MULTIPLY CHARGED CATIONIC PAIRING AGENTS FOR TRACE ANALYSIS OF ANIONIC SPECIES BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

by

JEFFREY WILLIAM REMSBURG

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ABSTRACT

MULTIPLY CHARGED CATIONIC PAIRING AGENTS FOR TRACE ANALYSIS OF ANIONIC SPECIESBY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Jeffrey William Remsburg, M.S.

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Supervising Professor: Daniel W. Armstrong

Electrospray ionization is one of the most broadly useful and successful approaches for coupling high performance liquid chromatography to mass spectrometry. Most of the research in electrospray ionization mass spectrometry (ESI-MS) has been limited to the positive ion mode, where lower detection limits are generally achieved, due several advantages over the more problematic negative ion mode. However, if one wishes to study anionic species, the negative ion mode traditionally had to be employed, and the problems associated with the negative ion mode had to be tolerated or ameliorated as much as possible.

Recently, novel work has been done to take advantage of the positive ion mode when studying anionic species. This is done by placing small amounts of a multiply-charged cationic reagent in the carrier stream. When an anion of lesser charge is injected into the carrier stream, a complex will form that contains a net positive charge that can be detected in ESI-MS in the positive ion mode. This method has been shown to be significantly more sensitive than detecting the anion in the negative ion mode.

The first two studies in this work have been done by using large, chaotropic dications to study singly-charged anions. A third study was done using trications to analyze dianions by ESI-MS. This method shows great promise for the analysis of anionic species by ESI-MS and should lead to lower detection limits for a wide variety of anionic species.

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CHAPTER 1

INTRODUCTION

The development of dicationic ionic liquids has led to advancement in many scientific fields. These materials were originally developed as ionic liquids with enhanced thermal stability compared to moncationic ionic liquids [1]. Many of these "multifunctional" ionic liquids were shown to be thermally stable to temperatures exceeding 400° [2]. The cations consisted of two charged moieties (i.e. imidazolium or pyrrolidinium) connected by an alkyl linkage chain of various lengths. These ionic liquids were subsequently used as solvents for high-temperature syntheses [3]. Recently, they have been used as coatings for solid phase microextraction (SPME) methods [4].

Most recently, the dicationic moieties were found to be essential for a special technique of anion analysis by electrospray ionization-mass spectrometry (ESI-MS) using the positive ion mode. Dasgupta, Armstrong, and co-workers took an interesting approach in incorporating the dicationic compounds described above into an ESI-MS study [5-8]. The purpose of the study was to improve the detection of perchlorate by ESI-MS. A dicationic ionic salt was placed in the mobile phase, and the perchlorate in the sample was injected into the carrier stream and subsequently detected as a dication-perchlorate complex containing a net positive charge. This approach was found to be extremely sensitive, reproducible, and was not difficult to use.

ESI itself has long been used for the analysis of a wide array of compounds. Originally developed by Fenn et al [9], ESI allows a continuous liquid flow to be subjected to analysis by mass spectrometry. The liquid flow is pumped through a capillary which has an applied voltage ($\underline{+}$2-6 kV). This voltage creates charge separation at the surface of the liquid, thereby producing a "Taylor cone" protruding from the capillary tip. Droplets that contain an excess charge (positive or negative depending on the polarity of the capillary voltage) will then detach from the end of the Taylor cone. These droplets eventually yield "naked" ions for analysis by mass spectrometry. This ionization source has allowed mass spectrometry to be coupled with liquid chromatography, thereby combining two of the most powerful tools in analytical chemistry [10]. The importance of ESI cannot be overstated, as it has been invaluable to the analytical chemistry field [11].

Although ESI can be used in both positive and negative ion modes, the positive ion mode is generally employed if possible. When analyzing complex molecules that contain both basic and acidic functional groups (i.e. proteins), the choice can be made to use the positive ion mode over using the negative ion mode. However, if the project entails the determination of simple anions in solution, then the positive ion mode traditionally cannot be used. When using the negative ion mode to study anions, it is generally done by adding a basic additive (i.e. NH_4OH) to the carrier stream (commonly methanol/water). Unfortunately, basic water/methanol solutions do not form a stable Taylor cone, thus greatly reducing the efficiency of the ESI process [11]. In fact, Straub and Voyksner reported that the number of ions produced in the negative ion mode gave only a 1-38 % relative response compared to the number of ions produced in the positive ion mode [12]. Obviously, switching from the positive ion mode to the negative ion mode is more complex than changing the additive and polarity of the voltages.

The main reason for this discrepancy is due to the prevalence of corona discharge in the negative ion mode. Corona discharge is an electrical discharge resulting from the ionization of a fluid surrounding a conductor. In ESI, corona discharge occurs when the high concentration of electrons on the capillary lead to ionization of the molecules around the capillary. Fenn and co-workers found that corona discharge occurs in the negative ion mode at a voltage several kilovolts lower than the onset of corona discharge in the positive ion mode [9, 13]. The large quantity of the ionized molecules then leads to significant background interferences and poor spray stability. Furthermore, if the corona discharge persists, it can lead to arcing. This is a substantial problem for ESI-MS, as it not only leads to a reduction of the current, but can damage the electrical components of the instrument [11].

Corona discharge can be "lessened" to some degree through the use of electron-scavenging gases [12-15] and/or halogenated solvents [14-16]. Halogenated solvents have a high relative electron affinity and can thus "capture" electrons, thereby inhibiting the occurrence of corona discharge. The same principle is operative when using electron scavenging gases such as oxygen or SF_6. While employing these methods may decrease corona discharge, the negative ion mode will still be more prone to it than the positive ion mode. Also, halogenated solvents are not commonly used solvents for liquid chromatography. Finally, introducing a bath gas such as SF_6 to the ionization source is not a routine task. Thus, a general, broadly useful method that allows for the analysis of anions in the positive ion mode using common solvents (especially HPLC solvents) would be ideal.

In this work I have attempted to develop and thoroughly evaluated the use of multivalent cationic additives for the ESI-MS analysis of a broad range of anions. Chapter 2 presents the results of a study involving over 30 anions using a single dicationic pairing reagent. This study provided insight into which anions are best suited to this approach of detection and successfully demonstrated how this method is superior to detecting anions in the negative ion mode. Also present in this study was the first reported application of tandem mass spectrometry when using pairing reagents for anion determination. The success from this work then led to another study. Obviously, structural differences in the dicationic reagent should lead to differing sensitivities for specific anions. Consequently the work presented in Chapter 3 showed how differences in the dicationic reagent affect the detection of different anions. Twenty-six different dications were evaluated to see how changes in their size, structure and geometry would affect the detection of complexed anions. Given the success of our ESI-MS studies in analyzing singly charged anions, an obvious question was raised as to how well trications would work in detecting complexed divalent anions. It was found that this approach also led to increased sensitivity for determining doubly charged anions. Seventeen different tricationic reagents were evaluated, and the results of that study are given in Chapter 4. Overall, this thesis presents cumulative studies on the detection of negatively charged species in the positive ion mode as a positively charged complex by ESI-MS.

CHAPTER 2

A GENERAL, POSITIVE ION MODE ESI-MS APPROACH FOR THE ANALYSIS OF SINGLY CHARGED INORGANIC AND ORGANIC ANIONS USING A DICATIONIC REAGENT

Renee J. Soukup-Hein, Jeffrey W. Remsburg, Purnendu K. Dasgupta, and Daniel W. Armstrong

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Abstract

Anion analysis continues to be of great importance to many scientific and technical fields. We propose here a general and sensitive method of detecting singly charged anions by ESI-MS and LC-ESI-MS as positive ions. This method utilizes a dicationic reagent to form a complex with the anion that retains an over all positive charge for analysis by MS. Nitrate, thiocyanate, perchlorate, perfluorooctanoic acid (PFOA), halogenated acetic acids, and various other inorganic and organic anions and are investigated. The use of tandem mass spectrometry to enhance the detection limits of some of the anions is demonstrated. Chaotropic anions provided the lowest detection limits, with PFOA detected at the hundreds of femtograms level. Indeed, this single approach provides the lowest reported detection limits for a variety of anions, especially PFOA, nitrate, monochloroacetic acid, dichloroacetic acid, and bromochloroacetic acid, among others. The integrated areas and signal to noise ratios for five ions during a chromatographic run in both the positive and negative ion modes are compared. The

ability of this method to detect differences in related ions is shown for four arsenic species. Finally, a tap water sample is analyzed for the anions in this study using the dicationic reagent method.

Introduction

The analysis of anions is essential in many areas of scientific and technical interest. Most commonly it is utilized in the analysis of environmental samples [5, 8, 17-29], especially water, human tissues and a variety of other fluids [6, 30-34]. In fact, the entire application area is diverse and includes the characterization of apple juice [35], marsala wines [36], and various foods and beverages from around the world [37]. Separation methods are often applied in anion analysis, especially when complex matrices are present. Ion chromatography is the most common separation method used [6-8, 20, 21, 24, 27, 29, 30, 34, 36-45], although reverse-phase chromatography is sometimes an option if ionization of the analyte is suppressed [35] or for ions with sufficiently hydrophobicity (e.g., perfluorooctanoic acid, PFOA) [23, 25, 31-32, 46]. Ion-pairing chromatography [33, 47] and capillary electrophoresis [48-50] also have been used. GC and GC-MS can provide sensitive and selective analysis methods for anions that have been converted to volatile derivatives before analysis. Such an approach was used in the analysis for trifluoroacetic acid [17-18] and thiocycanate [51]. Direct techniques for the determination of anions that do not involve separation techniques include mass spectrometry [19, 52-53], spectrophotometry (including ICP) [21, 24, 29, 54-56], and ion-selective potentiometry, and other electrochemical techniques [57-62].

In the never-ending search for more sensitive and selective methods of analysis, scientists have begun to examine electrospray mass spectrometry as an alternative choice for the analysis of some anions [5-8, 19, 23, 25, 27, 29, 32, 34, 37, 40, 45, 47, 52-53]. Given that anions are negatively charged, it is not surprising that most reported results have used the negative ion mode. When analyzing inorganic and organic anions via mass spectrometry, there are some generally acknowledged limitations. For example, small anions with masses below the mass cut-off of the mass spectrometer (specifically ion traps) cannot be detected. Small, very polar analytes tend to be more hydrated and reside in the more neutral interior of the electrospray droplets, which in turn can lead to lower than expected signals [11, 63]. Analytes in the low mass range that are above the cut-off generally reside in the region of high chemical noise [11]. In addition, these anions also can experience some reduced sensitivity compared to larger ions in some mass spectrometers (e.g., ion traps [64]). It is also known that negative mode electrospray conditions are inherently more prone to corona discharge than is the positive mode [11]. Both corona discharge and arcing are more likely in the negative mode due to the high negative voltages (i.e., electrons) being applied to form the electrospray [11, 12]. Corona discharge results in a higher background and poor spray stability [11]. With regards to anion analysis, the rather conductive solvents used for reverse phase and especially ion chromatography (water, buffers, methanol, etc.) contribute to corona discharge conditions when high negative voltages are applied to create the electrospray. Analyte signals can be stabilized by using halogenated solvents [15] and/or scavenging gases [12]. There are other factors (pKa, surface activity, etc.)

that influence how easily the analyte will form negative ions and how easily these are transferred to the gas phase [11, 63]. These are the same factors that affect the ease of ionization in the positive mode, but they can have different effects in the negative mode. For example, small acidic molecules that form negative ions in solution may become neutral in the gas phase due to reactions with weak gas-phase base solvents (i.e., water, methanol) [65]. These same weak gas-phase solvents allow for the beneficial protonation of analytes in the positive mode. Consequently, solvents such as propanol, 2-propanol, and butanol have been recommended for negative mode LC-ESI-MS [66], likely due to their higher gas-phase proton affinities [65]. However, these solvents have much different chromatographic selectivities and produce higher column operating pressures than methanol and acetonitrile. It makes some sense then that optimum sensitivity in the negative mode is not always achieved by just applying a negative voltage to the LC column eluent.

Since water, methanol, and acetonitrile are common separation solvents, it would seem desirable to detect anions in these solvents while avoiding the accompanying problems of operating in the negative ion mode. In order to detect the anions in the positive mode, the anions must be paired with another reagent that can produce two (or more) positive charges so that the adduct as a whole retains at least one positive charge. For small anions of very low mass to charge ratios (<100 m/z), the resulting adduct increases the m/z at which the anion is detected. This reduces the low mass bias experienced by these small anions [64]. This is also an effective means for detecting anions whose m/z fall below the low mass cutoff of the mass spectrometer.

Even for slightly larger anions, adducts formed with the reagent can move the mass of the adduct to a higher m/z region where there is less background noise. Organic bases [52] and cationic surfactants [19-20] were used to form negatively charged adducts with two perchlorate anions (or a perchlorate and another anion) for detection by ESI-MS in the negative mode. Recently, we first used dications, i.e.,hydrocarbon chains terminated by tetralkylammonium, substituted imidazolium, or substituted pyrrolidinium groups (which were originally synthesized for use as high-stability ionic liquids [2]), to detect perchlorate in the positive mode by ESI-MS [5]. The notable features of this work were (a) its ease of use, (b) ultra high sensitivity, and (c) elimination of background interferences. Indeed this method proved to be nearly as sensitive as any known method for perchlorate [34] and it eliminated the interference from ubiquitous sulfate. This method also is compatible with ion chromatography and has been subsequently been used for the determination of perchlorate, iodide, and thiocyanate in seawater and seaweed [6], bovine and human milk [7], and urine [8]. In this work, we examine the use of geminal organic dications as a general approach for the analysis of a wide variety of singly charged anions by ESI-MS and LC-ESI-MS in the positive mode. We also explore the possibility of using tandem mass spectrometry to further enhance the sensitivity of this method. Further, we compare the anion signals in the negative mode and in the positive mode with this geminal dicationic reagent for five anions separated in a chromatographic run. We also use this ESI-MS method to demonstrate the detection of several environmental arsenic contaminants in an aqueous sample in a single injection and analyze a tap water sample for anions.

Experimental

Materials

HPLC grade water and methanol were obtained from Burdick and Jackson (Honeywell Burdick and Jackson, Morristown, NJ). Sodium hydroxide and sodium fluoride were of reagent grade. The anions listed in Table 1 were purchased as the sodium/potassium/lithium salt or as the free acid and all were of reagent grade or better. The dicationic salt was synthesized according to Anderson et al. in the bromide form [2].

Methods

The dicationic reagent was exchanged into the fluoride form to maximize the amount available for adduct formation. This was achieved using ion-exchange. Four milliliters of Amberlite IRA-400 in the chloride form was packed into a disposible 10-mL syringe. The column was washed with ten column volumes of 1 M NaOH, ten column volumes of water, seven column volumes of 0.5 M NaF, and ten more column volumes of water to put the resin in the fluoride form. One milliliter of 0.1 M of the dicationic reagent in the bromide form dissolved in water was passed through the resin and eluted with water into a 10-mL volumetric flask. The resulting stock solution was then used to make up the working solutions in either water or methanol at the desired concentration to give a final dicationic reagent (entering the mass spectrometer) of 10μM.

ESI-MS analysis

The mass spectrometer used in this study was an LXQ (Thermo Fisher Scientific, San Jose, CA) with a six port injection valve used to make injections. The sample loop size was 2.0 µL. A carrier flow of 300 µL/min was provided by a Surveyor MS pump (Thermo Fischer Scientific, San Jose, CA) with a membrane degasser. Introduction of the dicationic solution into the sample stream was accomplished via a Y-type mixing tee. The flow rate of the dicationic solution was 100 µL/min and a Shimadzu LC-6A pump was used for this purpose. 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. In negative mode the conditions were: spray voltage: 4.7 kV; capillary temperature: 350ºC; capillary voltage: -25 V; tube lens: -6 V; sheath gas 37 arbitrary units (AU); auxiliary gas: 6 AU. The MS was operated in either single ion monitoring (SIM) or single reaction monitoring (SRM) acquisition mode. Normalized collision energy for SRM experiments was set at 25 and the activation time was set at 30 ms. Data was collected and analyzed using Xcalibur and Tune plus software.

The conditions reported here were optimized for the perchlorate adduct and used for all of the anions. This method could likely be further improved by optimizing the MS conditions for the specific anion of interest and to the specific type of mass spectrometer used. We believe it is likely that other mass spectrometers (e.g., triple quadrupoles) may achieve even lower detection limits when using this technique/reagent.

The precision of this technique is dependent on both the nature and the concentration of the analyte anion (precision decreases as the detection limit is approached). The experimental error for most determinations via this method were less than five percent and can likely be attributed to injection volume variation (± 5%). This would indicate this method is highly reproducible and the association of the anion with the dication is rapid. These results are in accord with previous studies on perchlorate, iodide, and thiocyanate [5-8].

Chromatography

The instrument configuration from above was modified slightly for chromatographic experiments. A Surveyor autosampler (Thermo Fischer Scientific, San Jose, CA) fitted with a 25 μL injection loop was used for sample introduction. The introduction of the dicationic solution in methanol (positive mode) or pure methanol (negative mode) was located between the column and mass spectrometer. A microbore Cylcobond I (250mm x 2.1mm i.d.) from Advanced Separation Technologies (Whippany, NJ) was used for the separation of anions. Cyclodextins have been used in the past to selectively bind ions in the absence of organic modifiers [67]. Also it has been shown that most ions, particularly those more chaotropic in nature, can include into the cavity of the cyclodextrin [67].

Water Analysis

The tap water sample was collected from the cold water tap of a laboratory sink. The water was allowed to run for 15 minutes before collecting the sample in a nalgene bottle. The same configuration used in the MS analysis was used for the determination of anions in water with one small difference. A five microliter sample loop was used for the determination of bromide and benzenesulfonate. SIM mode was used to identify anions present and SRM used to confirm the association with the dicationic agent. Quantification was performed on the anions individually in either SIM or SRM mode as specified in Table 2.

Results and discussion

In previous work [5] we found that an imidazolium-based dicationic reagent (shown in Figure 1A) paired well with perchlorate in the gas phase produced a very sensitive and interference free analysis for perchlorate. Here, we examine the possibility of using such a dicationic reagent as a general reagent for the sensitive detection of other singly charged anions in the positive ion mode.

Table 1 lists the anions included in this study in order of decreasing sensitivity (in the SIM mode). The included anions are of both inorganic and organic types. Also included are anions of broad research interests such perfluorooctanoic acid (PFOA), halogenated acetic acids, and a few environmentally important arsenic species. Additionally, there are several anions (chloride, cyanide, cyanate, thiocyanate, formate, nitrite, nitrate) that would be prone to discrimination by low mass bias or fall below the mass cut-off of certain types of mass spectrometers. Single ion and single reaction monitoring acquisition modes were used to find the lowest detectable levels of the anions. Sensitivity in SIM is important for all mass spectrometers, but especially those without the capability to perform MS/MS (single quadrupole mass spectrometers). SRM

Table 1: Positive Ion Limits of Detection for Anions Using Dicationic Reagent

Anion	SIM mass	SIM LOD (ng)	SRM mass	SRM LOD (ng)
Perfluorooctanoic acid (PFOA)	703	1.22E-04	289	7.32E-05
Nitrate (NO_3^-)	352	1.84E-03	289	1.38E-03
Tetrafluoroborate (BF_4^-)	376	1.96E-03	289	3.90E-01
Thiocyanate (SCN$^{-)}$	348	2.00E-03	289	2.00E-03
Benzenesulfonate (BZSN)	447	2.06E-03	289	4.12E-04
Trifluoromethanesulfonimide (NTF_2^-)	570	2.26E-03	289	2.26E-03
Hexafluorophosphate (PF_6^-)	435	4.28E-03	289	2.14E-03
Iodide (I^-)	417	6.00E-03	289	2.00E-01
Perchlorate(ClO_4^-)	389	1.02E-02	289	1.02E-02
Dichloroacetic acid (DCA)	417, 419	1.50E-02	289	2.00E-02
Monochloroacetic acid (MCA)	383, 385	1.50E-02	289	1.90E+00
Bromochloroacetic acid (BCA)	461, 463	1.54E-02	289	1.54E-02
Periodate (IO_4^-)	481	4.48E-02	289	1.12E+00
Bromate (BrO_3^-)	417, 419	5.00E-02	289	5.00E-02
Iodate (IO_3^-)	465	6.00E-02	289	1.39E-02
Bromide (Br^-)	369, 371	6.00E-02	289	6.00E-02
Bromooctanoic acid (BOA)	511, 513	6.00E-02	289	6.00E-02
Trifluoromethanesulfonate (TFO^-)	439	1.98E-01	207	1.98E-03
Trifluoracetic acid (TFA)	403	2.00E-01	289	2.00E-01
Malic acid	423	2.12E-01	289	6.36E-02
Bromoacetic acid (MBA)	427, 429	2.22E-01	289	1.11E-02
Benzoate	411	3.88E-01	289	9.72E-01
Monomethylarsonic acid (MMA^v)	429	6.00E-01	289	4.02E-02
Nitrite (NO_2^-)	336	6.24E-01	289	2.10E-01
Permanganate (MnO_4^-)	409	6.84E-01	N/A	N/A
Arsenate ($H_2AsO_4^-$)	431	1.00E+00	289	4.12E-02
Chloride (Cl^-)	325	1.77E+00	289	1.77E+00
Formate ($HCOO^-$)	335	4.40E+00	289	2.20E+00
Dimethylarsinic acid (DMA^v)	427	5.56E+00	289	1.00E+02
Trichloroacetic acid (TCA)	452	6.42E+00	289	1.96E+00
Cyanate (OCN^-)	332	6.42E+01	289	1.93E+01
Arsenite ($H_2AsO_3^-$)	415	1.00E+02	289	2.02E+01
Acetate (CH_3COO^-)	349	>2.00E+03	289	>2.00E+03
Cyanide (CN^-)	316	>2.00E+03	289	>2.00E+03

Limits were determined in ESI-MS analysis configuration. Limit of detection (LOD) defined as signal to noise ratio of 3.

could provide a way to reduce the noise when anions of interest are in complex matrices. While detecting the anions using SIM is fairly straightforward, SRM is slightly more complicated. In order for SRM detection to work, there must be a positively charged fragment of the dication-anion adduct remaining after dissociation. Thus, in MS/MS the final ion detected is not the anion at all, but a remnant of the dication. When the dication-anion adduct is excited and dissociated, the dominant positively charged fragment that results is usually the $[M-H]^+$ cation of the dicationic reagent. This fragment is formed from the dication by the loss of C2 hydrogen on one of the imidazole rings (see Figure 1B). The SRM signal results from plotting the intensity of each specific $[\text{dication+anion}]^+ \rightarrow [\text{dication-H}]^+$ reaction. In most cases, SRM achieved lower detection limits than SIM due to the characteristic reduction in noise. For those anions where the detection limits are the same or higher in the SRM mode, there may be other (unidentified) fragmentation pathways which reduce the abundance of the $[M-H]^+$ cation. Trifluoromethanesulfonate (triflate, TFO) was the only anion for which an alternate fragmentation pathway was identified. The most abundant dication fragment detected in MS/MS for TFO is m/z 207, which corresponds to the dication losing one imidazole group, thereby forming a 1-nonyl-3-methyl imidazolium monocation (see Figure 1C). SRM for this transition (m/z 439→207) lowered the detection limit for TFO by two orders of magnitude. While MS/MS has been used to increase the sensitivity of some anions [23, 25, 31, 32, 34, 37, 45], monoatomic anions cannot be fragmented. By using the mass transition of $[\text{dication+anion}]^+ \rightarrow [\text{dication-H}]^+$, a general MS/MS method can be used for all anions

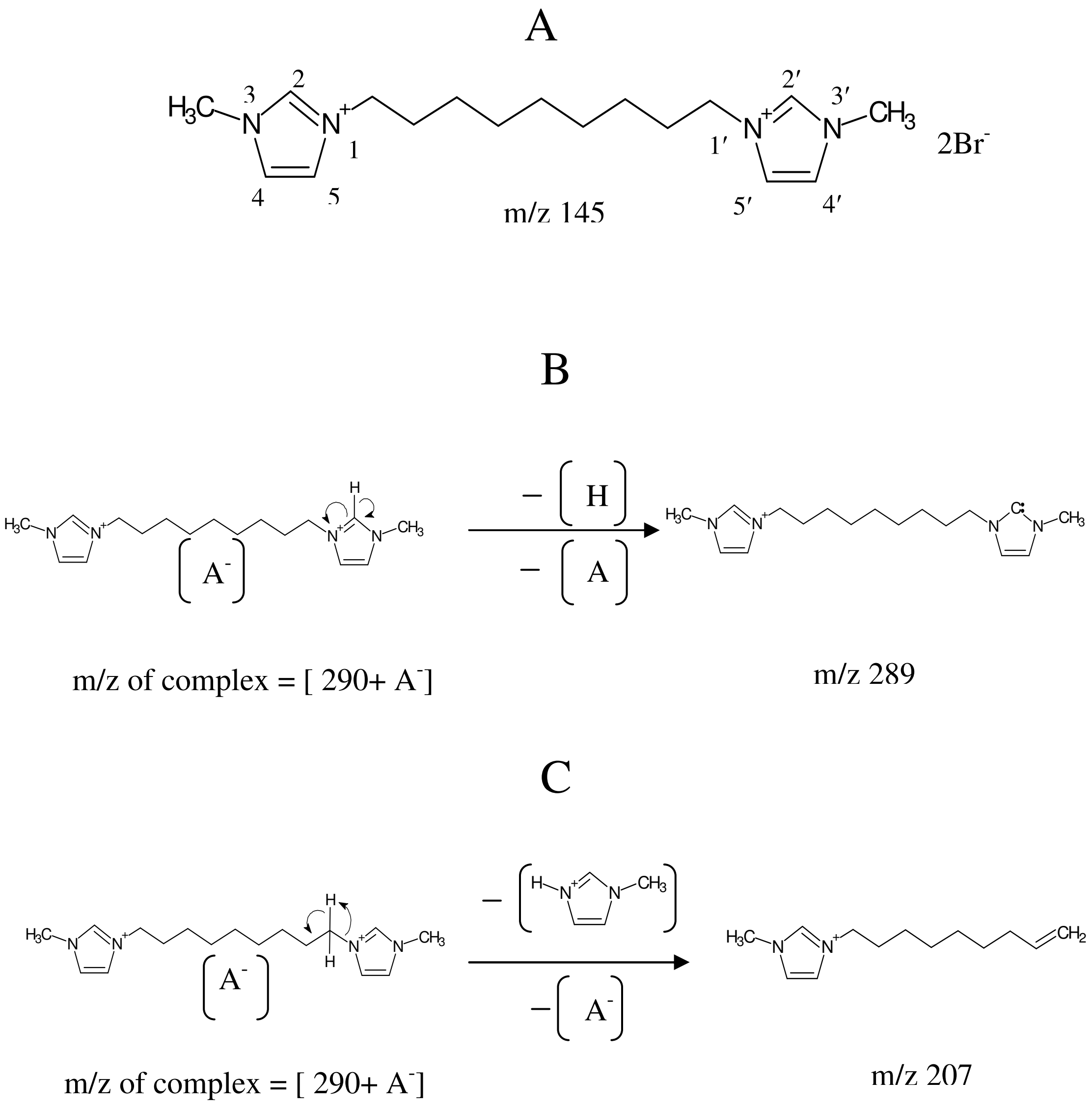

Figure 1: Structure of the dicationic reagent in its synthesized form (A) and proposed fragmentation pathways (B, C) for an anion (A⁻) of interest.

in an analysis. This is the first reported use of MS/MS for the analysis of anions using dicationic reagents.

The ions that show the lowest detection limits in the positive mode (SIM) loosely follow the Hofmeister series. The Hofmeister series orders anions according to their ability to stabilize or destabilize proteins in solution [68-69]. Anions that stabilize proteins in solutions are termed chaotropic. Here in our study, those anions that are more chaotropic have lower detection limits in the positive SIM mode than those that are less chaotropic. Nitrate and tetrafluoroborate seem to be the ions that do not fall into the order of the Hofmeister series. Nitrate and tetrafluoroborate both exhibit better limits of detection than predicted by the Hofmeister series, while the thiocyanate, hexafluorophosphate, iodide, and perchlorate generally performed as predicted by the Hofmeister series. There seems to be two possible reasons for this. First, the dicationic reagent may have slightly different affinity for nitrate and BF_4^- from the much more complex proteins used to determine the Hofmeister series. Secondly, these differences may have more to do with lack of other chemical noise near the m/z of the anion-dication adduct of nitrate and BF_4^- than with their place in the Hofmeister series. There also seem to be structural indicators that improve adduct formation with the dicationic reagent. Those anions that contain halogen atom(s) can be detected at lower levels than related anions that lack halogen atoms. The best example of this is the acetate family of anions. Acetate itself is not detected as an adduct (which makes it an ideal, non-interfering buffer component for LC-MS applications), but every acetate with at least one halogen atom can be seen adducted to the dicationic reagent (and with low detection limits, see Table 1.) In particular, those anions (PFOA, BF_4, and PF_6) with fluorine atoms usually exhibit exceptional sensitivity. In fact, PFOA was detected at the sub-picogram level, the lowest of the anions tested and lowest ever reported. Both SIM and SRM for PFOA detection limits were twice as low as that of an LC-ESI-MS/MS method with a preconcentration step [23]. Based on these properties, trifluoroacetic acid (TFA) and chloride were expected to have better than observed detection limits. In the case of chloride, its natural occurrence at high levels increases the noise level at the monitored mass of the dicationic-anion adduct, resulting in a higher detection limit. TFA also suffers from increased background levels, most likely from traces left in the solvent lines of the LC systems. Increasing the oxidation state of the central atom of an anion also seems to be beneficial in some cases. By adding oxygens, the anion increases in size and spreads the negative charge over a larger area. For example, the detection limit for nitrate is over two orders of magnitude lower than for nitrite. The same trend (but to lesser degrees) can be seen for periodiate/iodate/iodide, bromate/bromide, and perchlorate/chloride. Also interesting is the trio of anions: thiocyanate, cyanate, and cyanide. The ability to adduct with the dicationic reagent ranges from very good for thiocyanate to unobservable for cyanide.

For certain ions, the absolute detection limits determined with the dicationic reagent in positive mode by ESI-MS (Table 1) compare favorably with absolute detection limits of other methods reported in literature. The literature methods were varied and often included extraction steps that could be used to concentrate the anion of interest before analysis. Using the "dicationic reagent" ESI-MS method, PFOA could be determined at 122 fg (SIM) and 73 fg (SRM) compared to 250 fg by a LC-ESI-MS/MS method that used an eight-fold SPE concentration step [23]. The detection limit for nitrate (1.84 pg for SIM, 1.38 for SRM) was found to be well below that of an IC-ESI-MS/MS technique (1.25 ng) [34], and almost an order of magnitude lower than an ion chromatography method using a PVC membrane anion electrode as the detector (16 pg) [42]. The LOD of the dicationic reagent ESI-MS method was approximately four times lower than the lowest limits for nitrate of the other methods which was achieved by Hadamard transform CE (~8.70 pg) and required the signal averaging of twenty electropherograms [50]. Even though chloride suffers from a high background signal its detection limit was just below that of the same ion chromatography method used to determine nitrate [42]. Detection limits achieved using a combination of electrospray ionization and high-field asymmetric waveform ion mobility spectrometry (ESI-FAIM-MS [53] were lower for trichloroacetic and monobromoacetic acids but higher for monochloroacetic acid, dichlorocetic acid, and bromochloroacetic acid when compared to this method. The only literature values found for BF_4 and PF_6 were determined by attenuated total reflectance FTIR on thin film coatings [56] so absolute LODs could not be found. However, the concentration limit given in Hebert et al. [56] was in the same range as the concentration used in this ESI-MS analysis. Perchlorate [34,37] and thiocyanate [51] are the only two anions located near the top of Table 1 for which the absolute LODs reported here are higher than the lowest literature absolute LODs. However, in the case of thiocyanate in the previous literature reference, the anion had to be converted to the pentafluorobenzyl derivative for analysis by GC-MS. There are of course, some anions listed in Table 1 that were determined at the nanogram level while literature values were at the picogram level. The use of IC-ICP-MS for the speciation of the arsenic species included here is one such example [30]. For a few ions (NTF_2 and bromooctanoic acid), no literature values were found.

In many applications, chromatography is used to remove interferences present in sample matrices and provide a temporal displacement of the analytes of interest. Figure 2 is a comparison of five anions chromatographed in both positive (Figure 2A) and negative (Figure 2B) polarity modes where the masses of the anions or the dicationic adducts were monitored. Even though ten times more thiocyanate (SCN), triflate (TFO), and benzenesulfonate (BZSN) was injected in negative ion mode, these three anions displayed integrated peak areas and signal to noise ratios that were larger in the positive ion mode with the dicationic reagent than in the negative ion mode (without dicationic reagent). Thiocyanate (m/z 58) is a victim of low-mass bias in the negative mode. It resides so close to the low mass cut-off (m/z 50) that it is not detected at all in the negative ion mode, and shows the largest improvement in the positive ion mode. Despite the fact that five times more PFOA is injected in negative mode, PFOA also shows a marked increase in S/N ratios in positive mode. This is undoubtedly due to its high affinity for the dicationic reagent as discussed above. Trifluoromethanesulfonimide (NTF_2) is the only anion of these five to show comparable signal to noise ratios in the positive and negative ion modes. Part of the reason may lie in the structure of NTF_2. NTF_2 is a large anion and the negative charge is delocalized amongst the nitrogen and

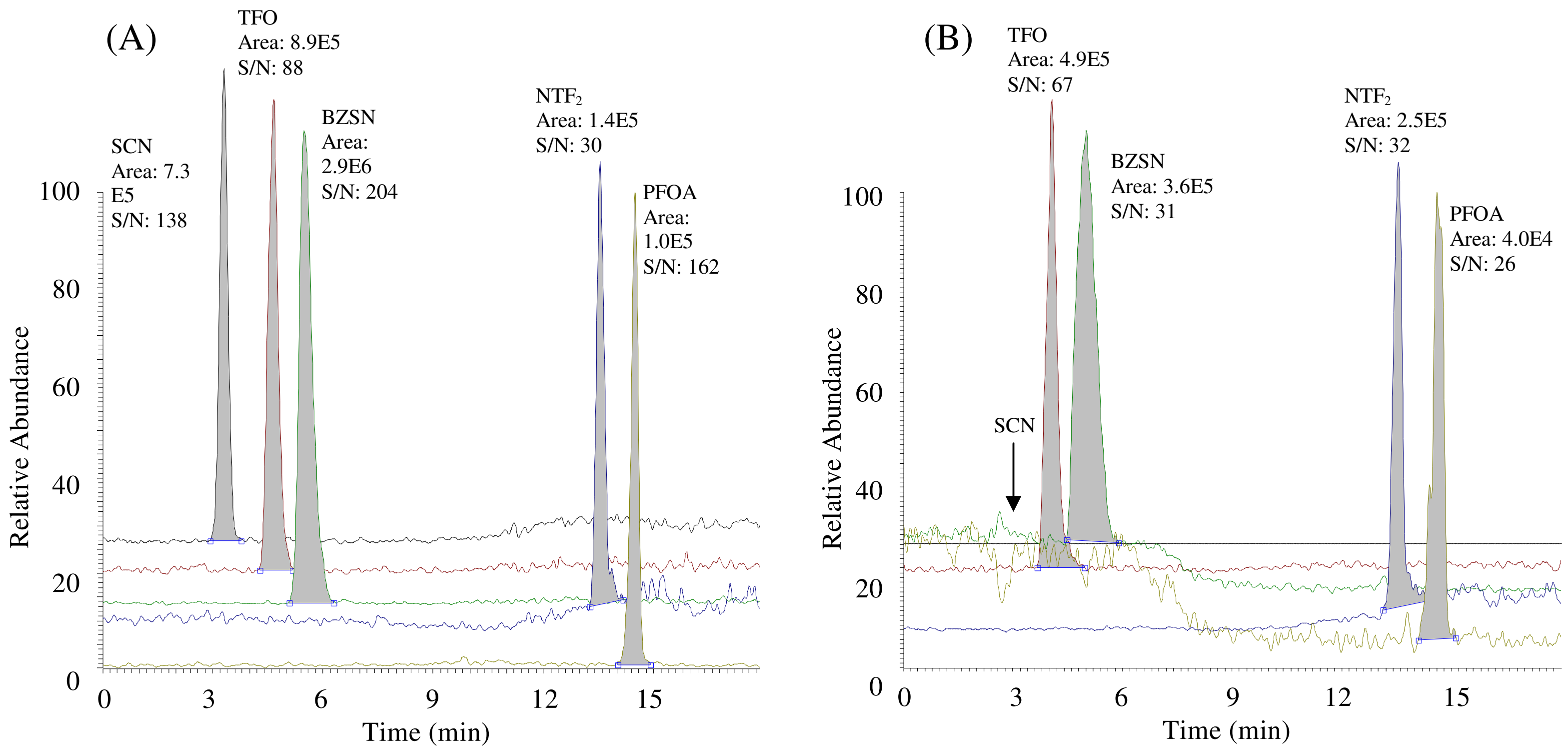

Figure 2: A comparison of the chromatographic separation and sensitivity of 5 anions on a Cyclobond I column detected in the (A) positive and (B) negative SIM modes. The mass injected in (B) is 10x that of (A) for SCN, TFO, and BZSN, 5x for PFOA, and the same for NTF$_2$. The mass injected in (A) is: 1.43 ng SCN, 9.92 ng TFO, 1.16ng BZSN, 0.68 ng NTF$_2$, and 1.30 ng PFOA. The column was equilibrated for 15 minutes with 100% Water with a linear gradient to 100 % MeOH beginning at 3 minutes and complete at 9 minutes. Flow rate was 300 µL/min. In (A) the dicationic salt solution (40 µM in MeOH) was added post-column at 100 µL/min where as in (B) it is methanol only. SCN: thiocyanate; TFO: triflate; BZSN: benzenesulfonate; PFOA: perfluorooctanoic acid; NTF$_2$: trifluoromethanesulfonimide.

sulfur atoms, with the oxygens and trifluoromethyl groups shielding its charge from other charges [70]. Benzenesulfonate and PFOA also are large anions, but in these cases the negative charge is more concentrated at one end of the structure. The more symmetrical NTF_2 anion may be more surface active [11], which leads to increased sensitivity in the negative ion mode as seen in Figure 2. NTF_2 can be detected at low levels in positive ion mode (see Table 1) because it would seem to be a fairly chaotropic anion.

The "dicationic reagent" approach to anion analysis uses the anion mass to discriminate against other related anions. Thus, this method may be useful in the detection of several related species without the use of a separation column. This is shown in Figure 3 with the detection of four arsenic species using the MS analysis configuration. The three compounds with arsenic in the higher oxidation state form a stronger adduct with the dicationic reagent as discussed above. ICP-MS is very specific for arsenic, but all speciation information is destroyed in the high temperatures of the ICP. Thus, it must be combined with HPLC for species-specific information. Due to differing toxicities, it is important to know not only total arsenic content, but also the level of the individual arsenic species [26]. This experiment clearly demonstrates the ability of the "dicationic reagent approach" to retain important structural information for related anions.

A tap water sample was analyzed by ESI-MS for the anions contained in Table 1. Five anions (chloride, nitrate, bromide, monochloroacetic acid (MCA), and benzenesulfonate) were found at levels higher than the detection limits and confirmed by the production of m/z 289 when SRM was used. Monochloroacetic acid is a known disinfection byproduct and is under regulation by the EPA [53]. Various benzenesulfonates are used in numerous industrial processes [71]. Quantitative results are shown in Table 2. The anions in this study were quantified separately (See Experimental and Table 2). Since an isotopically labeled internal standard was not available for all of the anions, no internal standard was used for this proof of concept screening. All anions showed good linearity over the calibration range with the common anions chloride and nitrate being the most abundant anions as would be expected.

Conclusions

A specific dicationic reagent formed adducts enabling detection of 32 of the 34 anions in the positive mode. For certain chaotropic anions (NO_3^-, BF_4^-, SCN^-, $BZSN^-$) absolute detection limits determined by ESI-MS were in the low picogram range, with PFOA at the femtogram level. Under gradient chromatographic conditions, PFOA adducted to the dicationic reagent gave approximately 30 times higher signal to noise ratios than it did alone in the negative ion mode. In fact, detecting the dication-anion adduct in the positive mode gave significantly better S/N and higher area counts than negative mode for four out of the five anions. From the determined detection limits, halogenated, oxidized, or other chaotropic anions not included here would also be expected to have low detection limits in the positive mode. Further information is needed about the characteristics of the dicationic reagent that affect adduct formation. This will be the subject of future studies.

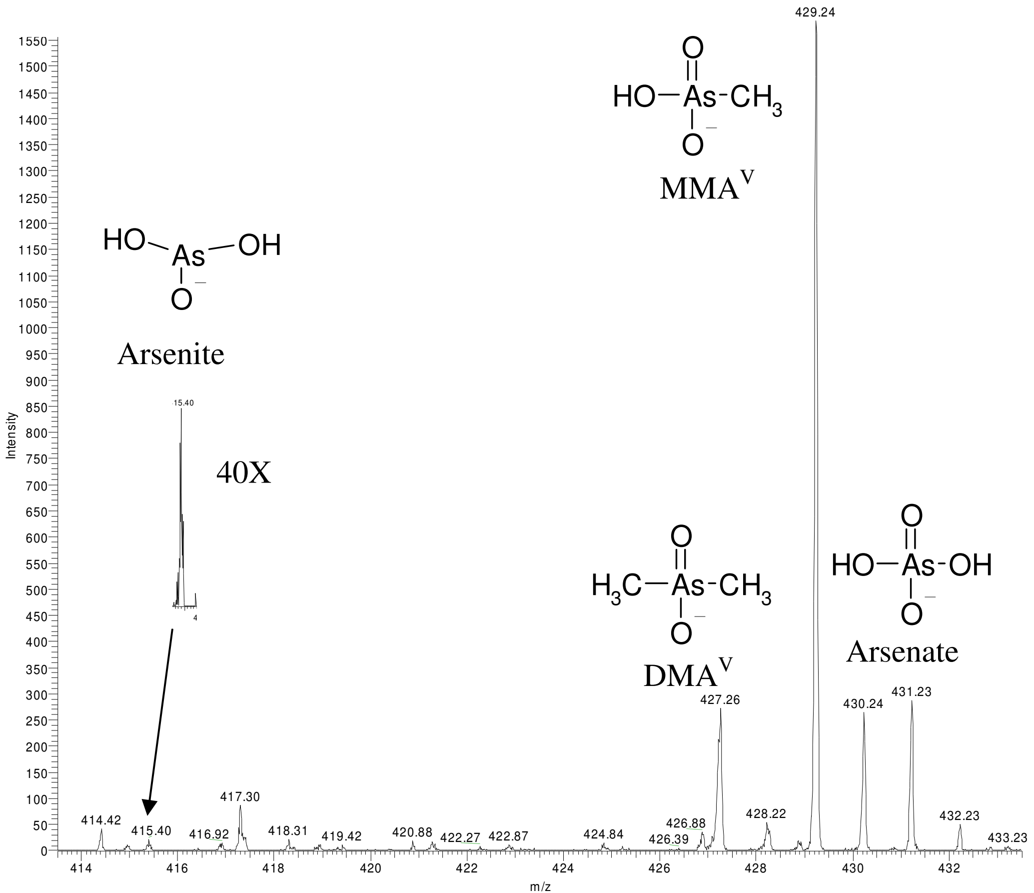

Figure 3: ESI-MS mass spectra of a water sample fortified with four arsenic species which are adducted with the dicationic reagent. MMA^V is monomethylarsonic acid and DMA^V is methylarsinic acid where the superscript V denotes the oxidation state of arsenic.

Table 2: Quantifiable Anions in Arlington Tap Water Sample

Water Analysis			
Anion	Concentration	Equation	R^2
Chloride	19.0 (± 3.6) ug/mL	y = 64956x + 100134	0.9913
Nitrate	574 (± 22) ng/mL	y = 268.46x - 2770.8	0.9972
MCA	49.0 (± 1.6) ng/mL	y = 85.778x + 5.84	0.9997
Bromide[1]	57.6 (±2.5) ng/mL	y = 398.23x + 13770	0.9994
BZSN	4.91(± 0.45) ng/mL	y = 78.103x + 281.44	0.9995

[1]:determined in SIM mode. All others determined in SRM mode and quantified separately.

Acknowledgements

The authors wish to thank Jeff Crank for synthesizing the dicationic reagent. The authors gratefully acknowledge the Robert A. Welch Foundation for its monetary support.

CHAPTER 3

EVALUATION OF DICATIONIC REAGENTS FOR THEIR USE IN DETECTION OF ANIONS USING POSITIVE ION MODE ESI-MS VIA GAS PHASE ION ASSOCIATION

Jeffrey W. Remsburg, Renee J. Soukup-Hein, Jeffrey A. Crank, Zachary S. Breitbach,
Tharanga Payagala, Daniel W. Armstrong

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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 perfluorooctanoate 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 twenty-three 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.

Introduction

Detection and quantitation of anions is of great importance 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 [72, 73], ion chromatography (IC) [74, 75], flow injection analysis (FIA) [76, 77] 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 [78]. 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 [11, 66, 79]. For positive mode analysis, an acidic additive commonly is 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 [11]. 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 [80]. Also, undesirable arcing is more prevalent in the negative ion mode. It has been suggested that halogenated solvents such as chloroform [16], hexafluoroisopropanol [81], and 2,2,2,-trifluororethanol [11] 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 [12] and halogenated solvents [14] have proven useful. While carefully choosing amongst the aformentioned 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 [5-8]. Most recently, it was shown to be advantageous for the analysis of over 30 different anions, proving its broad applicability and effectiveness [82]. 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 [82]. Only a small concentration of the 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 is severely hindered, it can be paired with a dication, thereby moving the detected m/z several hundred mass units higher, to a region of low background interference noise and few interfering peaks.

Apart from the original perchlorate study [5], 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 3) was consequently used for multi-anion study of Ref 82. 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 [2, 83-85]. Originally synthesized as ultra-stable ionic liquids [2], this

Table 3 – Structures and Masses of the Dications Used in this Study.

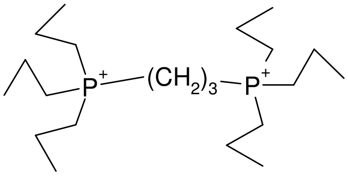
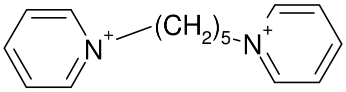
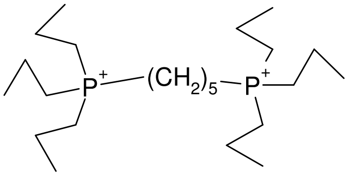
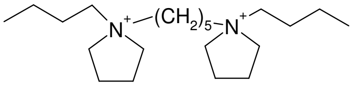
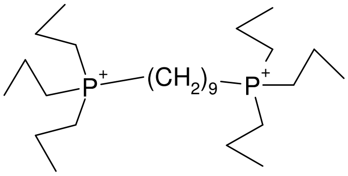
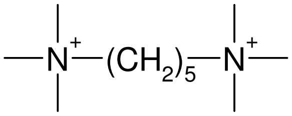
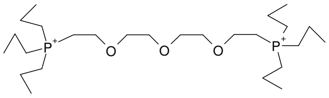
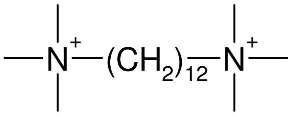
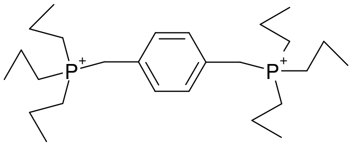
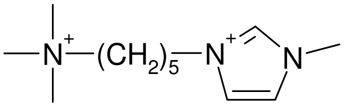
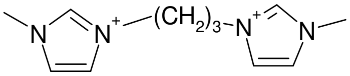
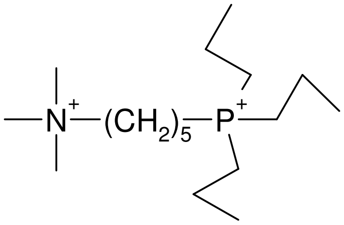
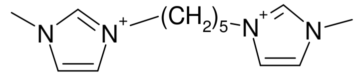
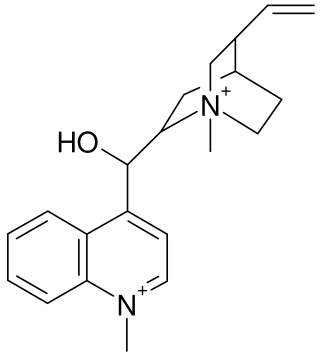
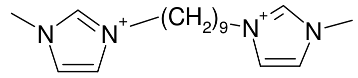
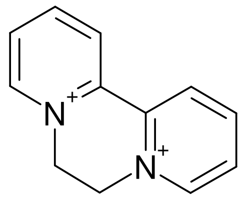
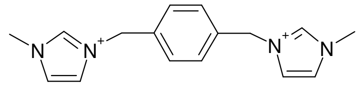
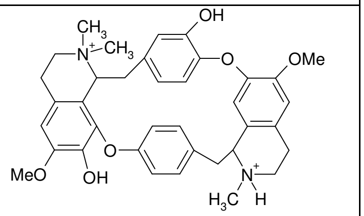
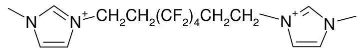
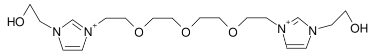
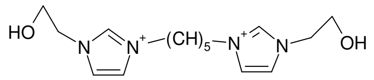
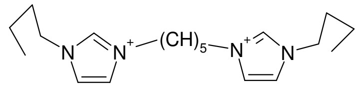
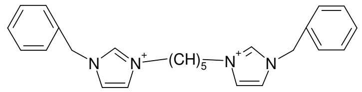
No.	Mass	Structure	No.	Mass	Structure
I	362.6		XV	228.1	
II	390.6		XVI	324.6	
III	446.6		XVII	188.4	
IV	480.6		XVIII	286.6	
V	424.8		XIX	211.2	
VI	206.3		XX	289.4	

Table 3 – *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				

research has led to the development of a wide array of dicationic compounds, including imidazolium [2, 83] and phosphonium [84] based dications, as well as those with differing linkage chains [83, 85] and even unsymmetrical species [85].

In this work, twenty three 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 in order 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 LC-ESI-MS analyses are done to illustrate the effect of using different dicationic reagents for anion analysis in the positive ion mode.

Experimental

Methanol and water were of HPLC grade and obtained from Burdick and Jackson (Morristown, MJ). Reagent grade sodium hydroxide and sodium fluoride were from Fisher Scientific. 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 3 was synthesized by dissolving one molar equivalent of 1,5-dibromopropane in isopropanol. To this solution, 3 molar equivalents of tripropylphosphine were added. The resulting mixture was stirred and heated to reflux for 48 hours. The solution was then cooled to room temperature and the solvent was removed by roto-evaporation. 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 roto-evaporation, 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 molar equivalent of (5-bromopentyl)-trimethylammonium bromide in isopropyl alcohol with 3 molar 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 molar equivalents of phosphorus tribromide. The reaction was then refluxed for 2hrs. Next, the reaction mixture was poured over ice to react the excess PBr_3. 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 over night 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° C. Four molar equivalents of methyl iodide were then added to the mixture and allowed to react for 48 hours. 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 anion exchange procedure is given in Ref. 5.

For direct injection analysis, a 40 μM dication-fluoride (DF_2) solution was directed into a Y-type mixing tee at 100 μL/min via a Shimadzu LC-6A pump. 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 Fischer Scientific, San Jose, CA). After the mixing tee, the final conditions were then 50/50 water/methanol with 10 μM DF_2 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: 3kV, capillary temperature: 350°C, capillary voltage: 11 V, tube lens voltage: 105 V, sheath gas: 37 arbitrary units (AU), auxiliary gas: 6 AU. For the negative ion mode analysis, voltage polarities are 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°C, capillary voltage: 35 V, tube lens voltage: 80 V, sheath gas: 25 AU, 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 10cm C-18 (3 μm particle size) obtained from Advanced Separations Technology (Whippany, NJ). In the chromatograph of the multi-anion sample used for Figure 1, the column was equilibrated with 100% water at 300 μL/min. At one minute, a linear gradient to 100% methanol began and was completed at three minutes. The addition of the DF_2 solution was done post-column at 100 μL/min via the mixing tee. For the chromatographs of the benzenesulfonate samples (Figure 3), the mobile phase consisted of 100% water at 300 μL/min for the entire analysis. To help with spray formation, the DF_2 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 DF_2 in methanol solution. The MS was again operated in SIM mode, monitoring the m/z values 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 was used for data collection and analysis.

The experimental parameters described above were adopted from reference 82. The authors strongly recommend further optimization when using a specific dication 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.

Results and Discussion

Table 3 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 4 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 4). The test anions were selected from to provide a cross-section of ions having different sizes and functionalities [82]. 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 [86]. Perfluorooctanoic acid (PFOA), a large, anionic fluorocarbon, is unlike any of the other anions. This, along with recent research interest in this

Table 4 – Absolute 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 4 – *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) as an environmental contaminant make it a good choice for inclusion in this study [87, 88].

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 alkyl-ammonium 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 (**VI-VIII**), 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 [89, 90].

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 phophonium based dication, a pyrrolidinium based dication, and two imidazolium based dications. Each of these has a different optimum hydrocarbon linkage chain length. It

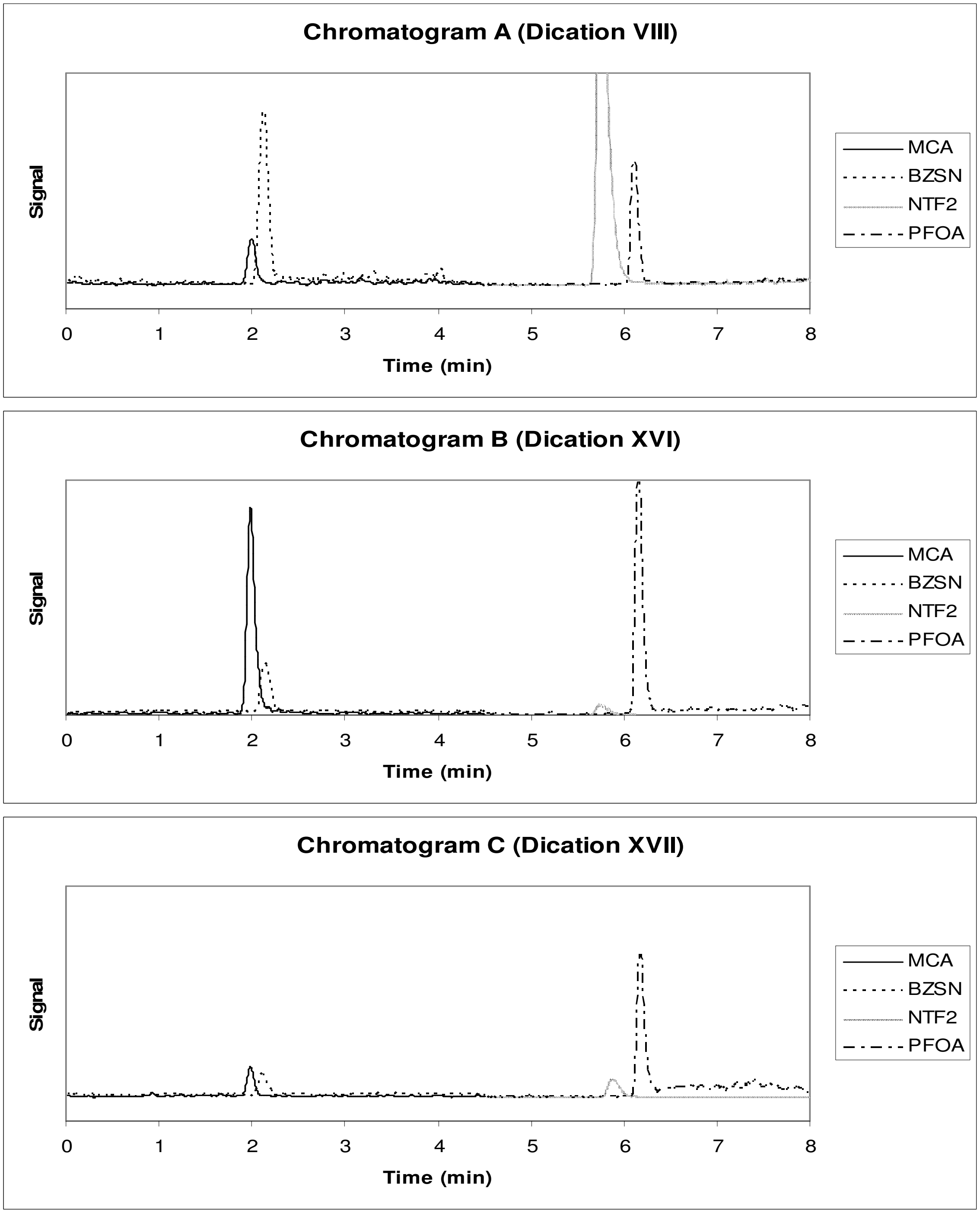


Figure 4 – Three separate chromatograms showing the separation of a sample containing four anions (150 ng/mL MCA, 50 ng/mL BZSN, 500 ng/mL NTF_2 and 75 ng/mL PFOA). The masses monitored are the sum of the mass of each anion and the mass of the corresponding dicationic reagent. Chromatograms A and B use recommended dications (**VIII** and **XVI**), while chromatogram C does not (**XVII**).

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.

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.

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 4). Two of the recommended dications are used (**VIII,** Figure 4A and **XVI,** Figure 4B), as well as a moderately successful but not recommended dication **XVII** (Figure 4C). 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 4) would produce peaks that could not be discerned under the conditions of Figure 4. Also apparent in Figure 4 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 Figure 5. The dication used in this instance is compound **XIV**. Several discernable fragments can be seen in the background even without excitation. The main fragments include the peaks at 227.3 (loss of benzyl imidazolium), 295.3 (loss of $[CH_2\text{-}C_6H_5]^+$), 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 6, 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

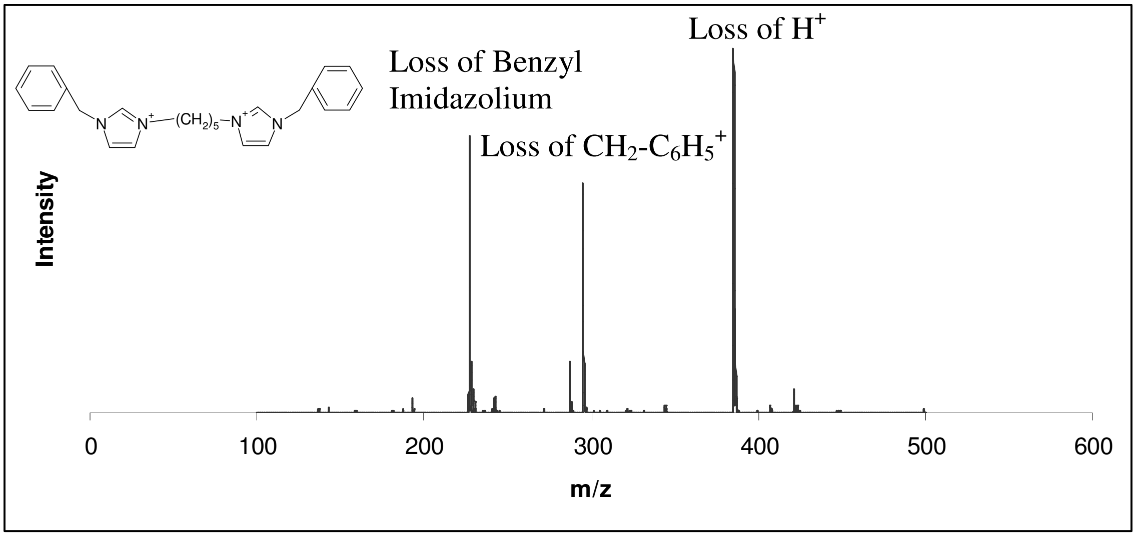


Figure 5 – Mass spectrum of the mobile phase containing the dicationic reagent under typical operating settings for chromatography. Notice the three most prominent peaks are actually fragments of the dicationic reagent (**XIV**). These fragments can be monitored after excitation of a dication-anion complex to typically lower detection limits.

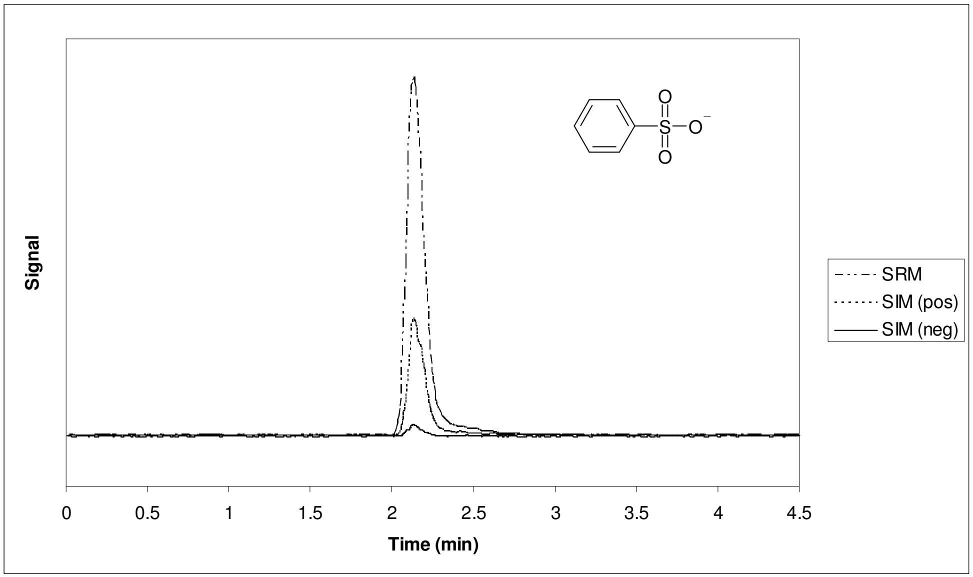


Figure 6 – Overlapping chromatograms of three separate injections of a 100 ng/mL sample of benzene sulfonate. The solid line represents the use of negative mode, monitoring the mass of the anion (methanol being added post column). When 40 µM of dication **XIV** in methanol is added post column, the mass of the dication-anion complex can be monitored and gives a significant increase in S/N (dotted line). Finally, when single reaction monitoring is used, an even further increase in S/N can be seen, as shown by the alternating line.

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.

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.

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CHAPTER 4

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

Renee J. Soukup-Hein, Jeffrey W. Remsburg, Zachary S. Breitbach, Pritesh S. Sharma, Tharanga Payagala, Eranda Wanigasekara, and Daniel W. Armstrong

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.

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 [42-43, 59, 62, 91] and spectrophotometric techniques [54-55] 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 [92]. Mass spectrometry is growing in popularity as a universal detector for anions and it can be used alone [19, 52] or in combination with a separation method [21, 29, 34, 92-93].

The negative ion mode is the most common way of detecting anions using ESI-MS. 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 [11, 80]. These effects can be suppressed by using electron scavenging gases [12] or halogenated solvents [14, 16, 80-81]. The substitution of isopropanol or butanol [66] 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 anions in the positive mode by ESI-MS [5, 82]. 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 [6], milk [7], and seawater [8]. 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 [94]. Thus, the goal of this note is two fold: (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.

Experimental

Tricationic Reagents

Figure 7 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 [5, 82] with some modifications. 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

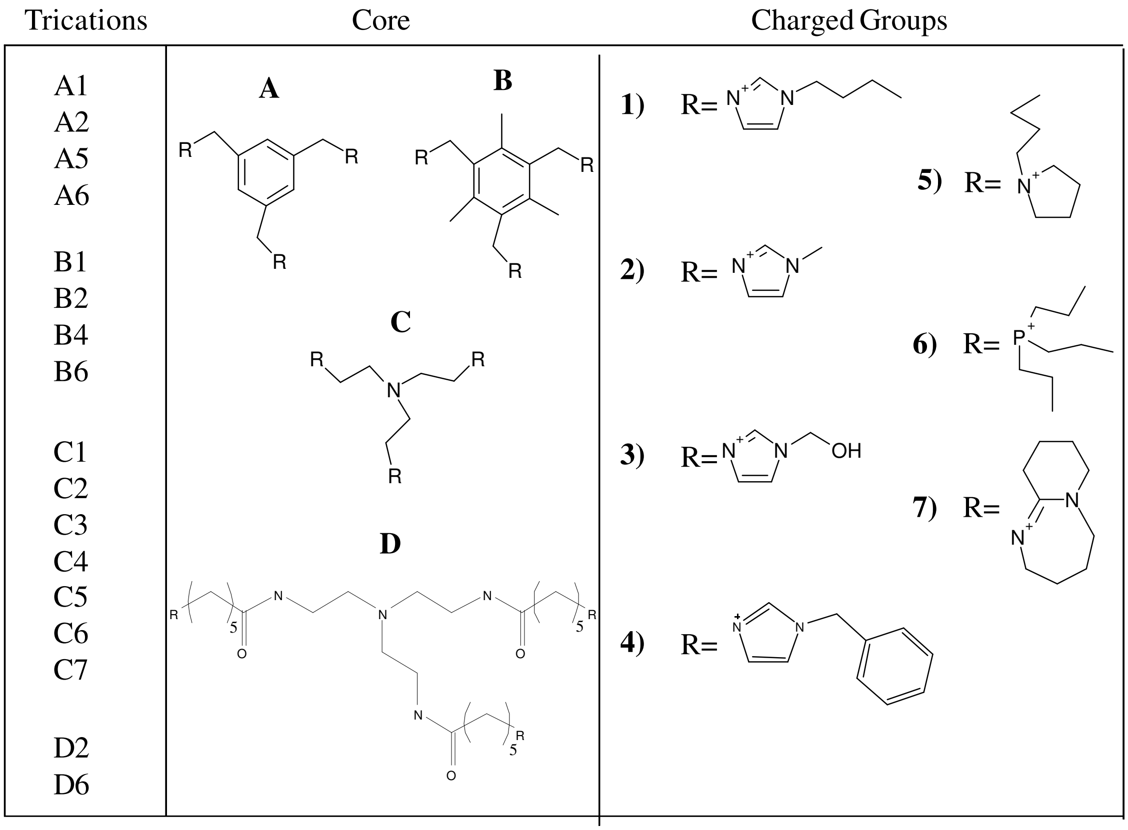

Figure 7: Structure of the tricationic reagents

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.

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.

Results and Discussion

Eleven divalent anions were used to evaluate seventeen different tricationic reagents (see Table 5). 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.7). **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 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 5 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)) methyl-2,4,6-trimethylbenzene trifluoride) also does well, but is in the top three less consistently than **A6**. Table 5 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.7). 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

Table 5: Detection limits of doubly charged anions with tricationic reagents

Sulfate	
Trication	LOD (ng)
B1	1.00E-01
B4	1.00E-01
A5	1.00E-01
C3	1.25E-01
D6	1.50E-01
C4	2.50E-01
B2	2.50E-01
A1	5.00E-01
A6	5.00E-01
A2	6.25E-01
D2	7.00E-01
C2	7.50E-01
C1	8.75E-01
B6	1.50E+00
C5	1.88E+00
C6	2.38E+00
C7	2.75E+00

Dichromate	
Trication	LOD (ng)
B1	4.58E-01
B4	2.00E+00
A6	1.00E+01
C4	1.00E+01
B2	1.00E+01
A1	1.02E+01
A2	1.25E+01
B6	1.49E+01
C2	1.73E+01
C1	1.75E+01
C3	2.00E+01
C5	2.50E+01
C6	4.50E+01
D6	4.88E+01
C7	4.96E+01
A5	1.75E+02
D2	2.50E+02

Oxalate	
Trication	LOD (ng)
C6	1.50E-02
A1	4.00E-02
B1	4.00E-02
B6	2.34E-01
A6	2.50E-01
C1	3.43E-01
C3	3.75E-01
C4	4.35E-01
A2	4.99E-01
A5	5.00E-01
B2	5.00E-01
C2	7.18E-01
B4	7.50E-01
D6	8.75E-01
C5	1.00E+00
D2	1.50E+00
C7	4.28E+00

Thiosulfate	
Trication	LOD (ng)
A6	1.25E-01
C1	1.25E-01
B2	1.50E-01
C5	1.53E-01
B4	1.61E-01
C4	2.00E-01
B1	2.41E-01
B6	2.60E-01
C6	4.50E-01
C3	4.99E-01
A2	7.50E-01
C2	7.80E-01
A1	1.00E+00
D2	1.38E+00
A5	2.14E+00
C7	5.20E+00
D6	1.50E+01

Bromosuccinate	
Trication	LOD (ng)
A6	7.50E-02
B6	4.99E-01
C3	5.00E-01
D6	5.00E-01
C6	7.50E-01
A5	1.50E+00
C5	1.63E+00
A2	4.99E+00
C1	5.00E+00
B2	5.00E+00
C2	7.00E+00
A1	7.50E+00
C4	8.75E+00
B4	1.00E+01
D2	1.25E+01
B1	1.75E+01
C7	4.50E+01

o-benzenedisulfonate	
Trication	LOD (ng)
A6	1.50E-02
C1	2.25E-02
B1	2.50E-02
B4	2.50E-02
C4	3.00E-02
C6	3.75E-02
A1	5.00E-02
C2	5.00E-02
B6	5.00E-02
C5	5.00E-02
A2	7.50E-02
C3	1.25E-01
D6	1.50E-01
A5	2.00E-01
C7	3.75E-01
B2	1.13E+00
D2	1.75E+00

Nitroprusside	
Trication	LOD (ng)
B4	3.22E-03
A6	7.50E-03
B1	8.55E-03
B6	1.38E-02
C4	2.00E-02
C1	2.73E-02
C5	2.73E-02
C3	4.25E-02
A1	4.29E-02
C2	4.42E-02
A2	4.86E-02
C7	6.00E-02
B2	1.00E-01
D6	1.25E-01
C6	2.00E-01
A5	3.15E-01
D2	8.75E-01

Hexachloroplatinate	
Trication	LOD (ng)
B4	2.60E-02
B1	3.90E-02
A6	7.50E-02
C1	1.00E-01
A1	1.30E-01
B6	1.58E-01
B2	2.00E-01
C4	2.50E-01
D6	5.00E-01
C5	8.75E-01
C3	1.00E+00
C2	1.05E+00
A2	1.58E+00
C7	1.58E+00
C6	2.00E+00
D2	2.13E+00
A5	2.25E+00

Table 5 – *Continued*

Dibromosuccinate		Fluorophosphate		Selenate	
Trication	LOD (ng)	Trication	LOD (ng)	Trication	LOD (ng)
B6	1.50E-01	A6	3.75E-02	A6	7.50E-02
A6	1.79E-01	D6	7.50E-02	B4	2.25E-01
C4	3.75E-01	B6	1.25E-01	B1	2.80E-01
C2	5.00E-01	D2	1.25E-01	C4	3.50E-01
C1	5.00E-01	B2	1.50E-01	C6	3.75E-01
C3	5.00E-01	C3	2.00E-01	B6	3.92E-01
C6	5.00E-01	B1	2.49E-01	D6	4.25E-01
C5	1.13E+00	B4	2.49E-01	C1	5.00E-01
B1	1.25E+00	C6	2.50E-01	C7	5.60E-01
B4	1.50E+00	C4	2.75E-01	C3	7.50E-01
C7	1.50E+00	C7	2.82E-01	D2	7.50E-01
D6	1.50E+00	C1	4.25E-01	C2	1.12E+00
A1	3.00E+00	C5	5.00E-01	A2	1.13E+00
A5	5.00E+00	A5	7.50E-01	B2	1.38E+00
A2	5.00E+00	C2	1.00E+00	A5	2.50E+00
B2	5.00E+00	A1	4.99E+01	A1	4.00E+00
D2	5.00E+00	A2	5.00E+01	C5	1.75E+01

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 [94]. 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 [94]. 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

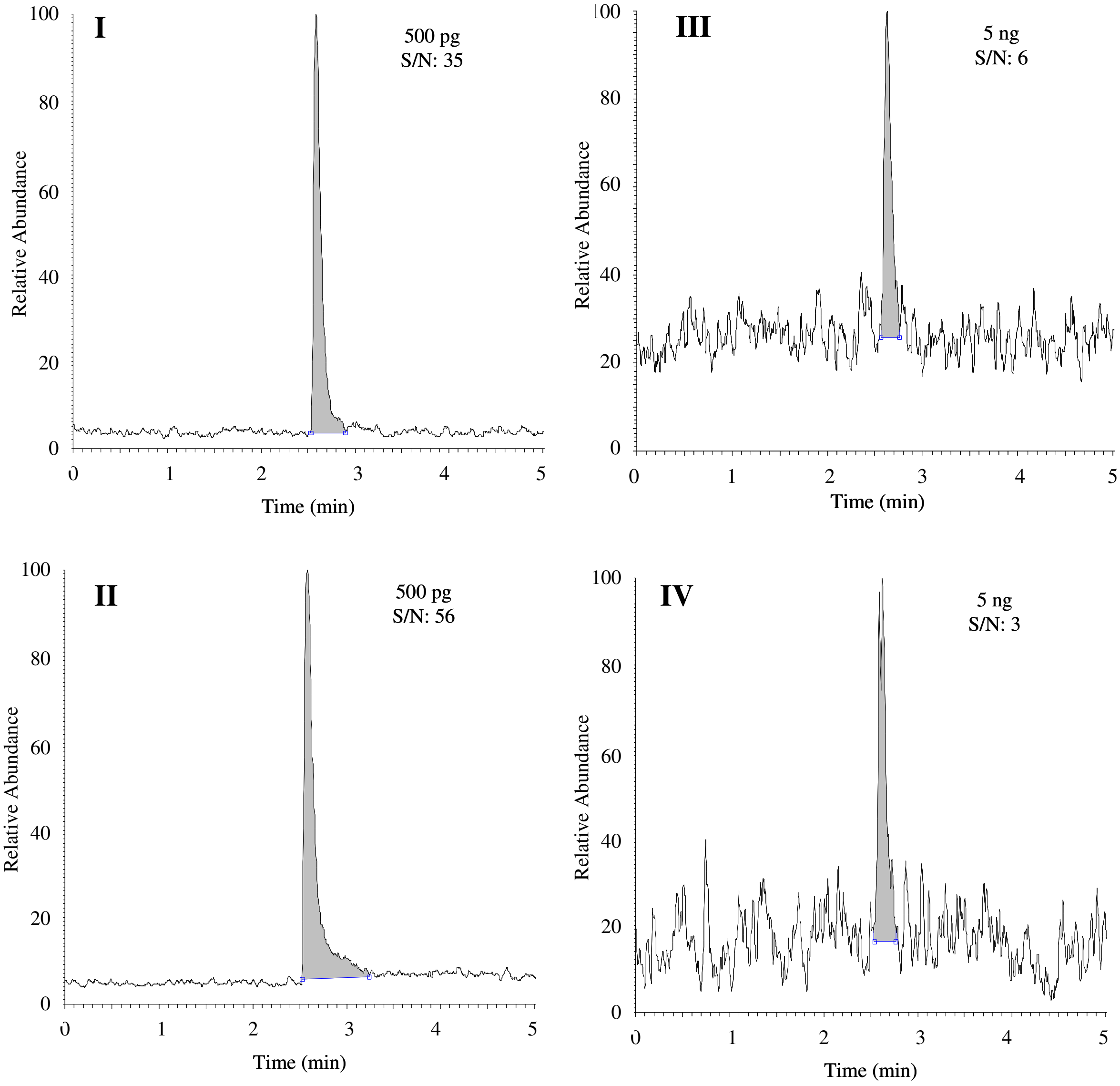

Figure 8: A comparison of positive (I, II) and negative modes (III, IV) for hexachloroplatinate (I, III) and o-benzenedisulfonate (II,IV). Tricationic reagents A6 (I) and B1 (II) in water were introduced into the carrier flow after anion injection.in positive ion mode while only water was used in negative ion mode (III, IV).

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 8 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.

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 o-benzenedisulfonate compared to negative mode even though ten times more sample was injected in the negative ion mode.

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CHAPTER 5

CONCLUSION

The studies described in this thesis demonstrate that by placing a multiply-charged cationic reagent in the carrier stream, an anion of lesser charge can be detected as a positively charged complex when using electrospray ionization mass spectrometry. Using this approach eliminates the need to use negative ion mode ESI-MS, which is significantly more prone to stability and sensitivity issues.

Detecting a wide variety of anions can be easily accomplished by employing this method. Using a single dicationic reagent, 32 different anions could be detected, most at sub-nanogram levels. Detection of several species was as sensitive as or better than other analytical techniques. Halogenated, oxidized, and other chaotropic anions were found to have the highest sensitivities. An ability to perform MS/MS experiments was demonstrated and generally provided increased sensitivity. Direct comparisons of the negative ion mode and positive ion mode showed that by using a dicationic reagent, significant increases in S/N could be seen when using the positive ion mode, even when less sample was injected [82].

Evaluation of 23 different dicationic reagents showed that significant differences arise when using a different dicationic reagent to detect anions. Differences in charged moieties, linker chain length, and linker chain type were studied and discussed. The empirical evidence shows that flexibility seems to be an important trait in a successful pairing reagent. A few dicationic reagents were found to have the most promising results and are recommended for using this method. It was also shown that the imidazolium based dications are best suited to analysis when MS/MS experiments are to be performed [94].

Finally, tricationic reagents were also shown to be advantageous in detecting doubly charged anions compared to using the negative ion mode. Differences in “core” structure and charged moieties were studied to find optimal tricationic reagents. As was the case with detecting singly charged anions, the larger, chaotropic doubly charged anions achieved the lowest detection limits [95].

Cumulatively, the studies in this thesis provide insight into using multiple charged cationic reagents to achieve increased sensitivity when using ESI-MS for anion analysis. Although this approach is relatively new, it has already been shown to be superior to the negative ion mode and shows much promise for future research. By extending the information found in these studies to other anions, increased sensitivity and thus, lower detection limits could be achieved and are expected. Ion mobility studies may also provide more information as to how the reagents and negatively charged species interact in the gas phase [89, 90]. Finally, the use of ESI-MS experiments have shown to be useful in studying noncovalent complexes and could give further insight into the considerable differences in sensitivity seen while using different cationic pairing agents [96-99].

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BIOGRAPHICAL INFORMATION

Jeffrey William Remsburg obtained his Bachelor of Science degree from Eastern Kentucky University majoring in Forensic Science. He then went on to study under Dr. Daniel Armstrong at Iowa State University and the University of Texas-Arlington. He earned his Master's of Science in December 2007 working on research in areas such as enantiomeric separations, liquid chromatography, and electrospray ionization mass spectrometry.