Synthesis of new classes of ionic liquids and polymeric ionic liquids and their applications in microextraction techniques

Manishkumar Dilipkumar Joshi
University of Toledo

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A Dissertation

entitled

Synthesis of New Classes of Ionic Liquids and Polymeric Ionic Liquids and their Applications in Microextraction Techniques

by

Manishkumar Dilipkumar Joshi

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Chemistry

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August 2013
An Abstract of

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Ionic liquids (ILs) are nonmolecular solvents that consist of organic cations and organic/inorganic anions. The chemical and physical properties of ILs can be tuned conveniently by changing the combination of cations and anions or tailoring the structure of cations. Recently, ILs has emerged as a novel media for various microextraction techniques. Three different applications involving ILs and polymeric ionic liquids (PILs) in the extraction and quantification of different analytes will be discussed.

In the first application, the extraction of boric acid/borate from water was achieved using glucaminium-based ILs. The synthesis of glucaminium-based ILs will be discussed. \(^{11}\)B NMR was used as a tool to confirm the complexation between glucaminium ILs and boric acid/borate. The effect of glucaminium IL concentration and pH on complex formation was studied. The application of in situ dispersive liquid-liquid microextraction for boric acid/borate extraction from water was successfully demonstrated. A comprehensive binding study of the glucaminium IL-borate complex was also performed using \(^{11}\)B NMR. Regeneration of the glucaminium-based IL will be discussed.

The compound 2-aminopyrimidine-5-ylboronic acid (2-APYBA) may be present
as an impurity in 2-aminopyrimidine-5-pinacolboronate ester (2-APPBE), a popular reagent used within the pharmaceutical industry in the Suzuki-Miyuara coupling reaction. The poor solubility of 2-APYBA in water and organic solvents poses a challenge for the separation and determination of 2-APYBA within the 2-APPBE reagent. This challenge was successfully addressed using two newly designed glucaminium-based ILs and separating the formed complex using reversed phase high performance liquid chromatography. The complexation between the two new glucaminium-based ILs and 2-APYBA was confirmed using $^{11}$B NMR. The effect of temperature and IL concentration on the complex was also studied. The method was successfully employed for the quantification of 2-APYBA in a real 2-APPBE sample used within the pharmaceutical industry.

Lastly, the use of PILs as sorbent coatings in solid-phase microextraction (SPME) for the analysis of polychlorinated biphenyls (PCBs) present in water and bovine milk will be discussed. The effect of different parameters such as extraction temperature, extraction time and salt concentration on the extraction of PCBs was studied. The analytical performance of the PIL-based SPME fibers was compared to the commercial polydimethylsiloxane (PDMS) fiber. A side-by-side comparison of detection limits was carried out using gas chromatography employing two detectors, namely, electron capture and mass spectrometry. The applicability of the PIL-based SPME method was evaluated in the analysis of two real sample matrixes, namely, ocean water and bovine milk.
This dissertation is dedicated to my parents: Dilip Joshi and Panna Joshi for their love, support and encouragement.
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Chapter 1

Overview: Ionic liquids in microextraction techniques

1.1 Ionic liquids (ILs):

ILs are entirely composed of organic cations and inorganic/organic anions. ILs are salts with melting point below 100 °C. ILs possessing melting points below 25 °C are known as room temperature ionic liquids (RTILs). The liquid nature of ILs or RTILs is believed to be due to weak interactions existing between the organic cation and anion. The history regarding the development of ILs stretches back to more than a century. The first IL, ethanolammonium nitrate, was developed by Gabriel and Weiner in 1888. After more than two decades, the first RTIL, ethylammonium nitrate was reported by Walden. The research regarding development of new ILs did not gain momentum for almost seven decades until Wilkes and co-workers introduced imidazolium-based RTILs with chloroaluminate anion. However, these RTILs were highly vulnerable to air and water due to the hydrolyzing nature of the chloroaluminate anion. This challenge was addressed successfully by Wilkes and co-workers by synthesizing first air and water stable pyridinium/imidazolium-based ILs containing acetate or trifluoroborate anion. This was the starting point of an era where ILs were recognized as a novel material possessing
endless opportunities to tailor the structure and eventually the physicochemical properties.

ILs possess fascinating physical and chemical properties such as higher thermal stability, negligible vapor pressure, tunable polarity, tunable viscosity, and wide liquid range. These properties are largely dependent on the combination of cations and anions comprising the IL. It has been reported that there are more than $10^{18}$ ILs possible based on different e of cation and anion. The most commonly used cations are imidazolium, pyridinium, phosphonium and ammonium, whereas common anion includes halides, hexafluorophosphate ($\text{PF}_6^-$), tetrafluoroborate ($\text{BF}_4^-$), and bis[(trifluoromethyl)sulfonyl]imide (NTf$_2^-$). The structures for common cations and anions are shown in Figure 1-1.

![Figure 1-1: Structures of common cations and anions comprising ionic liquids.](image-url)
The physical properties of ILs are highly dependent on the specific combination of cation and anion as well. The properties of ILs can be tuned conveniently by changing the combination of cation and anion or functionalizing them. For example, 1-butyl-3-methylimidazolium chloride (BMIM-Cl) IL is vulnerable to heat and tend to degrade if temperature is higher than 150 °C. The thermal stability increases significantly when chloride anion is replaced with NTf$_2^-$ anion. Similarly, dicationic ILs based on imidazolium cation are thermally more stable when compared to monocationic ILs, as described by Anderson and co-workers. ILs possessing halide anion are generally very hydrophilic and can be turned to be hydrophobic when anion is replaced with NTf$_2^-$ or PF$_6^-$. Due to highly tunable nature of ILs, they are widely applied in different fields such as synthesis, catalysis, chromatography, separation science, and mass spectrometry. ILs can undergo multiple interactions such as electrostatic, dispersive, π-π and, hydrogen bonding with different molecules making them ideal as a solvent, extraction media, ion pairing reagent and, stationary phase in chromatography. Due to the negligible vapor pressure and higher solvation capability, ILs have became popular as "Green solvents". However, extensive study for measuring the toxicity of ILs is highly desirable.

### 1.2 ILs as an extraction media:

Pre-concentration of analytes poses a great challenge and most of the time mandatory for extraction or detection of the analyte. Liquid-liquid extraction (LLE) is one of the most widely used conventional techniques for extraction. However, LLE is
often inefficient, lacks selectivity and requires large volume of extractant phase which makes it environmentally unfriendly. ILs have emerged as a novel media for the extraction due to the ability to solvate various analytes including organic species, metal ions, and biomolecules. Huddleston and co-workers performed a detailed study involving the partitioning of various analytes such as acids, bases, alcohols and benzene derivatives in [BMIM] [PF$_6$] IL.$^{11}$ Fadeev and co-workers investigated the extraction of butanol from fermentation broth using 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM] [PF$_6$]) IL.$^{12}$ Die and co-workers demonstrated the extraction of strontium ion using RTIL where crown ether was used as an extractant.$^{13}$ In another study, Visser and co-workers demonstrated the ability of urea and thiourea functionalized imidazolium-based ILs to extract various metal ions such as Hg$^{2+}$ and Cd$^{2+}$ from an aqueous phase.$^{14}$ The extraction of size and shape specific gold nanoparticle and gold nanorods was demonstrated by Wei and co-workers.$^{15}$

1.3. **IL-based Dispersive liquid-liquid microextraction (IL-DLLME):**

DLLME was introduced by Rezaee and co-workers in 2006.$^{16}$ This new method is similar to LPME except it includes a third component referenced to as the dispersive solvent. The detailed schematic of the DLLME method is described in Figure 3-2. Briefly, the extractive solvent was dissolved in a small quantity of dispersive solvent which are generally acetone, methanol or acetonitrile. This solution was then introduced to the aqueous phase. The presence of the dispersive solvent helps the generation of fine droplets of extractive solvent and eventually increases the surface area significantly. A
cloudy solution appears and is subjected to centrifugation. The analyte is pre-concentrated due to the selective partition to the extractant solvent phase. There are different parameters affecting the extraction efficiency of DLLME including the volume of dispersive solvent and volume of extracting solvent. It is very important that the dispersive solvent dissolves in both the extracting solvent and aqueous matrix. Commonly used extractive solvents are generally halogenated solvents such as chloroform and carbon tetrachloride. These organic solvents are often volatile and pose severe health hazards. Also, limitation of using halogenated nonpolar solvent can limit the type of analyte that can be extracted. To increase the extraction efficiency as well as versatility of the extraction, an alternative solvent are highly desirable.

ILs are known as green solvents as they have negligible vapor pressure. ILs can also be conveniently tuned to undergo multiple solvation interactions with analyte of interest and be hydrophobic enough to form separate layer from an aqueous phase. Due to the aforementioned advantages, ILs were found to be suitable alternative as an extraction solvent for DLLME. The use of 1-hexyl-3-methylimidazolium hexafluorophosphate ([C₆MIM] [PF₆]) IL as extraction solvent was demonstrated by Zhou and co-workers for the extraction of pyrethroid pesticides from water. However, due to the hydrophobic nature of the [C₆MIM] [PF₆] IL, heat was applied and IL phase was collected after cooling. Instead of heat, ultrasonication was also applied to IL-based DLLME. The need for heat and ultrasonication make the above mentioned IL-based DLLME relatively time consuming and less energy efficient. Therefore, Liu and co-workers came up with
the same approach as DLLME and used organic solvent as a disperser solvent to dissolve the IL.\textsuperscript{19} 

Baghdadi and Shemirani demonstrated the \textit{in situ} solvent formation microextraction method where 1-hexyl-3-methylimidazolium tetrafluoroborate IL was dissolved in the water.\textsuperscript{20} The extraction of mercury metal ion was achieved by addition of sodium hexafluorophosphate salt as an ion pairing reagent. A similar approach was introduced by Yao and co-workers using \textit{in situ} dispersive liquid-liquid microextraction (\textit{in situ} DLLME) in 2009.\textsuperscript{21} In this work, the hydrophilic BMIM-Cl IL (the extraction solvent) was dissolved in an aqueous matrix. The metathesis salt LiNTf$_2$ was dissolved in water and added to the IL as well as analyte containing aqueous phase. The anion chloride was exchanged with NTf$_2^-$ anion through \textit{in situ} metathesis and analyte was pre-concentrated to the [BMIM][NTf$_2$] IL phase. The enrichment factor defined as the ratio of analyte concentration in IL phase and analyte concentration in aqueous phase was calculated. Higher enrichment factors were achieved using \textit{in situ} DLLME when compared to conventional DLLME method where organic solvent was used to disperse IL into an aqueous phase. The successful application of \textit{in situ} DLLME was demonstrated by Joshi and co-workers for the extraction of boron species from water using glucaminium-based ILs.\textsuperscript{22} Li and co-workers demonstrated the extraction of DNA from an aqueous matrix using \textit{in situ} DLLME.\textsuperscript{23} The main advantage of \textit{in situ} DLLME is that the extraction and metathesis steps are simultaneous which results in increased extraction efficiency and decreased extraction time. Moreover, the use of organic solvent
as a disperser solvent can be avoided and make *in situ* DLLME environmentally benign method.

1.4. **Solid-phase microextraction:**

Solid-phase microextraction (SPME) was introduced by Pawliszyn and co-workers in early 1990s. SPME is very different than conventional solid phase extraction (SPE) method. In SPE the matrix containing analyte of interest passes through the sorbent bed for exhaustive extraction. It is followed by back extraction of analyte from the sorbent bed using organic solvent. SPE is relatively time consuming due to separate extraction and pre-concentration step and suffers from limited lifetime of the sorbent bed. In contrast, SPME is a nonexhaustive method combines sample preparation and pre-concentration in a single step. SPME has become very popular since its introduction due to the fact that it is simple, robust, inexpensive, rapid, solvent free and compatible to automation.

The SPME device consists of syringe containing fused silica capillary. The outer surface of the capillary contains a coating of sorbent material which can be solid or liquid. The film thickness of the sorbent coating is in the sub micron range and generally varies coating to coating. This fiber can be exposed to the matrix or the headspace for the extraction of analyte of interest. It is followed by thermal desorption of analytes from the sorbent coating to the gas chromatography (GC) inlet for the analysis. Two different modes of SPME, namely, direct immersion mode and headspace mode are shown in Figure 1-2. In direct immersion mode, the SPME fiber is dipped into the matrix. The
matrix is stirred using stir bar and analyte will partition to the sorbent coating from the matrix. Contrarily, in HS-SPME the fiber is exposed to the headspace and analytes partition between headspace and sorbent coating as well as matrix and headspace. Generally, for volatile analytes including acid, esters, alcohols, HS-SPME is preferred where DI-SPME is generally used for analytes which are less volatile and partition insignificantly to the headspace.
1.5. IL-based SPME:

The types of sorbent coatings available commercially for SPME are very limited. To increase the sensitivity of the method, tailoring the sorbent coating is required. Recently, ILs have emerged as a very promising alternative for the development of new sorbent
coatings. ILs can undergo multiple interaction with analyte based on the tailored structure of ILs and increase the sensitivity of the method significantly. In 2005, Liu and co-workers introduced IL-based disposal sorbent coating for the extraction of BTEX (benzene, toluene, ethylbenzene, and xylenes) in a paint using [OMIM] [PF₆] IL. However, the IL coating was removed after every extraction as it was physically coated to the fiber. Higher limits of detection (LOD) were achieved using IL sorbent coating compared to polydimethylsiloxane (PDMS) commercial coating. This gave momentum to find a way to stabilize the IL coating on to the fused silica fiber. In order to achieve stable IL sorbent coating Hsieh and co-workers pretreated the fused silica capillary using nafion. Detection of trace level of polyaromatic hydrocarbons were achieved in the water matrix. Even film of IL on to the surface of the fiber was achieved due to the nafion coating. However, the fiber still required to be changed after every extraction. The basic problem was associated with the removal of physically coated IL sorbent coating at elevated desorption temperature. This was justified the fact that density of IL decreases at higher temperature and IL flowed off the fiber.

Despite the fact that ILs proved to be more efficient coating for extraction, there was a bottleneck of IL flowing off the fiber and in some cases getting into the GC liner. There was a huge need for the generation of IL coating which can sustain elevated temperatures and significantly increase the fiber life time. In order to address this challenge, our group introduced polymeric ionic liquid (PIL)-based coatings as a novel sorbent material. The bulk synthesis of PIL was achieved using free radical polymerization method using AIBN as an initiator. This PIL was then dissolved in an organic solvent and fiber was dip
coated. Good film thicknesses (~12 µm) for PIL sorbent coatings were achieved. This approach allowed the good extraction efficiency as well as good reproducibility. Different PILs were designed based on the analyte of interest. The thermal stability of PIL-based sorbent coating was further enhanced when NTf₂⁻ anion was introduced to the PIL. The selectivity towards polar analyte was higher for PIL sorbent coating containing Cl⁻ anion due to the hydrogen bonding capability.²⁸

In order to further enhance the thermal stability and robustness of the PIL-based sorbent coatings generation of crosslinked PIL-based coating was highly desirable. The previous approach, involving bulk polymerization using AIBN, has certain limitations. The generation of crosslinked PIL-based sorbent coating was not possible as crosslinked PIL possess very limited or no solubility in most of the organic solvents. In order to address this challenge, an "on-fiber polymerization" approach was recently introduced by our group.²⁹ This approach involves preparation of a solution containing IL monomer, IL crosslinker and photo initiator in appropriate amount. The percentage of IL crosslinker can be varied. The fiber was dip coated using the above mentioned solution. This was followed by subjecting the fiber to UV (360 nm) exposure for half an hour to generate PIL-based sorbent coating. This approach is simple, required very small amount of coating material and because of crosslinked PIL-based coating the thermal stability was improved. This approach is used in the following work where polychlorinated biphenyls were extracted from ocean water and bovine milk, as shown in Chapter 5.
References:


Chapter 2

Synthesis of glucaminium-based ionic liquids and their application in the removal of boron species from water

A paper published in *Chemical Communications*\(^1\)

Manishkumar D. Joshi, Guillaume Chalumot, Yong-wah Kim, Jared L. Anderson

**Abstract**

A novel class of ionic liquids (ILs), exhibiting high selectivity towards boron species as well as the ability to phase separate from water, were synthesized from N-methyl-D-glucamine. The complexation of boric acid/borate with the ILs was confirmed using \(^{11}\)B NMR.

**2.1. Introduction**

The presence of boron (in the form of boric acid and borate) in water is a growing concern for the health of plants, animals, and humans. Although boron is a micronutrient necessary for plant growth, higher levels of boron species in water can adversely affect

\(^1\) Reprinted from *Chemical Communications*, 2012, 48, 1410-1412. Copyright © The Royal Society of Chemistry 2012
the crop by lowering the crop yield and eventually result in plant death.\(^1\) It is known that exposure to higher levels of boron species can cause reproductive hazards and be teratogenetic to humans.\(^2\) Due to the health consequences of elevated levels of boron species, the World Health Organization (WHO) has set the tolerance limit for boron species in drinking water to 0.5 mg/L.\(^3\) Due to the scarcity of fresh water, it is becoming essential to recycle used water or purify seawater. The average concentration of boron species in seawater ranges from 4-5 mg/L. There are several methods available including precipitation-coagulation,\(^4\) adsorption on activated carbon,\(^5\) fly ash,\(^6\) and oxides\(^7\) in addition to reverse osmosis\(^8\) (RO) that are capable of removing boron species from water. However, these methods lack efficiency. The widely used RO technique employs polyamide membranes that are commercially affordable. The major bottleneck encountered when removing boron species from water using membranes is poor rejection of uncharged molecules such as boric acid. While the rejection rate can be increased by working at pH 10, this leads to high costs and corrosion effects.\(^9\) Removal of boron species can also be increased by passing the water through multiple membranes, but this increases the cost of water purification by 10-20\%.\(^{10}\) These challenges can be overcome by modification of the membrane surface by boron-selective adsorbents.

It is well-known that compounds possessing adjacent hydroxyl groups (i.e., cis diols) are efficient in the selective binding of boron species. One example, N-methyl-D-glucamine (NMDG), possesses multiple adjacent hydroxyl groups which are very selective in the extraction of boron from water. Organic and inorganic polymeric
materials were functionalized using NMDG to develop various adsorbents.\textsuperscript{11} Nevertheless, these adsorbents are either expensive or less efficient for the removal of boron species from water. Commercially available adsorbents such as Amberlite IRA-743 resin suffer severe losses in efficiency every time it is regenerated.\textsuperscript{11b} Hence, it is important to develop an adsorbent material which is highly selective for boron species while possessing efficient extraction capabilities in addition to being commercially affordable.

The development of ionic liquids (IL) capable of strongly interacting with boron species and sequestering it from aqueous solution provides a new approach for removing boron species from water. ILs are a useful class of solvents due to their widely tuneable and varying physicochemical properties (e.g., melting point, viscosity, as well as miscibility with water and various organic solvents).\textsuperscript{12} Their use as potentially green and reusable solvent systems has presented many opportunities when employed in liquid-liquid extraction.\textsuperscript{13} In our quest to identify a boron-selective IL suitable for a broad range of applications, it is imperative that the IL candidate possess cis diol functional groups capable of binding the boron species as well as the capability of being structurally tuned/modified so that it can form a biphasic system with water. The N-methyl-D-glucamine molecule is an ideal starting material since the amine group can be functionalized with hydrophobic alkyl moieties (avoiding interference with borate-diol complexation) to mitigate the polar carbohydrate portion of the molecule thereby permitting phase separation when paired with a hydrophobic anion. For the first time, we
report a new class of ILs which can efficiently extract boron species from water. The complexation of these ILs with boron species was confirmed using $^{11}$B NMR.

### 2.2 Experimental

The synthesis of glucaminium-based ILs was performed as shown in Figure 2-1. Specific synthesis conditions are provided in electronic supplementary information (ESI). Compound 1 was obtained by reacting two moles of N-methyl-D-glucamine with one mole of 1-bromodecane in the presence of sodium carbonate in methanol. N,N-didecyl-N-methyl-D-glucaminium bromide (2) was synthesized by reacting equimolar amounts of compound 1 with 1-bromodecane in isopropanol. In order to obtain N-decyl-N-methyl-D-glucaminium chloride (3), one mole of compound 1 was reacted with 1.1 moles of hydrochloric acid at room temperature. The synthesis of peracetylated N-decyl-N-methyl-D-glucaminium chloride (4) was performed by reacting one mole of compound 3 with 8 moles of acetic anhydride in pyridine with a catalytic amount of 4-dimethylaminopyridine at room temperature. All of the above ILs were characterized by $^1$H NMR, $^{13}$C NMR, mass spectrometry and elemental analysis.
Figure 2-1: Synthesis of functionalized N-methyl-D-glucaminium-based ionic liquids: 
*Reagents and conditions*: (i) 1-bromodecane, Na₂CO₃, methanol, 50-55°C, 48 hrs.; (ii) 1-bromodecane, isopropanol, 50-55°C, 72 hrs; (iii) HCl, water, r.t., 12 hrs.; (iv) Pyridine, 4-dimethylaminopyridine, acetic anhydride, r.t., 12 hrs.

### 2.3 Results and Discussion

The mechanism for adsorption of boron species was described in detail by Ishihara and co-workers where it has been shown that borate forms anionic complexes with adsorbants possessing two or more hydroxyl groups. Additionally, Yoshimura and co-workers studied the complexation of N-methyl-D-glucamine with borate in water using $^{11}$B NMR. A similar approach was taken to confirm the complexation of IL 3 with
borate in water. All $^{11}$B NMR measurements were performed at room temperature (19 ± 1°C) using a 5 mm quartz tube at a resonance frequency of 128.0 MHz, 75° flip angle, pulse repetition time of 0.06 s, and a spectral width of 27 KHz. The peak for the neutral boron species in a 0.02 M free boric acid solution was taken as the external standard and set to 0 ppm, as shown in Figure 2-2(a). The concentration of boric acid was chosen as 0.02 M since polyborate species are known to form above this concentration. IL 3 and borate formed a bischelate complex which was observed as a single broad peak at -10 ppm when the pH was adjusted to 4.8. The effect of concentration of IL 3 was studied at pH 4.8, where the concentration of IL 3 was varied from 0.06 M to 0.4 M and the concentration of boric acid was kept constant at 0.02 M, as shown in Figure 2-2(b)-(d). It is important to note that as the concentration of IL 3 was increased, the peak area for the IL 3-borate complex increased significantly and the peak area for free boric acid decreased. The high selectivity of these ILs for borate is attributed to the presence of multiple hydroxyl groups present within the carbohydrate portion of the ILs. In order to confirm this, all of the hydroxyl groups present within IL 3 were acetylated, as shown in Figure 2-1, to obtain IL 4. A $^{11}$B NMR study using 0.4 M IL 4 suggests that there is no complex formation between IL 4 and borate (See Figure 2-2(e)). A single peak appeared at 0 ppm corresponding to free boric acid. These results prove that the hydroxyl groups play an active role in the extraction of boron species from water.

The effect of pH on the complexation was investigated using $^{11}$B NMR (as shown in Figure 2-3) where the concentration of IL 3 and boric acid were kept constant at 0.4 M
and 0.02 M, respectively. The pH was varied from 2 to 10 using different buffer solutions. At pH 2, no complex peak was observed and a single peak was observed at 0 ppm corresponding to the free boric acid, as shown in Figure 2-3(a). At pH 4.8, a broad peak appears at -10 ppm which corresponds to the bischelate complex with a small free boric acid peak at 0 ppm. As the pH was increased to 7 and 10, three peaks were observed which include the free boric acid peak (0 ppm), bischelate complex peak (-10 ppm), and monochelate complex peak (-12 ppm). These values are in good agreement with the literature. The peak corresponding to the free or uncomplexed borate anion appears at approximately -18 ppm, as shown in Figure 2-3(c)-(d) where at higher pH boron exists exclusively as borate anion.
Figure 2-2: $^{11}$B NMR spectra for solutions containing 0.02 M boric acid (ionic strength was set to 0.1 M using NaCl and pH=4.8 using NH$_4$OAc buffer) where (a) with no IL (b) 0.06 M [C$_{10}$-NMDG] [Cl] (c) 0.2 M 3 (d) 0.4 M 3 and (e) 0.4 M peracetylated [C$_{10}$NMDG] [Cl] 4.
Figure 2-3: $^{11}$B NMR spectra for the solution containing 0.4 M IL 3, 0.02 M boric acid and 0.1 M NaCl at (a) pH=2, (b) pH=4.8, (c) pH= 7, (d) pH=10. Spectrum (e) represents a solution containing 0.006 M boric acid at pH=10 with no IL.

Figure 2-3(e) represents the single peak at -15 ppm which corresponds to tetrahydroxyborate anion in the absence of IL 3. It is well known from the literature that polyborate species form in a solution of free boric acid at high concentration and high
pH. Therefore, the concentration of boric acid was decreased to 0.006 M with no IL 3 present.

To examine the extraction performance of these ILs as well as the ability to form a water-immiscible phase capable of pre-concentrating boron species, an *in-situ* dispersive liquid-liquid microextraction (DLLME) method was employed. A schematic representation of *in-situ* DLLME is shown in Figure 2-4. *In-situ* DLLME was performed by dissolving the hydrophilic, halide form of an IL in the aqueous phase containing the analyte of interest. A water immiscible IL phase is generated by performing a metathesis reaction in which the halide anion is changed to the hydrophobic tris(pentafluoroethyl)trifluorophosphate (FAP) anion. As the IL phase separates from the aqueous phase, the boron species are pre-concentrated into the small volume of the IL phase. After extraction, the aqueous layer was withdrawn and subjected to high performance liquid chromatography (HPLC) to determine the remaining boric acid concentration. The HPLC method employed chromotropic acid which forms an anionic borate ester complex which was separated using an anion exchange column. The amount of boric acid extracted from the aqueous layer by the IL can be calculated by taking the difference of boric acid concentration before and after the extraction.
Addition of boric acid solution

i) Vortex 1 min

ii) Addition of 1 mL aq. solution of KFAP

i) Vortex 1 min

ii) Centrifuged 10-30 min

Aqueous phase

IL phase

(a)

(b)

(c)

(d)

Figure 2-4: In-situ dispersive liquid-liquid microextraction of boric acid from water using glucaminium-based ILs where ■ = IL, * = free boric acid and ○ = IL-borate complex.

As shown in Table 2.1, a significant amount of boric acid was extracted from water by ILs 2 and 3 using in-situ dispersive liquid-liquid microextraction. The extraction efficiency of IL 2 for boric acid was found to be slightly higher than IL 3 which can be attributed to the more hydrophobic nature of IL 2.

Table 2.1: Results for in situ dispersive liquid-liquid microextraction of boric acid using boron-selective ILs as the extraction phase

<table>
<thead>
<tr>
<th>IL</th>
<th>Metathesis salt</th>
<th>Initial boric acid concentration (ppm)</th>
<th>Concentration of boric acid remaining in aqueous layer using HPLC (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>KFAP</td>
<td>9</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>KFAP</td>
<td>9</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>
2.4 Conclusion

In summary, a new class of ILs was synthesized for the extraction of boron species from water. The results obtained from in situ dispersive liquid-liquid microextraction are very promising and show significant decrease in boric acid concentration following extraction. Data obtained from a $^{11}$B-NMR study provides insight into how the IL selectivity can be modulated in terms of increasing IL-borate complexation as well as how complexation can be reversed, which is essential in the stripping of the boron species and re-use of the IL. The versatility of these compounds make them particularly attractive in membrane technologies, such as supported ionic liquid membranes (SILM), where the liquid nature, low vapour pressure, low water solubility, and high selectivity for boron species are essential characteristics. On-going research in our group is focused on the recycling and re-use of these ILs as well as their application in a broader range of liquid-based separations, including SILMs.
References


Chapter 3

Evaluating the complexation behavior and regeneration of boron selective glucaminium-based ionic liquids when used as extraction solvents

A paper published in *Analytica Chimica Acta*¹

Manishkumar D. Joshi, Daniel J. Steyer, and Jared L. Anderson

Abstract

Glucaminium-based ionic liquids are a new class of solvents capable of extracting boron-species from water with high efficiency. The complexation behavior of these ILs with borate was thoroughly studied using $^{11}$B-NMR. Two different complexes, namely, monochelate complex and bischelate complex, were observed. $^{11}$B-NMR was used extensively to determine the formation constants for monochelate and bischelate complexes. The IL concentration was observed to have a significant effect on the IL-

¹ Reprinted from *Analytica Chimica Acta* 740 (2012) 66–73. Copyright © 2012 Elsevier B.V
borate complexes. Using an *in-situ* dispersive liquid-liquid microextraction (*in-situ* DLLME) method, the extraction efficiency for boron species was increased dramatically when lithium bis[(trifluoromethyl)sulfonyl]imide (LiNTf₂) was used as the metathesis salt in an aqueous solution containing 0.1 M sodium chloride. IL regeneration after extraction was achieved using 0.1 M hydrochloric acid. The extraction efficiency of boron species was consistent when the IL was employed after three regeneration cycles. The selectivity of the IL for boron species in synthetic seawater samples was similar to performing the same extraction from Milli-Q water samples.

### 3.1. Introduction

Boron is a naturally occurring element which is most commonly found as boric acid or borate ion in the soil and water. Depending upon the pH of water, the ratio of boric acid to borate ion varies significantly. Boron exists primarily as boric acid at pH lower than 7 and as borate ion at pH higher than 10.¹ Boron is a known micronutrient in plants which helps in plant growth. However, higher levels of boron in irrigation water possess detrimental effects which can eventually result in plant death.² Preliminary studies have suggested that higher levels of boron in drinking water can lead to teratogenic effects in humans.³ Due to the aforementioned threat posed by higher boron concentrations, the World Health Organization (WHO) has set a threshold of 0.5 ppm in drinking water and 0.3-0.5 ppm in irrigation water.⁴
The significant presence of boron in water is a consequence of increased use of boron in industrial products such as borosilicate glass, surfactants, and electronics. Many parts of the world experience water shortage which requires recycling or processing of seawater. Desalination is a primary method which is used to process the seawater. The usual concentration of boron in the seawater is approximately 5 ppm. There are several different methods available to remove boron from the water such as reverse osmosis (RO),\textsuperscript{5} electrodialysis,\textsuperscript{6} and precipitation-coagulation.\textsuperscript{7} Among all these methods, RO is the most widely used methods for water purification. The removal of boron using RO is challenging as it possesses very low rejection rate for uncharged boric acid which is primarily present in water at neutral pH. The rejection rate for boric acid can be significantly increased by raising the pH or passing the water multiple times through the RO membrane. However, this leads to corrosion and an approximate 10-20\% increase in the water purification cost.\textsuperscript{8} These conventional methods have failed to achieve the desired low threshold limits. Alternative methods include removal of boron by adsorption using fly ash,\textsuperscript{9} activated carbon,\textsuperscript{10} oxides and hydrotalcite-like compounds.\textsuperscript{11}

The high complexation ability of boric acid/borate with compounds containing two adjacent hydroxyl groups has given rise to their development as sorbent materials. Surface modification of membranes using polyhydroxy compounds is also performed for the removal of boron from water.\textsuperscript{12} Several organic and inorganic based polymeric materials containing polyol moieties have been developed and employed for boron removal from water.\textsuperscript{13} So far, the best results have been obtained for removal of boron using different ion exchange resins functionalized with carbohydrate moieties such as N-
methyl-D-glucamine. Mesoporous supports have also been functionalized with different carbohydrates such as mannose\textsuperscript{14} and N-methyl-D-glucamine.\textsuperscript{15} However, higher cost of the material and low efficiency make it difficult to implement them on commercial scale.\textsuperscript{16} There is a huge need for the new sorbent materials which are robust, inexpensive, and highly efficient for the removal of boron from water.

Ionic liquids (ILs) are a class of “designer solvents” due to the fact that various properties of ILs can be tuned by the combination or simple structural modifications to the cation and/or anion. They possess a number of unique properties including high thermal stability, negligible vapor pressure at ambient temperatures, and variable viscosities. Due to their low vapor pressures, some classes of ILs are considered as environmentally benign solvents as they exhibit a number of advantages to organic solvents which are typically very volatile and highly flammable. These unique properties of ILs have been widely exploited in organic synthesis,\textsuperscript{17} catalysis,\textsuperscript{18} and electrochemistry.\textsuperscript{19} In the last decade or so, ILs have investigated as a novel extraction media.\textsuperscript{20}

In our previous work, we demonstrated the synthesis of a new class of glucaminium-based ILs and highlighted their application in the removal of boron from water.\textsuperscript{21} Using different microextraction techniques such as \textit{in-situ} dispersive liquid-liquid microextraction (\textit{in-situ} DLLME) and dispersive liquid-liquid microextraction (DLLME), glucaminium-based ILs demonstrated promising results for the efficient removal of boron species from water. In order to efficiently use these glucaminium-based boron-selective ILs in extractions/separations, insight into the mechanism of
complexation is needed. In addition, it is highly desirable to re-use the ILs following extraction. A knowledge and understanding of how these compounds can be employed to achieve the desired selectivity as well as how the substrate can be effectively removed from the sorbent is imperative if these materials are to be used in membrane-based separations.

In this study, we examine the complexation of the glucaminium-based ILs with boron species in aqueous solution by reporting the formation constants for monochelate and bischelate complexes. Enhanced extraction efficiency was observed when the metathesis salt lithium bis[(trifluoromethyl)sulfonyl]imide (LiNTf$_2$) was introduced into the in-situ DLLME experiment and the ionic strength adjusted to 0.1 M using sodium chloride. IL regeneration after extraction of boron species was systematically studied. Effective and rapid IL regeneration was achieved by treating the IL complex with an acidic aqueous solution for only 60 seconds. The extraction efficiency achieved when using the same IL after multiple extraction and regeneration cycles was very stable indicating that the IL can be effectively re-used after stripping the borate ion from the IL complex during regeneration.

3.2. Experimental

3.2.1. Materials and chemicals

N-methyl-D-glucamine, 1-bromodecane, chromotropic acid, ethylenediaminetetraacetic acid, sodium perchlorate, boric acid, magnesium chloride
hexahydrate, sodium monosilicate nonahydrate, strontium chloride hexahydrate, and iron (III) phosphate tetrahydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid, methanol, isopropanol, acetone, chloroform, ethyl acetate, sodium carbonate, ammonium acetate, disodium phosphate, sodium chloride, sodium sulfate, calcium chloride hexahydrate, potassium chloride, potassium bromide, sodium fluoride, ammonium nitrate were purchased from Fisher Scientific, (Fair Lawn, NJ, USA). Octyltrimethylammonium chloride was purchased from TCI America, (Portland, OR), and Lithium bis[(trifluoromethyl)sulfonyl]imide was obtained from SynQuest Labs. Inc., (Alachua, FL, USA). All solutions were prepared using Milli-Q water (18 MΩ.cm resistivity).

3.2.2. Synthesis of N-methyl-D-glucaminium-based ILs

Glucaminium-based ILs 1 and 2, as shown in Figure 3-1, were synthesized according to our previous work. Briefly, N-decyl-N-methyl-D-glucamine was synthesized by reacting N-methyl-D-glucamine, 1-bromodecane, and sodium carbonate in methanol for 48 hours at 50-55 °C. N-decyl-N-methyl-D-glucamine was obtained by vacuum filtration followed by crystallization in ethanol. IL 1 was synthesized by reacting N-decyl-N-methyl-D-glucamine and hydrochloric acid in water at room temperature for 12 hours. IL 2 was synthesized by reacting N-decyl-N-methyl-D-glucamine and 1-bromodecane in isopropanol for 72 hours at 70 °C.
3.2.3. *In-situ* dispersive liquid-liquid microextraction

In a 15 mL polypropylene tube, 0.05 g of IL 2 was dissolved in 8.1 mL deionized water containing 0.1 M sodium chloride. This was followed by the addition of 0.9 mL of 100 ppm boric acid solution. The resulting solution was vortexed for 60 seconds followed by the addition of 0.028 g of lithium bis[(trifluoromethyl)sulfonyl]imide (LiNTf₂) in 1 mL of water. Due to the metathesis reaction, a cloudy solution immediately appeared and the solution was vortexed for 60 seconds. The solution was then subjected to centrifugation for 15 minutes. Two separate layers were observed; the top and bottom layer corresponded to the aqueous and [(C₁₀₂-NMDG)[NTf₂]] IL layers, respectively. A total volume of 10 mL was obtained and the initial concentration of boric acid was 30 ppm. The aqueous phase was subjected to HPLC measurements for the determination of remaining boric acid. The amount of boric acid extracted was determined by the difference in the initial boric acid and remaining boric acid concentration.

Figure 3-1: Structures of the N-methyl-D-glucaminium-based ILs examined in this study.
3.2.4. Dispersive liquid-liquid microextraction

In a 15 mL polypropylene tube, 10 mL of 30 ppm boric acid solution was prepared where the ionic strength was set to 0.1 M using NaCl. In a separate glass vial, 0.068 g [(C_{10})_2-NMDG][NTf_2] IL was dissolved in 1 mL of chloroform. This IL solution was added directly to the aqueous solution. A cloudy solution immediately appeared and was vortexed for 60 seconds followed by centrifugation for 15 minutes. Two separate layers, namely, the aqueous and IL layers appeared in which boric acid was preconcentrated in the IL phase. The total aqueous phase volume obtained was 10 mL and the initial concentration of boric acid was 30 ppm. The amount of remaining boric acid in the aqueous phase after extraction was determined by HPLC. The amount of boric acid extracted was determined by the difference in the initial boric acid and remaining boric acid concentration.

3.2.5. regeneration of N-methyl-D-glucaminium-based IL

After extraction, the IL phase containing the borate ester complex was dissolved in 1 mL chloroform. This was followed by the addition of 4 mL of 0.1 M HCl solution containing 0.1 M NaCl. The binary system was vortexed for 60 seconds followed by centrifugation for 15 minutes. Two clear layers appeared. The upper water layer contains boric acid generated through breaking of the complex. The bottom chloroform layer was withdrawn and chloroform was removed completely under reduced pressure. The sample was dried in a vacuum oven at 70 °C for 24 hours. The resulting regenerated IL N,N-
didecyl-N-methyl-D-glucaminium bis-[(trifluoromethyl)sulfonyl]imide \([([\text{C}_{10}]\text{2-NMDG})[\text{NTf}_2])\] was re-used for the extraction of boric acid from water using DLLME.

### 3.2.6. HPLC method for the detection of boric acid in water

Detection of boric acid was performed according to a previously reported HPLC method developed by Jun et al.\textsuperscript{22} All measurements were performed using a Shimadzu LC20-AT HPLC. A TSK gel IC-Anion-PW anion exchange column (5.0 cm x 4.6 mm (ID)) was obtained from Tosoh Bioscience (Montgomeryville, PA, USA). The calibration curve was generated where the concentration of boric acid was varied from 0.1 to 15 ppm. In order to determine the amount of boric acid in the aqueous sample, 5.0 mL of the aqueous phase was placed in a polypropylene tube. This was followed by the addition of 0.25 mL of solution A (0.016 M chromotropic acid and 0.1 M EDTA) and 0.25 mL of solution B (2.0 M octyltrimethylammonium chloride and 1.0 M sodium acetate). The sample was stirred well and kept in the dark for 2.5 hours. A sample volume of 10 µL was injected onto the column using a mobile phase consisting of 0.2 M sodium perchlorate and 1 mM sodium acetate. The retention time for chromotropic acid and the anionic complex was 1 min and 3 min, respectively. The anionic complex peak was observed at 350 nm.

### 3.2.7. \(^{11}\text{B}-\text{NMR Experiments}\)

All \(^{11}\text{B}-\text{NMR}\) experiments were performed at room temperature (19 ± 1 °C) on a Varian VXRS 400 MHz NMR spectrometer. The NMR spectrometer was tuned for \(^{11}\text{B}\)
nuclei using a resonance frequency of 128.0 MHz. All measurements were performed in a 5 mm quartz tube obtained from Wilmad, (Vineland, NJ, USA). Stock solutions of 0.1 M IL and 0.1 M boric acid were prepared in deionized water. The sample for $^{11}$B-NMR analysis was prepared by taking different volumes of stock solutions where D$_2$O was present at 10% of the total sample volume. The sample pH was adjusted to 7 or 4.8 using phosphate or ammonium acetate buffer solutions, respectively. All $^{11}$B-NMR measurements were performed with 75 ° flip angle, 0.06 second pulse repetition time, and 27.0 KHz spectral width, with a total number of 8064 scans being recorded.

3.2.8. Preparation of synthetic seawater

The synthetic seawater was prepared as per literature by dissolving 24 g NaCl, 11 g MgCl$_2$.6H$_2$O, 4 g Na$_2$SO$_4$, 2 g CaCl$_2$.6H$_2$O, 0.7 g KCl, 0.1 g KBr, 0.005 g NaSiO$_3$.9H$_2$O, 0.04 g SrCl$_2$.6H$_2$O, 0.003 g NaF, 0.002 g NH$_4$NO$_3$, 0.001 g Fe$_3$PO$_4$.4H$_2$O in 850 mL of DI water.$^{23}$ The pH was adjusted to 7.8 followed by the addition of DI water to make a total volume of 1 liter.

3.3. Results and Discussion

3.3.1. In-situ DLLME using different metathesis salts

The in-situ DLLME technique using LiNTf$_2$ metathesis salt was introduced by our group in 2009.$^{24}$ A schematic representing the in-situ DLLME method is shown in Figure 3-2(a). The method involves dissolving a hydrophilic IL in an aqueous solution
containing the analyte(s) of interest. An *in-situ* metathesis reaction is achieved by the addition of a metathesis salt to the aqueous solution where typically the halide anion is replaced with a hydrophobic anion. The newly formed hydrophobic IL undergoes phase separation resulting in the analyte being preconcentrated in the hydrophobic IL phase.

The detailed procedure for the *in-situ* DLLME method used for removal of boron species from water is described in the experimental section.

![Diagram](image)

**Figure 3-2:** Schematic representation of (a) *in-situ* DLLME and (b) DLLME used in this work. The original DLLME method (b) utilizes an organic dispersive solvent whereas the *in-situ* DLLME method eliminates the requirement of an organic solvent.

The structures of the two glucaminium-based ILs (N-decyl-N-methyl-D-glucaminium chloride 1 and N,N-didecyl-N-methyl-D-glucaminium bromide 2) used for
**in-situ** DLLME are shown in Figure 3-1. In our previous work, IL 2 was employed with the potassium tris-(pentafluoroethyl)trifluorophosphate (KFAP) metathesis salt where the bromide anion of IL 2 was replaced with the hydrophobic FAP anion. Due to the fact that the KFAP salt is not currently commercially available, it is important to explore other metathesis salts. In this work, the LiNTf₂ metathesis salt was examined for **in-situ** DLLME using IL 2. The extraction performance was compared to the KFAP salt, as shown in Table 1. Higher extraction efficiency of the boron species was observed for IL 2 when LiNTf₂ was used as the metathesis salt. This is attributed to the use of 0.1 M sodium chloride when employing the LiNTf₂ metathesis salt. Due to the presence of the quaternary ammonium cation and five hydroxyl groups, IL 2 undergoes significant hydrogen bonding with water. This results in difficulty in forming two separate layers when using LiNTf₂ as the metathesis salt. This challenge was addressed by the addition of 0.1 M sodium chloride. Addition of sodium chloride to the aqueous phase resulted in the formation of binary system.

**Table 3.1: Extraction of boric acid from water using in-situ DLLME**

<table>
<thead>
<tr>
<th>IL</th>
<th>Metathesis Salt</th>
<th>Initial boric acid concentration (ppm)</th>
<th>Remaining boric acid in aqueous phase (ppm) N=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>KFAP</td>
<td>9.0</td>
<td>2.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>LiNTf₂</td>
<td>30.0</td>
<td>1.4 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data was obtained from reference [21]. <sup>b</sup> 0.1 M NaCl was used.
Furthermore, the effect of the NTf$_2^-$ anion on the extraction of the boron species was studied. IL 2 was replaced with the 1-decyl-3-methylimidazolium bromide IL (a cation that lacks the ability to form a complex with boric acid/borate) followed by the use of the LiNTf$_2$ salt during *in-situ* DLLME. An initial concentration of 10 ppm boric acid was employed. Following extraction, the amount of boric acid found in the aqueous phase was 10.4 ± 0.08 ppm (N=5). These results confirm that the NTf$_2^-$ anion does not play an active role in the extraction of boron species from water and that the selectivity originates from the cation. The significant increase in the extraction efficiency of IL 2 can be explained by the better phase separation of the N,N-didecyl-N-methyl-D-glucaminium bis[(trifluoromethyl)sulfonyl]imide IL from water due to the presence of sodium chloride.
3.3.2. Formation constant determination of IL-borate complexes

Yoshimura and co-workers have previously described the formation of monochelate, tetrade-tate, and bischelate complexes between N-methyl-D-glucamine (NMDG) and boric acid/borate.\textsuperscript{25} The complexation between IL 1 and boric acid/borate was studied using $^{11}$B-NMR. There are mainly two different types of complexes namely monochelate and bischelate complexes. In monochelate complex, one molecule of IL 1 is involved in the complexation with one molecule of boric acid/borate. The bischelate complex is formed by complexation of one molecule of boric acid/borate with two
molecules of IL 1. The type of complexation depends on the concentration ratios between IL 1 and boric acid/borate. All $^{11}$B-NMR measurements were performed at pH 7 in which the boric acid concentration was kept constant at 0.02 M and the concentration of IL 1 was varied. As shown in Figure 3-3(a), the peak intensity for the monovalent complex at -12 ppm was much higher than the peak intensity for the bischelate complex at -10 ppm when the concentration of IL 1 is half the concentration of boric acid. The bischelate complex becomes more prevalent when IL 1 and boric acid are present in the same molar concentration (Figure 3-3(b)). The bischelate complex becomes the dominant species when the concentration of IL 1 is increased by an order of magnitude to 0.4 M, as shown in Figure 3-3(c). It should be noted that no free boric acid is observed under the latter condition.

\[
\text{B(OH)}_3 + \text{H}_2\text{O} \rightleftharpoons \text{B(OH)}_4^- + \text{H}^+ \quad (1)
\]

\[
K_1 = \frac{[\text{B(OH)}_4^-][\text{H}^+]}{[\text{B(OH)}_3]} = 10^{-9.05}
\]

\[
\text{B(OH)}_4^- + \text{IL}^+ \rightleftharpoons \text{IL}^+ - \text{B(OH)}_2^- + 2\text{H}_2\text{O} \quad (2)
\]

\[
K_2 = \frac{[\text{IL}^+ - \text{B(OH)}_2^-]}{[\text{B(OH)}_4^-][\text{IL}^+]} \]

\[
\text{IL}^+ - \text{B(OH)}_2^- + \text{IL}^+ \rightleftharpoons (\text{IL}^+)_2 - \text{B}^- + 2\text{H}_2\text{O} \quad (3)
\]

\[
K_3 = \frac{[(\text{IL}^+)_2 - \text{B}^-]}{[\text{IL}^+ - \text{B(OH)}_2^-][\text{IL}^+]} \]

The determination of formation constants for monochelate and bischelate complexes of IL 1 were performed as per the equilibria represented by equations 1-3, where B(OH)$_3$ and B(OH)$_4^-$ represent boric acid and borate ion, respectively. IL$^+$ represents the N-decyl-N-methyl-D-glucaminium cation. The equilibrium between boric
acid and borate ion is shown in equation 1. The equilibrium constant (K₁) for equation 1 was obtained from the literature. Monochelate complex formation is described in equation 2 where one mole of IL 1 chelates to one mole of borate ion. The formation constant for monochelate complex is represented by K₂, where IL⁺-B(OH)₂⁻ denotes the monochelate complex. The bischelate complex, (IL⁺)₂-B⁻, was formed through complexation of another mole of IL 1 with the monochelate complex. The formation constant for the bischelate complex is represented by K₃ (see equation 3). Literature precedence strongly suggests that a tetradentate complex is not formed while NMDG is in solution. Hence, the formation of a tetradeinate complex with the studied glucaminium-based IL was ignored in the above equilibria.
A series of $^{11}$B-NMR experiments were performed at pH 7 in which the boric acid concentration was kept constant at 0.02 M and the concentration of IL 1 was varied from 0.002 M to 0.02 M. The equilibrium concentration of IL 1 was calculated from above equilibria using equations 1 and 2. Briefly, ratio of peak intensities of monochelate complex and free boric acid in $^{11}$B NMR and, total concentration of boric acid added were used to obtained equilibrium concentration of free boric acid using equilibria 2. Here it was assumed that the ratio of equilibrium concentration of monochelate complex and boric acid is the same as the ratio of peak intensity of these two species in $^{11}$B NMR. Once equilibrium concentration of free boric acid was obtained, it has been used in calculating equilibrium concentration of borate ion using equation 1. The equilibrium
concentration of monochelate complex was achieved by subtracting the equilibrium concentration of free boric acid from total concentration of the boric acid. Similarly the equilibrium concentration of bischelate complex was calculated from the ratio of the peak area of bischelate complex and monochelate complex and equilibrium concentration of ionic liquid using equation 3. The equilibrium concentration of IL 1 was plotted against the ratio of equilibrium concentrations of monochelate complex and borate ion as shown in Figure 3-4. The least-squares-fit method was used to obtain a straight line passing through the origin. The slope of the line obtained is defined as the formation constant (K2) for the monochelate complex. Similarly, the equilibrium concentration of IL 1 was plotted against the ratio of equilibrium concentrations of the bischelate and monochelate complexes. The slope of the line passing through the origin was taken as the formation constant (K3) of the bischelate complex. (This data is presented in the electronic supplementary information). It was found that a very narrow linear range was obtained for the neat IL 1 samples, as shown in Figure 3-4(a), which limits the determination of formation constants for IL 1-borate ion complexes. This leveling off behavior was not observed when studying NMDG-borate complexation using free NMDG, as shown in Figure 3-4(b). It is known from the literature that the stability of the NMDG-borate complex is due to the electrostatic attraction between the negatively charged boron atom and the positively charged nitrogen atom formed after complexation. In the case of NMDG, the proton released during complexation is likely abstracted by the tertiary amine group to form a quaternary ammonium cation. In the case of IL 1, it is already a quaternary ammonium moiety due to functionalization with the long alkyl chain
substituent. The stability of the quaternary ammonium cation of IL 1 containing the alkyl chain is higher than quaternary ammonium cation of NMDG which possesses exchangeable protons.\textsuperscript{26} To further confirm this behavior, NMDG was mixed at different proportions with IL 1, as shown in Figure 3-4(c)-(d). These plots resulted in a straight line with reasonable fitting of the data points ($R^2 = 0.91-0.98$). Formation constants for NMDG-borate complexes (using free NMDG) were obtained as per the above procedure.

The obtained formation constants for NMDG-borate complexes as well as previously reported literature values are shown in Table 2.\textsuperscript{25} The logarithmic values of formation constants for NMDG/IL 1-borate ion complexes are reported in Table 2. It can be observed that the value for the formation constant for monochelate ($\log \beta_{1:1}$) and bischelate ($\log \beta_{1:2}$) complexes decreased as the percentage of IL 1 was increased in the sample. The average value for $\log \beta_{1:1}$ was 3.53 when 100 \% NMDG was present in the sample. The value for $\log \beta_{1:1}$ was reduced to 3.37 and 3.18 when the percentage of IL 1 was increased to 50 \% and 75 \%, respectively. Similarly, the value for $\log \beta_{1:2}$ was decreased to 5.07 and 5.03 when the amount of IL 1 was increased to 50 \% and 75 \% compared to 5.40 when 100 \% NMDG was used. These results prove that the formation of the bischelate complex is more favorable compared to the monochelate complex.
Table 3.2: Formation constants for monochelate and bischelate complexes of boric acid with N-methyl-D-glucamine (NMDG) and/or IL 1 using $^{11}$B-NMR. The determined formation constants are the average of three separate experiments. 

\[ a \log \beta_{1:1} = \log K_2; \ b \log \beta_{1:2} = \log K_2 K_3. \ c \] Data from reference.\textsuperscript{21}

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$\log \beta_{1:1}^{a}$</th>
<th>$\log \beta_{1:2}^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMDG</td>
<td>3.53 ± 0.30</td>
<td>5.40 ± 0.32</td>
</tr>
<tr>
<td>NMDG</td>
<td>4.90 ± 0.05\textsuperscript{c}</td>
<td>6.32 ± 0.11\textsuperscript{c}</td>
</tr>
<tr>
<td>NMDG:IL 1/50:50</td>
<td>3.37 ± 0.02</td>
<td>5.07 ± 0.13</td>
</tr>
<tr>
<td>NMDG:IL 1/25:75</td>
<td>3.18 ± 0.18</td>
<td>5.03 ± 0.10</td>
</tr>
</tbody>
</table>
3.3.3. Regeneration of glucaminium-based ionic liquid

Figure 3-5: $^{11}$B-NMR spectra of a solution containing 0.4 M IL 1 and 0.02 M boric acid (ionic strength was 0.1 M using NaCl and pH was 4.8 using ammonium acetate buffer). The effect of HCl concentration on IL 1-borate complexation using (a) No HCl, (b) 0.1 M HCl, (c) 0.2 M HCl, (d) 0.4 M HCl, (e) 0.6 M HCl, (f) 0.8 M HCl, (g) 1.0 M HCl, (h) 1.4 M HCl, and (i) 2.0 M HCl.

The ability to regenerate sorbent materials is an important aspect into determining their reusability. An ideal sorbent material is the one that can be easily regenerated multiple times while exhibiting similar extraction efficiencies for the desired substrate after every regeneration step. High stability of the sorbent material under acidic pH conditions as well as minimum weight loss during regeneration are key factors to
consider for sorbent materials with potential in commercial applications or when using the extraction phase after multiple extractions. The regeneration of glucaminium-based ILs was studied in this work. In our previous study, it was shown using $^{11}$B-NMR that the stability of the complex formed between IL 1 and borate is pH dependant. Borate ester complexes are very stable at pH 4.8 and above, but are not stable at lower pH. Previous literature reports have utilized hydrochloric acid (HCl) to regenerate NMDG-based sorbent materials by stripping off the borate ion. $^{16}$ $^{11}$B-NMR experiments were performed to study the effect of HCl concentration on the complexation of IL 1 to boron species, as shown in Figure 3-5. The concentration of boric acid and IL 1 was kept constant at 0.02 M and 0.4 M, respectively. The ionic strength was adjusted to 0.1 M using sodium chloride and the pH was adjusted to 4.8 using ammonium acetate buffer. The concentration of HCl was varied from 0.1 M to 2.0 M. Figure 5(a) shows the $^{11}$B-NMR spectrum when no HCl is added. The complex peak is observed at -10 ppm and free boric acid is observed at 0 ppm. As the concentration of HCl was increased from 0.1 to 1.4 M (Figure 3-5(b)-(h)), the peak area for free boric acid increased and the peak area of the complex decreased significantly. When the concentration of HCl was increased to 2.0 M (Figure 3-5(i)), the complex peak completely disappeared and only a peak for free boric acid was observed. These results demonstrated the effective dissociation of the IL 1-borate complex at lower pH values.
Figure 3-6: Extraction/regeneration cycle describing the extraction of boric acid from water following regeneration of IL 2.

Figure 6 represents a schematic diagram demonstrating the extraction/regeneration cycle employed in this study. Firstly, *in-situ* DLLME was performed using IL 2 ([(C\(_{10}\))\(_2\)-NMDG][Br]) where LiNTf\(_2\) was used as the metathesis salt. During *in-situ* DLLME, the bromide anion of IL 2 was replaced with the NTf\(_2^-\) anion. The resulting hydrophobic IL phase separates and contains the IL 2-borate complex (see Figure 2(a)). This was followed by the regeneration of IL phase using 0.1 M HCl where the borate ion was stripped from the N,N-didecyl-N-methyl-D-glucaminium cation to generate the uncomplexed NTf\(_2^-\) salt. The detailed regeneration procedure is discussed in the experimental section. Due to the metathesis reaction that takes place during the *in-situ* DLLME step, the bromide anion was replaced with the
NTf$_2$ anion making it impossible to use the regenerated [(C$_{10}$)$_2$-NMDG][NTf$_2$] IL for the specific in-situ DLLME approach. Therefore, the traditional DLLME method, as shown in Figure 2(b), was used for the extraction of boric acid using the regenerated IL. Using this method, a small amount of IL (extraction solvent) was dissolved in 1 mL of chloroform (dispersive solvent) and added to 10 mL of the aqueous phase. This resulted in the formation of a cloudy solution which was vortexed and centrifuged. The boric acid was subsequently preconcentrated in the IL layer. The remaining boric acid in the aqueous phase was determined using the HPLC method.

Table 3.3: Dispersive liquid-liquid microextraction of boric acid using regenerated glucaminium-based ILs. Chloroform was used as the dispersive solvent and the ionic strength was set to 0.1 M using sodium chloride.

<table>
<thead>
<tr>
<th>Ionic liquid</th>
<th>Boric acid concentration (ppm) N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>1$^{\text{st}}$ Regeneration</td>
<td>[(C$_{10}$)$_2$-NMDG][NTf$_2$]</td>
</tr>
<tr>
<td>2$^{\text{nd}}$ Regeneration</td>
<td>[(C$_{10}$)$_2$-NMDG][NTf$_2$]</td>
</tr>
<tr>
<td>3$^{\text{rd}}$ Regeneration</td>
<td>[(C$_{10}$)$_2$-NMDG][NTf$_2$]</td>
</tr>
</tbody>
</table>

Extraction and regeneration steps were performed multiple times to study the reusability of the glucaminium-based ILs. Three regeneration cycles were performed in order to study the effect of regeneration on the extraction efficiency of IL 2. Results for the extraction of boric acid from water using the regenerated IL are shown in Table 3.3. It
is evident from the results that even after three extraction-regeneration cycles, the extraction performance of the regenerated IL was consistent in the removal of boron species from water. However, the extraction efficiency was slightly lowered after third regeneration cycle. This may be due to the presence of residual HCl which accumulates after multiple extraction-regeneration cycles. The appearance of the IL recovered after the first two regeneration cycles was colorless contrary to the yellowish IL observed after the third regeneration. Overall, an approximate 10-13% loss in the weight of the regenerated IL was observed after the \textit{in-situ} DLLME extraction-regeneration cycle. The weight loss of the regenerated IL was found to be lower (7-9%) after the subsequent DLLME extraction-regeneration cycle. The short regeneration time of 60 seconds is evidence of a quick regeneration method for the glucaminium-based IL compared to other polymeric adsorbent materials. In contrast to existing sorbent materials,\textsuperscript{16} to the glucaminium IL-based solvent exhibit consistent extraction efficiency even after multiple extraction/regeneration cycles. Ongoing work in our group is focused on optimizing the regeneration method to further minimize sample loss while maintaining short regeneration cycles.

3.3.4. Boron removal from synthetic seawater

An ultimate goal of this study is to use these ILs in simple, rapid, and robust methods for the removal of boron species from water or seawater. One major challenge lies in the possible interference of other ionic species that are often present in real seawater samples. To examine the selectivity of the IL-based solvents in the removal of
boron species from seawater, extractions were carried out using synthetic seawater prepared from a previously published method. To eliminate any matrix effects, a separate HPLC calibration curve was generated using synthetic seawater by spiking incremental amounts of boric acid from 0.1 ppm to 15 ppm (see ESI). In-situ DLLME extractions were performed using IL 2 in synthetic seawater. The initial concentration of boric acid in the seawater was 30 ppm. Following extraction, the amount of boric acid was reduced to 1.2 ± 0.4 ppm (N=5). Despite the presence of different anions such as chloride, nitrate, and sulfate, similar extraction performance was observed compared to extractions performed in Milli-Q water by the new glucaminium-based IL. This preliminary study shows the feasibility of applying glucaminium-based ILs in the removal of boron species from seawater samples.

3.4. Conclusions

The results obtained in this study demonstrate the successful use of glucaminium-based ILs for the efficient and rapid removal of boron species from water. The use of LiNTf₂ as metathesis salt was demonstrated. A significant increase in the extraction efficiency of boric acid/borate from water was observed when 0.1 M sodium chloride was employed in the extraction method. The extensive study of the IL-borate complex was performed using ¹¹B NMR to determine the formation constants of IL-borate complexes. N,N-didecyl-N-methyl-D-glucaminium bis[(trifluoromethyl)sulfonyl]imide was successfully regenerated multiple times and used for the extraction of boron species from water. The regeneration process was found to be quick and effective. The extraction
efficiencies for the regenerated IL were found to be consistent after three regeneration cycles. Ongoing studies are focused on the use of these materials in membrane-based separations.

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References


Chapter 4

Determination of polychlorinated biphenyls in seawater and bovine milk using crosslinked polymeric ionic liquid sorbent coatings by solid-phase microextraction

A paper submitted to *Analytical and Bioanalytical Chemistry*

Manishkumar D. Joshi, Tien D. Ho, William T. S. Cole, Jared L. Anderson

Abstract

Crosslinked polymeric ionic liquid (PIL)-based sorbent coatings were employed in the extraction of twenty one polychlorinated biphenyls (PCBs) from water and bovine milk using solid-phase microextraction (SPME). The extraction temperature, time, and concentration of sodium chloride added to the matrix were optimized in order to determine the best extraction conditions for the extraction of PCBs. The analytical performance of the crosslinked PIL-based SPME fibers were compared with a commercial 7 μm polydimethylsiloxane (PDMS) fiber using gas chromatography (GC) employing electron capture detection (ECD) and mass spectrometric detection (MS). Higher sensitivities for PCBs were achieved using PIL-based fibers when compared to PDMS fiber due to the incorporation of benzyl moieties into the PIL structures. The
limits of detection (LOD) for all PCBs were determined to be in the sub ng L−1 range using the three studied coatings. Recovery studies were performed for PCBs in ocean water and bovine milk to validate the applicability of the current SPME method.

4.1. Introduction

Polychlorinated biphenyls (PCBs) are a class of chlorinated hydrocarbons possessing various degrees of chlorination on a biphenyl ring. The number of chlorine atoms present can vary from two to ten resulting in the generation of different isomers. PCBs were widely used in industry as fluid for transformers and capacitors, plasticizers, adhesives, and fire retardants due to their unique properties such as chemical inertness, thermal stability, and inflammability.1

The presence of PCBs in the environment was first observed by Risebrough and co-workers in 1968.2 PCBs in the environment can cause adverse health effects to all living organisms. Additionally, PCBs are known to be toxic and carcinogenic to humans.3 Elevated levels of PCBs can also alter the levels of the thyroid hormone in infants and pregnant women.4 Due to their environmental and health hazards, the production and use of PCBs has been banned since 1977. However, due to their good thermal and chemical stability, as well as hydrophobic nature, PCBs are well-retained in the environment and can be found in trace levels within water and soil. Therefore, it is of great importance to develop analytical methods that are sensitive, rapid, and robust to quantify the trace levels of PCBs present within the environment.

The analysis of PCBs in environmental matrixes, such as soil or water, typically requires a pre-concentration or sample preparation step. Two techniques most often used
for pre-concentration of PCBs are liquid-liquid extraction (LLE) and solid phase extraction (SPE). However, these pre-concentration methods can be tedious and time consuming. LLE typically requires the use of copious volumes of organic solvents, which makes it environmentally unfriendly. SPE usually requires less organic solvent but may need larger sample volumes and can suffer from breakthrough.

Solid-phase microextraction (SPME) is an additional sample preparation technique first described by Pawliszyn and co-workers in 1990. SPME is widely used due to the fact that it is a simple, rapid, inexpensive, and solvent-free microextraction method. In previous studies, Potter and co-workers demonstrated SPME in the pre-concentration of PCBs from water using the PDMS sorbent coating. Llompart and co-workers showed the application of SPME in the extraction of PCBs from water samples using direct-immersion and headspace extraction modes. Yang and co-workers reported the analysis of PCBs in water using SPME with gas chromatography employing electron capture detection (GC-ECD). It was shown that headspace SPME is more sensitive than direct-immersion SPME for the analysis of PCB congeners in water. SPME was also exploited in the analysis of PCBs from complex matrixes such as human serum, blood plasma, milk, soil, and ash. Augusto and co-workers performed the analysis of PCBs in human milk samples. All of the above methods involved the analysis of PCBs using the commercial PDMS fiber. However, the development of new SPME sorbent materials as coatings is necessary to enhance the sensitivity and selectivity of the method, particularly when performing analysis from complex matrixes. Recently, Wang and co-workers developed fluorinated polyaniline-based (PANI) SPME sorbent coatings for the analysis of various PCB congeners. The limit of detection (LOD) obtained for various
PCBs using the PANI-based fibers were in the sub ng L$^{-1}$ range and relatively lower than the 100 µm PDMS fiber.

Recently, ionic liquids (ILs) have emerged as novel sorbent materials in various microextraction methods including single drop microextraction, liquid phase microextraction, dispersive liquid-liquid microextraction and SPME.$^{11}$ Our group has been particularly interested in designing polymeric ionic liquid (PIL)-based sorbent coatings for SPME.$^{12}$ The recent introduction of an "on-fiber" photo-initiated polymerization approach enables the generation of crosslinked PIL-based sorbent coatings.$^{13}$ Crosslinked PIL-based sorbent coatings possess enhanced mechanical and thermal stability in addition to being highly robust due to the crosslinking and covalent bonding of IL monomers to the silica support.

In this work, two structurally diverse PIL-based crosslinked SPME sorbent coatings were generated for the analysis of PCBs. The PIL-based crosslinked sorbent coatings were fabricated by mixing the 1-vinylbenzyl-3-hexadecylimidazolium bis[(trifluoromethyl)sulfonyl]imide [VBHDIM] [NTf$_2$] IL monomer individually with two different IL crosslinkers, namely, 1,12-di(3-vinylimidazolium)dodecane dibis[(trifluoromethyl)sulfonyl]imide ([DVIM)$_2$C$_{12}$] 2[NTf$_2$]) and 1,12-di(3-vinylbenzylimidazolium)dodecane dibis[(trifluoromethyl)sulfonyl]imide ([DVBIM)$_2$C$_{12}$] 2[NTf$_2$]), respectively. The analytical performance of the novel PIL-based crosslinked coatings was compared to a PDMS coating by using two different detectors, namely, ECD and MS in conjunction with GC. Method validation was performed via recovery experiments in an environmental seawater and a biological bovine milk matrix. This is
the first report to exploit crosslinked PIL-based SPME sorbent coatings for the analysis of PCBs in two complex sample matrixes.

4.2 Experimental

4.2.1 Instrumentation:

A RPR-100 UV reactor containing 16 lamps and a spinning carousel was obtained from Southern New England Ultraviolet Company (Bradford, Connecticut). UV polymerization was performed at a wavelength of 360 nm. An Agilent Technologies 7890 gas chromatograph (Santa Clara, CA, USA) equipped with an electron capture detector as well as a 5975C inert XL MSD with a Triple Axis detector (GC-MS) was used. The separation of 21 PCB congeners by GC-ECD was achieved using a HP-5 capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) obtained from Agilent Technologies (Santa Clara, CA, USA). The separation of analytes by GC-MS was performed using a HP-1 column (30 m × 0.25 mm I.D., 0.25 μm film thickness).

4.2.2 Materials

1-Vinylimidazole, 1,12-dibromododecane, vinyltrimethoxysilane (VTMS), ammonium hydrogen difluoride, and 2-hydroxy-2-methylpropiophenone (DAROCUR 1173) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The PCB mixture containing 100 g mL⁻¹ of twenty one different congeners in acetone was purchased from Accustandard (New Haven, CT, USA). Acetonitrile, acetone, chloroform, methanol, isopropanol, dichloromethane, ethyl acetate, and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A 10 μL Hamilton syringe was obtained from
Hamilton (Reno, NV, USA). A 7 μm PDMS fiber was obtained from Supelco (Bellefonte, PA, USA). Untreated fused silica capillary tubing (0.5 mm I.D), amber glass vials (20 mL), and polytetrafluoroethylene (PTFE) septa caps were purchased from Supelco. Milli-Q water (18.2 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Bovine milk containing one percent fat was purchased from a local market (Toledo, OH, USA). Seawater was obtained from Charleston Bay (Charleston, SC, USA).

4.2.3 Synthesis of ionic liquid monomer and cross-linkers:

The structures of the three ILs used to synthesize the PIL-based sorbent coatings are shown in Table 1. The synthesis of the [VBHDIM] [NTf₂] IL monomer and the \([[(DVIM)_2C_{12}] 2[NTf_2]\) IL cross linker were performed according to previously reported procedures. The synthesis of the IL cross linker \([(DVIM)_2C_{12}] 2[NTf_2]\) was accomplished as shown in supplementary information. Firstly, imidazole was stirred with acrylonitrile in methanol at 45 °C under nitrogen for 5 hours. Compound 1 was obtained by complete removal of methanol under reduced pressure. Two equivalents of compound 1 were then reacted with 1,12-dibromododecane in isopropanol for 24 hours at 70 °C. This was followed by the removal of isopropanol under reduced pressure to obtain compound 2. Compound 2 was then dissolved in 40 mL water and washed 5 times with 10 mL of ethyl acetate.
Table 4.1: IL monomer and crosslinkers used in this study to generate PIL-based sorbent coatings.

<table>
<thead>
<tr>
<th>Sorbent coating</th>
<th>IL monomer + IL crosslinker (2:1)</th>
<th>Approximate Film thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIL 1</td>
<td>![Image of PIL 1 structure]</td>
<td>5</td>
</tr>
<tr>
<td>PIL 2</td>
<td>![Image of PIL 2 structure]</td>
<td>2</td>
</tr>
<tr>
<td>PDMS</td>
<td>![Image of PDMS structure]</td>
<td>7</td>
</tr>
</tbody>
</table>

Chloroform was used to extract compound 3 from the reaction mixture. The chloroform layer was washed multiple times with water to remove excess base. The reaction then proceeded by reacting compound 3 with two equivalents of p-(vinylbenzyl)chloride in chloroform at reflux temperature for 12 hours. The excess solvent was removed under reduced pressure to obtain compound 4, which was then dissolved in 40 mL water and washed five times with 10 mL ethyl acetate. Compound 4 was then reacted with LiNTf₂ in water in a 1:3 stoichiometric ratio for 3 hours at room temperature to obtain compound 65.
5. \( [(DVIM)_{2} C_{12}] 2[NTf_{2}] \). The resulting ionic liquid-based cross linker layer was collected and dried in a vacuum oven at 70 °C for 24 hours. Characterization of the product was performed by \(^1\)H-NMR and ESI-MS, as shown in supporting information.

### 4.2.4 Preparation of SPME fiber:

The PIL-based sorbent coating was prepared by an on-fiber photoinitiated copolymerization approach recently developed by our group.\(^{13}\) Briefly, 1 cm of the fiber's polyimide coating was removed. This was followed by immersing the bare fiber in a 5% (w/v) methanolic solution of ammonium hydrogen difluoride for 30 mins, dried in air for 30 mins, and conditioned in the GC injector port for 1 hour at 250 °C. The etched fiber surface was derivatized by immersing the etched portion of the fiber into 10 mL of VTMS solution for 30 minutes. The derivatized fiber was conditioned by exposing the fiber in the GC injection port at 200 °C for 5 minutes. The etched and derivatized fiber was dip-coated with a mixture containing the IL monomer, crosslinker (50% of monomer weight) and the photoinitiator DAROCUR 1173 (3% by weight). This was followed by exposing the fiber to 360 nm UV light for 30 minutes. The fiber was then conditioned several times at 280 °C for 5 minutes each. Two different PIL-based sorbent coatings were generated, namely PIL 1 and PIL 2. As shown in Table 1, the \([\text{VBHDIM}] [\text{NTf}_2]\) IL monomer was used for both PILs while the IL crosslinkers \( [(DVIM)_{2} C_{12}] 2[\text{NTf}_2] \) and \( [(DVIM)_{2} C_{12}] 2[\text{NTf}_2] \) were employed in PIL 1 and PIL 2, respectively.

### 4.2.5 Headspace solid-phase microextraction of water samples:

It has been shown previously that HS-SPME provides higher extraction efficiencies compared to DI-SPME at elevated extraction temperatures.\(^{7a}\) Therefore, HS-
SPME was chosen as a preferred method for the extraction of PCBs. The mixture of 21 different PCB congeners containing 100 µg mL\(^{-1}\) of each PCB congener was diluted using acetone to prepare a series of stock solutions at concentrations of 10 ng mL\(^{-1}\), 100 ng mL\(^{-1}\) and 1000 ng mL\(^{-1}\). A 20 mL amber glass vial was filled with 15 mL of an aqueous sodium chloride solution (30 % w/v). A certain volume of the PCB stock solution was spiked into the vial and the vial immediately immersed in an oil bath thermostated at 65 °C. After 10 mins, the fiber was exposed to the headspace of the sampling vial for 45 mins. The fiber was then withdrawn and exposed to the GC injection port for 5 mins at 280 °C. The temperature program used for GC-ECD was as follows: initial temperature was set at 60 °C and ramped to 130 °C at 25 °C/min. The temperature was then increased to 300 °C at 8 °C/min and held for 2 mins. The µ-ECD detector temperature was set to 300 °C and the argon/methane make-up flow was set to 60 mL/min. The temperature program used for GC-MS was as follows: initial temperature was set at 60 °C hold and ramped to 130 °C at 15 °C/min. The temperature was then increased to 200 °C at a rate of 5 °C/min and held for 15 mins. Finally, the temperature was increased to 280 °C at 8 °C/min and held for 5 mins. The SIM ions chosen for each PCB during GC-MS analysis are listed in Table 2. It should be noted that no agitation methods employing stir bars were used for the analysis of PCBs in the water samples. It has been shown that PCBs tend to adsorb to the Teflon-coated surface of stir bars and can severely affect the reproducibility due to analyte-to-stir bar carryover.\(^8\)
### Table 4.2: Names, structures, and SIM ions chosen for all studied PCBs.

<table>
<thead>
<tr>
<th>PCB</th>
<th>Name</th>
<th>Structure</th>
<th>SIM ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2,4'-dichlorobiphenyl</td>
<td>![Structure Image]</td>
<td>75, 152, 222</td>
</tr>
<tr>
<td>18</td>
<td>2,2',5-trichlorobiphenyl</td>
<td>![Structure Image]</td>
<td>186, 221, 256</td>
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<tr>
<td>28</td>
<td>2,4,4'-trichlorobiphenyl</td>
<td>![Structure Image]</td>
<td>150, 186, 258</td>
</tr>
<tr>
<td>52</td>
<td>2,2',5,5'-tetrachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>150, 220, 292</td>
</tr>
<tr>
<td>44</td>
<td>2,2',3,5'-tetrachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>220, 257, 292</td>
</tr>
<tr>
<td>66</td>
<td>2,3',4,4'-tetrachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>150, 220, 292</td>
</tr>
<tr>
<td>101</td>
<td>2,2',4,5,5'-pentachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>254, 291, 326</td>
</tr>
<tr>
<td>77</td>
<td>3,3',4,4'-tetrachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>150, 220, 292</td>
</tr>
<tr>
<td>118</td>
<td>2,3',4,4',5-pentachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>184, 256, 326</td>
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<tr>
<td>153</td>
<td>2,2',4,4',5,5'-hexachlorobiphenyl</td>
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<td>2,3,3',4,4'-pentachlorobiphenyl</td>
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<tr>
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<tr>
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<td>3,3',4,4',5-pentachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>184, 256, 326</td>
</tr>
<tr>
<td>187</td>
<td>2,2',3,4',5,5',6-heptachlorobiphenyl</td>
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<td>324, 360, 396</td>
</tr>
<tr>
<td>128</td>
<td>2,2',3,3',4,4',-hexachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>145, 290, 360</td>
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<td>179, 360, 430</td>
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<tr>
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<td>![Structure Image]</td>
<td>324, 360, 394</td>
</tr>
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<td>![Structure Image]</td>
<td>324, 360, 394</td>
</tr>
<tr>
<td>195</td>
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<td>![Structure Image]</td>
<td>179, 360, 430</td>
</tr>
<tr>
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<td>![Structure Image]</td>
<td>392, 430, 464</td>
</tr>
<tr>
<td>209</td>
<td>2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl</td>
<td>![Structure Image]</td>
<td>179, 214, 498</td>
</tr>
</tbody>
</table>
4.2.6 Headspace solid-phase microextraction of milk samples:

To prepare milk sample solutions for analysis, 5 mL of bovine milk (stored at 4 °C) was transferred to a 20 mL amber glass vial and spiked with 400 µL of methanol. The bovine milk sample was then diluted with Milli-Q water at a 1:1 v/v ratio. The sample was then incubated for 24 hours at 4 °C. After incubation, 2.0 g of sodium chloride was added to the sample and the sample was vortexed for 5 mins followed by shaking for an additional 2 mins. A Teflon-coated stirbar was also added to the sample vial to agitate the sample at a rate of 500 rpm during the extraction step. Prior to extraction, the sample was equilibrated at 65 °C for 10 minutes. The fiber was exposed to the headspace for 45 minutes where the temperature was kept constant at 65 °C. After extraction, the fiber was exposed to the GC injector for desorption at 280 °C for 5 minutes. All milk samples were analyzed using GC-ECD.

4.3 Results and Discussion

4.3.1 Effect of extraction temperature and time:

In HS-SPME, elevated temperatures can significantly increase the amount of analytes that partition to the headspace and can increase the overall extraction efficiency of the system. However, an increase in temperature may also decrease the partition coefficient of analytes sorbed to the fiber coating while also increasing the likelihood of analytes leaking from the vial cap, leading to a loss of analyte. Thus, optimizing the extraction temperature is critical for obtaining high analyte extraction efficiency and low LODs. In this study, the PIL 1 fiber was used to evaluate the effect of temperature on the extraction efficiency of PCBs in water by HS-SPME. Three different extraction
temperatures, namely, 45 °C, 65 °C, and 85 °C were evaluated. An extraction time of 45 minutes and salt concentration of 30 % (w/v) were employed. As shown in Figure 4-1, the extraction efficiency of PCBs increased significantly as the extraction temperature was increased from 45 °C to 65 °C. However, as the temperature was increased from 65 °C to 85 °C, the extraction efficiency of all 21 PCBs decreased. This may be due to a combination of the decrease in the coating-to-analyte partition coefficient from high temperatures and leaking of analyte from the sample vial. It is important to note that the variation in the extraction efficiencies of PCB congeners 8, 18, 118, 153, 138, 201 was found to be negligible when the temperature was varied from 45 °C to 65 °C. There is no specific trend correlating to the structure of the PCB but may attributed to the difference in the interaction of the PCB with the fiber sorbent coating. An optimized extraction temperature of 65 °C was used throughout this study.
Figure 4-1: Effect of temperature on the extraction efficiency of all PCBs using the PIL 1 sorbent coating. A concentration of 1 µg L⁻¹ PCBs was spiked in 15 mL aqueous solution containing 30 % (w/v) sodium chloride. Separation and detection was achieved by using GC-ECD. (A) 8 (▲), 18 (○), 28 (▲), 52 (△), 44 (■), 66 (●), 101 (□), 77 (◇), 118 (◇), 153 (+) and (B) 105 (○), 138 (□), 126 (○), 187 (○), 128 (▲), 201 (※), 180 (+), 170 (■), 195 (●), 206 (▲), 209 (△).

The optimization of extraction time can have profound effects on the extraction efficiency of an analyte. With an increase in extraction time, higher amounts of analytes
can be sorbed to the fiber coating until a steady-state is reached. The effect of extraction time on the extraction performance of 21 PCBs was evaluated by examining four different extraction times (i.e., 15, 30, 45, and 60 min). As shown in Figure 4-2, the extraction efficiencies of all PCBs increased significantly as the extraction time increased from 15 to 45 min. An extraction time longer than 45 min resulted in a slight decrease in the extraction efficiencies, a likely result of analyte adsorption to the sample vial surface.\textsuperscript{16} Hence, 45 minutes was used as an optimized extraction time for subsequent HS-SPME measurements.
Figure 4-2: Effect of extraction times on the extraction efficiency of different PCBs using the PIL 1 sorbent coating. (A) 8 (○), 18 (■), 28 (▲), 52 (△), 44 (■), 66 (●), 101 (▲), 77 (★), 118 (×), 153 (●) and (B) 105 (○), 138 (×), 126 (★), 187(△), 128 (▲), 201 (■), 180 (□), 170 (■), 195 (●), 206 (●), 209 (+). A PCB concentration of 1 µg L$^{-1}$ in 30 % (w/v) aqueous sodium chloride solution was chosen for the extraction. The extraction was performed at 65 °C.
4.3.2. Effect of salt concentration:

It is well-known that the addition of kosmotropic salts to an aqueous sample solution can decrease the solubility of organic analytes, especially non-polar compounds, and increase the partitioning of these compounds to the sample headspace. Using the commercial PDMS fiber, the effect of salt concentration on the extraction efficiency of various PCBs has been controversial within the literature. Llompart and co-workers saturated an aqueous PCB sample with potassium chloride and observed no effect on the extraction efficiency while using a 100 µm PDMS fiber. A decrease in the extraction efficiency of PCBs from water samples was observed by Shu and co-workers when sodium chloride was added and the extraction performed using a 100 µm PDMS fiber. Contrarily, Wang and co-workers observed an increase in the extraction efficiency of PCBs with an increase in sodium chloride concentration when using the fluorinated PANI-based sorbent coating. Since a number of mixed results have been reported from the addition of salt to the matrix, the effect of salt concentration using the crosslinked PIL-based coatings was investigated.

As shown in Figure 4-3, sodium chloride was added to the aqueous samples at various concentrations (0 to 35 % w/v) to evaluate the effect on the PCB extraction efficiency using the PIL 1 fiber. When the concentration of sodium chloride was increased from 0 to 20 % (w/v), no significant increase in the extraction of PCBs was observed. This is in agreement with the observations of Shu and co-workers. However, the extraction efficiencies increased significantly for all PCBs when the salt concentration was increased from 20 to 30 % (w/v). The extraction efficiency of most PCBs leveled off and remained constant when the salt concentration was increased from
30 to 35 % (w/v). Therefore, a 30 % (w/v) salt concentration was applied for all subsequent studies involving SPME analysis of water samples.

Figure 4-3: Effect of sodium chloride concentration on the extraction efficiency of all PCBs using the PIL 1 sorbent coating. (A) 8 (Δ), 18 (○), 28 (▲), 52 (x), 44 (○), 66 (■), 101 (+), 77 (●), 118 (■), 153 (●) and (B) 105 (■), 138 (★), 126 (○), 187(x), 128 (+), 201 (○), 180 (▲), 170 (○), 195 (■), 206 (●), 209 (▲). A PCB concentration of 1 µg L\(^{-1}\) in 30 % (w/v) aqueous sodium chloride solution was chosen for the study. The extraction time and temperature were maintained at 45 min and 65 °C, respectively.
4.3.3. Analytical performance of selected coatings in the extraction of PCBs using headspace SPME

The analytical performance was studied for all coatings in order to explore their differences in selectivity for the PCBs. Calibration curves of the PCBs, obtained by GC-ECD, in an aqueous sample solution containing 30 % (w/v) sodium chloride were constructed for the PIL 1, PIL 2, and PDMS coatings. As shown in Table 5.1, the approximate film thicknesses of PIL 1 (5 μm) and PIL 2 (2 μm) are smaller than that of the 7 μm PDMS coating. As shown in Tables 4.3, 4.4 and 4.5, the linear range of all fibers varied from 2.5 ng L^{-1} to 100 ng L^{-1}. The LODs were determined by decreasing the analyte concentration until a 3:1 signal: noise (S:N) ratio was achieved. In the case of the PDMS coating, the LOD ranged from 1-20 ng L^{-1} when using ECD and 2.5 ng L^{-1} when using MS detection. The precision of the method using the PDMS coating was slightly higher than the PIL-based coatings and ranged from 1.6-21.5 % and 4.9-22.4 % using ECD and MS detection, respectively. Similar to the PDMS coating, the crosslinked PIL-based coatings were highly applicable in the determination of trace-level PCBs from a simple water matrix. The LODs of all PCBs using PIL 1 ranged from 1-2.5 ng L^{-1} and 2.5-25 ng L^{-1} while the precision ranged from 2.0-19.1 % and 0.3-19.5 % for ECD and MS detection, respectively. The analytical performance of PIL 2 fiber for the extraction of PCBs in terms of LOD ranged 1-7.5 ng L^{-1} using ECD detection while a LOD of 2.5 ng L^{-1} was achieved using MS detection. The precision for PIL 2 fiber for the extraction of PCBs ranged from 0.7-20.7 % and 4.1-18.8 % using ECD and MS detection, respectively.
Table 4.3: Figures of merit for the PDMS fiber in the extraction of PCBs from deionized water using headspace SPME GC/MS and GC/ECD at 65 °C.  

<table>
<thead>
<tr>
<th>PCB</th>
<th>Linear Range</th>
<th>LOD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LOD&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% RSD&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% RSD&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>(ng L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td>(ng L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10-100</td>
<td>5.38 ± 0.130</td>
<td>0.999</td>
<td>7.5</td>
<td>21.3</td>
<td>13.7</td>
</tr>
<tr>
<td>18</td>
<td>7.5-100</td>
<td>10.7 ± 0.259</td>
<td>0.998</td>
<td>5</td>
<td>12.2</td>
<td>15.2</td>
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<tr>
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<td>7.5-100</td>
<td>20.3 ± 0.872</td>
<td>0.995</td>
<td>5</td>
<td>9.5</td>
<td>13.7</td>
</tr>
<tr>
<td>52</td>
<td>2.5-100</td>
<td>13.6 ± 0.349</td>
<td>0.997</td>
<td>1</td>
<td>3.1</td>
<td>16.2</td>
</tr>
<tr>
<td>44</td>
<td>2.5-100</td>
<td>28.3 ± 0.953</td>
<td>0.995</td>
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<td>2.0</td>
<td>13.9</td>
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<td>5.0</td>
<td>13.6</td>
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<td>9.4</td>
<td>12.2</td>
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<td>9.1</td>
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<td>8.3</td>
<td>11.8</td>
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<td>1</td>
<td>5.1</td>
<td>8.0</td>
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<td>50.5 ± 0.792</td>
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<td>9.7</td>
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<td>0.998</td>
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<tr>
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<td>166 ± 2.65</td>
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<td>1</td>
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<td>209</td>
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<td>125 ± 1.35</td>
<td>0.999</td>
<td>1</td>
<td>4.8</td>
<td>22.4</td>
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<sup>a</sup> SD: Error of the slope for n = 7.  
<sup>b</sup> Determined by decreasing the analyte concentration until a 3:1 S:N ratio was achieved.  
<sup>c</sup> Determined by performing repeated experiments at 100 ng L<sup>-1</sup> (n = 4).  
<sup>†</sup> Data obtained by GC/ECD.  
<sup>‡</sup> Data obtained by GC/MS.
Table 4.4: Figures of merit for the PIL 1 fiber chosen in the extraction of PCBs from deionized water using headspace SPME GC/MS and GC/ECD at 65 °C.  

\(^{a}\) Error of the slope for n = 7.  
\(^{b}\) Determined by decreasing the analyte concentration until a 3:1 S:N ratio was achieved.  
\(^{c}\) Determined by performing repeated experiments at 100 ng L\(^{-1}\) (n = 4).  
\(^{†}\) Data obtained by GC/ECD.  
\(^{‡}\) Data obtained by GC/MS.  
(-) Data not available.

<table>
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<tr>
<th>PCB</th>
<th>Linear Range ((\text{ng} \text{ L}^{-1}))</th>
<th>Slope ± SD (^{†})</th>
<th>(R^{†})</th>
<th>LOD (^{‡}) ((\text{ng} \text{ L}^{-1}))</th>
<th>% RSD (^{‡})</th>
<th>LOD (^{‡}) ((\text{ng} \text{ L}^{-1}))</th>
<th>% RSD (^{‡})</th>
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<tr>
<td>8</td>
<td>5-100</td>
<td>11.5 ± 0.226</td>
<td>0.999</td>
<td>2.5</td>
<td>6.7</td>
<td>2.5</td>
<td>19.5</td>
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<td>5-100</td>
<td>12.6 ± 0.279</td>
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<td>2.5</td>
<td>10.2</td>
<td>2.5</td>
<td>16.6</td>
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<td>30.1 ± 0.863</td>
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<td>8.1</td>
<td>2.5</td>
<td>12.6</td>
</tr>
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<td>36.1 ± 0.808</td>
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<td>24.2 ± 0.577</td>
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<td>17.1</td>
<td>2.5</td>
<td>7.5</td>
</tr>
<tr>
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Table 4.5: Figures of merit for the PIL 2 fiber in the extraction of PCBs from deionized water using headspace SPME GC/MS or GC/ECD at 65 °C. a SD: Error of the slope for n = 7. b Determined by decreasing the analyte concentration until a 3:1 S:N ratio was achieved. c Determined by performing repeated experiments at 100 ng L⁻¹ (n = 4). † Data obtained by GC/ECD. ‡ Data obtained by GC/MS.

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<th>LODb†</th>
<th>% RSD†</th>
<th>LODb‡</th>
<th>% RSD‡</th>
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<td>9.1</td>
<td>2.5</td>
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<td>1</td>
<td>12.3</td>
<td>2.5</td>
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<td>206</td>
<td>2.5-100</td>
<td>90.5 ± 2.82</td>
<td>0.996</td>
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<td>20.4</td>
<td>2.5</td>
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<td>209</td>
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<td>64.1 ± 2.51</td>
<td>0.994</td>
<td>1</td>
<td>17.3</td>
<td>2.5</td>
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</table>

A bar graph describing the sensitivities obtained for all coatings is shown in Figure 4-4. The sensitivity, defined as the slope of the calibration curve, varied significantly when different coatings were used. Compared to the crosslinked PIL-based coatings, lower sensitivities were obtained using the PDMS coating for congeners with lower degrees of chlorine substitution. As the degree of substitution increased to more than four chlorine atoms, the PDMS coating possessed noticeably higher sensitivities compared to PIL 1. The PIL 1 coating showed the highest sensitivity for PCBs containing...
the highest and lowest degrees of substitution, namely PCBs 8, 18, 28, 206, and 209. However, the sensitivities of many other congeners, such as those containing four to six chlorine atoms, were relatively lower to those obtained with the PDMS and PIL 2 coatings.

![Figure 4-4: Comparison of the sensitivities obtained for all studied PCBs using the (□) PDMS, (◼) PIL 1, and (■) PIL 2 sorbent coatings.](image)

The PIL 2 coating exhibited up to a two-fold increase in sensitivity for congeners containing four to eight chlorine atoms when compared to the other coatings. This may be due to the aromatic moieties specifically tailored within the structure of the dicationic IL crosslinker in addition to the benzyl moiety present in the monocationic IL monomer. Analogous to previous studies employing PILs comprised of aromatic substituents for the extraction of aromatic analytes 16, 19, the benzyl moieties tailored to both the monomer and crosslinker can enhance π-π interactions with the PCBs, leading to higher analyte sensitivity and selectivity. Although PIL 2 exhibited superior sensitivities for most PCBs, this coating showed relatively lower sensitivity for congeners containing the highest
degrees of chlorine substitution, namely PCBs 206 and 209. Overall, both PIL 1 and PIL 2 coatings exhibit unique selectivity and often similar or better sensitivity for all PCB congeners compared to the PDMS coating, even though they possess smaller film thicknesses.

4.3.4. Method validation and recovery in real-world samples

Ocean water and bovine milk were chosen as real-world matrixes for the extraction of the PCBs in order to demonstrate the applicability of the proposed method. Ocean water samples were prepared by spiking 30 ng L\(^{-1}\) of PCBs into a 20 mL sample vial containing 15 mL ocean water with the addition of 30 % (w/v) NaCl. Extractions were performed at 65°C via headspace SPME-GC/MS. Prior to the analysis, blank extractions were performed to ensure no analyte was present in the sample matrix. As shown in Table 4-6, the relative recoveries of the PCBs from ocean water ranged from 89.7-136.1 %, 61.2-115.6%, and 76.0-135.7% for the PDMS, PIL 1, and PIL 2 fibers, respectively. The proposed method proves to be highly applicable in real-water matrixes as the concentration of PCBs chosen (in parts-per-trillion range) approaches the actual concentrations of PCBs found in various water sources.\(^{20}\)
Table 4.6: Relative recoveries of all PCBs from ocean water using selected SPME sorbent coatings. An analyte concentration of 30 ng L\(^{-1}\) was chosen for the analysis using HS-SPME GC/MS. (−) Data not available.

<table>
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<tr>
<th>PCB</th>
<th>PIL 1</th>
<th>PIL 2</th>
<th>PDMS</th>
</tr>
</thead>
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<td>8</td>
<td>-</td>
<td>109.0 ± 14.4</td>
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<td>18</td>
<td>61.9 ± 9.5</td>
<td>117.3 ± 6.6</td>
<td>99.0 ± 22.4</td>
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<td>72.9 ± 21.3</td>
<td>112.9 ± 6.5</td>
<td>93.2 ± 12.7</td>
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<td>61.2 ± 27.1</td>
<td>115.5 ± 10.9</td>
<td>101.0 ± 17.1</td>
</tr>
<tr>
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<td>79.1 ± 12.5</td>
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<td>105.1 ± 10.4</td>
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<td>97.8 ± 8.5</td>
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<td>98.3 ± 14.3</td>
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<td>81.1 ± 15.8</td>
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<td>111.5 ± 14.2</td>
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</table>

Various experimental parameters were modified in the extraction of bovine milk samples. Unlike ocean water, bovine milk contains proteins, carbohydrates, and lipids which can severely interfere with analysis. In an effort to decrease the effects of matrix interference\(^{21}\) as well as increase the extraction efficiency of the PCBs, milk samples were diluted with de-ionized water at a 1:1 (v:v) ratio. Furthermore, agitation using a Teflon-coated stir bar was employed to ensure thorough mixing of the sample components during extraction and to also prevent coagulation of the milk samples. Finally, 4 % methanol (v/v) was added to sample solution to minimize any non-specific analyte adsorption to the sampling vial wall.\(^{10d}\) Samples were prepared by spiking 15 or 60 g L\(^{-1}\) of PCBs into a 20 mL sample vial containing 10 mL of the milk/water mixture.
with the addition of 20 % (w/v) NaCl. Headspace SPME was performed under agitation at 65°C with ECD detection. As shown in Table 4.7, the relative recoveries of most PCBs from the milk/water mixture were in acceptable ranges for all three coatings at a spiking concentration of 60 μg L⁻¹. The recovery of all PCBs at this concentration ranged from 81.9-110.4 %, 96.7-132.5 %, and 89.3-120.2 % for the PDMS, PIL 1, and PIL 2 coatings, respectively. It is also interesting to note that recoveries could not be determined for congeners 201 to 209 using the PDMS coating since this spiking concentration exceeded the linear range of this fiber. At the lower spiking concentration (15 μg L⁻¹), the PDMS coating produced much better recoveries. The recoveries ranged from 94.3-121.6 %, 68.9-131.1 %, and 60.4-138.4 % for the PDMS, PIL 1, and PIL 2 coatings, respectively.
Table 4.7: Relative recoveries of all PCBs from bovine milk using selected SPME sorbent coatings. Analyte concentrations of 15 and 60 g L$^{-1}$ were chosen for the analysis using HS-SPME GC/ECD. (-) Data not available.

<table>
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4.4 Conclusions

The successful application of HS-SPME using crosslinked PIL-based sorbent coatings was demonstrated for the extraction of PCBs from water and bovine milk samples. The crosslinking of IL monomer significantly increased the structural integrity and thermal stability of PIL-based sorbent coating making it applicable to more complex matrixes. The crosslinked PIL-based SPME fibers proved to be superior in terms of sensitivity and exhibited comparable LODs to the commercial PDMS fiber with significantly lower coating film thickness. The enhanced selectivity towards PCBs exhibited by the PIL-based coatings can be partly attributed to π-π interactions due to the introduction of aromatic groups to the IL monomer and IL crosslinker. The LODs for PCBs in aqueous samples were found in the sub ng L\(^{-1}\) range using ECD and MS detection. Recovery studies in both environmental and complex biological matrixes were in acceptable ranges. Based on this work, the benzyl-functionalized crosslinked PIL-based SPME coatings have proven to be useful sorbent coatings in the analysis of higher boiling compounds. Future investigations into the robustness of the SPME sorbent coatings will be explored by examining the extraction performance within biological samples for a broader range of analytical applications.

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Systems Division from the National Science Foundation for a CAREER grant (CHE-0748612).
References


Chapter 5

Overview: Suzuki-Miyuara reaction and detection of 2-APYBA in pinacolboronate ester using HPLC

5.1 Suzuki-Miyaura coupling reaction

The carbon-carbon cross coupling reaction is one of the most important and challenging reactions in industry for preparing various organic compounds including drugs. The Suzuki-Miyaura coupling reaction was introduced in 1979.\(^1\) This reaction facilitates the carbon-carbon bond formation between organoboron compounds and organohalides in the presence of palladium catalyst and an appropriate base. The detailed catalytic cycle is demonstrated in Figure 5-1. This cross coupling reaction is consist of three main steps. The first step is a oxidative addition reaction of organohalide with Pd(0) to form adduct 1. It is also considered to be a rate limiting step of the reaction depending on the type of organohalide. The general reactivity trend described by Suzuki et al. in decreasing order is as follows: I > OTf > Br >> Cl.\(^2\) Adduct 1 then undergoes the transmetalation step with organoboron compound to generate adduct 2. The last step involves the reductive elimination where carbon-carbon cross coupling is achieved.
The Suzuki-Miyaura reaction is highly preferred by the synthetic community and industry due to the following reasons. 1) the reaction conditions required for this reaction are relatively mild, 2) this reaction can tolerate many different groups attached to organoboron compound and organohalide and, 3) this reaction generates the least toxic impurities. The main factors affecting the reaction are the type of catalyst, type of base, different organohalides and organoborons. The types of organohalides than used are numerous. Similarly, several different types of organoboron compounds, namely, boronic acids, organoboranes, organotrifluoroborates and boronate esters are being used to generate various organic structures. The boronic acids are known to form boroxine in the solution which can make it difficult to obtain completely pure product. Boronic acids are difficult to purify and can undergo multiple degradation pathways such as oxidation, protodeboronation, and polymerization to generate undesired side products. Organoborane compounds are vulnerable to air and must be generated in situ for the cross coupling reaction.
coupling reaction which makes it less viable option. Boronate esters are often the first choice as they are very stable at ambient condition as well as stable in the presence of air and moisture. However, boronate esters are generated from corresponding boronic acids and can be hydrolysed back if exposed to water at high pH condition.

5.2 HPLC method for determination of pinacolboronate ester purity

The Suzuki-Miyaura cross coupling reaction is one of the most important reactions employed in the pharmaceutical industry for the generation of different drug molecules. However, achieving desired purity of the drug is very important. One of the organoboron compounds used widely in this reaction is pinacolboronate ester. Due to the hydrolysis of pinacolboronate ester, the corresponding boronic acid is generated. The hydrolysis process is shown in Figure 6-2. If the pinacolboronate ester containing boronic acid as an impurity is used in Suzuki-Miyaura reaction, it can generate significant impurity in the final product which is an active pharmaceutical ingredient (API). In order to prepare impurity free API, the purity of pinacolboronate ester (one of the starting material in Suzuki-Miyaura reaction) should be determined. This represents a great analytical challenge as there are several challenges associated with creating robust analytical separation methods. First of all, the pinacolboronate ester can be hydrolyzed on to the column to generate corresponding boronic acid. In order to circumvent this challenge Duran and co-workers developed a fast reversed phase HPLC method for the analysis of boronic acid and boronate esters. However, some of the boronic acid impurities such as 2-aminopyrimidine-5-ylboronic acid (2-APYBA) will not dissolve in any organic solvents as well as water. The solubility of 2-APYBA can be achieved using
very high pH (~ 12) buffer only. Recently, Zhong and co-workers have demonstrated the successful separation of 2-aminopyrimidine-5-pinacolboronate ester and its impurity 2-APYBA. Various HPLC columns were examined but no separation was achieved. Due to the requirement of very basic condition for solubilizing 2-APYBA, reversed phase silica-based column cannot be used. Finally the poly(styrene-divinyl benzene)-based HPLC column was able to separate them in a reversed phase mode. However, the impurity 2-APYBA was not retained on this column as it was very hydrophilic in high pH phosphate buffer. Efforts were made to retain 2-APYBA using ion pairing reagent. However, the retention was hardly improved insignificantly.

The following Chapter describes the detailed study performed for the use of newly designed glucaminium-based ionic liquids as a derivatizing agent for 2-APYBA. The IL-(2-APYBA) complex was successfully separated using HPLC and complexation was confirmed using $^{11}$B NMR.
References


Chapter 6

Chromatographic detection of 2-aminopyrimidine-5-ylboronic acid as an impurity in suzuki coupling reagent pinacolboronate ester using glucaminium-based ionic liquids

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Abstract

The HPLC method was developed for the detection of boronic acid impurity present in pinacolboronate ester. Pinacolboronate ester is one of the key reagents in Suzuki-Miyaura coupling reaction which undergoes hydrolysis to generate the corresponding boronic acid. The retention of this boronic acid on column was achieved using newly synthesized glucaminium-based ILs \text{N,N-bis(4-(methoxycarbonyl)benzyl)-N-methyl glucaminium bromide} and \text{N,N-dibenzyl-N-methyl-D-glucaminium bromide.} The complexation of glucaminium-based ILs with 2-aminopyrimidine-5-ylboronic acid was thoroughly studied using $^{11}$B-NMR. The complexes formed between glucaminium ILs and 2-aminopyrimidine-5-ylboronic acid were retained significantly unlike free 2-
aminopyrimidine-5-ylboronic acid which eluted at column dead time. The analytical performance was also evaluated for this HPLC method. The quantification of 2-aminopyrimidine-5-ylboronic acid in real pinacolboronate ester sample was achieved successfully using this newly developed HPLC method based on glucaminium ILs.

6.1 Introduction

Carbon-carbon bond formation or biaryl coupling is important for the generation of complex organic molecules. The Suzuki-Miyaura coupling reaction, discovered in 1979, is one of the most widely used carbon-carbon bond formation reactions used by the pharmaceutical industry due to its high efficiency as well as the low toxicity of the reagents.\(^1\) This involves a palladium catalyzed reaction where organoboron compounds (i.e., organoboranes, boronic acids, boronate esters or organotrifluoroborates) react with organohalides or pseudohalides. Among these organoboron compounds, organoboranes are air sensitive, difficult to purify, and often require \textit{in situ} generation which generally limit their use.\(^2\) Boronic acids are less appealing as they can undergo cyclization to form boroxines, which ultimately may affect the reaction stoichiometry and require an excess amount of the boronic acid.\(^2\) In addition, boronic acids can undergo multiple degradation pathways such as oxidation, protodeboronation, and polymerization to generate undesired side products.\(^3\) To overcome this, the free hydroxyl groups can be masked by the pinacol moiety to generate pinacolboronate esters.

Pinacolboronate esters are relatively stable to air, easy to purify, and require no deprotection step. However, pinacolboronate esters are vulnerable to hydrolysis in aqueous solution and generate the corresponding boronic acid. The extent of hydrolysis
depends greatly upon the presence of electron withdrawing or electron donating groups as well as the pH of the solution.\textsuperscript{4} Therefore, it is very important to know the extent of hydrolysis (i.e., the amount of boronic acid present) within the corresponding pinacolboronate ester. Boronic acids are less volatile and may have very limited solubility in organic solvents compared to boronate esters. The difference in solubility of boronate esters and boronic acids leads to a very challenging analytical problem when it is desired to analyze both species using high performance liquid chromatography (HPLC). Duran and co-workers developed a fast reversed phase HPLC method for the analysis of boronic acid and boronate esters used in the pharmaceutical industry.\textsuperscript{5} However, some of the boronic acids, such as 2-aminopyrimidine-5-ylboronic acid (2-APYBA), exhibit very limited solubility in many organic solvents and therefore require a different approach. Zhong and co-workers developed a reversed phase HPLC method using a polymeric column and gradient program employing two solvent systems consisting of phosphate buffer (pH=12) and acetonitrile/water (80/20).\textsuperscript{6} The different impurities within 2-aminopyrimidine-5-pinacolboronate ester (2-APPBE) were well separated using this method. However, the boronic acid impurity, 2-APYBA, eluted at the dead time of the column. The retention of 2-APYBA was improved by only 0.2 minutes by the use of an ion pairing reagent. The low retention of this analyte leads to challenges in the quantification of 2-APYBA within 2-APPBE. Therefore, the development of new methodologies capable of modulating the retention of 2-APYBA is greatly needed.

Recently, it has been shown by our group that glucaminium-based ionic liquids can form monochelate and bischelate complexes with boric acid at pH 4.8 and higher.\textsuperscript{7} Using
\(^{11}\)B NMR, it was shown that the borate ion undergoes complexation with the cis-diol moiety within the glucaminium cation to form the aforementioned complexes.\(^8\) The similarities of the borate ion and the boronate ion led us to examine the possibility of modulating the retention of the glucaminium-boronate complex to allow for quantitative determination of free boronic acid. Two new ILs, N,N-bis(4-(methoxycarbonyl)benzyl)-N-methyl glucaminium bromide (IL 1) and N,N-dibenzyl-N-methyl-D-glucaminium bromide (IL 2), were synthesized and evaluated in this study. This method is simple, rapid, and requires very small amounts of IL to be added to the diluent to allow for complex formation prior to separation using reversed phase chromatography. As opposed to ion pairing reagents, the IL is not added to the mobile phase due to high stability of the IL-boronate complex.

### 6.2. Experimental

#### 6.2.1 Materials

N-methyl-D-glucamine, methyl-4-(bromomethyl)benzoate and benzyl bromide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, N,N-dimethylformamide, sodium carbonate, tripotassium phosphate, and sodium chloride were purchased from Fisher Scientific, (Fair Lawn, NJ, USA). 2-aminoypyrimidine-5-ylboronic acid and 2-aminoypyrimidine-5-pinacolboronate ester were provided by Genentech, (San Francisco, CA, USA). All solutions were prepared using Milli-Q water (18 MΩ.cm).
6.2.2 Instrumentation

High-performance liquid chromatographic analysis was performed using a LC-20A liquid chromatograph (Shimadzu, Japan) with two LC-20AT pumps, a SPD-20 UV/VIS detector, and a DGU-20A3 degasser. All separations were carried out using a PLRP-S polymeric reversed phase column (styrene divinyl benzene copolymer, 150 × 4.6 mm i.d, 3 µm particle size, 100 Å pore size) with a guard column (styrene divinyl benzene copolymer, 5 × 3 mm i.d, 3 µm particle size, 100 Å pore type) from Agilent (Santa Clara, CA, USA).

6.2.3 Synthesis of glucaminium-based ILs

The synthesis of the functionalized glucaminium-based ILs utilized in this study was performed according to our previous report, with a few minor modifications. The synthetic scheme is described in Figure 6-1. Firstly, 5.0 g of N-methyl-D-glucamine (0.026 mol) and 13.6 g of sodium carbonate (0.13 mol) were mixed and stirred in methanol (100 mL) at 45 °C for 30 minutes. A methanolic solution of methyl-4-(bromomethyl)benzoate or benzyl bromide was prepared by dissolving 0.08 moles of each in 10 mL of methanol. This solution was slowly added to the reaction mixture over the course of one hour. The reaction mixture was stirred at 45 °C for 72 hours. The mixture was then filtered to remove sodium bromide and excess sodium carbonate followed by removal of methanol under reduced pressure. The dried product was then
dissolved in ethanol (50 mL) and filtered to remove the salt followed by the removal of ethanol under reduced pressure at 40 °C for 3 hours.

An extra purification step was performed for IL 2. The crude IL 2 product was dissolved in chloroform (50 mL) and filtered to further remove the salt. This was followed by the removal of chloroform under reduced pressure at 30 °C for 4 hours. Finally, IL 1 and IL 2 were dissolved in water (50 mL) and washed five times with 15 mL of ethyl acetate to remove excess starting material. After purification, water was removed under reduced pressure for 5 hours at 70 °C and the product subsequently dried in a vacuum oven for 24 hours to obtain the highest purity. Both ILs were characterized using ¹H NMR, ¹³C NMR and ESI-MS. All NMR and mass spectra are provided as supplementary data.

Figure 6-1: Synthetic scheme used to generate the two functionalized glucaminium-based ionic liquids employed in this study.
6.2.4 Sample preparation and chromatographic conditions

Mobile phase A was prepared by mixing 20 mL of 0.5 M tripotassium phosphate solution in 980 mL Milli-Q water. The pH of the phosphate buffer was adjusted to 12.4 by dissolving 4.5 g of sodium hydroxide. Stock solutions of 2-APYBA (10 mM) and IL 1 (10 mM) were individually prepared in 10 mM potassium phosphate buffer. The stock solution of IL 2 (10 mM) was prepared in acetonitrile/water (67/33, v/v). The samples for HPLC analysis were prepared by mixing different volumes of 2-APYBA and IL stock solutions in a 2.0 mL microcentrifuge tube. The sample solutions were kept at room temperature for 30 minutes before injection. A sample volume of 5 µL was injected onto the column. A flow rate of 0.5 mL/min was used throughout the study. The column temperature was maintained using a thermostatted water bath.

The different chromatographic parameters used for the separation of the (2-APYBA)-IL complex in the presence of IL 1 and IL 2 are described within Table 1, unless otherwise noted within the text. In both cases, 10 mM phosphate buffer (pH=12) was used as the diluent. The major difference in chromatographic
Table 6.1: Chromatographic conditions employed for the analysis of 2-aminopyrimidine-5-ylboronic acid (2-APYBA) and boronic acid-IL complex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Part I: Condition for analysis of (2-APYBA)-IL 1 complex</th>
<th>Part II: Condition for analysis of (2-APYBA)-IL 2 complex</th>
<th>Part III: Condition for analysis of 2-APYBA in 2-APPBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>PLRP-S 100A column, 150 mm × 4.6 mm, 3 μm</td>
<td>PLRP-S 100A column, 150 mm × 4.6 mm, 3 μm</td>
<td>PLRP-S 100A column, 150 mm × 4.6 mm</td>
</tr>
<tr>
<td>Column temperature</td>
<td>35 °C</td>
<td>35 °C</td>
<td>35 °C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.5 mL/min</td>
<td>0.5 mL/min</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 μL</td>
<td>5 μL</td>
<td>5 μL</td>
</tr>
<tr>
<td>Ionic liquid used</td>
<td><strong>IL 1</strong></td>
<td><strong>IL 2</strong></td>
<td><strong>IL 2</strong></td>
</tr>
<tr>
<td>Sample diluent</td>
<td>2-APYBA and IL 1: 10.0 mM potassium phosphate buffer (pH=12)</td>
<td>2-APYBA: 10.0 mM potassium phosphate buffer (pH=12)</td>
<td>2-APPBE and <strong>IL 2</strong>: DMF</td>
</tr>
<tr>
<td></td>
<td><strong>IL 2</strong>: acetonitrile/water (67/33, v/v)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nominal concentration</td>
<td>2-APYBA: 2.0 mM</td>
<td>2-APYBA: 2.0 mM</td>
<td>2-APPBE: 9.0 mM</td>
</tr>
<tr>
<td></td>
<td><strong>IL 1</strong>: 2.0-20.0 mM</td>
<td><strong>IL 2</strong>: 2.0-6.0 mM</td>
<td><strong>IL 2</strong>: 4.5-18.0 mM</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>293 nm</td>
<td>293 nm</td>
<td>293 nm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A: 10 mM potassium phosphate buffer (pH=12)</td>
<td>A: 10 mM potassium phosphate buffer (pH=12)</td>
<td>A: 10 mM potassium phosphate buffer (pH=12)</td>
</tr>
<tr>
<td></td>
<td>B: Water</td>
<td>B: acetonitrile/water (80/20, v/v)</td>
<td>B: acetonitrile/water (80/20, v/v)</td>
</tr>
<tr>
<td>Gradient program</td>
<td>Time (min) A (%) B (%)</td>
<td>Time (min) A (%) B (%)</td>
<td>Time (min) A (%) B (%)</td>
</tr>
<tr>
<td></td>
<td>0 99 1</td>
<td>0 90 10</td>
<td>0 99 1</td>
</tr>
<tr>
<td></td>
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<td>2 99 1</td>
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<td>22 70 30</td>
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<td>32 70 30</td>
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<td>45 100 0</td>
<td>35 100 0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50 100 0</td>
</tr>
</tbody>
</table>
conditions involve the use of different mobile phase B and a different gradient program. In the case of IL 1, water was used as the mobile phase unlike 80/20 ACN/water, which was used for IL 2. In the analysis of the real 2-APPBE sample, N,N-dimethylformamide was used as the diluent. The chromatographic conditions employed for the real sample were similar to the conditions used for IL 2 except for a different gradient program.

6.2.5 Acquisition of $^{11}$B NMR spectra

$^{11}$B-NMR experiments were performed using a Varian VXRS 400 MHz NMR spectrometer at room temperature (19 ± 1 °C). All measurements were performed in a 5 mm quartz tube obtained from Wilmad (Vineland, NJ, USA) using a resonance frequency of 128.0 MHz. Stock solutions of 0.1 M IL and 0.1 M 2-APYBA were prepared in Milli-Q water and phosphate buffer (pH=12.4), respectively. Samples for $^{11}$B-NMR analysis were prepared by mixing 2-APYBA with different volumes of IL stock solution. The amount of D$_2$O was maintained at 10% of the total sample volume. Other parameters used in acquiring the $^{11}$B-NMR spectra included a 0.06 second pulse repetition time, 75 ° flip angle and 27.0 KHz spectral width with a total number of 8064 scans being recorded.

6.3. Results and Discussion

6.3.1 $^{11}$B NMR study of the (2-aminopyrimidine-5-ylboronic acid)-glucaminium IL complex

In aqueous solution, 2-APPBE can undergo hydrolysis to generate the corresponding boronic acid, 2-APYBA, as shown in Figure 6-2. The pKa of 2-APYBA has been estimated to be 10.9.$^6$ Depending on the pH, the boron atom of 2-APYBA exists
in the neutral trigonal form (A) and/or the tetrahedral boronate ion (B) in aqueous solution, as shown in Figure 3.

![Diagram of hydrolysis reaction](image)

**Figure 6-2:** Hydrolysis of 2-aminopyrimidine-5-pinacolboronate ester to form the corresponding boronic acid.

In our previous study, complexation of N-decyl-N-methyl-D-glucaminium bromide ([C_{10}-NMDG][Br]) and boric acid was studied using $^{11}$B NMR.\textsuperscript{7,8} Hence, a similar approach was undertaken to examine whether or not complexation occurs between 2-APYBA and the [C_{10}-NMDG][Br] IL. A complex peak was observed and increased significantly as the concentration of the [C_{10}-NMDG][Br] IL was increased within the NMR tube (data not shown). However, when subjected to HPLC, the complex was not well retained and overlapped with the free 2-APYBA peak, which eluted at the dead time. In an effort to increase retention on the PLRP-S stationary phase, the glucaminium-based ILs were functionalized with aromatic groups (see Figure 6-1) to enhance $\pi-\pi$ interactions between the IL and stationary phase.
Figure 6-3: Equilibria of free 2-aminopyrimidine-5-ylboronic acid, 2-aminopyrimidine boronate ion, and glucaminium IL-boronate complex in aqueous solution

The complexation behavior of IL 1 with 2-APYBA is shown in Figure 6-4 using $^{11}$B-NMR. The $^{11}$B NMR spectra demonstrate the formation of the (2-APYBA)-IL 1 complex. As shown in Figure 6-3, the boronate ion (B) can readily form covalent bonds with the cis-diol groups of the glucaminium-based IL to generate the complex. In the absence of IL 1 (Figure 6-4(a)), 2-APYBA (0.02 M) appears as a single sharp peak which was used as the external standard and set to 0 ppm. The concentration of 2-APYBA was kept constant at 0.02 M and the concentration of IL 1 was varied from 0.02 M to 0.06 M. The (2-APYBA)-IL 1 complex peak appeared at 4 ppm and increased significantly as the concentration of IL 1 was increased, as shown in Figure 6-4(b)-(d). Similarly, complexation between IL 2 and 2-APYBA was also investigated, as shown in Figure 6-5. The single peak at 0 ppm represents 2-APYBA (0.005 M) which is used as an external standard (see Figure 6-5(a)). The (2-APYBA)-IL 2 complex peak appeared at 5 ppm when IL 2 was added. As shown in Figure 6-5(b)-(d), the peak intensity for the (2-
APYBA)-\textbf{IL 2} complex peak increased significantly when the concentration of \textbf{IL 2} was varied from 0.0025 to 0.0075.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6-4.png}
\caption{$^{11}$B NMR spectra for solutions containing 0.02 M 2-APYBA where the concentration of \textbf{IL 1} was varied from (a) No \textbf{IL 1} (b) 0.02 M \textbf{IL 1} (c) 0.04 M \textbf{IL 1} and (d) 0.06 M \textbf{IL 1}. All measurements were performed at pH 12.4 using phosphate buffer and an ionic strength of 0.1 M using NaCl.}
\end{figure}

M. The free 2-APYBA peak completely disappeared when \textbf{IL 2} was present in excess (Figure 6-5(d)). It is important to note that when the 2-APYBA to \textbf{IL 1} ratio was 3, most of the 2-APYBA present in the aqueous solution was complexed to \textbf{IL 1}. However, most of the 2-APYBA was complexed with \textbf{IL 2} when the ratio of 2-APYBA to \textbf{IL 2} was 2.
These results suggest that **IL 2** is more efficient at undergoing complexation with 2-APYBA.

![Diagram](image)

Figure 6-5: $^{11}$B NMR spectra for solutions containing 0.005 M 2-APYBA where the concentration of **IL 2** was varied from (a) No **IL 2** (b) 0.0025 M **IL 2** (c) 0.005 M **IL 2** and (d) 0.0075 M **IL 2**. All measurements were performed at pH 12.4 using phosphate buffer and an ionic strength of 0.1 M using NaCl.

### 6.3.2 Complexation of 2-APYBA with IL and separation using HPLC

As mentioned previously, the extremely hydrophilic nature of 2-APYBA results in low retention times on the PLRP-S polymeric column. This column was used due to
the fact that a high pH mobile phase is required to solubilize 2-APYBA and 2-APPBE. The results shown in Section 6.3.1 prove that 2-APYBA forms a complex with both functionalized ILs, namely \textbf{IL 1} and \textbf{IL 2}. However, the stability of the complex was unknown. More importantly, it was also not known if the complex is sufficiently stable throughout the timeframe of the chromatographic separation. To study this, the (2-APYBA)-\textbf{IL} complex was formed outside the column by mixing stock solutions of 2-APYBA and either \textbf{IL 1} or \textbf{IL 2} in a 2.0 mL microcentrifuge tube. After 30 minutes, this mixture was injected onto the HPLC column where free 2-APYBA was separated from the 2-APYBA-IL complex.

As shown in Figure 6-6(a), free 2-APYBA eluted at the column dead time (3.5 minutes) when no IL was added to the diluent containing the sample. As \textbf{IL 1} was added to the diluent, free 2-APYBA formed a complex with the IL and was retained for nearly 9 minutes. The peak area of the (2-APYBA)-\textbf{IL 1} complex increased significantly as the concentration of \textbf{IL 1} was varied from 0.002 M to 0.006 M, as shown in Figure 6-6(b)-(d). In an analogous approach, the (2-APYAB)-\textbf{IL 2} complex was formed outside the column and then subjected to chromatographic separation. In this case, the (2-APYBA)-\textbf{IL 2} complex retained for approximately 22 minutes. The peak area for the (2-APYBA)-\textbf{IL 2} complex increased significantly as the concentration of \textbf{IL 2} was increased from 2 mM to 6 mM, as shown in Figure 6(b)-(d).
Figure 6-6: HPLC chromatograms demonstrating the complexation of 2-APYBA with IL 1. The concentration of 2-APYBA was constant at 2 mM and the concentration of IL 1 added to the sample was varied from (a) No IL 1 (b) 0.002 M IL 1 (c) 0.004 M IL 1 and (d) 0.006 M IL 1. The chromatographic conditions are shown in Table 1 except that the column temperature was maintained at 40 °C.

The chromatographic conditions were also optimized individually for IL 1 and IL 2 to provide the best separation of the complex peak from free 2-APYBA. Firstly, mobile phase A (10 mM phosphate buffer) and mobile phase B (80 / 20 ; ACN / water) were employed for the separation of free 2-APYBA, IL, and (2-APYBA)-IL complex. Significantly higher retention of the (2-APYBA)-IL 2 complex was achieved compared to the (2-APYBA)-IL 1 complex. This is
Figure 6-7: HPLC chromatograms demonstrating complexation of 2-APYBA with IL 2. The concentration of 2-APYBA was constant at 2 mM where the concentration of IL 2 added to the sample was varied from (a) No IL 2 (b) 0.002 M IL 2 (c) 0.004 M IL 2 and (d) 0.006 M IL 2. The chromatographic conditions are shown in Table 1 except that the column temperature was maintained at 40 °C.

likely due to the presence of the methyl benzoate functional group within the glucaminium cation of IL 1 which allowed the (2-APYBA)-IL 1 complex to partition more to the mobile phase resulting in lower retention. Contrarily, the benzyl groups within IL 2 makes the (2-APYBA)-IL 2 complex more hydrophobic allowing for higher retention. Although both (2-APYBA)-IL complexes retained well under the aforementioned separation conditions, the (2-APYBA)-IL 1 complex peak overlapped with the free IL 1 peak (see Figure S7 in supporting information). Mobile phase B (80 / 20 ; ACN / water) was varied to 100 % water in order to achieve separation of the (2-APYBA)-IL 1 complex from IL 1.
6.3.3 Effect of temperature on complexation

It has been shown by Duran and co-workers that high separation temperatures can affect the stability of boronate esters.\textsuperscript{5} The effect of temperature on the complexation of 2-APYBA and the two glucaminium-based ILs was studied by varying the column temperature from 35 °C to 50 °C. The concentration of 2-APYBA and ILs was kept constant at 0.002 M. As shown in Figure 4-8, the peak area for both IL complexes with 2-APYBA decreased significantly as the column temperature was increased from 35 °C to 50 °C. This observation indicates the instability of the (2-APYBA)-IL complexes on the column at high temperatures. A decrease in peak area for the (2-APYBA)-IL \textbf{2} complex was more prominent compared to the (2-APYBA)-IL \textbf{1} complex. This may be due to the fact that the retention time of the (2-APYBA)-IL \textbf{2} complex is nearly double that of the (2-APYBA)-IL \textbf{1} complex. It was also observed in this study that, a significant increase in column back pressure was observed when separations were performed at an ambient temperature. This issue was addressed by elevating the column temperature in an effort to minimize the column back pressure while retaining the stability of the (2-APYBA)-IL complexes on the column. Therefore, the optimum column temperature was found to be 35 °C.
Figure 6-8: Effect of column temperature on the peak area of the 2-(APYBA)-IL complex: (◇) 2-(APYBA)-IL 1 and (□)2-(APYBA)-IL 2. The concentration of 2-APYBA, IL 1, and IL 2 were maintained at 2 mM.

6.3.4 Analytical performance

As shown in the Table 6.2, the analytical performance of the HPLC method using IL 1 and IL 2 for the detection of 2-APYBA was evaluated in terms of linear range, standard deviation of the regression line, limit of detection (LOD), and correlation coefficient (R). Calibration curves for both ILs were generated by plotting the peak areas of the (2-APYBA)-IL complex with 11 different concentration levels of 2-APYBA. The concentration of IL 1 and IL 2 were kept
Table 6.2 Figures of merit of the calibration curves for analysis of 2-APYBA using glucaminium-based ILs.

<table>
<thead>
<tr>
<th>Ionic liquid</th>
<th>Slope ± error</th>
<th>$S_{yx}$</th>
<th>Linear range (µM)</th>
<th>Correlation coefficient (R)</th>
<th>LOD (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL 1</strong></td>
<td>1147.5 ± 5.5</td>
<td>43410</td>
<td>4.0 - 8000</td>
<td>0.999</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>IL 2</strong></td>
<td>1123.7 ± 16.9</td>
<td>66482</td>
<td>6.0 - 4000</td>
<td>0.999</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a Condition: **IL 1** concentration: 20.0 mM; **IL 2** concentration: 6.0 mM. Other Separation parameters are the same as listed Table 1.

b Standard deviation of regression constant at 20.0 mM and 6.0 mM, respectively. At these IL concentrations, complete conversion of free 2-ABYBA to the (2-APYBA)-IL complex was observed. The linear range of the calibration curves for 2-APYBA were from 0.004 to 8.0 mM using **IL 1** and from 0.006 to 4.0 mM using **IL 2**. In both cases, good correlation coefficients (R = 0.999) were achieved. The small errors of the slope indicate the exceptional linearity for the HPLC method using both ILs. The LODs were determined based on the ratio of peak intensity of the lowest sample concentration three times higher than that of the system noise. The obtained LODs for 2-APYBA were 0.003 mM for **IL 1** and 0.004 mM for **IL 2**. These LODs are similar to a previously reported HPLC method for the analysis of 2-APYBA in which a LOD of 0.005 mM was reported. The reproducibility of both **IL 1** and **IL 2**-based HPLC methods for the analysis of 2-APYBA was determined in triplicate. The relative standard deviation (RSD) values ranged from 0.6-7.4 % and 1.1-8.5 % for **IL 1** and **IL 2**, respectively.
6.3.5 Analysis of 2-aminopyrimidine-5-pinacolboronate ester for the determination of 2-aminopyrimidin-5-yl boronic acid impurity

To validate the proposed IL-based HPLC method for real sample analysis, 2-APPBE was taken as a model compound and analyzed to detect the level of 2-APYBA impurity. To prevent the hydrolysis of 2-APPBE, DMF was chosen as the diluent. IL 2 was found to be a good candidate as it possessed higher solubility in DMF compared to IL 1. In order to separate these four compounds (2-APPBE, 2-APYBA, IL 2 and (2-APYBA)-IL 2 complex), the separation program was modified (see Table 6.1, part III). Firstly, the 2-APPPE solution (9 mM) without IL was subjected to HPLC separation. As shown in Figure 6-9(a), the peaks for 2-APYBA and 2-APPBE appeared at approximately 3.5 minutes (dead time) and 20 minutes, respectively. The presence of 2-APYBA as an impurity in 2-APPBE was confirmed by this result. IL 2 was then added to the 2-APPBE solution and kept at room temperature for 30 minutes before injection. Using the aforementioned modified separation condition for the analysis of 2-APYBA within 2-APPBE, the retention time of the (2-APYBA)-IL 2 complex was 31 minutes. The concentration of IL 2 was varied from 4.5 mM to 18.0 mM while keeping the concentration of 2-APPPE constant at 9.0 mM. The peak area for the (2-APYBA)-IL complex increased significantly as the amount of IL 2 was increased. It is important to note that the addition of IL 2 to the diluent had no effect on the observed peak area for 2-APPBE. As shown in Figure 6-9(b)-(d), peak area of 2-APYBA decreased with increasing amounts of IL 2 added to the diluent. When 18.0 mM of IL 2
was added to the diluent, 2-APYBA was completely converted to the (2-APYBA)-IL complex, which is evident from the disappearance of 2-APYBA peak (see Figure 6-9(d)).

The accuracy of the IL 2-based 2-APYBA HPLC analysis method was evaluated in term of relative accuracy ($R_a$), as shown in Equation 1,

$$R_a = \left( \frac{C_{cc}}{C_{real}} \right) \times 100\%$$

(1)
where $C_{cc}$ is the concentration of 2-APYBA in 2-APPBE obtained using this IL 2 based method, and $C_{\text{real}}$ is the real concentration of 2-APYBA present in 2-APPBE. In this study, $C_{cc}$ can be calculated by applying the peak area of the (2-APYBA)-IL 2 complex to the calibration curve of the IL 2-based 2-APYBA analysis method (see section 6.4 analytical performance). However, since $C_{\text{real}}$ is unknown, the following method was used to calculate this value. The percentage of 2-APYBA in 2-APPBE can be obtained by directly comparing the peak area between the 2-APYBA and 2-APPBE, which allows for the calculation of $C_{\text{real}}$ by Equation 2.  

$$C_{\text{real}} = C_{\text{st}} \times \left( \frac{P_{\text{real}}}{P_{\text{st}}} \right)$$  

Here, $C_{\text{st}}$ is the concentration of 2-APPBE, $P_{\text{real}}$ and $P_{\text{st}}$ is the peak area of 2-APYBA and 2-APPBE from the 2-APPBE sample chromatogram without adding IL 2, respectively. Using the calibration curve, 9.0 mM of 2-APPBE and 18.0 mM of IL 2 was used to calculate $C_{cc}$. Equation 2 was used to determine $C_{\text{real}}$ using 9.0 mM of 2-APPBE ($C_{\text{st}} = 9.0$ mM). After determining $C_{cc}$ and $C_{\text{real}}$, a $R_a$ of 112.2 $\pm$ 6.2 % of was determined in triplicate using Equation 1. It is evident from the results that the IL-based HPLC method is suitable for the analysis of 2-APYBA in the 2-APPBE sample. This method can very well be extended for the purity analysis of different boronate esters, provided that the analytes are capable of complexing with the IL.

### 6.4 Conclusions

In this study, two functionalized glucaminium-based ILs were synthesized and employed as complexing agents for the reversed phase HPLC analysis of a boronic acid impurity that elutes near the dead time of the column. The retention times achieved for
the two complexes using different ILs were 9 mins and 22 mins, respectively. $^{11}$B-NMR confirmed the complexes formed using the boronic acid (2-APYBA) and glucaminium-based ILs. The IL containing benzyl group on glucaminium cation was found to be more efficient in complexing boronic acid. Higher column temperatures were found to diminish the stability of the (2-APYBA)-IL complexes on the column. The limit of detection was found to be 3-4 µM. The developed HPLC method was employed in the quantification of 2-APYBA in a real 2-APPBE sample. This method requires very small amounts of glucaminium ILs and is rapid and robust. This method is applicable to other boronic acids that present challenges in seaparation due to the susceptibility of the boronate ester undergoing hydrolysis or the hydrophilic nature of the boronic acid that leads to low retention.
References


Chapter 7

General conclusions

The first part of the dissertation describes the development of new glucaminium-based ILs and their application in the removal of boron species from water. The structure of glucaminium-based ILs were tuned to be hydrophobic by functionalization of the tertiary nitrogen on the N-methyl-D-glucamine molecule. The in situ dispersive liquid-liquid microextraction method was employed successfully using these ILs. Significant extraction of boric acid was achieved from water. The complexation was confirmed using $^{11}$B NMR. The mechanism of complexation was also confirmed using peracetylated glucaminium-based IL with the help of $^{11}$B NMR. It was confirmed that the hydroxyl groups present on glucaminium-based ILs are actively involved in the complex formation with boron species.

In the follow up study, Chapter 3, two main goals were achieved. The first goal involved the understanding of binding between N-decyl-N-methyl-D-glucaminimium chloride IL and boric acid using $^{11}$B NMR. The binding constants were achieved for monochelate and bischelate complexes. Secondly, an important question regarding the regeneration of glucaminium-based IL was addressed. Successful regeneration of N,N-didecyl-N-methyl-D-glucaminium bis[(trifluoromethyl)sulfonyl]imide was achieved using 0.1 M hydrochloric acid solution. Due to the hydrophobic nature of IL, it was
collected and reused for the extraction of boron species. Consistent extraction performance was achieved up to three regeneration cycles. Higher extraction efficiencies were achieved in the presence of 0.1 M sodium chloride using LiNTf₂ as a metathesis salt.

The use of crosslinked polymeric ionic liquids (PILs) as new sorbent coatings in solid-phase microextraction was demonstrated in Chapter 4. The recent approach introduced by our group was successfully used for an "on-fiber polymerization" of IL monomers using 360 nm UV light. Two different PIL-based sorbent coatings with different structures were achieved using this method. The thickness of the PIL-based sorbent coatings were estimated 5 µm and 2 µm for PIL 1 and PIL 2, respectively. Successful headspace solid-phase microextraction (HS-SPME) was performed for polychlorinated biphenyls (PCBs) from Milli-Q water, ocean water as well as bovine milk samples. Different parameters such as extraction temperature, extraction time and salt concentration were optimized. The extraction performance of PIL-based fibers was compared with commercial 7 µm PDMS fiber. Equivalent or superior extraction performance was obtained using PIL-based sorbent coatings. Two different gas chromatography systems, namely, gas chromatography-electron capture detection (GC-ECD) and gas chromatography-mass spectrometric detection (GC-MS) were employed and the limits of detection for all twenty one PCBs were compared. GC-ECD found to be equally sensitive to GC-MS where LODs were obtained in sub ng L⁻¹.

The second part of the dissertation describes the use of newly designed glucaminium-based ILs as a derivatizing agents for the chromatographic detection of 2-aminopyrimidine-5-ylboronic acid (2-APYBA) using HPLC. The complexation between
glucamininium-based ILs and 2-APYBA was confirmed using $^{11}$B NMR. The successful retention of IL-(2-APYBA) complex was achieved. The complex formed between IL 1 and 2-APYBA was retained for approximately 9.0 minutes where the complex between IL 2 and 2-APYBA was retained for 22.0 minutes. A wide linear range was achieved. The limit of detections were 3 µM and 4 µM for IL 1 and IL 2, respectively. The quantification of 2-APYBA in a real pinacolboronate ester received from Genentech Inc. (South San Francisco, CA) was performed.
References

Chapter 1

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22. (a) Joshi, M. D.; Chalumot, G.; Kim, Y.; Anderson, J. L., Chem. Commun. 2012,
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**Chapter 2**


Chapter 3


Chapter 4


**Chapter 5**


**Chapter 6**


Appendix A

Supplemental Figures Accompanying Chapter 2
Experimental

Materials:
N-methyl-D-glucamine, boric acid, 1-bromodecane, chromotropic acid, ethylenediaminetetraacetic acid and sodium perchlorate was obtained from Sigma-aldrich, St.louis, MO, USA. Hydrochloric acid, methanol, isopropanol, sodium carbonate, ammonium acetate, disodium phosphate, ethyl acetate and toluene were purchased from Fisher Scientific, (Fair lawn, NJ). Pyridine and 4-dimethylaminopyridine (DMAP) was obtained from Acros, (Morris Plains, NJ, USA). N-octyltrimethyl ammonium chloride was purchased from TCI America, (Portland, OR). Potassium tris-(pentafluoroethyl)trifluorophosphate was obtained from Merck KGaA, (Darmstadt, Germany).
Synthesis of N-decyl-N-methyl-D-glucamine (1):

N-decyl-N-methyl-D-glucamine 1 was synthesized by combining two moles of N-methyl-D-glucamine with one mole of 1-bromodecane and 0.5 mole of sodium carbonate in 150 mL of methanol and stirred at 50-55 °C for 48 hours. To this reaction mixture, 250 mL methanol was added and the temperature increased to 50-55 °C and kept for 45 minutes. Methanol was completely removed under reduced pressure and product suspended in 300 mL of DI water to remove unreacted N-methyl-D-glucamine. To this, 150 mL of ethyl acetate was added. The solid product was filtered using vacuum filtration and dried at room temperature. The product was recrystallized using ethanol and dried under vacuum for 24 hours at 70 °C. Melting for compound 1 was found to be 85 °C. The percent yield was 88.6 %.

$^1$H-NMR (d$_6$-DMSO, 600 MHz): 0.8 ppm (t, 3H, J = 6.8, -CH$_3$), 1.2 ppm (m, 14H, -CH$_2$), 1.3 ppm (t, 2H, J = 6.4, -CH$_2$), 2.1 ppm (s, 3H, -CH$_3$), 2.3 ppm (d, 2H, J = 2.4, -CH$_2$OH), 4.3 ppm (t, 2H, J = 5.2, -NCH$_2$), 4.4 ppm (d, 2H, J = 5.6, -NCH$_2$), 2.4, 3.4, 3.5, 3.6 ppm (m, 1H, -CHOH), 4.6 ppm (s, 1H, -OH).

$^{13}$C-NMR (d$_6$-DMSO, 400 MHz): 14.0 ppm (-CH$_3$), 22.1 ppm (-CH$_2$), 26.6, 26.9, 28.7, 29.0, 29.0, 29.1, 31.3 ppm (-CH$_2$), 42.5 ppm (N-CH$_3$), 58.0, 60.2 ppm (N-CH$_2$), 63.5 ppm (-CH$_2$OH), 70.3, 70.4, 71.4, 72.0 ppm (-CHOH).
Synthesis of N,N-didecyl-N-methyl-D-glucaminium bromide (2):

Synthesis of N,N-didecyl-N-methyl-D-glucaminium bromide 2 included reaction of one mole of N-decyl-N-methyl-D-glucamine 1 with one mole of 1-bromodecane in isopropanol for 72 hours at 70 °C. Isopropanol was completely removed under reduced pressure to obtain the final product. The product was dried under vacuum at 70 °C for 24 hours. The percent yield was 83.4 % reported.

$^1$H-NMR (d$_6$-DMSO, 600 MHz): 0.8 ppm (t, 6H, J = 6.8, -CH$_3$), 1.2 ppm (m, 29H, -CH$_2$), 1.6 ppm (t, 2H, J = 6.8, -CH$_2$), 2.9 ppm (s, 3H, -CH$_3$), 3.4 ppm (m, 2H, -NCH$_2$), 3.4, 3.5, 3.6, 3.9 ppm (m, 1H, -CHOH), 5.3 ppm (d, 1H, J = 4.8, -NCH$_2$), 4.8 ppm (d, 1H, J = 6.0, -NCH$_2$), 2.4, 3.6, 3.7, 4.1 ppm (s, 1H, -OH), 4.6 ppm (d, 2H, J = 5.2, -CH$_2$OH).

$^{13}$C-NMR (d$_6$-DMSO, 400 MHz): 13.9 ppm (-CH$_3$), 21.4, 22.1 ppm (-CH$_2$), 25.4, 25.8, 26.2, 28.5, 28.7, 28.8, 28.9, 31.3 ppm (-CH$_3$), 48.5 ppm (N-CH$_3$), 58.0, 60.2 ppm (N-CH$_2$), 63.5 ppm (-CH$_2$OH), 70.3, 70.4, 71.4, 72.0 ppm (-CHOH).

Experimental result for elemental analysis: C 57.31%, H 10.79%, N 2.89%

Theoretical values for elemental analysis: C 58.26%, H 10.50%, N 2.52%
Synthesis of N-decyl-N-methyl-D-glucaminium chloride (3):

N-decyl-N-methyl-D-glucaminium chloride 3 was synthesized by reacting one mole of N-decyl-N-methyl-D-glucamine 1 with 1.1 mole of 12.1 M hydrochloric acid in water for 12 hours at room temperature. Water was completely removed under vacuum and the product was dried under vacuum at 70 °C for 24 hours. The percent yield was 98.9 %.

$^1$H-NMR (d$_6$-DMSO, 600 MHz): 0.8 ppm (t, 3H, J = 3.6, -CH$_3$), 1.2 ppm (m, 14H, -CH$_2$), 1.6 ppm (m, 2H, -CH$_2$), 2.7 ppm (d, 3H, J = 18.4, -CH$_3$), 2.9 ppm (m, 2H, -NCH$_2$), 3.0 ppm (m, 2H, -NCH$_2$), 3.4 ppm (m, 1H, -CHOH), 3.5 ppm (d, 1H, J = 7.2, -CHOH), 4.8 ppm (d, 1H, J = 11.6, -CHOH), 3.9 ppm (m, 1H, -CHOH), 3.4, 3.6, 4.4, 5.4 ppm (s, 1H, -OH), 4.6 ppm (d, 2H, J = 17.6, -CH$_2$OH), 9.1 ppm (s, 1H, -NH).

$^{13}$C-NMR (d$_6$-DMSO, 400 MHz): 14.0 ppm (-CH$_3$), 22.2, 23.6, 26.2, 28.7, 28.8, 29.0, 29.0, 31.4 ppm (-CH$_2$), 40.6 ppm (N-CH$_3$), 55.9, 57.9 ppm (N-CH$_2$), 63.5 ppm (-CH$_2$OH), 67.6, 70.4, 71.2 (-CHOH).

Experimental result for elemental analysis: C 54.67%, H 10.59%, N 3.99%

Theoretical values for elemental analysis: C 54.90%, H 10.30%, N 3.77%
Synthesis of peracetylated N-decyl-N-methyl-D-glucaminium chloride (4):

One mole of N-decyl-N-methyl-D-glucaminium chloride 3 was dissolved in 10 mL of pyridine followed by the addition of eight moles of acetic anhydride. A catalytic amount of DMAP was added. The reaction was stirred for 12 hours at room temperature. Pyridine was completely removed by co-distillation with toluene. The resulting solid was dissolved in water and the product isolated using ethyl acetate. Ethyl acetate was completely removed under reduced pressure and the product was dried under vacuum for 24 hours at 70 °C. The percent yield was found to be 89.9%.

$^1$H-NMR (d$_6$-DMSO, 600 MHz): 0.8 ppm (t, 3H, J = 6.6, -CH$_3$), 1.2 ppm (m, 14H, -CH$_2$), 1.6 ppm (m, 2H, -CH$_2$), 1.9 ppm (s, 6H, -COCH$_3$), 2.0 ppm (s, 9H, -COCH$_3$), 3.3 ppm (s, 3H, -CH$_3$), 4.0, 4.3 ppm (m, 1H, -NCH$_2$), 5.3 ppm (d, 2H, J = 5.4, -NCH$_2$), 3.3, 5.0 ppm (m, 1H, -CHOCONH), 10.4 ppm (s, 1H, -NH).

$^{13}$C-NMR (d$_6$-DMSO, 400 MHz): 13.9 ppm (-CH$_3$), 21.4, 20.4, 20.5, 20.5 (-COCH$_3$), 22.6, 25.4, 26.1, 28.5, 28.7, 28.8, 28.9, 31.3 ppm (-CH$_2$), 40.9 ppm (N-CH$_3$), 54.3, 56.9 ppm (N-CH$_2$), 61.2 ppm (-CH$_2$OCOCH$_3$), 68.5 (-CHOCONH), 169.4, 169.6, 170.0, 171.9 ppm (-C=O).

Experimental result for elemental analysis: C 57.78%, H 8.72%, N 2.59%

Theoretical values for elemental analysis: C 55.71%, H 8.31%, N 2.41%
$^{11}$B NMR Experiments:

The $^{11}$B NMR experiments were performed at room temperature (19 ± 1 °C) on a Varian VXRS 400 MHz NMR spectrometer with a 5 mm broadband probe at a resonance frequency of 128.0 MHz. All measurements were performed in a 5 mm quartz tube obtained from Wilmad, (Vineland, NJ). Stock solutions of 0.1 M IL and 0.1 M boric acid were prepared in DI water. All buffer solutions were prepared in deionized water. The sample for $^{11}$B NMR was prepared by taking different volumes of stock solutions where D$_2$O was present 10-30 % of the total sample volume. The sample pH was adjusted by using different buffer solutions. The parameters for $^{11}$B NMR were as follows:

The flip angle: 75 °

Pulse repetition time: 0.06 Sec

The spectral width: 27.0 KHz

Number of scans received: 8064
Sample preparation and Calibration curve for HPLC method:

All measurements were performed on Simadzu LC20-AT HPLC instrument. A TSK gel anion exchange column IC-Anion-PW (5.0 cm X 4.6 mm (ID)) was obtained from Tosoh Bioscience (Montgomeryville, PA, USA). 5.0 mL of aqueous phase was placed in a polypropylene tube. In this, 0.25 mL of solution A (0.016 M chromotropic acid and 0.1 M EDTA) and 0.25 mL of solution B (2.0 M octyltrimethylammonium chloride and 1.0 M sodium acetate) were added. The mixture was stirred well and kept in the dark for 2.5 hours. 10 µL aqueous phase was injected using a mobile phase consisting of 0.2 M sodium perchlorate and 0.001 M sodium acetate was used. The anionic complex peak was observed at 350 nm. The retention time for chromotropic acid and the anionic complex was 1 min and 3 min, respectively. The calibration curve was generated as shown below where the concentration of boric acid was varied from 0.1 to 15 ppm.

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<th>Average Peak Area (N=6)</th>
<th>% Relative Standard Deviation</th>
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<td>82550</td>
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</tr>
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<td>0.8</td>
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</tr>
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</table>
$y = 150815x + 59807$

$R^2 = 0.9997$

Peek Area versus Boric acid concentration (ppm).
In-situ dispersive liquid-liquid microextraction:

IL is dissolved in deionized water as shown in Figure 2(a). An aqueous solution of boric acid is then added to this IL solution as shown in Figure 2(b). The resulting solution is vortexed followed by the addition of an aqueous solution of metathesis salt potassium tris-(pentafluoroethyl)trifluorophosphate (KFAP). A cloudy solution immediately appears (Figure 2(c)). This will be followed by the vortexing and centrifugation of the sample. As shown in Figure 2(d), two separate layers can be observed where the top and bottom layer correspond to the aqueous and IL layers, respectively. Here, 0.05 g (9.78 x 10^{-5} moles) of IL was dissolved in 9.0 mL of a 10 ppm boric acid solution in a polypropylene tube. The solution was vortexed for 1 min. A cloudy solution was observed upon addition of 1.0 mL of 9.78 x 10^{-5} M potassium tris-(pentafluoroethyl)trifluorophosphate (KFAP) solution. The solution was vortexed for 1 min and centrifuged for 10 to 30 minutes. Two separate layers namely aqueous and IL layer were observed.
Figure A-1: $^1$H NMR of N-decyl-N-methyl-D-glucamine (I):
Figure A-2: $^{13}$C NMR of N-decyl-N-methyl-D-glucamine (1):
Figure A-3: $^1$H NMR for N,N-didecyl-N-methyl-D-glucaminium bromide (2):
Figure A-4: $^{13}$C NMR for N,N-didecyl-N-methyl-D-glucaminium bromide (2):
Figure A-5: ESI mass spectrum of N,N-didecyl-N-methyl-D-glucaminium bromide (2):
Figure A-6: $^1$H NMR for N-decyl-N-methyl-D-glucaminium chloride (3):
Figure A-7: $^{13}$C NMR for N-decyl-N-methyl-D-glucaminium chloride (3):
Figure A-8: ESI mass spectrum for N-decyl-N-methyl-D-glucaminium chloride (3):
Figure A-9: $^1$H NMR for peracetylated N-decyl-N-methyl-D-glucaminium chloride (4):
Figure A-10: $^{13}$C NMR for peracetylated N-decyl-N-methyl-D-glucaminium chloride (4):
Figure A-11: ESI mass spectrum for peracetylated N-decyl-N-methyl-D-glucaminium chloride (4):
Figure A-12: UV-Vis measurements for glucaminium-based ILs in ethanol
Appendix B

Supplemental Figures and Tables Accompanying Chapter 3
Figure B-1: Formation constant for bischelate complex
Table B.1: Calibration curve for seawater samples

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Figure B-2: Calibration curve for seawater samples
Appendix C

Supplemental Figures Accompanying Chapter 4
Figure C-1: Synthetic scheme used to generate 1,12-di(3-vinylbenzylimidazolium)dodecane dibis[(trifluoromethyl)sulfonyl]imide [(DVBIM)₂C₁₂]₂[NTf₂]
Figure C-2: $^1$H NMR of the 1,12-di(3-vinylbenzylimidazolium)dodecane dibis[(trifluoromethyl)sulfonylimide [(DVBIM)$_2$C$_{12}$] 2[NTf$_2$] IL

$^1$H-NMR (600 MHz, DMSO): 9.24 (s, 2H), 7.82 (d, 4H), 7.53 (d, 4H), 7.38 (d, 4H), 6.71 (m, 2H), 5.90 (d, 2H), 5.41 (s, 4H), 5.33 (d, 2H), 4.17(t, 4H), 1.71 (m, 4H), 1.23 (m, 16H)
Figure C-3: ESI mass spectrum of 1,12-di(3-vinylbenzylimidazolium)dodecane dibis[(trifluoromethyl)sulfonyl]imide [(DVBIM)$_2$C$_{12}$] 2[NTf$_2$]:
Appendix D

Supplemental Figures Accompanying Chapter 6
Figure D-1: $^1$H NMR for N,N-bis(4-(methoxycarbonyl)benzyl)-N-methyl glucaminium bromide (IL 1)

$^1$H-NMR (d$_6$-DMSO, 600 MHz): 7.44, 7.57, 7.83, 7.90, 8.01 ppm (8H, -CH), 5.76 ppm (1H, -OH), 4.73, 4.65 ppm (4H, -NCH$_2$), 5.42, 4.48, 3.76, 3.47 ppm (4H, -OH), 4.85, 4.80, 4.55, 3.57 ppm (4H, -CHOH), 3.86 ppm (6H, -OCH$_3$), 2.96 ppm (3H, -NCH$_3$)
Figure D-2: $^{13}$C NMR for N,N-bis(4-(methoxycarbonyl) benzyl)-N-methyl glucaminium bromide (IL 1)

$^{13}$C-NMR ($d_6$-DMSO, 600 MHz): 165.70, 166.09 ppm (-C=O), 127.75, 148.23 ppm (-C-CH), 126.21, 128.90, 130.84, 133.90 ppm (-CH), 71.13, 70.06, 69.90, 63.10 ppm (-CHOH), 66.72 ppm (-NCH$_2$), 63.10 ppm (-NCH$_2$), 62.16 ppm (-CH$_2$OH), 51.32, 52.32 ppm (-OCH$_3$), 46.86 ppm (-NCH$_3$)
Figure D-3: ESI-MS spectrum of N,N-bis(4-(methoxycarbonyl)benzyl)-N-methyl glucaminium bromide (IL 1)
Figure D-4: $^1$H NMR for N,N-dibenzyl-N-methyl-D-gluaminium bromide (IL 2)

$^1$H-NMR ($d_6$-DMSO, 600 MHz): 7.65 ppm (4H, -CH), 7.48-7.54 ppm (6H, -CH), 5.52, 4.90 ppm (2H, -NCH$_2$), 4.70, 4.61 ppm (4H, -NCH2), 4.56, 4.50 ppm (2H, -CHOH), 4.46 ppm (2H, -CH$_2$OH), 3.75 ppm (1H, -OH), 3.59, 3.53, 3.44, 3.42 ppm (4H, -CHOH), 2.91 ppm (3H, -NCH$_3$)
Figure D-5: $^{13}$C NMR for N,N-dibenzyl-N-methyl-D-glucaminium bromide (IL 2)

$^{13}$C-NMR ($d_6$-DMSO, 600 MHz): 133.54 ppm (-C-CH), 130.20, 128.90, 128.01 ppm (-CH), 70.23, 71.30 ppm (-NCH$_2$), 67.0, 65.83, 65.56, 62.82 ppm (-CHOH), 63.27 ppm (-CH$_2$OH), 46.62 ppm (-NCH$_3$)
Figure D-6: ESI-MS spectrum of N,N-dibenzyl-N-methyl-D-glucaminium bromide (IL 2)
Figure D-7: Chromatograms demonstrating the separation of 2-APYBA, IL 1 and (2-APYBA)-IL 1 complex employing chromatographic conditions used in part II where mobile phase B was 80:20/ACN:water. Detection was performed at two different wavelengths, namely, 293 nm (--) and 254 nm (---).