Several key observations can be made from Table 11.2. First, the $\Delta\Delta\gamma$ values for bromide are usually quite low. This directly correlates to the finding that bromide detection by positive mode ESI-MS was not as sensitive as for the other anions. However, BzO, which also was not particularily sensitive, had $\Delta\Delta\gamma$ values that were fairly comparable to BZSN and iodide. Next, it was observed that the DiPhosC3/I complex resulted in very high $\Delta\Delta\gamma$ values. In fact, after the addition of 3 molar equilavents of iodide, precipitation occurred. This means that this complex is very surface active and eventually becomes not only chaotropic but completely insoluable in water at higher concentrations. Clearly this is a factor that led to the very low LOD found for DiPhosC3 and iodide. Another system that yielded large $\Delta\Delta\gamma$ values was the DiImC9/BZSN complex. Again this was one of the more successful systems tested in terms of LODs. Yet, there is not a direct correlation between all of these results and the LOD values. For example, BZSN had lower detection limits with DiPhosC3 than with DiImC9 or MixedC5, yet the $\Delta\Delta\gamma$ values for DiPhosC3 and BZSN are slightly lower than the $\Delta\Delta\gamma$ with the other systems.

Table 11.2 ΔΔγ values for all the systems studied.*

Complex	$\Delta\Delta\gamma_{5eq}$	$\Delta\Delta\gamma_{1eq}$	$\Delta\Delta\gamma_{2eq}$	$\Delta\Delta\gamma_{3eq}$	$\Delta\Delta\gamma_{4eq}$	$\Delta\Delta\gamma_{5eq}$
DiImC9 + BZSN	5.8	8.5	10.0	10.9	10.5	10.1
DiImC9 + BzO	3.8	5.6	5.0	6.5	6.7	7.0
DiImC9 + I	2.5	4.6	5.9	7.6	8.4	8.6
DiImC9 + Br	0.7	1.4	2.9	4	5.4	5.6
DiPhosC3 + BZSN	3.7	5.4	6.8	7.7	7.7	7.5
DiPhosC3 + BzO	4.4	6.3	7.5	8.5	8.7	9.3
DiPhosC3 + I	6.1	9.5	12.3	precip	precip	precip
DiPhosC3 + Br	1	1.5	2.6	3.4	4.1	5.4
MixedC5 + BZSN	4.3	5.9	6.7	7.6	7.6	7.2
MixedC5 + BzO	3.7	5.6	6.3	7.1	6.7	6.7
MixedC5 + I	3	4.9	6.7	8.7	9.2	9.7
MixedC5 + Br	0.2	1.1	2.1	2.1	3.7	4.5

*For a detailed descripton of how ΔΔγ was calculated, see the preceeding text and Fig 11.7. For other parameters used to obtain these values, see the Experimental section.

Most importantly, there is clearly an increase in the surface activity of all these systems when the complex forms. This will cause a signifigant partitioning of the complex to the outter region (surface) of the droplet. This increase in surface activity is identified as a major factor that leads to the low LODs seen with these systems. Furthermore, the partitioning of the complex to the outer phase of the droplet will cause a depletion in the concentration of the complex in the inner phase. If the binding kinetics are faster than the desolvation process, more complex will be formed to satisfy the association constant. Due to the high surface activity of these complexes, this process may occur several times during the desolvation process, thus enhancing the degree of association of these systems.

Lastly, it can be concluded that the formation of a surface active complex may be part of the reason why it was observed previously that flexible dications yield lower LODs than rigid agents. It was hypothesized that the ability of the ion-paring agents to “wrap around” the anions could stabilize the gas phase complex. Here we have shown that another possible reason for

the success of flexible ion-paring reagents is their ability to form surface active complexes. A scenario that would be less likely with more rigid dications.

11.4 Conclusions

In this study, three dicationic ion-pairing agents and four mono-valent anions were used as models to further understand the mechanism by which dication-anion complexes lead to ultra-high sensitivity in ESI-MS. First, the solution phase binding constants were evaluated using NMR. This resulted in the confirmation that these ions indeed bind in solution. The binding constants were also evaluated using ESI-MS. These results did not follow the same trend as the NMR data. More importantly, it was observed that the association constants obtained via ESI-MS were approximanetly 2 orders of magnitude greater than the solution values determined by NMR. This leads to the conclusion that there is a enhancement in the binding which occurs during the desolvation process. Both the shrinking droplet and the EPM models have been used to better describe the process that occurs during desolvation. Surface tension measurements were completed to better understand the relative surface activity of the complexes. The results showed that the complexes greatly decrease the surface tension of water. This leads to the conclusion that as the complexes form they will have a greater affinity for the surface of the droplet, thus they have greater ionization efficiencies. Indeed this may be the driving force for the low LODs reported for these systems. However, neither the binding constants nor the surface tension alone can be directly correlated with the LODs. It is a combination of these effects that controls the obsereved sensitivities. Additional information concerning the rate at which these binding system equilibrate and the rate at which they partition to the outer phase of the droplet will be needed for a more complete explanation of the mechanism. Also, the response factors were assumed to be unity. Future work should be done to develop a method by which the actual response factors can be determined. Nevertheless, a better understanding as to how these dicationic ion-paring reagents achieve their “sensitivity enhancement effect” has been gained.

CHAPTER 12

GENERAL SUMMARY

Room Temperature Ionic liquids (RTILs) are a novel class of solvents that are composed entirely of ions. Throughout this dissertation several new uses for RTILs have been discussed. In Chapter 2, newly synthesized phosphonium based monocationic and dicationic RTILs have been used successfully as gas chromatographic (GC) stationary phases. It was determined that the new phosphonium based salts possessed superior thermal stability than many of the nitrogen based RTILs. Additionally, the solvation parameters of these phosphonium based RTILs were found to be unique and also differed from commonly used imidazolium based RTILs. In Chapter 3, one of these novel phosphonium RTIL based GC columns was used as a primary column for comprehensive two-dimensional GC (GCxGC). This work showed that the new phosphonium type stationary phase offered great orthogonality when compared to existing commercially available columns. Using the phosphonium IL based GC phase resulted in highly selective GCxGC separations. This column allowed for exceptional group separations of the components present in diesel fuel. In Chapter 4, the first tricationic RTIL GC stationary phases were described. LSERs proved that the solvating properties of these new phases were unique. These phases also showed exceptional thermal stability. The major finding in this study was that one of the tricationic IL GC phases was able to produce excellent peak shape for hydrogen bonding analytes.

In the later portion of this dissertation, the use of RTIL based salts as ion-pairing agents was examined. In Chapter 5, a plethora of dicationic ion-pairing agents were tested for their efficacies in pairing with singly charged anions. This study resulted in the discovery of four dicationic ion-pairing reagents that outperformed the rest. Several other empirical observation centering the structural motifs which make for good dicationic paring agents were discussed.

Chapter 6 presented the first use of tricationic ion-pairing agent for the detection of doubly charged anions. It was found that tricationic ion-pairing reagents could be used to detect -2 anions in a similar manner as dicationic pairing agents and singly charged anions. Two of the most successful tricationic ion-paring reagents were identified as well. However, the structures of these tricationic ion-pairing agents were all a bit rigid. Earlier in Chapter 5, it was observed that flexibility is a key structural component to a good paring agent. Hence we proceeded to test synthesize and test more linear, flexible, tricationic ion-pairing reagents. In Chapter 7, the results of using linear trications was described. It was determined that in general, the use of the more flexible ion-paring reagents yielded greater sensitivity for the detection of di-valent anions. Two linear tricationic ion-paring agents have were as most successful. In Chapter 8, the two best trigonal and two best linear tricationic ion-paring agents were used to detect a plethora of doubly charged anionic species. Also in Chapter 8, the use of SRM for the trication-anion complexes was described. Chapter 9 showed the applicability of this ion-pairing method when used in conjunction with CE-MS. For these experiments it was determined that the ion-pairing reagent could be added three ways. First, the reagent could be added to the anion analyte solution. Second, it could be added to the background electrolyte. Third, it could be added to the sheath liquid flow that is used to join CE and ESI. It was determined that the addition of the ion-paring reagent to the run buffer was the optimum method. In Chapter 10, the ion-pairing technique was extended to the use of tetracationic ion-pairing agents for the detection of tri-valent anions in the positive mode ESI-MS. It was determined that these tetracationic ion-pairing reagents allowed more sensitive detection of tri-valent anions. In the penultimate chapter, the mechanism by which dicationic ion-pairing agents produce such low limits of detections for anions was considered. It was determined that the formation of surface active complexes is one major driving force for the success of this method.

APPENDIX A

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CHAPTER 11: Zachary S. Breitbach, Eranda Wanigasekara, Edra Dodbiba, Kevin A. Schug, and Daniel Armstrong

APPENDIX B

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BIOGRAPHICAL INFORMATION

Zachary S. Breitbach obtained his Bachelor of Science degree in chemistry from Clarke College in 2005. He then chose to further his education by joining Dr. Daniel Armstrong's research group in 2005 at Iowa State University. After one year at Iowa State, he moved with Dr. Armstrong to the University of Texas at Arlington, where he obtained his Doctor of Philosophy degree in analytical chemistry in 2010. His research interests include all forms of chiral and achiral separations, including the development of new chiral stationary phases. He is also interested in developing sensitive detection strategies using ESI-MS and discovering new uses for ionic liquids in analytical chemistry.