FUNCTIONAL IONIC LIQUIDS FOR USE IN PHARMACEUTICAL APPLICATIONS

by

WHITNEY LAUREN HOUGH

A DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Chemistry
in the Graduate School of
The University of Alabama

TUSCALOOSA, ALABAMA

2010

ABSTRACT

For years, the pharmaceutical industry has relied heavily on crystalline active pharmaceutical ingredients (APIs) that can be approved by the Federal Drug Administration (FDA) as neutral compounds, salts, or solvates of said neutral compounds and salts. Yet, the solid crystalline form can have unexpected and unfavorable effects on properties such as solubility, bioavailability, efficacy, etc., due to different polymorphic forms of the API. A drug can be present in multiple forms and interconvert between forms during isolation, manufacturing, storage, and transport of the end product. These unwelcome problems could be alleviated or even eliminated by the formation of a liquid drug, which possesses no crystal structure. Unfortunately, research in this area has been limited to solubilization of solid drugs into various drug delivery vehicles such as emulsions, suspensions, and liposomes. However, it is possible for a drug to crystallize from these vehicles during the manufacturing, storage, and transportation. Thus, a new method to liquefy pharmaceuticals, thereby reducing problems associated with the solid-state, is needed.

A potential solution is the use of ionic liquids (IL), defined as salts that melt below 100 °C. Since ILs are salts it is possible to combine a pharmaceutical ion with any desired counter ion, thereby, providing a level of tunablity that is not possible with current techniques. This IL modular strategy was the basis for the research discussed here, in which APIs with known problems were combined with GRAS (generally regarded as safe) compounds or FDA-approved APIs, which resulted in ILs displaying dual biological functionality.

This strategy was successful in producing a wide range of ILs, all containing at least one pharmaceutically active ion. The physical property set for these synthesized ILs was varied, as it is difficult to predict how two ionic organic compounds will interact. However, common trends regarding melting point depression, thermal stability, and solubility were determined. The most exciting results were exhibited during the biological testing, as several of the synthesized ILs demonstrated improved biological activity over the precursor ions. Additionally, the drug mechanism, at a cellular level, was found to be modified when contained within an IL. This indicates that ILs behavior differently in the body than simple halide containing salts. Overall, the obtained results signify that ILs can serve as pharmaceuticals, in which these liquid salts eliminate problems associated with the solid-state and displayed to synergistic physical and biological properties.

DEDICATION

This dissertation is dedicated to my family, David Hough, Nancy Hough and Alex Hough, for their support during both the good and bad times. My parents have shown me that anything is possible with hard work and persistence. They taught me that you have to find your own opportunities because no one will hand them to you. My brother, Alex, has reminded me to not sweat the small stuff, the things in life you can not control and for that I am grateful.

LIST OF ABBREVIATIONS AND SYMBOLS

A	Absolute coefficient of deterrency
A^-	Anion
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
AUC	Analgesic curve
[BA][Ace]	Benzalkonium acesulfamate
[BA][Cl]	Benzalkonium chloride
[BA]Ibuprofenate	Benzalkonium ibuprofenate
[BA][Sac]	Benzalkonium saccharinate
[BA]Sulfacetamide	Benzalkonium sulfacetamide
BL	Baseline
C	One control disc; average weight of food consumed in choice test
C^+	Cation
CC	Two control discs; average weight of the food consumed in the control
cfu	Colony-forming unit
[DDA][Ace]	Didecyldimethylammonium acesulfamate
[DDA][Br]	Didecyldimethylammonium bromide
[DDA][Sac]	Didecyldimethylammonium saccharinate

[DDA]Sulfacetamide	Didecyldimethylammonium sulfacetamide
[DDA]Ibuprofenate	Didecyldimethylammonium ibuprofen
DI	Deionized
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
ES-MS	Electrospray mass spectrometry
FDA	Federal Drug Administration
GIT	Gastrointestinal tract
GRAS	Generally regarded as safe
[Hex][Ace]	Hexadecylpyridinium acesulfamate
[HEX][Cl]	Hexadecylpyridinium chloride
[HEX][Sac]	Hexadecylpyridinium saccharinate
IL	Ionic liquid
K[Ace]	Potassium acesulfamate
$KMnO_4$	Potassium permanganate
LD	Lidocaine docusate
LHCl	Lidocaine hydrochloride
MBC	Minimum biocidal concentration
mg/kg b.w.	Milligram of substance per kilogram of body weight
MIC	Minimum inhibitory concentration
MO_6	Hexamolybdate
Na[Sac]	Sodium saccharinate
NSAID	Non-steroidal anti-inflammatory drug

NGF	Nerve growth factor
NMR	Nuclear magnetic resonance
PC12	Pheochromocytoma
PCM	Polish Collection of Microorganisms
PHCl	Procaine hydrochloride
PI-BL	Post injury baseline
ppm	Parts per million
QACs	Quaternary ammonium compounds
R	Relative coefficient of deterrency
R&D	Research and development
RTIL	Room-temperature ionic liquid
SEDDS	Self-micro emulsifying drug delivery systems
SHE	Safety, health, and environment
Sodium Docusate	Sodium dioctylsulfosuccinate
T	One treated disc; average weight of food consumed in choice test
T_c	Temperature of crystallization
T_g	Temperature of glass transition
TGA	Thermogravimetric analysis
TLC	Thin layer chromatography
T_m	Temperature of melting
T_{onset}	Temperature onset to total mass loss
$T_{onset5\%}$	Temperature onset to 5 weight percent mass loss
T_{s-s}	Temperature of solid-solid transition

TT	Two treated discs; average weight of food consumed in the no-choice test
TW	Tail withdrawal
UV	Ultraviolet
VOC	Volatile organic compound

ACKNOWLEDGMENTS

I would like to acknowledge my advisor, Dr. Robin D. Rogers, for his guidance and support during my years as both an undergraduate and graduate student. He has opened my eyes to what a career in chemistry can be for me. I appreciate all of the time and patience he provided to model me into the chemist and person I am today. I would also like to thank Dr. Dan Daly for providing a graduate assistantship throughout my years as a graduate student. I appreciate all his mentoring in both chemistry and entrepreneurship. I would also like to thank Renae Sullivan for always helping me during computer and printer malfunctions. She always had enough time to assist me in any way she could.

I thank my committee members, Dr. Shane Street, Dr. David Nikles, Dr. Stephen Woski, and Dr. Chris Brazel, for their involvement and advice throughout my graduate career. In addition to excellent committee members, I would like to thank them for also being great scientific educators.

I would like thank at research scientists at AIME, Dr. Scott Spear, Dr. Rachel Frazier, and Dr. Ying Qin, for helping me in any way possible from troubleshooting equipment to opinions on data interpretation to listening to presentations.

Furthermore, I would like to acknowledge the Rogers' research group members, both past and present, for their many years of advice, support, and assistance. Specifically, I would like to thank Dr. Rick Swatloski, Dr. Keith Gutowski, Dr. Meghna Dilip, Dr. Megan Turner, Dr. W.

Matthew Reichert, Dr. Marcin Smiglak, Dr. Julia Shamshina, and Parker McCrary for their countless hours of moral, academic, and personal support that I will always remember. They have become great friends over the years and I hope to continue our friendship for years to come.

I would like to thank my friends who have been with me from the start, Tara Shinholster Sexton, Whitney Mims Swatloski, and Megan Suggs. They provided unwavering love and support throughout my experiences as a student and, above all, as the best friends I could ever ask for. Thank you for always having the time to be there and listen when I needed you the most.

Finally, I would like to acknowledge The University of Alabama for awarding me a National Alumni Association License Tag Fellowship. Thank you to Dr. Rogers and Dr. Daly for providing me the means to complete my degree. Lastly, I would like to thank you to The University of Alabama and the Department of Chemistry for providing the surroundings and support for my research and development as a chemist.

CONTENTS

ABSTRACT ii

DEDICATION iv

LIST OF ABBREVIATIONS AND SYMBOLS v

ACKNOWLEDGMENTS ix

LIST OF TABLES xvi

LIST OF FIGURES xvii

CHAPTER 1 INTRODUCTION 1

1.1 Pharmaceuticals 1

1.1.1 Emulsions 3

1.1.2 Co-solvents 4

1.1.3 Particle Size Reduction 5

1.1.4 The Solid State 5

1.1.5 Problems with the Solid State – Polymorphs, Solvates, and Hydrates 7

1.2 Ionic Liquids 12

1.2.1 Properties of Ionic Liquids 16

1.2.2 Applications of Ionic Liquids 17

1.3 Research Strategy 19

CHAPTER 2 GENERAL EXPERIMENTAL PROCEDURES 23

2.1 Thermogravimetric Analysis (TGA) 23

2.2 Differential Scanning Calorimetry (DSC) Analysis 24

2.3 Water Content Determination 25

2.4 Nuclear Magnetic Resonance (NMR) 25

2.5 X-Ray Diffraction 25

CHAPTER 3 SWEET AND ANTI-MICROBIAL QUATERNARY AMMONIUM-BASED IONIC LIQUIDS 27

3.1 Introduction 27

3.2 Experimental 29

3.2.1 Chemicals and Microorganisms 29

3.2.2 General Synthesis 29

3.2.3 Anti-Microbial Characteristics 32

3.2.4 Acute Oral Toxicity Test 32

3.2.5 Skin Irritation Tests 33

3.2.6 Feeding Deterrent Activity Tests 33

3.3 Results and Discussion 34

3.3.1 Synthesis 34

3.3.2 Crystal Structure 34

3.3.3 Thermal Behavior 40

3.4 Biological Properties 43

3.4.1 Anti-microbial, Anti-bacterial, and Anti-Fungal Activities 43

3.4.2 Acute Oral Toxicities 44

3.4.3 Skin Irritation 46
3.4.4 Deterrent Activity 46
3.5 Conclusions 48

CHAPTER 4 PRODUCTION OF IONIC LIQUIDS CONTAINING DUAL FUNCTIONALITY 49
4.1 Introduction 49
4.2 Experimental 53
4.2.1 Chemicals 53
4.2.2 General Synthesis 53
4.3 Results and Discussion 55
4.3.1 Synthesis 55
4.3.2. Thermal Analysis 55
4.4 Conclusions 57

CHAPTER 5 LOCAL ANESTHETIC IONIC LIQUID FOR TRANSDERMAL APPLICATIONS 59
5.1 Introduction 59
5.2 Experimental 62
5.2.1 Chemicals 62
5.2.2 Synthesis and Purification 62
5.2.3 Solubility 63
5.2.4 Mouse Antinociception Tests 64
5.2.5 Suppression of PC12 Neuritic Outgrowth 65

5.3 Results and Discussion 66
5.3.1 Synthesis and Purification 66
5.3.2 Solubility and Thermal Stability 68
5.4 Biological Properties 69
5.4.1 Antinociception Tests 69
5.4.2 Suppression of PC12 Neuritic Outgrowth 73
5.5 Conclusions 76

CHAPTER 6 CONTROL OF LIDOCAINE AND PROCAINE SOLUBILITY WITH AN IONIC LIQUID APPROACH 77
6.1 Introduction 77
6.2 Experimental 80
6.2.1 Chemicals 80
6.2.2 Synthesis and Purification 81
6.2.3 Solubility 84
6.2.4 Mass Spectrometry 85
6.3 Results and Discussion 85
6.3.1 Synthesis and Purification 85
6.3.2 Thermal Behavior 88
6.3.3 Solubility 92
6.3.4 Ion Pairing 93
6.4 Conclusions 100

CHAPTER 7 CONCLUSIONS 101

REFERENCES 107

LIST OF TABLES

1.1 Commonly used additives in the crystallization process 10

1.2 Biological factors affected by salt formation 20

3.1 Thermal properties of anti-bacterial sweetener ILs 42

3.2 Minimal inhibitory concentration values 45

3.3 Minimum biocidal concentration values 45

3.4 Criteria for the estimation of the deterrent activity based on the total coefficients 47

3.5 Feeding deterrent activity 47

4.1 Synthesized IL-APIs 56

5.1 Comparison of IL properties with precursor anion and cation 68

6.1 Structures and functions of the utilized pharmaceuticals 79

6.2 Column chromatography solvent elution gradients and resulting purified ILs 87

6.3 Physical Properties of Target and Starting Salts 90

6.4 Comparison of ion pairs/clusters in the positive mode within aqueous solutions of lidocaine ILs, as determined by ES-MS. Ratios were calculated from the intensity of the C^+ and the intensity of the ionic species 97

6.5 Comparison of ion pairs/clusters in the positive mode within aqueous solutions of procaine ILs, as determined by ES-MS. Ratios were calculated from the intensity of the C^+ and the intensity of the ionic species. 99

LIST OF FIGURES

1.1 Structures of some commonly used co-solvents 4

1.2 Solid state drugs in the pharmaceutical industry 6

1.3 Structures of cations and anions found in many ILs 14

1.4 General scheme for IL metathesis 15

3.1 Structures of the synthesized anti-bacterial sweetener ILs 35

3.2 Packing diagram along the *a* crystallographic axis for [HEX][Ace] 36

3.3 Overlay of the two cations of [HEX][Ace] in the asymmetric unit including the anions with close contacts to each.. 37

3.4 One cation in [HEX][Ace] π-stacks in a polymeric fashion (interplanar spacing 3.5 and 3.6 Å). 39

3.5 Second cation in [HEX][Ace] forms π-stacked dimers (interplanar spacing 3.4 Å) with acesulfamate anions capping both sides. 39

4.1 Cations and anions found in biological active ILs 51

5.1 IL composed of lidocaine and docusate 61

5.2 General TLC spotting during the purification of LD 67

5.3 Effects of topical LD and LHCl on the warm-water mouse tail withdrawal (TW) latency response at 1 mM 71

5.4 Effects of topical LD and LHCl on the warm-water mouse tail withdrawal (TW) latency response at 100 mM**.** 71

5.5 Effects of topical LD and LHCl on the warm-water tail-flick latency response of mice bearing a tail wound about a 1 cm^2 was placed in a 49 ^oC water bath (BL = baseline, PI-BL = post injury baseline). Drugs were administered 20 min after PI-BL 72

5.6 LHCl or LD suppression of neuritic outgrowth in PC12 cells treated with 50 ng/mL NGF 74

6.1 TGA analysis of synthesized caine ILs 91

6.2 ES-MS spectra of **1d** (50 μM) with labeled species in this positive mode. 96

6.3 ES-MS spectra of **2d** (50 μM) with labeled species in the positive mode 98

CHAPTER 1

INTRODUCTION

1.1. Pharmaceuticals

A pharmaceutical is defined simply by the Merriam-Webster Dictionary as a medicinal drug.[1] These medicinal drugs take an average of 6 years from initial discovery to final product before becoming available for consumers.[2] Unfortunately, it is predicted that research and development (R&D) costs and time associated with the both discovery and manufacturing of pharmaceuticals will increase in the future. Currently, R&D costs border on approximately $1.50 billion for a single drug while the number of new drugs approved by the Federal Drug Administration (FDA) is decreasing.[2] There are several factors that contribute to this high cost – low output problem.[2]

- the pharmaceutical pipeline (potential drug compounds) is low and the number of possible drug candidates is declining;
- the rising R&D costs, which are driven by more complex clinical studies along with the use of new and potentially untested enabling technologies; and
- the increased length of clinical trials required by the FDA, which reduces commercial life of patented drugs.

Many drugs that undergo years of clinical trials with millions or billions of dollars spent to ensure its' safety and efficacy, fail in phase III of the FDA's clinical trials.[3] Phase III is divided into two sections, in which Phase IIIa establishes the drug's effectiveness and Phase IIIb

compares the efficacy of drug candidate to currently known drugs for the same application. Failure in Phase III is defined as a drug that prematurely exited the FDA's clinical trials or did not give the necessary results to secure FDA approval. Of the drugs that have passed both Phase I and II clinical trials, only 60% will ever be available in the marketplace, where 40% of drugs fail in Phase III.[3] Within this 40% failure rate, 50% of these drugs will not demonstrate an increase in efficacy over placebo, while 30% will be considered unsafe for human consumption. The last 20% will not be safer or more effective than currently used drugs; therefore, there was no need to continue funding R&D on that specific drug.[3] It is interesting to note that half of the failed drugs had problems related to efficacy, as one of the key objectives of Phase II clinical trials is to establish proof of efficacy.

A major factor affecting a drug's efficacy is bioavailability, which is defined as the rate and level of the active drug absorbed from the dosage form, once it becomes accessible to the active site. However, a portion of the drug will be eliminated due to absorption, distribution, metabolism, and excretion issues.[4] A drug's bioavailability is a complex property that hinges on several factors including solubility in an aqueous environment and the ability of the drug to permeate liphophilic membranes.[5] A high aqueous solubility allows for a concentration gradient in the stomach or intestinal fluids, which drives the absorption of orally delivered drugs. The drug can then be readily distributed to the site of action, thereby inducing the desired pharmacological response.[6] Only solublized drug molecules can be absorbed by the cellular membranes, as any precipitated drug will not interact with the active site, and therefore, will be excreted from the body unchanged.[4] Poor aqueous solubility can lead to a host of problems *in vivo* such as fed/fasting effects, variable bioavailability, and difficulties determining a safety margin for dosing.[6] In addition to *in vivo* problems, low solubility drugs pose formulation

challenges which increases the overall R&D cost. However, researchers have several tools to overcome poor drug water solubility such as emulsions, co-solvents, and particle size reduction, etc., described below.[7]

1.1.1 Emulsions

Microemulsions are employed for practically insoluble (< 0.1 mg/mL solubility) drugs and the administration of proteins for oral, parenteral, and percutaneous/transdermal application.[8] The insoluble drug is combined with a surfactant, a mixture of surfactants, and/or co-surfactants along with water and oil. The surfactant can be non-ionic, cationic, anionic, or zwitterionic. This solubilization method is moderately tunable, as a variety of surfactants can be used to obtain the desired solubility level. However, due to the high concentration of surfactant, microemulsions can not be utilized for intravenous administration. Dilution below the critical micelle concentration of the employed surfactant can cause precipitation of the drug.[4,9]

Another type of emulsion used to enhance a drug's water solubility is self-emulsifying or self-micro emulsifying drug delivery systems (SEDDS).[10] A mixture of oil, surfactant, co-surfactant, one or more hydrophilic solvents, and a co-solvent forms a transparent isotropic solution in the absence of water, which allows for the *in situ* formation of an emulsion in the gastrointestinal tract (GIT).[11,12] This method is best used to increase an already lipophilic drug's dissolution and absorption in the GIT. SEDDS forms spontaneously upon mixing of all constituents with only gentle shaking and is thermodynamically stable, thereby providing a longer shelf life than other delivery vehicles. However, drugs can experience chemical instabilities in this system and there is a high concentration, 30-60%, of surfactant. This large amount of surfactant can irritate the GIT and cause diarrhea if used long term.[4] Due to its liquid

nature, SEDDS are limited to oral administration form, as they must be contained within a lipid-filled soft or hard-shelled gelatin.

1.1.2 Co-solvents

Co-solvency is defined as increasing the solubility of a drug by adding water miscible organic solvent, which said drug is known to be soluble.[13] This technique can be employed for both solids and liquids, in which a solid is formed during the spray freezing of a liquid to produce a powder. Due to the irritating effects of surfactants and the low toxicity of many organic solvents, drugs utilizing co-solvents are reserved for parenteral administration. Figure 1.1 shows some of the most commonly used co-solvents, which must possess a large solubilization capacity and low toxicity.[4] The choice of co-solvent can impact the chemical stability and the acid dissociation constant of the drug, as well as the overall viscosity and surface tension of the entire solution.[14] Therefore, a change in these properties can have a large affect on the bioavailability of the drug.

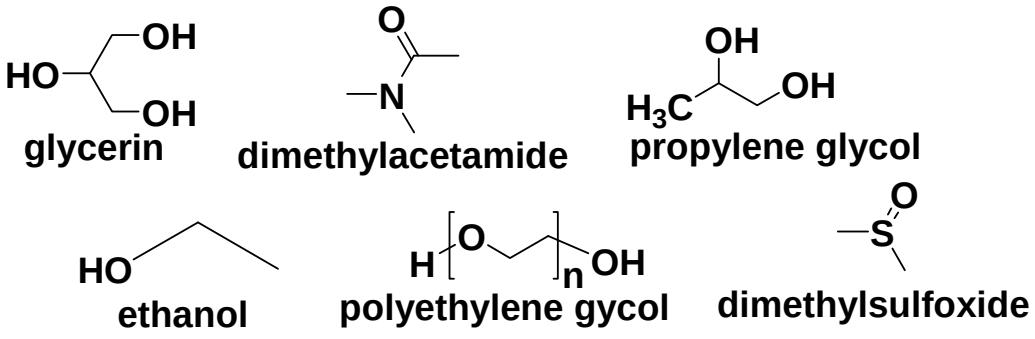


Figure 1.1. Structures of some commonly used co-solvents

1.1.3 Particle Size Reduction

The drug particle size is intrinsically related to the bioavailability, thus by reducing the particle size, the surface area is increased, thereby improving the dissolution of the drug.[15] Traditional methods to reduce particle size include milling/grinding and spray drying. However, since milling/grinding depends on mechanical forces to reduce particle size, the large amount of physical stress can cause drug degradation.[4] Both milling/grinding and spray drying can generate a substantial amount of heat, which can decompose thermosensitive or unstable drugs. However, problems with milling/grinding and spray drying have led researchers to develop alternative methods, namely microionization and nanosuspension, for very insoluble pharmaceuticals. Microionization employs jet mills and rotor stator colloid mills to increase surface area. Nanosuspensions combine an insoluble drug with a surfactant to produce a sub-micron colloidal dispersion.

1.1.4 The Solid State

The arrangement of a drug's molecules within a crystal structure can substantially affect the stability, ease of manufacture, and biopharmaceutical performance.[16] Drug molecules typically contain multiple functional groups along with complex chemical structures, which can exist in various conformations. Molecular packing with small local free energy minima differences create conformers such as polymorphs, co-crystals, or solvates/hydrates. Interestingly, each new material created from the various conformers will have unique physical, chemical, mechanical and biopharmaceutical properties.[16] The development of an unexpected or undesired crystal form may result in production failures and product performance problems. As

seen in Figure 1.2, the solid state of pharmaceuticals reaches into every facet of the discovery, development, and manufacturing of the desired drug.

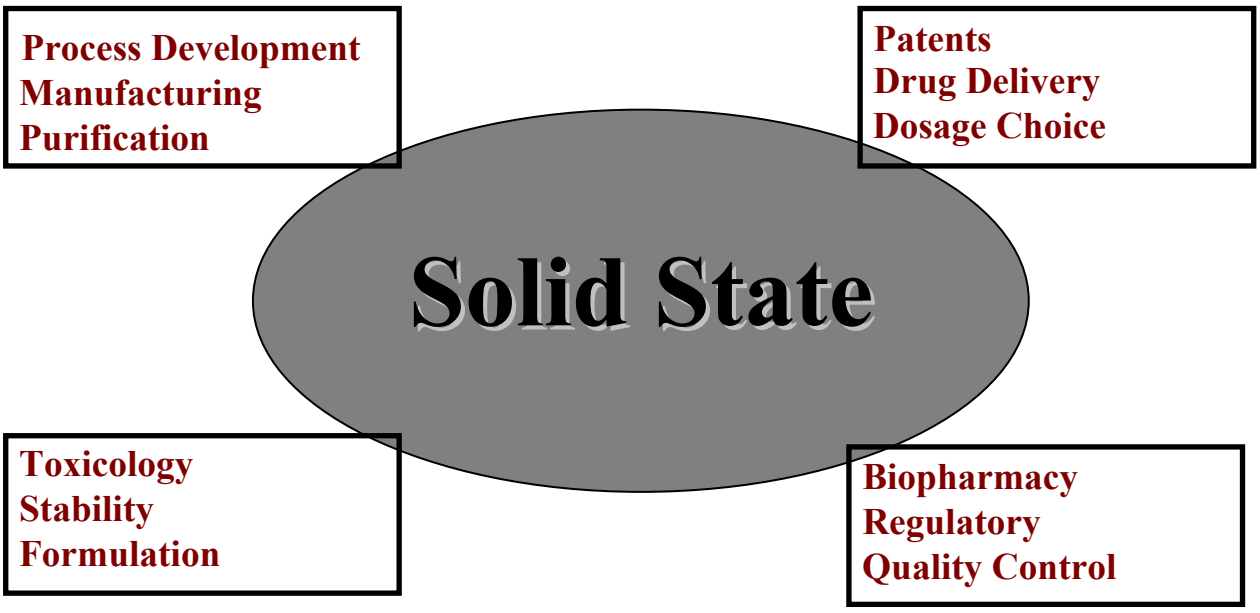


Figure 1.2. Solid state drugs in the pharmaceutical industry[17]

Crystal habit, defined as a crystal with identical chemical composition and structure, but different shape,[17] is a direct manifestation of both point group symmetries and crystallization conditions.[16] As stated by the Law of Rational Indices, low energy crystals are more stable, thus these crystals are typically observed in nature, as they are produced from a slower rate of crystallization.[18] Moreover, crystal shape can be influenced by the relative growth rate of the crystal faces, which can be impacted by mass and heat transfer, the drug manufacturing process, and interaction with crystallization solvents.[19] Unfortunately, crystal habit and size are not frequently considered when the desired crystal structure of a drug candidate is finalized for further development. Crystal shape may cause problems in the following:[16]

- mixing and de-mixing, flow, and milling;
- tablet lamination or capping as a result of particle slippage particle bonding, preferred orientations, and pressure transmission;
- dissolution; and
- aerodynamic properties of respiratory particles.

Although, different packing and habits of drug crystals alone or with other components can have major effects on a number of drug properties, crystal forms are mostly discovered by empirical approaches or serendipity. This makes predicting crystal different packing and habits difficult.[20]

1.1.5. Problems with the Solid State - Polymorphs, Solvates, and Hydrates

As discussed previously, a minute difference in crystal structure can change melting point, solubility, density, hardness, crystal shape, dissolution rate, etc, thereby affecting the biological properties of pharmaceutically activity compounds.[17] Interestingly, it has been found that out of 245 known pharmaceuticals, 89% of these possessed two or more different crystalline forms. Within this 89%, 50% of the drugs were found to be polymorphs (two crystals that have the same chemical composition, yet different molecular packing (crystal structure)),[17] 27% hydrates (crystal with same chemical composition but also containing molecule(s) of water),[17] and 23% solvates (crystal with same chemical composition but also containing molecule(s) of solvent).[17,21] unfortunately, detecting a new crystal structure of a drug might not happen for years, which could occur after a pharmaceutical has passed all FDA clinical trials.

An example of this problem is the active pharmaceutical ingredient (API) ritonavir (Norvir™).[22] Norvir™ was developed by Abbott Laboratories and was marketed in 1996 as

both an oral liquid and a semi-solid capsule. Throughout the development and clinical trails, only one crystalline type of ritonavir was identified. However, after 240 successful batches of ritonavir, it was found that ritonavir could convert to another polymorphic form (form II) during the manufacturing process. Form II of ritonavir was found to be more stable and less soluble than the originally FDA approved and tested form I. Since form II was less soluble, the bioavailability was poor; thereby the required dose of ritonavir was not achieved. Abbott Laboratories terminated manufacturing of Norvir™ and recalled all products from the market. Although the case of Norvir™ is extreme, identification of different crystalline forms can occur at all levels of a pharmaceutical's development, FDA clinical trials, and even during the manufacturing of an FDA-approved drug.

It is challenging to control the crystallization process to generate only the desired crystalline form, even when it seems that the system is understood. The prevention and/or prediction of crystalline transformations during the manufacturing process hinge on the crystals mutual interconversion.[22] If only one crystal form is thermodynamically favored, all other forms will be converted to the most stable crystal at equilibrium crystallization conditions. This process is exactly what occurred for Abbott Laboratories. Once the thermodynamically favorable conditions have been established, it is then possible to isolate the desired stable form of the crystal.[22] What form will be generated and how long the form will be present is reaction-scale dependent and cannot be easily predicted or controlled.[23] There are a number of controlling factors that can influence the crystallization structure of a drug such as saturation, temperature, solution concentration, cooling rate, type of solvent, agitation, pH, additive, impurities, etc.[24] Additionally, the temperature affects the intermolecular interactions, solubility, the collision frequency, etc. between the molecules of a drug. This is one of the predominant

factors that affect nucleation, growth, and transformation of polymorphs.[17] These factors have been categorized by Kitamaura[25] as either primary (basic factors) or secondary depending on the importance:

- Primary – supersaturation, temperature, seeds, stirring rate, and addition rate of anti-solvent.
- Secondary – types of solvent, additives, and interfaces.

Solvent selection is considered to be a secondary factor, as it is commonly used as a final step to isolate the purified API. Researchers often attempt to crystallize a drug from several types of solvent, ranging in polarity and proton-donating ability, to determine any and all polymorphs before development continues. The kinetic control of the crystallization depends greatly on the choice of solvent, as the solvent molecules can be selectively adsorbed onto the crystal face of the drug molecule. This can inhibit the nucleation growth of specific polymorphic forms.[26] The type of polymorph form can be influenced by the solvent-solute interactions, thereby manipulating the nucleation, crystal growth, and polymorphic transformation.[27] Seeding can also be used to control a drug's polymorphic form by adding seeds of the desired polymorph during the nucleation phase, thereby overriding the spontaneous nucleation.[24] However, the effectiveness of this method is directly related to the amount of solid added and the crystallization rate of the desired polymorph.

An additive can cause a dramatic effect on the crystallization of polymorphs, even though it is considered to be a secondary factor. Additives can play a role in the nucleation and crystal growth of polymorphs, by influencing the structure, shape, crystal size distribution, purity, stability, shelf life, etc.[28] Table 1.1 shows the variety of potential additives used in the

pharmaceutical industry. Unfortunately, the mechanism by which additives effect the crystallization of polymorphs is not well understood; however, several probable explanations of these mechanisms have been developed. The additives can alter the solubility, thus changing the solution structure. They can also be adsorbed onto the surface of formed or forming crystals, thereby blocking growth of other polymorphic forms. Lastly, the additives can be incorporated in the crystal lattice hindering the production of the desired polymorph.

Table 1.1 Commonly used additives in crystallization process[24]

Class of Additive	**Additive**
Metal Ions	Cr^{3+}, Fe^{3+}, Al^{3+}, Cu^{+}, Cd^{2+}, Mn^{2+}, etc.
Surfactants	Sodium dodecyl benzenesulfonate, surfactant gels, etc.
Salts	Sodium chloride, ammonium chloride, etc.
Organic Compounds	Urea, oxalate, alcohols, amino acids, etc.

Even though researchers make every attempt to control the polymorphic form of APIs, there are some drugs with desired therapeutic activity that cannot be isolated in the correct crystalline form by the above techniques. Thus, researchers have developed methods to reduce or eliminate the crystallinity of the drug all together. A solid dispersion system is used for APIs that will only be delivered orally with a formulation goal of preventing or predicting nucleation and growth of the drug crystal in both the drug-excipient matrix and the dissolution solvent.[16] Furthermore, the crystal growth of the drug is manipulated by the rate of diffusion. A solid dispersion is only thermodynamically stable if the desired drug dosage does not surpass the drug's overall solubility within the solid dispersion matrix.[16] To overcome this, the use of a solubilization agent or surfactant and liquid-filled hard gelatin capsules can be employed. In an

effort to decrease the solid-state transformation, i.e., change from amorphous to crystalline or from one polymorphic form to another, and thereby prolong the product shelf-life, a solid dispersion formulation needs to have the highest glass transition temperature (T_g) possible.[16] This can be achieved by adding high T_g excipients and storing the solid dispersion formulation at a temperature much lower than the T_g of the drug. However, the role that interacting excipients has on the overall stability cannot currently be predicted. Solid dispersion formulation should be kept under dry conditions, as water is a known destabilizer of these formulations.[29] However, an understanding of the temperature dependence of drug solubility also needed in addition to the T_g. to predict the overall physical stability of the API within the solid dispersion.[16] For amorphous drugs in a solid dispersion formulation, the temperature dependence of both the molecular mobility and activation energy of the nuclei formation should be identified to assist in the prediction of the possible crystallization rate.[30] Solid dispersion formulations are still relatively new, thus most of the testing has been performed on bench scale samples. The results obtained in these studies have not translated well into manufacturing; consequently more research is needed on solid dispersion formulations.[31]

To eliminate the crystalline structure, an API can be converted into an amorphous material, which can be defined as a supercooled liquid with considerably reduced molecular movement below its known T_g.[16] The thermodynamic characteristics, entropy and enthalpy are the driving forces for the phase transitions of an amorphous solid; however, the kinetic stability is controlled by the rate of nucleation and crystallization.[16] A commonly used measure for the creation of an amorphous drug is the 'T_g-50K Rule', where it is thought that crystallization is less expected if the drug is stored at 50 K below the T_g.[16] Unfortunately, no predictive model has been developed to link the crystalline stability to the shelf-life of the amorphous solid.

Additionally, the assessment of the biopharmaceutical efficacy and processing capacity must be correlated to the solid-state properties of the amorphous drug. As with any solid-state drug, the permeability and aqueous solubility should be evaluated, as it needs to be high enough for the drug to be able to reach the active site and be absorbed into the body.[32] Lipinski *et al.*[33] has developed the 'rule of 5' to provide a preliminary assessment for the possible permeability of the amorphous drug. By this rule, it is predicted that drugs will have low absorption or permeation if they possess (a) more than 5 hydrogen bond donors, (b) a molecular weight over 500, (c) a log P (partitioning ratio of octanol and water) value over 5, and (d) more than 10 hydrogen bond acceptors. Therefore, all the above factors must be taken into account when preparing amorphous drugs. Overall, there are several problems associated with the solid-state that can hinder the isolation, manufacture, and delivery of needed APIs. An additional strategy to overcome problems associated with the solid-state is currently needed.

1.2. Ionic Liquids

Classified as liquids exclusively composed of ions, ionic liquids (ILs) were first isolated by Walden[34] in 1914, when he synthesized a room temperature liquid, ethylammonium nitrate. Initially, this definition not only included ILs but also molten salts or fused salts that typically have a melting point over 100 °C.[35] Recently, ILs have become distinguished from molten/fused salts by displaying a melting point or T_g below 100 °C. Thus, aqueous solutions of salts cannot be classified as ILs as only ions must be present. Research continued steadily in the field of molten/fused salts, yet many applications of these salts were hindered by the high melting point. The high melting point was overcome by utilizing both pyridinium and imidazolium-based salts, which had a wide liquid range with some liquid at room temperature.[36] However, the early

attempts to synthesize room temperature salts resulted in problems with water-and air-stability, thus these first liquid salts had limited applications. After analyzing the structures of the cation and anion, it was found that dialkylimidazolium cations paired with tetrafluoroborate, nitrate, sulfate, and acetate counter ions yielded a room temperature liquid possessing water-and air-stability.[37] Researchers now know the vast potential of compounds that can be tuned for desired properties, thereby igniting research in the field of ILs.[38,39] Since the late 1990's, research within the IL field has increased at a staggering rate as both the types of ions, from simple imidazolium to complex, multi-substituted cations, and applications encompassing physical, chemical, and/or biological properties have become more widespread.[35]

Historically, room-temperature ILs (RTILs), ionic compounds that are liquid at room temperature, have been composed of large nitrogen- or phosphorous containing organic cations. These cations often include a linear alkyl chain of variable length. The most popular cation type is 1-alkyl-3-methyimidazolium with an alkyl chain length varying from methyl to decyl.[35] This cation can be paired with small inorganic ions such as chloride, bromide, and iodide, in addition, to larger anions like nitrate, dicyanamide, among others. However, in recent years, the types of both cations and anions have grown in complexity and variety as shown in Figure 1.3.

Several synthetic methods are utilized in the production of ILs, depending on the availability of the precursor cations and anions. Alkylation is primarily used with the majority of ammonium, imidazolium, pyridinium, and phosphonium containing ILs, where an alkylating agent such as an alkylhalide or a dialkyl sulfate is employed, thereby allowing for customization of the alkyl chain on the cation.[35] The desired alkylating agent plus an amine, phosphine, sulfide, or imidazole are then reacted to form the IL containing a halide counter ion. This reaction is attractive to many researchers as there are a wide range of inexpensive haloalkanes

available and the reaction conditions are mild, yet, produce good yields.[40] However, there are a limited number of counter ions available with this type reaction, which has prompted the use of anion exchange reactions.

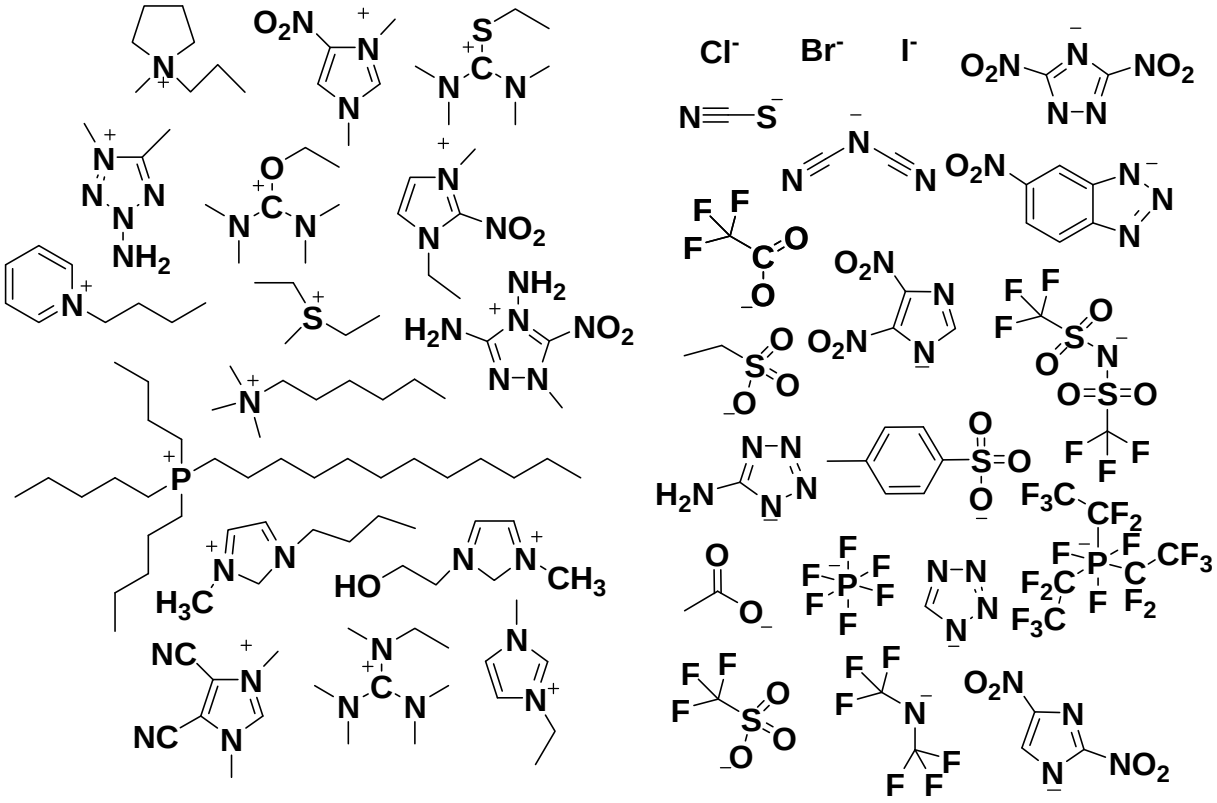


Figure 1.3. Structures of cations and anions found in many ILs

Anion exchange reactions are performed in two-steps, where the halide containing IL synthesized in the alkylation reaction will be used as the starting material. The halide anion is exchanged for the new, preferred anion by a simple anion metathesis reaction, normally removing the simple halide for an organic ion. The anion exchange can also take place by utilizing an ion-exchange resin preloaded with the desired anion.[35] An ion exchange (metathesis) reaction can be employed if the desired cation and anion are available as salts themselves, commonly paired with simple metal or halide ions.[37,41] When the precursor cation and anion

compounds are combined together, an ion exchange occurs resulting in the formation of the IL and a salt byproduct such as NaCl, NaBr, KCl, etc. (Figure 1.4) Unfortunately, this reaction generates a halide salt by-product that must be removed from the IL. The halide salt is typically precipitated by addition of solvent and then removed by filtration, however, it has been reported that even after several precipitation/filtration steps that trace amounts of halide salt can still be present. Even parts per million (ppm) quantities of halide salt and/or solvent can impart a marked effect on the physical properties. Halide impurities can also deactivate catalysis when ILs are used as reaction solvents.[35]

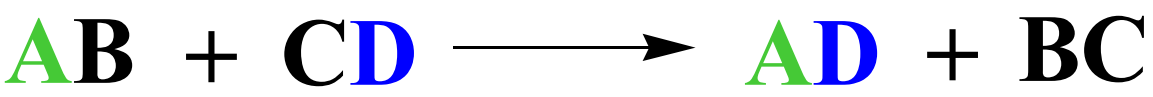


Figure 1.4. General scheme for IL metathesis reaction

Obviously, purification of the IL is needed once the synthesis has occurred. A general rule in the IL field is to remove as many impurities as possible from the starting materials, as the purification techniques for ILs are limited. It is best to employ synthetic methods which produce fewer side products. When utilizing liquid cation precursors such as 1-methylimidazole, it is possible to use distillation to remove impurities, which can result in colorless ILs.[42] For anion- or ion-exchange reactions, any solvents employed should be previously distilled and dried. However, the major impurity of this reaction type is halide salts, which must be monitored by either ion-sensitive electrode or a chemical method such as Volhand procedure.[43] This problem is more predominate in water-soluble ILs, as water-miscible ILs can not be washed with water to remove the halide salt.[35]

1.2.1 Properties of Ionic Liquids

The knowledge base regarding the physical, chemical, and biological properties of ILs is quite limited when compared to conventional organic solvents. Over the years, generalizations have been made relating to ILs properties, which simply cannot be true for all compounds within a class. The key trait of ILs is the inherent tunablity due to modification of cation and/or anion, thereby allowing for a wide variety of properties.[35] Obviously, ILs are best known for large liquid ranges that cannot be achieved with traditional molecular solvents, which is classified as the range between the melting point or T_g and thermal decomposition temperature. Low or negligible vapor pressure is another property that is often exploited in many IL applications, which is due to the strong Coulombic interactions between the ions.[35]

ILs also exhibited low melting points, as many were liquids at room temperature. The melting point of ILs is influenced by the size of the anion or cation, which, in turn, controls the magnitude of the Coulombic attraction and the packing efficiency. As the size or shape of either ion increases, the charge becomes more delocalized while the ability of the ions to closely pack is also diminished, thereby reducing the melting point.[44] Not only do the size and shape of the ions have an effect, but the symmetry of the cation also plays a significant role. When the symmetry is increased, the ions are able to more efficiently pack into the crystal lattice, thereby increasing the melting point.[44]

Since ILs can be composed of various cations and anions, the solubility and solvating power is controlled by the ion features. Since many researchers have intensely studied ILs based on imidazolium, ammonium, pyridinium, or phosphonium cations, it is possible to make general statements regarding the overall properties. These ILs are normally regarded as highly polar, yet, weakly coordinating solvents and have polarities similar to short-chain alcohols and polar,

aprotic solvents.[45,46] ILs often exhibit stronger solvating ability than traditional organic solvents, as solvation in ILs occurs through a multitude of interactions i.e., ionic, dipole-dipole, hydrogen bonding, van der Waals forces, and π-π interactions.[35] However, the magnitude of the various interactions depends not only on the cation and anion structure but also on the solute.

Lastly, the most controversial property of ILs is their 'greenness' or lack there of. In the late 1990's – early 2000's, several researchers, intrigued by ILs' lack of vapor pressure, labeled the entire class as 'green solvents'.[47,48] Indeed, the ILs utilized in these studies eliminated the need for volatile organic solvent and hazardous catalyst disposal. Thus, in this specific situation, ILs were a 'green' option to traditional methods, however, ILs can also possess 'non-green' properties such as toxicity. Unfortunately, information regarding ILs impact on the environment, animals, and humans is less than complete. Thus, the extent that ILs can be considered 'green' depends entirely on the choice of the ions.[35]

1.2.2 Applications of Ionic Liquids

The most popular application of ILs, based primarily on the physical properties, is solvent replacement and catalytic activity. The number of reactions that ILs have been used as solvents and/or catalysts is numerous and widespread across many disciplines of chemistry. ILs have been shown to increase both the reactivity and/or selectivity of reactions.[49-52] To determine a solution's chemical reactivity, the ability of the solvent to interact with substrates, intermediates, and transitions states must be assessed.[53] The inherent physical and chemical properties of the reacting solute(s) must be known. ILs have been found to interact with solutes via dipole-dipole and dispersion forces, as well as acting like strong hydrogen-bond acceptors, which is controlled by the anion.[45,54] Due to ion-ion interactions within the IL, there is a three

dimensional supermolecular network of anions and cations linked by hydrogen bonds and/or Coulombic interactions.[55,56] Therefore, ILs provide an entirely unique environment for the reaction to take place when compared to traditional molecular solvents. Initially, applications involving ILs focused on the physical properties, however, this inherent tuanbility of ILs allows for utilization of desired physical properties, large liquid range, negligible vapor pressure, etc. in addition to customized chemical properties.

However, in recent ILs have been used for their chemical application as high energy density (energetic) materials such as explosives, propellants, etc.[57-59] Energetic materials are defined as compounds that possess a large amount of energy in a small volume.[35] The majority of propellants are composed of a fuel and an oxidizer within the same compound, such as solid ammonium nitrate. Upon detonated, this compound explosively decomposes to generate by-products, nitrogen gas, oxygen gas, and water vapor. However, since ammonium nitrate is a solid, it is difficult to turn off denotation once it has begun, thus all of the propellant must be consumed once it is detonated. This is not a problem with liquid propellants, which can be turned off and on easily, but have much lower performances. ILs can possess many desired physical properties such as large liquid range, low or negligible vapor pressure, high thermal stability, etc., while allowing the researchers to tune the cation and anion for chemical applications.

Since both physical and chemical properties have been utilized in tandem to produce energetic materials, research within the IL field has shifted to encompass another property, biological. The synthesis and application of ILs has now moved to include biological properties, while still possessing the traditional physical and chemical attributes of ILs. It has been found that some imidazolium and pyrdinium-containing ILs, those previously utilized for physical

properties, are antimicrobial agents against bacteria and fungi.[60-62] The relationship between IL structure and antimicrobial activity was found to be dependent on the alkyl chain length, as the chain increased the biological activity also increased. Interestingly, ILs have also found use in embalming and tissue preservation, as a replacement to the commonly used formalin. Pernak *et al.*[60] reported that ILs containing long alkoxymethyl chains on the imidazolium cation were able prevent biological decomposition of the tissue. After two years of tissue immersion in IL, the tissue showed no signs of decay.

The above mentioned examples of biologically active ILs do not include API ions. If two different pharmaceuticals are combined into an IL, it is possible have a single compound with two biological functions along with the typical IL physical property. MacFarlane *et al.*[63] has the synthesized of several RTILs composed solely of API ions that exhibited decreased melting point from the precursor salts. Additionally, the liquidification of aspirin has also been reported, in which, aspirin was combined with several analgesic molecules to produce dual functioning (possessing two different APIs) salts.[64] However, the knowledge base regarding the formation of pharmaceutically based API is small, as much research in this field is needed.

1.3. Research Strategy

Drugs can exist as either neutral compounds or salts, depending on the desired physical, chemical, and biological properties. When a neutral drug is converted into a salt form not only do the solid-state properties change, but the biological activity can also be substantially modified. Table 1.2 lists the differences in biological efficacy with drug salts.

Table 1.2. Biological factors affected by salt formation

Factors Influenced by Salt Formation	Example
Dissolution rate	Transformed from quick release to slow-release drug[65]
Common-ion effect	Solubility of chloride containing salt can have decreased solubility in HCl versus water[66]
Solvation	Anhydrous form of API is freely water soluble in water while hydrate is only slightly soluble[67]
Solubilization	Different salts produce higher or lower bioavailability due to micellar solubilization[68]
Ion pair	Ion pair formation can increase the transport of a drug through the skin[69]

It has been determined that approximately half of all therapeutic drugs are currently used as salts; therefore solid salt forms are commonly utilized.[70] However, as previously discussed, there are many problems associated with the solid-state that can lead to undesired problems. Thus, a liquid salt drug form would eliminate difficulties with the solid state, while providing tunability in the cation and anion structure that may affect the compound's physical, chemical, and biological properties. To explore the IL modular strategy, this dissertation will discuss the formation of ILs from known APIs with the hope to understand how the structure of the ions and the interactions between the ions influence the physical and biological properties.

Chapter 2 describes the general characterization procedures that are used to determine the physical properties of the synthesized ILs. To begin, Chapter 3 focuses on the formation of antibacterial sweetener ILs from known IL forming ions. Although both the cation and anion molecules have previously been utilized in the synthesis of ILs, the combination of anti-bacterial and sweetener has not been reported. We hypothesized that since these ions had found prior use

in ILs, this pairing would also result in an IL. Artificial sweeteners do not possess pharmacological activity, but find application in food products; therefore these compounds are included on the GRAS list. The physical properties along with the anti-microbial activity was assessed and compared with the starting materials to determine positive or negative synergistic effects.

The results obtained from Chapter 3 provided valuable information regarding the influence of ion structure on the physical and biological properties. However, these synthesized ILs do not contain two biological active compounds, therefore research regarding the combination of two APIs was the subject of Chapter 4. The same anti-bacterial cations utilized in Chapter 3 were also employed for this study, due to their known IL-forming ability. However, in this project, these cations were paired with the commonly used pain reliever, ibuprofen, and anti-acne agent, sulfacetamide. By combining two drugs into a single compound, it was hypothesized that these new ILs could potentially be dual functional in nature i.e., possess two separate biological functions while still exhibiting typical IL physical attributes i.e., low melting point. Since the selected anions had previously not been employed to form ILs, it was unclear if an IL would result. Thus, Chapter 4 centered on the production of liquid salts from two biological active compounds coupled with an assessment of the resulting physical properties.

The same synthetic strategy employed in Chapter 4 was also utilized in Chapter 5 to produce dual functional liquid salt based on the popular local anesthetic, lidocaine. However in addition to understanding the physical properties, the IL was subjected to biological testing to determine the analgesic power and the cellular mechanism of action. Due to interesting biological results obtained in Chapter 5, more knowledge regarding the solution behavior of these ILs was needed. Thus, the research discussed in Chapter 6 attempted to determine

solubility, thermal stability, and melting point depressing trends, which could be used in the prediction of ILs' properties, by the synthesis of structurally similar ILs based on lidocaine. Finally, Chapter 7 concludes the information obtained in this dissertation and examines the future research needed to understand the ion influences on the IL.

CHAPTER 2

GENERAL EXPERIMENTAL PROCEDURES

The experimental procedures discussed below were general analytical techniques used in the research presented in this work.

2.1. Thermogravimetric Analysis (TGA)

To determine the range of thermal decomposition temperatures for each synthesized IL, TGA was employed using TA Instruments (New Castle, DE) model 2950 thermogravimetric analyzer. The samples were analyzed on a platinum pan with a purge gas of dried air. The samples were heated over a temperature range of 30-600 °C with a constant heating rate of 5 °C min^{-1} in ensure the compound was completely decomposed. The sample is initially heated to 75 °C with a constant heating rate of 5 °C min^{-1}. To guarantee all water or residual solvent is removed from the compound, it was held at a constant temperature of 75 °C for 60 minutes. Then the IL was heated to a final temperature of 600 °C, at constant heating rate of 5 °C min^{-1}. The decomposition profiles of the compounds were established from the onset temperatures for 5% decomposition of the sample, and the value was reported as a single temperature.

2.2. Differential Scanning Calorimetry (DSC)

TA Instruments (New Castle, DE) model 2920 differential scanning calorimeter was employed to determine melting points, glass transitions, and crystallization temperatures. Approximately 3-10 mg of the sample was placed in closed, but not sealed aluminum pan (KLD-202, KEtecand Lab Devices, Inc., Mount Berry, GA) with a pin-hole in the lid. The pin-hole was needed to allow any water or residual solvent to escape from the sample during the first heating cycle. The instrument was temperature calibrated with separate samples of indium and DI water before data was collected on IL samples. An empty closed pan was utilized as a reference for each experiment, where the ramp temperature was 5 °C min^{-1}. After each interval of cooling to heating or heating to cooling, the sample was allowed to equilibrate for 5 minutes before a new process was started. Each sample was heated and cooled at least twice to confirm the transitions and temperatures (double cycle run). All transition temperatures were determined from the onset temperatures for the observed melting point, glass, or liquid-liquid transitions.

Solid samples were initially heated past the melting point temperature to a temperature no greater than 30 °C below the onset temperature for 5% decomposition of the sample, which was pre-determined by TGA analysis. Once the maximum heating temperature was reached, the 5 minute interval was applied, and then the sample was cooled at 5 °C min^{-1} to a minimum temperature of -100 °C. The 5 minute equilibrate interval was applied and the entire process was repeated two twice. Liquid samples were subjected to a different process than solid samples. The liquid was first cooled to -110 °C and then heated to a maximum temperature 30 °C below the onset temperature for 5% decomposition of each liquid as previously established by TGA. The sample was allowed to equilibrate for 5 minutes before cooling at 5 °C min^{-1}. This process was repeated twice.

2.3. Water Content Determination

Water has been known to have adverse effects on the physical and chemical properties of ILs, therefore it is necessary to account for the amount of water before any testing of physical, chemical, or biological properties can occur.[71,72] Parts per million quantities of water determined via Karl-Fisher (volumetric) titration by EM Science Aquastart V1B volumetric titrator. Calibration of the titrator with pure deionized (DI) water was conducted prior to water content determination of the ILs. Each sample was titrated three times, and if found in good correlation, the values were averaged to obtain the water content.

2.4. Nuclear Magnetic Resonance (NMR)

All NMR spectra, ^{1}H or ^{13}C, were collected utilizing [d_6] DMSO as the solvent, unless otherwise stated, with TMS as the internal standard. ^{1}H NMR measurements were taken at either 360 MHz or 500 MHz while ^{13}C NMR measurements were recorded at 90 MHz or 125 MHz. All chemical shifts are given in δ (ppm).

2.5 X-Ray Diffraction.

Crystalline samples were mounted on a glass fiber on a goniometer head of a Siemens SMART CCD diffractometer equipped with a MoKα source (λ = 0.71073 Å) and a graphite monochromator. Data collection was conducted at -100 ^{o}C which was achieved by streaming cold nitrogen over the crystal. Final unit cell parameters were determined by least squares refinement of the hemispherical data set obtained from 20 second exposures. Data were corrected for Lorentz and polarization effects and absorption using SADABS.[73] The initial

structure solution was carried out using the direct methods option in SHELXTL version 5.[74] The positions of all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were added and allowed to refine unconstrained in order to obtain proper close contact interactions.

CHAPTER 3

SWEET AND ANTI-MICROBIAL QUATERNARY AMMONIUM-BASED IONIC LIQUIDS

Taken in part from a published manuscript: Troutman-Hough, W. L.; Smiglak, M.; Griffin, S.; Reichert, W. M.; Mirska, I.; Jodynis-Liebert, J.; Adamska, T.; Nawrot, J.; Stasiewicz, M.; Rogers, R. D.; Pernak, J, "*New J. Chem*. **2009**, 33(1), 26-33.

Acknowledgment: Griffin, S.; Reichert, W. M (The University of Alabama, Tuscaloosa, AL) for collecting and analyzing the crystal structure. Mirska, I.; Jodynis-Liebert, J.; Adamska, T.; Nawrot, J.; Stasiewicz, M.; Pernak, J (Poznań University of Technology, Poznan, Poland) for biological testing.

3.1. Introduction

The anti-bacterial properties of quaternary ammonium compounds (QACs) were first discovered during the late 19th century.[75] Initially, QACs were found to be most effective against gram-positive organisms, until Jacobs and Heidelberger[76-79] further exploited their anti-bacterial properties against other types of organisms. It was not until 1935 that the full potential of QACs was recognized by the chemical community, when the synthesis of benzalkonium chloride, a long-chain QAC, by Domagk[80] and further characterization of its anti-bacterial activities, proved that QACs were effective against a wider variety of bacterial strains.

Later, in the 20th century, researchers became more interested in the synthesis of water soluble QACs for potential applications as surfactants,[81,82] anti-electrostatic agents,[83] anti-corrosive agents,[84] disinfectants,[85] and phase-transfer catalysts.[86] These newly developed water soluble QACs showed anti-bacterial action against not only gram-positive and gram-negative

bacteria, but also pathogenic species of fungi and protozoa.[87] These discoveries led to applications for QACs in wood preservation[88-90] and as preservatives in common household products,[91] especially for general environmental sanitation in hospitals and food production facilities. Furthermore, QACs have been used as penetration enhancers for transnasal and transbuccal drug delivery, such as nasal vaccinations.[92] The ability of QACs to penetrate and open cell membranes has been widely used in drug delivery such as liposomes, which consists of long alkyl chain QACs, and non-viral gene delivery.[93]

There was specific interest in employing the IL concept to pair the biological activity of a class of compounds such as QACs, with a second biological activity inherent in the counter ion.[94] One such class of ions, which has also seen independent use in preparing 'edible' ILs, includes non-nutritive sweeteners such as saccharinate and acesulfame.[95,96] Salts of these anions (sodium and potassium, respectively) are currently used in food products and are approved as food additives by most national and global health agencies. In addition to providing a non-caloric sweet taste, these artificial sweeteners are also utilized to increase the shelf life of some sweetened products.[97] High artificial sweetener thermal stability is needed, as long time at high cooking temperatures can cause decomposition with the potential of new compound formation.[98] The combination of QACs and artificial sweeteners has been reported in the literature.[99] However, these compounds were used in phase separation and not for their biological functions. It is expected that the pairing of a long chain anti-bacterial cation with a solid sweetener would yield a liquid with increased thermal stability (from the QAC cation) while still retaining both the anti-bacterial activity and sweet taste. We envision that these ILs could find applications in various food products specifically sweetened products such as cakes, icing, candies, etc. where increased shelf life would reduce the amount of spoilage. Thus, we will report the synthesis of

six new ILs along with the physical properties, anti-microbial activities, toxicity, and insect deterrent activity.

3.2. Experimental

3.2.1 Chemicals and Microorganisms.

Benzalkonium chloride [BA][Cl] (molecular formula $C_6H_5CH_2N(CH_3)_2RCl$ with a mixture of R groups (R is $C_{12}H_{25}$ (60%) and $C_{14}H_{29}$ (40%)), didecyldimethylammonium bromide [DDA][Br] (tech., 75 wt% gel in water), hexadecylpyridinium chloride [HEX][Cl] (monohydrate, minimum 99%), and sodium saccharinate Na[Sac] (hydrate, minimum 98%) were purchased from Sigma-Aldrich (St. Louis, MO). Potassium acesulfamate K[Ace] (≥ 99%) was purchased from Fluka.

The following microorganisms were used: bacteria *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* (MARSA) ATCC 43300, *Enterococcus faecium* ATCC 49474, *Escherichia coli* ATCC 2592,2 *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 4352, and fungi *Candida albicans* ATCC 10231, *Rhodotorula rubra* PhB and *Streptococcus mutans* PCM (Polish Collection of Microorganisms) 2502. The *Rhodotorula rubra* was obtained from the Department of Pharmaceutical Bacteriology, Poznań University of Medical Sciences, Poland.

3.2.2 General Synthesis.

Solid (1 mmol) Na[Sac] or K[Ace] was dissolved in 50 mL distilled water and added to hot aqueous solutions containing 1 mmol of [BA][Cl], [DDA][Br], or [HEX][Cl]. The mixtures were stirred at 60 ^{o}C for 1 hour and then cooled to room temperature. The hydrophobic product

was extracted with 100 mL chloroform and washed with DI water washes. The halide impurities (NaCl or NaBr) were monitored by silver nitrate ($AgNO_3$) test, in which one or two drops of 0.1 M $AgNO_3$ aqueous solution was added to the DI water washes. Insoluble salts i.e., silver chloride and silver bromide were formed, which precipitate from the aqueous solution as a white and white-yellow solid, respectively. The chloroform phase was washed with DI water until the water washings tested negative for NaCl or NaBr via $AgNO_3$ test. The chloroform was evaporated, and the IL was dried under high vacuum for at least 12 hours with gentle heating (50-80 °C). Karl-Fischer analysis indicated the water content of all dried ILs to be less than 500 ppm.

Benzalkonium saccharinate [BA][Sac]: White solid; yield 98%; ^{1}H NMR (360 MHz; DMSO-d_6; Me_4Si) 0.85 (3H, t), 1.24 (20H, m), 1.77 (2H, m), 2.95 (6H, s), 3.24 (2H, m), 4.53 (2H, s), and 7.59 (9H, m); ^{13}C NMR (90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 21.7, 22.0, 25.7, 28.4, 28.6, 28.7, 28.8, 28.9, 31.2, 49.0, 63.4, 66.1, 118.9, 122.3, 128.0, 128.8, 130.1, 131.4, 132.8, 134.8, 145.2, and 167.7.

Didecyldimethylammonium saccharinate [DDA][Sac]: Viscous liquid, yield 95%, ^{1}H NMR (360 MHz; DMSO-d_6; Me_4Si) 0.85 (6H, t), 1.24 (28H, m), 1.63 (4H, m), 3.00 (6H, s), 3.22 (4H, m), and 7.58 (4H, m); ^{13}C NMR (90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 21.6, 22.0, 25.6, 28.4, 28.6, 28.7, 28.8, 31.2, 49.9, 62.7, 118.9, 122.3, 130.8, 131.3, 134.8, 145.2, and 167.7.

Hexadecylpyridinium saccharinate [HEX][Sac]: White solid, yield 86%, ^{1}H NMR (360 MHz; DMSO-d_6; Me_4Si) 0.85 (3H, t), 1.25 (26H, m), 1.89 (2H, m), 4.59 (2H, t), 7.57 (4H, m), 8.16

(2H, t), 8.61 (1H, t), and 9.11 (2H, d); ^{13}C NMR (90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 21.9, 25.2, 28.2, 28.6, 28.8, 28.9, 29.0, 30.6, 31.1, 60.6, 99.5, 118.9, 122.3, 127.9, 130.7, 131.3, 134.8, 144.6, 145.3, and 167.6.

Benzalkonium acesulfamate [BA][Ace]: White solid, yield 95%, δ_H(360 MHz; DMSO-d_6; Me_4Si) 0.85 (3H, t), 1.25 (20H, m), 1.78 (2H, m), 1.90 (3H, s), 2.95 (6H, s), 3.24 (2H, m), 4.53 (2H, s), 5.28 (1H, s), and 7.52 (5H, m); δ_C(90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 19.3, 21.7, 22.0, 25.7, 28.4, 28.6, 28.7, 28.8, 28.9, 31.2, 49.0, 63.4, 66.1, 102.0, 128.0, 128.8, 130.1, 132.8, 159.5, and 167.6.

Didecyldimethylammonium acesulfamate [DDA][Ace]: Viscous liquid, yield 94%, ^{1}H NMR (360 MHz; DMSO-d_6; Me_4Si) 0.86 (6H, t), 1.26 (28H, m), 1.63 (4H, m), 1.89 (3H, d), 2.99 (6H, s), 3.22 (m, 4H), and 5.26 (1H, q); ^{13}C NMR (90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 19.3, 21.6, 22.0, 25.7, 28.4, 28.6, 28.7, 28.8, 31.2, 49.8, 62.7, 102.0, 159.4, and 167.6.

Hexadecylpyridinium acesulfamate [HEX][Ace]: White solid, yield 88%, ^{1}H NMR (360 MHz; DMSO-d_6; Me_4Si) 0.85 (6H, t), 1.23 (26H, m), 1.89 (5H, m), 4.58 (2H, t), 5.26 (1H, s), 8.15 (2H, t), 8.61 (1H, t) and 9.08 (2H, d); ^{13}C NMR (90.6 MHz; DMSO-d_6; Me_4Si) 13.8, 19.3, 21.9, 25.2, 28.2, 28.5, 28.6, 28.7, 28.8, 28.9, 31.1, 60.6, 101.9, 127.9, 144.6, 145.3, 159.3, and 167.5

3.2.3 Anti-microbial Characteristics.

Anti-microbial activity was determined by the tube dilution method. Bacteria strains were cultured in Mueller-Hinton broth for 24 h, and fungi were cultured on Sabouraud agar for 48 h. Suspensions of the above microorganisms, at a concentration of 10^6 colony-forming units (cfu)/mL, were prepared from each culture. Two milliliters of serial twofold dilutions of IL were inoculated with the above-mentioned suspension to obtain a final concentration of (1 to 5) × 10^5 cfu/mL.

Growth of the microorganism was determined visually after incubation for 24 hour at 35 ^{o}C (bacteria) or 48 hour at 22 ^{o}C (fungi). The lowest concentration at which there was no visible growth (turbidity) was determined to be the minimal inhibitory concentration (MIC). Then, from each tube content, 10 mL (calibrated loop) was smeared on an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80, and 0.1% L-cysteine) and incubated for 48 hour at 35 ^{o}C (bacteria) or for 5 days at 22 ^{o}C (fungi). The lowest concentration of the IL that killed 99.9% or more of the microorganism was defined as the minimum biocidal concentration (MBC).

3.2.4 Acute Oral Toxicity Test.

The toxicity was tested according to the method of acute toxic class.[100] Three male (250 ± 25 g) and three female (170 ± 17 g) Wistar rats were used for each IL tested and at each dosage. The ILs were first suspended in distilled water and then administered intragastrically at doses of 300 mg/kg b.w. or 2000 mg/kg b.w. After the dose was administered, the rats were observed for 14 days.

3.2.5 Skin Irritation Tests.

Each IL was tested on 3 male New Zealand albino rabbits, where the fur was previously removed from the back of the rabbit. Half a milliliter of the ILs (100%, pure) was distributed on two 6 cm^3 sites of the same animal. The application site was then covered with a porous gauze dressing and secured in place with tape. After a 4 hour exposure, the dressing was removed, and the application site was gently washed with water. Observations were then conducted at 1, 24, 48, and 72 h, where the test sites were evaluated for erythema and edema using a prescribed scale.[101]

3.2.6 Feeding Deterrent Activity Tests.

Three species of insects were selected for testing: *Tribolium confusum* Duv. (larvae and beetles), *Sitophilus granarius* L. (beetles), and *Trogoderma granarium* Ev. (larvae). Insects were grown on a wheat grain or whole-wheat meal diet in laboratory colonies which was maintained at 26±1 ^{o}C and 60±5% relative humidity. The laboratory assay was conducted according to the method developed and standardized for storage insects feeding activity for both choice and no-choice test.[102]

Wheat wafer discs (1 cm in diameter × 1 mm thick) were saturated by dipping in either ethanol (96%) only (control) or in a 1% ethanol solution of [DDA][Ace] or [DDA][Sac]. After evaporation of the solvent by air-drying (30 min), the wafers were weighed and offered as the only food source for the insects over a five day period. The feeding of the insects was recorded under three conditions: (a) control test (two control discs (CC)), (b) choice test (a choice between one treated disc (T) and one control disc (C)), and (c) no-choice test (two treated discs (TT)). Each of the three experiments was repeated five times with 3 beetles of *Sitophilus granarius*, 20

beetles and 10 larvae of *Tribolium confusum*, and 10 larvae of *Trogoderma granarium*. The number of individual insects depended on the intensity of their food consumption. The beetles utilized in the experiments were unsexed, 7-10 days old, and the larvae were 5-30 days old. After five days of feeding, the discs were reweighed. The data from the experiments have been statistically corrected by an analysis of variance.

3.3. Results and Discussion

3.3.1 Synthesis.

[DDA][Sac], [DDA][Ace], [BA][Sac], [BA][Ace], [HEX][Sac], and [HEX][Ace] (Figure 3.1) were prepared in yields from 86% ([HEX][Sac]) to 95% ([BA][Ace]) as hydrophobic salts from commercially available QACs. Each cation precursor was paired with sodium saccharinate or potassium acesulfamate by a stoichiometric metathesis reaction in aqueous solution. The hydrophobic nature of these ILs allowed for easy extraction from the aqueous phase into chloroform, in which the majority of the halide salt remained in the aqueous phase. To ensure the complete removal of the halide salts, the chloroform phase was washed with several aliquots of water until $AgNO_3$ test was negative. The synthesized ILs were found to be only sparingly soluble in cold and hot water, but freely soluble and stable in many organic solvents i.e., chloroform, methanol, ethanol, ethyl acetate, N,N-dimethylformamide, and DMSO.

3.3.2 Crystal Structure[103]

Only one crystal structure, [HEX][Ace], was obtained from the synthesized salts. The crystal was isolated from the room temperature solid after drying on the high vacuum line for 12 hours at 50 °C. Interesting packing behavior was observed that may provide clues to the low

melting nature of these synthesized ILs particularly and QAC ILs in general. The packing diagram for [HEX][Ace] (Figure 3.2 & Figure 3.3) reveals that the cation tails interdigitate to create charge-rich and hydrophobic regions.

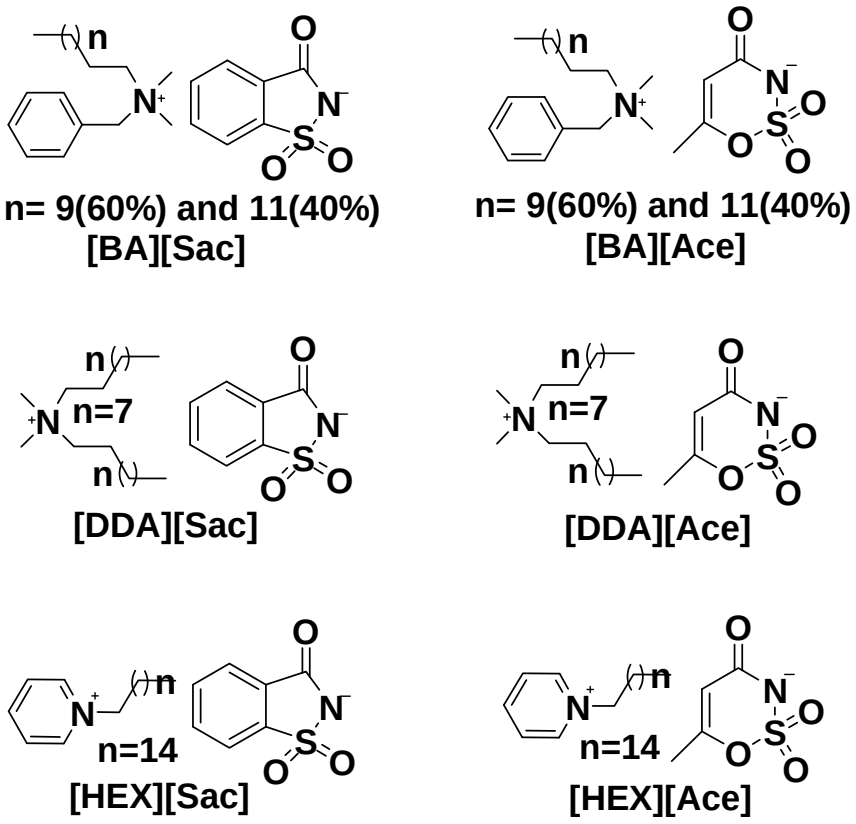


Figure 3.1. Structures of the synthesized anti-bacterial sweetener ILs.

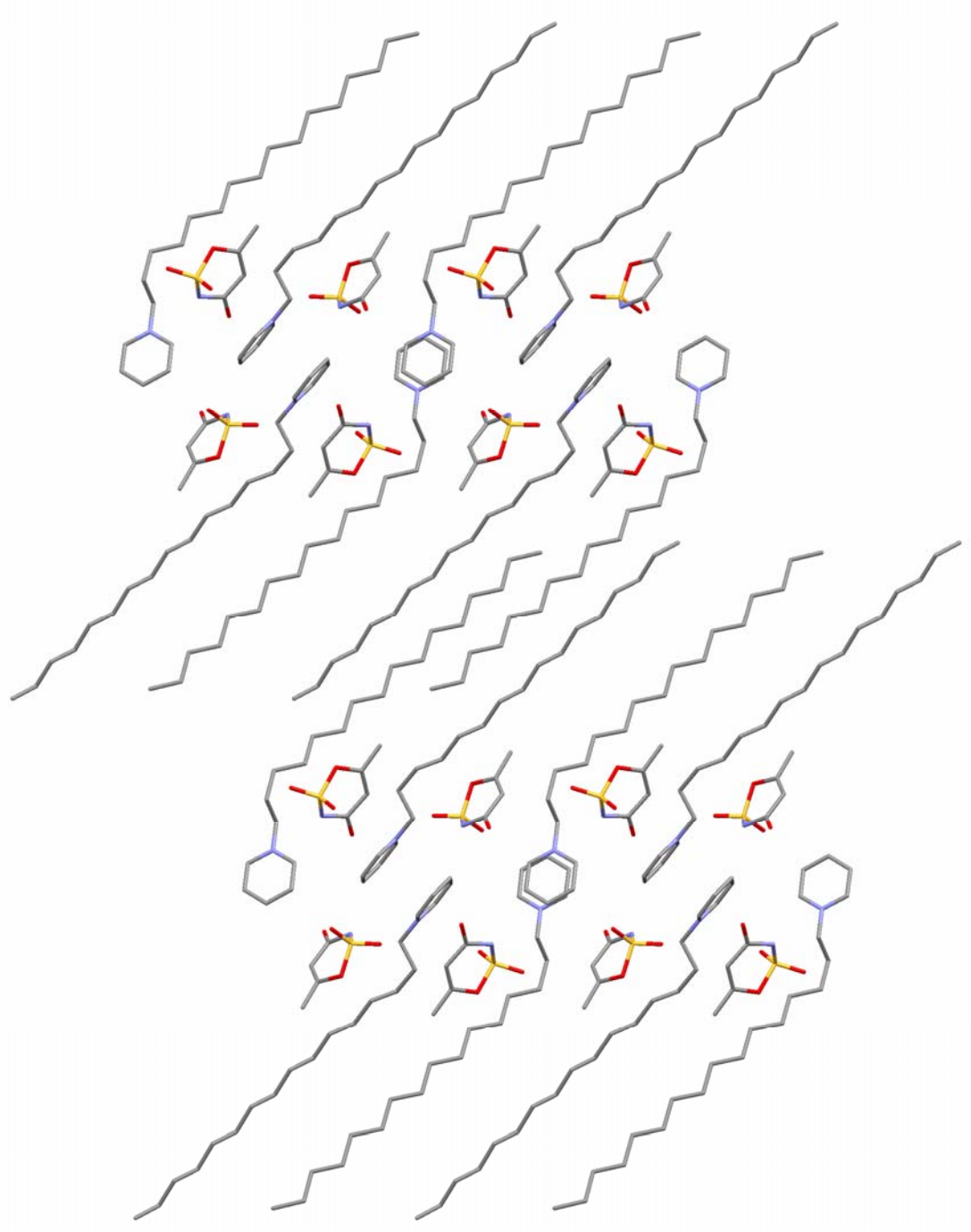


Figure 3.2 Packing diagram along the *a* crystallographic axis for [HEX][Ace].

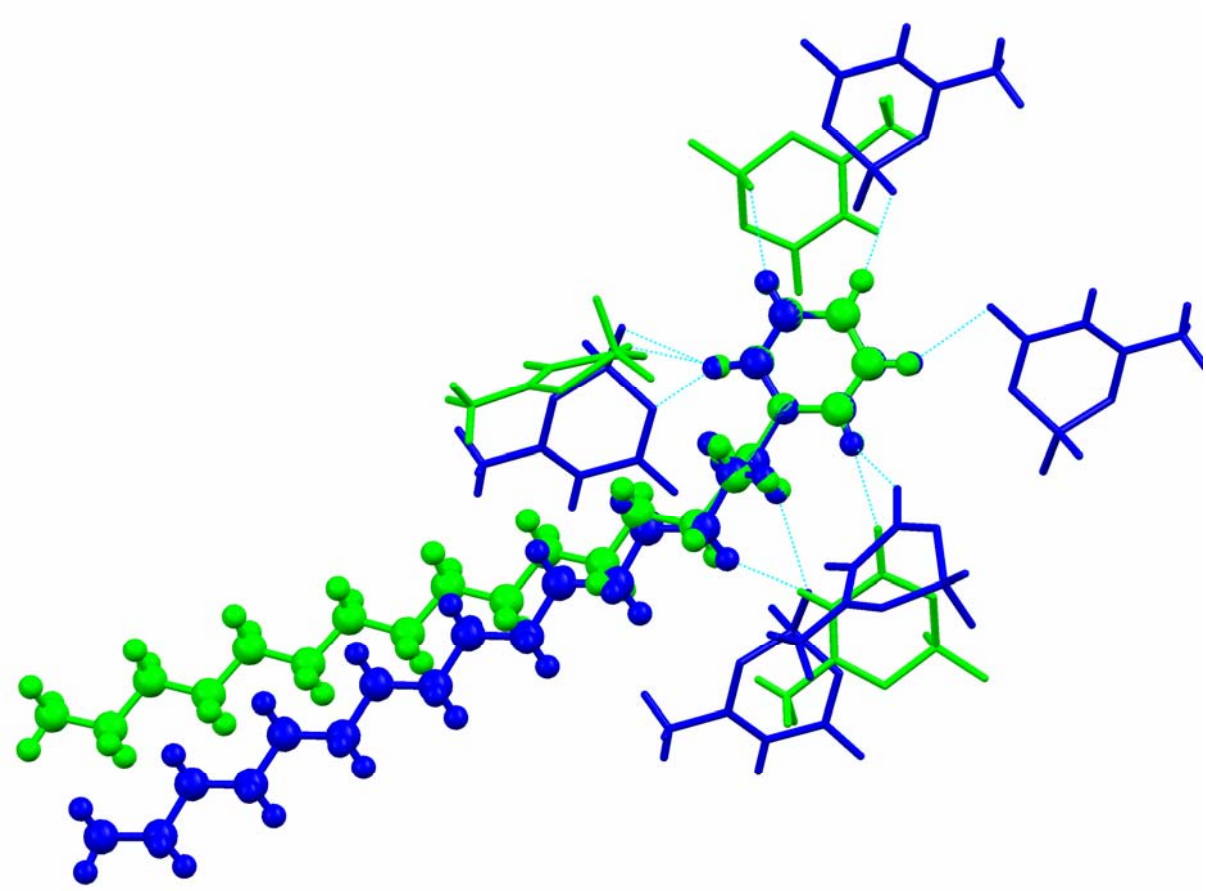


Figure 3.3. Overlay of the two cations of [HEX][Ace] in the asymmetric unit including the anions with close contacts to each.

This behavior has also been observed in other crystal structures containing [HEX], however none have been ILs. Ito *et al.*[104] found crystals of [HEX] with the polyoxometalate hexamolybdate, MO_6, to possess alternating inorganic monolayers of MO_6 and organic bilayers of [HEX] cations. As observed for [HEX][Ace], the hydrophobic hexadecyl chains interdigitate with the bilayers of [HEX] in [HEX]MO_6 with the hydrophilic heads of [HEX] associated to the MO_6 anions.[104] Additionally in both crystal structures, π-π stacking of the pyridine ring of [HEX] is seen, which stabilizes the overall structure (Figure 3.3). [104] Thus, the cation [HEX] exhibits similar crystal structure if paired with an organic cation, [Ace] or an inorganic cation, MO_6; therefore, the [HEX] influences the overall structure of the crystal.

However on closer examination of [HEX][Ace], it was found that the [HEX] cations in the [HEX][Ace] crystal are not equivalent, as slight differences in the orientation of the hexadecyl tail groups are observed. This modest difference in orientation leads to completely different packing environments. One cation π-stacks in a polymeric fashion and has only three close contacts with the anions (Figure 3.4). The second cation forms a π-stacked dimer with anions capping each open face. In addition to π stacking, the [HEX] cations have five close contacts with the anions (Figure 3.5). This phenomenon was not seen in the [HEX]MO_6, as hydrogen bonding in not present in this compound, however, it is possible for the cation and anion to hydrogen bond in [HEX][Ace].

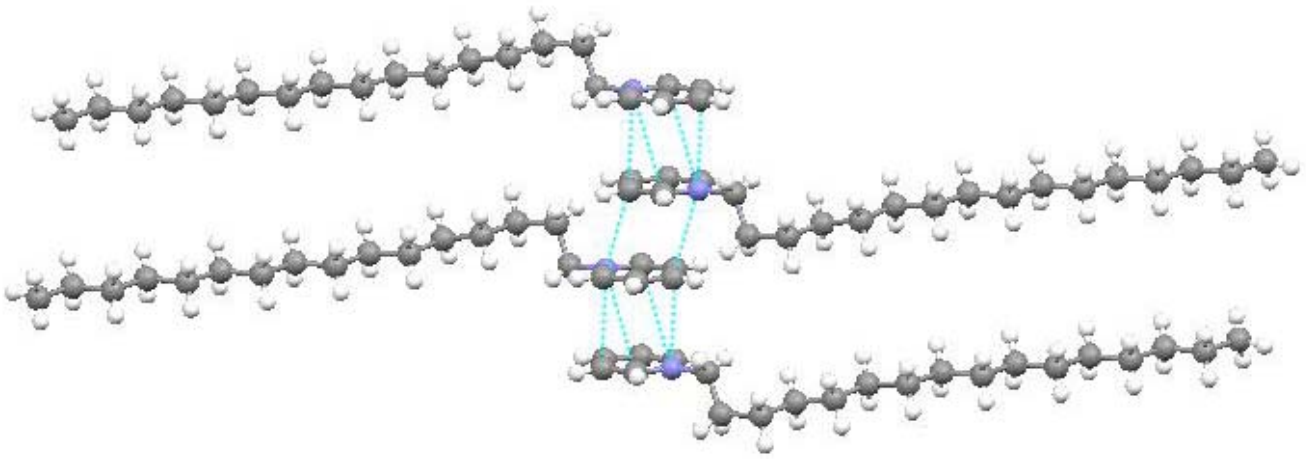


Figure 3.4 One cation in [HEX][Ace] π-stacks in a polymeric fashion (interplanar spacing 3.5 and 3.6 Å).

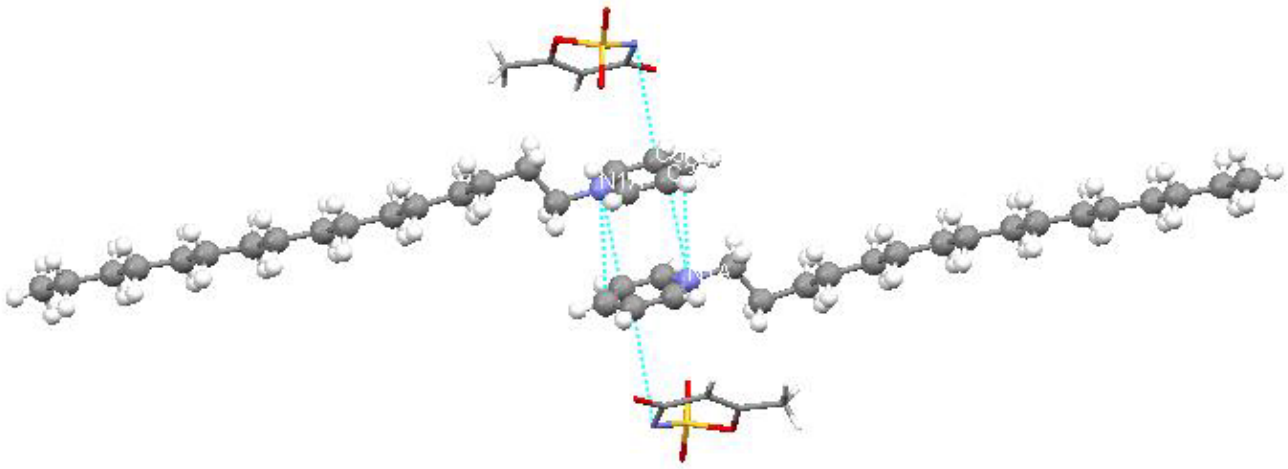


Figure 3.5 Second cation in [HEX][Ace] forms π-stacked dimers (interplanar spacing 3.4 Å) with acesulfamate anions capping both sides.

3.3.3 Thermal Behavior

The thermal properties of the synthesized ILs (Table 3.1) were determined by DSC and TGA and were compared with the precursor salts. All of the synthesized salts exhibited melting points below 100 ^{o}C, ranging from 16 °C ([DDA][Sac]) to 90 °C ([BA][Ace]), which allow them to be classified as ILs. All of the newly prepared ILs were found to be low melting solids at room temperature with the exception of [DDA]-containing salts, the only cation without an aromatic ring, which were found to be liquid at room temperature. However, only [DDA][Ace] did not exhibit a melting point, but only a T_g Additionally only two other ILs, [DDA][Sac] and [HEX][Ace], possessed T_g, at -53 ^{o}C, and -11 ^{o}C, respectively. Following the glass transition temperature, [DDA][Sac] and [HEX][Ace] both displayed consecutive crystallization and melting transitions. However, [HEX][Ace] had two crystallization temperatures, one observed on the heating cycle and one on the cooling cycle. This observation is reasonable since the cation can possess two different orientations in the crystal structure.

Additionally, Pernak *et al.*[105] have synthesized [BA] and [DDA] lactate ILs that have also exhibited a loss of melting points, as only a T_g at -36 °C and -56 °C, respectively, were displayed. Although, [BA] lactate exhibited only a T_g, when the counter ion was changed to either [Ace] or [Sac], a discernable melting point was obtained. Also both the lactate containing ILs were obtained as viscous liquids, whereas [BA][Ace] and [BA][Sac] were isolated as waxy solids. This behavior could be attributed to the structural similarities of the cation and anion, as the aromatic rings can π stack. This interaction is not possible with the lactate counter ion that does not contain an aromatic ring.

As seen in Table 3.1, the ILs were thermally stable to temperatures ranging between 160 ^{o}C and 210 ^{o}C. One-step decomposition was found for [BA][Sac] and [DDA][Sac], however, the

thermal stability was only slightly higher than [K][Ace] and substantially lower than the [Na][Sac]. The precursor anion salt normally displays a two-step decomposition, suggesting that the cations play a larger role in the decomposition of these ILs, thus resulting in the single decomposition step observed.

Two-step decomposition was observed for samples [HEX][Sac], [DDA][Ace], and [HEX][Ace]. Increase in the thermal stability (first decomposition step) in these ILs, over the thermal stabilities of the starting materials, may indicate an anion stabilizing effect. Similarly, the stabilizing effect of the anion can be observed for the sample of [BA][Ace], which is the only sample that exhibits a three-step decomposition pathway. Unfortunately, only one IL, [HEX][Ace], possessed a decomposition temperature greater, approximately 25 °C, than the precursor salts,. Thus, the combination of anti-bacterial cations with sweetener anion did not yield increased thermal stability for the majority of the synthesized ILs.

Table 3.1 Thermal properties of anti-bacterial sweetener ILs*

Ionic Liquids	T_g	T_c	T_{s-s}	T_m	$T_{onset5\%}$	T_{onset}
[BA][Sac]	--	16 [a]	--	74	164	204
[DDA][Sac]	-33	15 [b]	--	16	187	214
[HEX][Sac]	--	30 [b]	--	66	207	253/412
[BA][Ace]	--	30 [b]	-36	90	184	187/249/394
[DDA][Ace]	-53	--	--	--	189	232/426
[Hex][Ace]	-11	5 [a] 18 [b]	--	57	212	267/494
Starting Materials						
Na[Sac]	--	98 [b]	--	120	431	459/541
K[Ace]	--	--	--	68	190	192/260
[BA]Cl	--	16 [a,c]	--	--	143	169
[DDA]Br[d]	--	--	--	--	166	196
[Hex]Cl	--	45 [b]	--	73	184	213

*T_g - glass transition temperature; T_c - crystallization temperature; T_{s-s} - solid-solid transition temperature on heating; T_m - melting point on heating. Decomposition temperatures were determined by TGA, heating at 5 °C min^{-1} under air atmosphere and are reported as ($T_{onset\ 5\%}$) onset to 5 wt% mass loss and (T_{onset}) onset to total mass loss.
[a]Transition measured on heating cycle; [b]Transition measured on cooling cycle; [c]Transition only during first heating; [d]Multiple transitions due to presence of water in starting material.

3.4 Biological Properties

3.4.1 Anti-microbial, Anti-bacterial, and Anti-fungal Activities

The MIC (Table 3.2) and MBC (Table 3.3) values were determined for [BA][Sac], [DDA][Sac], [BA][Ace], and [DDA][Ace]. The starting materials, [BA][Cl] and [DDA][Cl], which inherently exhibit anti-microbial, anti-bacterial, and anti-fungal activities, are included in the tables for comparison. The overall activities of the ILs were found to be less than those of the precursor cations. The average MIC value for [BA]-containing ILs were approximately 50% more than those for [BA]Cl, thus the anion structure hinders the QACs biological property. However, there were a few bacteria strains that [BA] ILs performed similarity to the precursor cation. Additionally, [BA][Sac] was 20 times more effective than [BA]Cl better than [BA]Cl against *S. mutans*, while [BA][Ace] was 2 times more effective. Unfortunately, [DDA] ILs only exhibited a decrease in effectiveness with only 40% the activity of [DDA]Cl, regardless of the bacteria type. MBC values echoed the trends seen with MIC values in which the ILs performed similarly or better in only a few cases. Again, the MBC values of the tested ILs were found to exhibit less anti-bacterial activity than the precursor cation on average. This indicates that the IL can have either negative or positive synergistic effects, which is dependent on the type of bacteria. However, in previous literature,[106] it was found that the anti-microbial activities for imidazolium chlorides, tetrafluoroborates, and hexafluorophosphates were independent of the counter ion.

3.4.2 Acute Oral Toxicities

The acute oral toxicities of [DDA][Ace] and [DDA][Sac] were determined in three male and three female Wistar rats, where the rats received a dosage of 300 mg/kg b.w. (mg of substance per kg of body weight) or 2000 mg/kg b.w. of each IL. The ILs were suspended in water prior to intragastric administration. After receiving the dosage of 300 mg/kg b.w. for [DDA][Ace] or [DDA][Sac], one male rat died during the first 24 h, while the other 5 rats remained alive. But when the dosage was increased to 2000 mg/kg b.w., all of the rats died between 24 and 96 h after administration. Death was preceded by decrease in spontaneous motor activity, excessive excretion from nostrils, and difficulty of breathing. Thus, the above results indicate the acute toxicity range for both ILs was between 300-2000 mg/kg b.w. in male and female rats. These ILs would be classified as category 4 (harmful) toxins according to standard OECD grading.[101]

Although it appears that toxicity is a negative property of these ILs, the cation precrusor, [DDA][Br], has an oral toxicity of 84 mg/kg b.w.[107] Thus, the IL demonstrated a decrease in the toxicity over the commonly used bromide salt with change of acute oral toxicity from 216 – 1916 mg/kg b.w. However, the IL toxicity is greater than that for [Ace], 7431 mg/kg b.w., or [Sac], 17,000 mg/kg b.w.[107] These ILs will not find application in food products, as the toxicity is too high for consumption by humans; yet, this example illustrate that toxicity can be modified by choice of ion.

Table 3.2. Minimal inhibitory concentration values[a]

Strain	**Ionic Liquids**				**Starting Materials**	
	[BA][Sac]	**[DDA][Sac]**	**[BA][Ace]**	**[DDA][Ace]**	**[BA][Cl]**	**[DDA][Cl]**
S. aureus	4	4	4	8	2	2
S. aureus (MRSA)	4	4	4	4	2	2
E. faecium	8	8	8	8	4	4
E. coli	16	16	31	16	8	8
M. luteus	8	4	8	8	4	2
S. epidermidis	4	4	4	4	2	2
K. pneumoniae	4	4	8	4	4	4
C. albicans	16	16	16	16	8	8
R. rubra	16	16	16	16	8	4
S. mutans	0.1	31	1	16	2	2
Mean value	8.0	10.7	10.0	10.0	4.4	3.8

[a]In ppm.

Table 3.3. Minimum biocidal concentration values[a]

Strain	**Ionic Liquids**				**Starting Materials**	
	[BA][Sac]	**[DDA][Sac]**	**[BA][Ace]**	**[DDA][Ace]**	**[BA][Cl]**	**[DDA][Cl]**
S. aureus	31.2	62.5	31.2	16	62.5	31.2
S. aureus (MRSA)	31.2	31.2	31.2	31.2	31.2	31.2
E. faecium	16	16	31.2	31.2	31.2	31.2
E. coli	62.5	16	125	62.5	62.5	31.2
M. luteus	62.5	31.2	62.5	62.5	31.2	31.2
S. epidermidis	31.2	16	62.5	31.2	16	31.2
K. pneumoniae	62.5	16	31.2	31.2	31.2	16
C. albicans	31.2	16	31.2	31.2	16	16
R. rubra	62.5	31.2	62.5	62.5	31.2	31.2
S. mutans	0.5	62.5	16	125	16	16
Mean value	39.1	29.9	48.5	48.5	32.9	26.6

[a]In ppm.

3.4.3 Skin Irritation

Skin irritation of [DDA][Ace] and [DDA][Sac] was determined on New Zealand albino rabbits. All of the exposed animals exhibited defined erythema after 1 hour. The erythema had increased to severe and severe eschar formation was also observed after 24 hour. Although no edema occurred, the skin irritation of these ILs is defined as category 4 (the highest) by standard OECD grading.[101]

3.4.4 Deterrent Activity

The deterrent activity of [DDA][Ace] and [DDA][Sac] toward *Tribolium confusum* (larvae and beetles), *Sitophilus granarius* (beetles), and *Trogoderma granarium* (larvae) was determined by using a known method, in which the amount of food consumed is monitored over a specific time interval. Three deterrent coefficients had to be calculated from the average amount of food consumed: a) the absolute coefficient of deterrency A = (CC – TT)/(CC + TT) x 100, b) the relative coefficient of deterrency R = (C – T)/(C + T) x 100, and c) the total coefficient of deterrency, which is the sum of the absolute and the relative coefficients, T = A + R.[108] In these equations, CC is the average weight of the food consumed in the control, TT is the average weight of the food consumed in the no-choice test, and T and C are the average weights of the food consumed in the choice test.

The total coefficient value, T, is compared to standard values for deterrent activity in Table 3.4, where a value of 0 equals neutral activity and a value of +150 to +200 corresponds to very high deterrent activity. The results of deterrent activity for [DDA][Ace] and [DDA][Sac] are compared to a natural deterrent, azadirachtin, in Table 3.5. The ILs received either ‘very good’ or ‘good’ deterrent activity for all tested insects. In particular, [DDA][Sac] exhibited the

same deterrent activity toward *Tribolium confusum* (larvae and beetles) as azadirachtin and thus, could be classified as a potential synthetic insect deterrent.

Table 3.4. Criteria for the estimation of the deterrent activity based on the total coefficient

Total Coefficient	**Deterrent Activity**
200 – 151	Very good
150 – 101	Good
100 – 51	Medium
50 – 0	Weak

Table 3.5. Feeding deterrent activity

Ionic Liquid	**Relative Coefficient (R)**	**Absolute Coefficient (A)**	**Total Coefficient (T)**	**Deterrent Activity**
	Sitophilus granarius (beetles)			
[DDA][Ace]	97.5	57.9	155.5	Very good
[DDA][Sac]	57.8	56.6	114.5	Good
Azadirachtin[a]	100.0	74.3	174.3	Very good
	Trogoderma granarium (larvae)			
[DDA][Ace]	94.0	85.0	179.0	Very good
[DDA][Sac]	94.2	86.1	180.3	Very good
Azadirachtin[a]	100.0	94.2	194.2	Very good
	Tribolium confusum (beetles)			
[DDA][Ace]	96.2	19.1	115.3	Good
[DDA][Sac]	95.0	90.7	186.6	Very good
Azadirachtin[a]	100.0	85.0	185.0	Very good
	Tribolium confusum (larvae)			
[DDA][Ace]	95.0	64.1	159.1	Very good
[DDA][Sac]	95.3	88.8	184.1	Very good
Azadirachtin[a]	100.0	88.4	188.4	Very good

[a]Natural deterrent.

3.5. Conclusions

The synthesis of ILs containing two biologically active ions by pairing anti-microbial QACs cations with sweetener anions was successful with the isolation of six salts. [HEX][Ace] the only IL found to crystallize and exhibited interesting orientation, in which the cation could form either π stacked dimers or polymers. This unique crystal structure is also thought to influence the thermal properties, as this IL possessed two crystallization temperatures. Only two ILs, both possessing [DDA] cations, were found to be liquids at room temperature. This property is mostly likely due to the lack of π stacking, as [DDA] contains no aromatic ring. However, these liquid salts were found to be both toxic and irritating to the skin, which will reduce the potential applications. Unfortunately, the majority of the new ILs exhibited lower thermal stability than the anion precursor salts, thereby limiting the use in food products. The [BA] and [DDA] containing ILs were found to possess greater MIC and MBC values than the commonly used [BA][Cl] and [DDA][Br], therefore indicating that a negative synergistic effect. Although, anti-bacterial activity was decreased for the synthesized ILs, these ILs were found to be better suited for use as insect deterrents. Hopefully, as our fundamental understanding of IL behavior increases, it will be possible to predict the resultant properties of the salts.

CHAPTER 4

PRODUCTION OF IONIC LIQUIDS CONTAINING DUAL FUNCTIONALITY

Taken in part from a published manuscript: Hough, W. L.; Rogers, R. D., *Bull. Chem. Soc. Jpn*., **2007**, 80(12), 2262-2269.

4.1. Introduction

In Chapter 3, it was found that ILs could be produced from an anti-bacterial cation and a sweetener anion. However, the anticipated properties such as liquid at room temperature and high thermal stability were not obtained. On the other hand, it is found that the sweetener anion [Ace] could negatively influence the biological activity of both [BA] and [DDA], thereby resulting in high MIC and MBC values. Although with "very good" or "good" outcomes in the insect deterrent experiments, the application of these ILs was altered from food products to insect deterrent. These were paired with traditional IL-forming counter ions to control physical properties, especially melting point along with preparing biologically active salts. But ILs are composed of a minimum of two ions, which both may impart biological activity into the resulting salt.

The synthesis of these pharmaceutically active salts is not the key to the IL-API approach; it is making the proper choices of ions. Our first goal was to choose a specific function for each ion and then combine them to form an IL. This approach requires not only knowledge of specific biological function possessed by each ion, but also what ion combinations will produce ILs. The knowledge base for the latter is still incomplete, and predicting ion combinations that

will lead to ILs is, unfortunately, a hit-or-miss proposition. Nonetheless, it did not escape our notice that: 1) many IL-forming cations bear similarity with many APIs or API-precursors, and 2) that many biologically active ions are large, charge diffuse, and asymmetric - all characteristics which should lead to low melting salts. Figure. 4.1 represents several different biologically active cations and anions, which would appear to be likely candidates for IL formation.

Ibuprofen (Figure 4.1) is a common, non-steroidal anti-inflammatory drug (NSAID) used to treat arthritis symptoms, fever, and can also have use as an analgesic.[109] This API is racemic with the pharmacological activity residing in the *S*-enantiomer. However, in the body the *R*-enantiomer can be converted into the biologically active version after oral administration, so ibuprofen is commercially sold as a racemic mixture.[110] However, the oral administration of ibuprofen can have adverse side effects, particularly in the gastrointestinal tract. Ibuprofen inhibits prostaglandin synthesis, which is the primary mechanism of action to reduce inflammation and pain.[111] Unfortunately, this inhibition of prostaglandin synthesis also allows for a wide variety of problems in the gastrointestinal tract.[112] It has been determined that topical administration of ibuprofen would allow for faster pain relief, in addition to lower side effects due to lower plasma concentrations.[113] Therefore, the pairing of ibuprofen with a QAC anti-bacterial (discussed in Chapter 3) could lower the melting point, thereby producing a room temperature liquid salt.

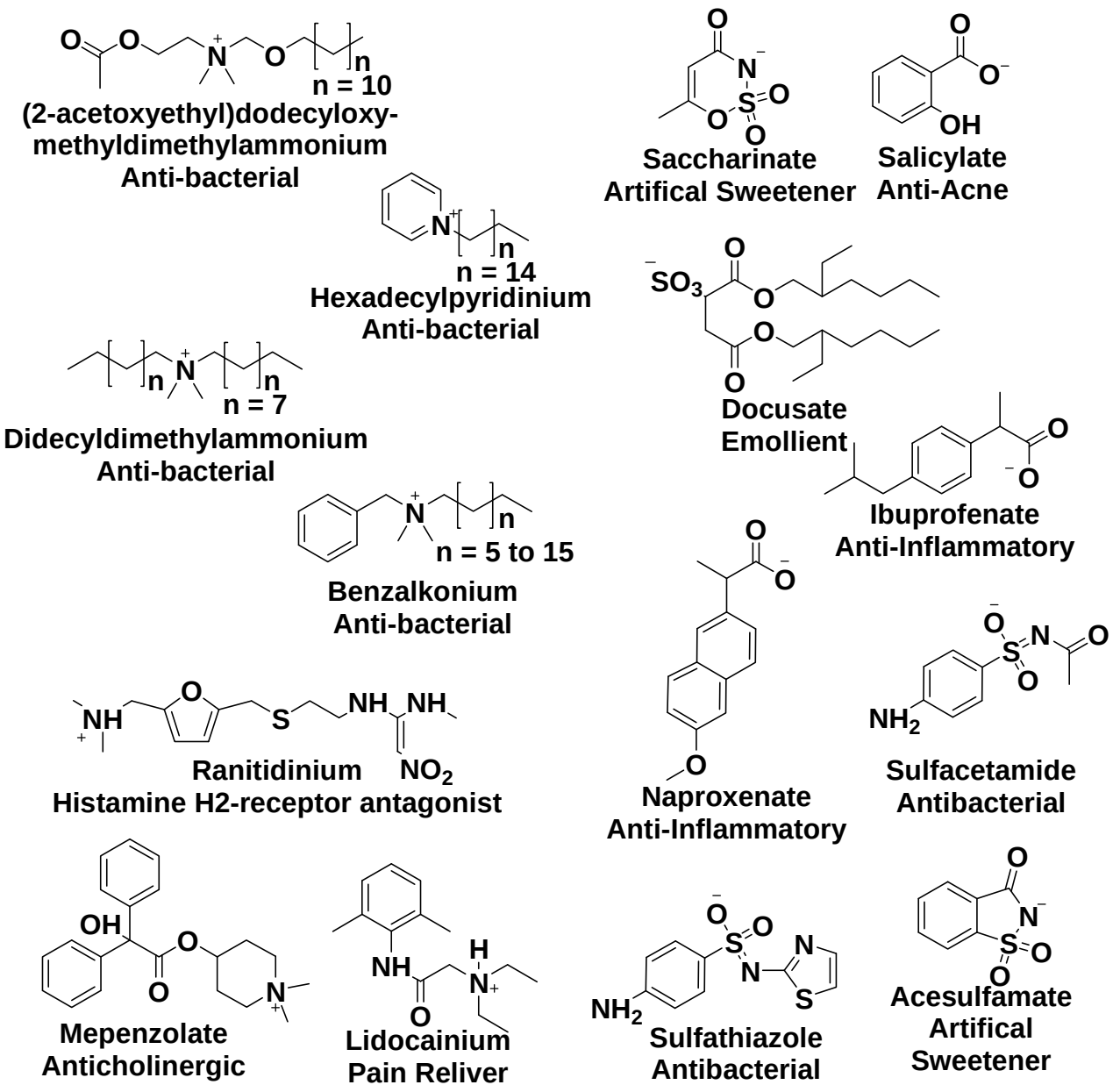


Figure 4.1. Cations and anions found in biological active ILs

We previously had success in melting point depression when utilizing anti-bacterial cations (discussed in Chapter 3), therefore we hypothesized that this combination will produce an IL. Also, a single compound containing an anti-bacterial ion to combat inflammation caused by bacterial infections could find many applications in the medical and military fields.

In addition to the synthesis of ibuprofen containing ILs, another anion, sulfacetamide (Figure 4.1), was selected to combine with anti-bacterial cations. Sulfacetamide is a sulfonamide antibiotic that is used in both skin[114] and ophthalmic[115] applications and is prescribed exclusively as the sodium salt. This antibiotic class blocks the folic acid synthesis in bacteria, thereby destroying the bacteria.[116] However, this class of drugs has interesting solid-state properties due to amount of acidic protons and electronegative atoms that can form hydrogen bonds.[117] This results in all sulfonamide drugs existing in at least 2 or more polymorphic forms.[118] In addition to polymorphic formation, sulfonamide compounds can easily hydrogen bond with one or more guest molecules to form a co-crystal.[117] Since sulfonamide drugs contain a large number of hydrogen-bonding atoms with an excess of acceptors along with confirmation mobility, therefore it is difficult to determine the hydrogen-bond patterns that will lead to a crystalline structure.[117] In the typical sulfonamide crystal structure, the chain of the sulfonamide compounds are joined by hydrogen-bonding between the amino proton and the sulfonyl oxygen.[117] Overall, it has been established that the amido proton on the sulfonamide molecule is the best hydrogen donor while the sulfonyl and activated aromatic nitrogen groups are the best acceptors.[117] To circumvent these solid-state issues, it would be advantageous to have a liquid form of sulfacetamide. By pairing the anionic form of this compound with a quaternary ammonium cation, the lack of hydrogen bond donors/acceptors should prevent the formation of

the crystalline form. Also, it might be possible to increase the antibiotic activity with this IL approach, as positive synergistic biological effects could increase the spectrum of bacteria for usage. This combination of antibiotic and anti-bacterial could also potentially reduce bacterial resistance, which is commonly seen in anti-acne treatments. Here we report the synthesis of two ibuprofen-and two sulfacetamide-containing ILs along with determination of thermal properties.

4.2. Experimental

4.2.1 Chemicals

Benzalkonium chloride (molecular formula $C_6H_5CH_2N(CH_3)_2RCl$ where $R=C_{12}H_{25}$ (60%) and $C_{14}H_{29}$ (40%)), didecyldimethylammonium bromide (tech., 75 wt% gel in water), sodium ibuprofen. sodium sulfacetamide were purchased from Sigma-Aldrich (St. Louis, MO).

4.2.2 General Synthesis.

Solid (1 mmol) [BA][Cl] or [DDA][Br] was dissolved in 50 mL distilled water with gentle heating (40-60 °C). 1 mmol of sodium ibuprofen or sodium sulfacetamide was dissolved in 50 mL distilled water by gentle heating and stirring. The two solutions were combined; the reaction mixture was heated to approximately 80 °C and stirred for 30 min. The reaction mixture was then cooled to room temperature and 60 mL of chloroform was added. The mixture was then stirred for an additional 30 min. The two phases were separated, in which the chloroform phase was washed three times with cool distilled water to remove any inorganic salt. The presence of chloride anions in the aqueous washings was monitored by $AgNO_3$ test. The chloroform phase was washed with DI water until the water washings tested negative for NaCl or NaBr via $AgNO_3$ test. The chloroform was then evaporated, and the IL was dried under high

vacuum for at least 12 hours with gentle heating (50-80 °C). Karl-Fischer analysis indicated the water content of all dried ILs to be less than 500 ppm.

Benzalkonium Ibuprofenate: Yellow Gel; yield 87%; ^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si): 0.84 (9H, dd), 1.13 (3H, d), 1.23 (23H, m), 1.74 (2H, m), 2.09 (2H, d), 2.34 (1H, d), 2.94 (6H, s), 3.19 (1H, m), 3.27 (2H, m), 4.60 (2H, s), 6.92 (2H, d), 7.15 (2H, d), 7.57 (5H, m)

Didecyldimethylammonium Ibuprofenate. Yellow gel, yield 91%; ^{1}H NMR 500MHz (DMSO-d_6): 0.85 (m, 12H), 1.19 (d, 3H), 1.25 (m, 28H), 1.58 (m, 4H), 1.77 (sept, 1H), 2.35 (d, 2H), 3.00 (s, 6H), 3.22 (m, 4H), 3.86 (s, 1H), 6.92 (d, 2H), 7.14 (d, 2H); ^{13}C NMR 500MHz (DMSO-d_6): 13.8, 19.9, 21.5, 22.0, 22.1, 25.6, 28.3, 28.5, 28.7, 28.8, 29.6, 31.2, 44.2, 49.7, 49.8, 50.2, 62.6, 127.0, 127.9, 137.3.

Benzalkonium Sulfacetamide: Yellow Gel; yield 76%; ^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si): 0.85 (3H, t), 1.25 (18H, m), 1.58 (3H, s), 1.76 (2H, m), 2.94 (6H, s), 3.23 (2H, m), 4.52 (2H, s), 5.33 (2H, s), 6.42 (2H, d), 7.36 (2H, d), 7.54 (5H, m); ^{13}C NMR (500 MHz; DMSO-d_6; Me_4Si): 13.7, 21.2, 21.8, 25.6, 26.5, 28.2, 28.4, 28.5, 28.7, 31.0, 48.8, 54.5, 63.2, 65.9, 111.4, 127.9, 128.6, 130.0, 132.6.

Didecyldimethylammonium Sulfacetamide: Yellow Gel; yield 87%; ^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si): 0.85 (6H, t), 1.25 (28H, m), 1.582 (3H, s), 1.625 (4H, m), 2.97 (6H, s), 3.22 (4H, m), 6.43 (2H, d), 7.36 (2H, d), ^{13}C NMR (500 MHz; DMSO-d_6; Me_4Si): 13.82, 21.52, 21.97, 25.63, 28.32, 28.54, 28.66, 28.77, 31.16, 63.42, 128.13, 173.13

4.3. Results and Discussion

4.3.1 Synthesis

[BA] ibuprofenate, [DDA] ibuprofenate, [BA] sulfacetamide, and [DDA] sulfacetamide were prepared in yields from 76% ([BA] sulfacetamide) to 91% ([DDA] ibuprofenate), where a stoichiometric metathesis reaction in aqueous solution was utilized. The ILs were extracted from the aqueous phase into chloroform, in which the majority of the halide salt remained in the aqueous phase. However as indicated by lower obtained yield, [BA] sulfacetamide is more water soluble than the ibuprofenate-containing ILs. Therefore, during the water washings to remove the halide impurity, some amount of the IL was solublized and lost in the washings. However, [DDA] sulfacetamide was isolated in approximately 10% greater yield than the corresponding [BA] IL. This would signify that the [DDA] cation, with long alkyl chains, influences the hydrophobicity of the IL more than the [BA] cation, containing shorter chains and an aromatic ring. Regardless of the composition, all synthesized ILs were found to exist as yellow gels at room temperature.

4.3.2 Thermal Analysis

All synthesized ILs were gels at room temperature, with values of T_g ranging from -77 °C ([BA] ibuprofenate) to 46 °C ([BA] sulfacetamide) (Table 4.1). When compared to the starting materials, the ILs displayed a dramatic decrease in melting point with a ΔT_m of over 200 °C. [BA] ibuprofenate was the only IL to possess a melting point. Both ibuprofenate ILs had very similar T_g, thereby indicating that the anion influences the melting point depression regardless of the cation. [DDA] ibuprofenate, [BA] sulfacetamide, and [DDA] sulfacetamide exhibited no melting points, as only T_g's were seen. As previously discussed (Chapter 3), the lack of melting

point for [DDA] ibuprofenate and [DDA] sulfacetamide is most likely attributed to the lack of an aromatic ring in the cation, which could π stack with both ibuprofenate and sulfacetamide anions. Also, since [DDA] contains only long alkyl chains, there is no electronegative atoms available for hydrogen-bonding, thus no lattice structure is formed. However, [BA] contains an aromatic ring which could readily π stack with the rings of sulfacetamide and ibuprofen. Yet, only [BA] ibuprofenate displayed a melting point, although quite low, as the alkyl chain could disrupt the π stacking interactions between the cation and anion.

Table 4.1. Synthesized IL-APIs*

Ionic Liquids	T_g	T_m	$T_{onset5\%}$	T_{onset}
[BA] Ibuprofenate	-77	-41	134	153
[DDA] Ibuprofenate	-73	--	147	168
[BA] Sulfacetamide	46		164	181
[DDA] Sulfacetamide	27	--	183	200
Starting Materials				
[BA][Cl]	--	--	143	169
[DDA][Br]	--	--	166	196
Na Sulfacetamide	--	183	323	--
Na Ibuprofenate	--	200	222	--

*T_g - glass transition temperature; T_m - melting point on heating. Decomposition temperatures were determined by TGA, heating at 5 °C min^{-1} under air atmosphere and are reported as ($T_{onset\ 5\%}$) onset to 5 wt% mass loss and (T_{onset}) onset to total mass loss

As observed in Chapter 3, all of the obtained ILs possessed lower melting points than the precursor anion salts with ibuprofenate salts averaging ΔT_m of 81 °C and sulfacetamide salts averaging ΔT_m of 159 °C, respectively. However, only [BA] ibuprofenate was found to be lower than both the cation and anion precursor salts, although the ΔT_m was quite small, only 9 °C. This would indicate that the ibuprofenate anion has no greater stabilizing effect than a simple halide counter ion. Both [BA] sulfacetamide and [DDA] ibuprofenate showed a slight increase in thermal stability over the precursor cation, however, as stated with [BA] sulfacetamide it seems the organic counter ion has little effect on the stabilization of the IL. Only [DDA] sulfacetamide displayed a double digit increase in thermal stability over the [DDA][Br] salt with a ΔT of 17 °C.

4.4. Conclusions

A modular IL strategy could potentially revolutionize the pharmaceutical and medical industries to provide a platform for improved activity and new treatment options. This IL-API strategy has the possibility to overcome problems such as polymorphism, solubility, bioavailability and adverse side effects that have hindered the use of many pharmaceuticals. As discussed here, we were able to take two solid ion precursors and convert the combination into a liquid form. For ibuprofen, the liquid salt can provide a way to bypass the side effects associated with the oral delivery. The transdermal application of this IL could also provide the combination of two biological activities, which could be affected either positively or negatively. Sulfacetamide, on the other hand, has a tendency to form crystalline structures due to the amount of hydrogen bond donors/acceptors. By pairing this antibiotic with a long chain QAC that possessed no hydrogen-bonding sites, it was possible to effectively depress the melting point, in which only a T_g was displayed for both [BA] sulfacetamide and [DDA] sulfacetamide. Now,

this pharmaceutical is no longer hindered by the crystalline form and the problems associated with polymorphism. Yet, thermal stability still remains an issue with pharmaceutically based ILs, as only one synthesized ILs displayed an increased thermal stability over either of the precursor cation and anion salts. More knowledge is needed to determine how the thermal stability can be efficiently tuned.

CHAPTER 5

LOCAL ANESTHETIC IONIC LIQUID FOR TRANSDERMAL APPLICATION

Taken in part from: Hough, W. L.; Smiglak, M.; Rodriguez, H. ; Swatloski, R. P.; Spear, S. K.; Daly, D. T.; Grisel, J. E.; Carliss, R.; Soutullo, M. D.; Davis Jr., J. H.; Rogers, R. D.; *New J. Chem*, **2007**, 31(8), 1429-1436.

Acknowledgement: Grisel, J. E.; Carliss, R. (The University of South Alabama, Mobile, AL) for the biological testing.

5.1. Introduction

As research progresses, we have demonstrated the ability to form ILs from anti-bacterials and artificial sweeteners, which displayed favorable biological effects as insect deterrents (Chapter 3). However, in this case, only one ion possessed true therapeutic activity while the sweetener anion was utilized as a known IL former. In Chapter 4, pharmaceuticals that had not previously been employed in the formation of ILs were exploited to produce ILs with containing two biological active molecules. The chosen anions had known problems that could be alleviated by the liquid salt form. The IL-API strategy was successful in depressing the melting point; however, no biological data was collected. Therefore, information regarding the biological activity, pharmacokinetics, biodistribution, etc., is needed to understand how the combination of ions affects not only the physical properties, but also the overall pharmaceutical activity of the IL.

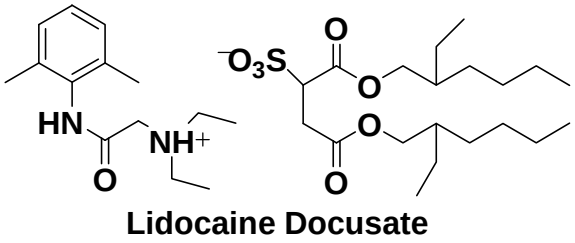
Care must be taken when choosing appropriate IL-forming ion pairs. Many of the important APIs are not permanent ions, but rather are protonated or deprotonated to form the

commonly used salts; thus suitable pK_a differences need to be considered.[119] Although MacFarlane and Seddon[120] have recently proposed that protic ILs only be considered 'ILs' if the pK_a difference is such that 99% or more of the salt exists in ionized form. For an API, such a distinction may not be needed since there may be advantages in having the ability to tune the exact amount of API present in ionized and neutral form. Additionally, IL-APIs might require a salts that are liquid at room temperature or at or below body temperature.

Local anesthetics have been known since the late 1800's with the isolation of cocaine from the Peruan *khoka* (*coca*) plant.[121] Unfortunately, cocaine usage results in several toxic side effects, which affect both the central nervous and cardiovascular systems and could ultimately lead to death.[122] This prompted development of new and substantially less toxic local anesthetics based on cocaine, thereby resulting in a class of drugs known as 'caines' which consists of a lipid soluble aromatic head group attached to a hydrophilic tertiary amine via an intermediate chain of one to three carbons such as lidocaine. The combination of both lipophilic and hydrophilic properties prevents the influx of sodium ions across the axolemma,[122] thus reducing the potential and inhibiting electric impulses, producing a lack of sensation in a localized area.[123 -126] Although the mechanism of caine drugs is under debate, the factors affecting the drug's overall efficacy are known. The efficacy hinges on the ability of the caine drug (1) to be at the target nerve in adequate concentration, (2) to completely diffuse through the lipid bilayer, and (3) to convert into the cationic form.[127]

Lidocaine (Figure 5.1), the first amide local anesthetic, is one of the most widely known of all the 'caine' drugs. Its desirable properties of rapid onset of anesthetic power and effectiveness for infiltration have allowed it to be used for all types of anesthesia, however, its potency is considered to be low, only a 4 out of 16 on the potency scale.[121] Lidocaine is

typically utilized in pharmaceutical formulations as the low melting solid, lidocaine hydrochloride (LHCl) However, LHCl is highly water soluble and has an anesthetic duration of only 120 minutes.[129] We hypothesize that a reduction in water solubility will increase the anesthetic duration, as when exposed to aqueous environments it will have longer resident time on the skin.



Lidocaine Docusate

Figure 5.1. IL composed of lidocaine and docusate

Transdermal dosage forms have gained popularity in the past years, as these formulations possess multiple advantages such as controlled absorption, simple administration mode, and easy removal of drug source if adverse side effects were seen.[130] Unfortunately, the barrier properties of skin can vary greatly, so a major hurdle regarding this administration route is the reproducibility of drug flux. However, transdermal enhancers can be used to overcome these problems and increase the drug flux to clinically benefice levels.[131] To increase lidocaine's ability to enter the body by transdermal administration, it was paired with known IL-former[132] and transdermal-enhancer, sodium docusate (Figure 5.1, sodium dioctylsulfosuccinate). Sodium docusate is also FDA-approved as a dispersing agent (emollient), which has been shown to be absorbed in epithelial intestinal cells.[133] In addition to the synthesis of the IL, lidocaine docusate, an assessment of the physical and biological properties were determined to provide information regarding the synergistic effects.

5.2. Experimental

5.2.1 Chemicals

Sodium dioctylsulfosuccinate (sodium docusate) and lidocaine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO).

5.2.2 Synthesis and Purification

Lidocaine Docusate (LD). LHCl (1 mmol) and sodium docusate (1 mmol) were each dissolved in 50 mL of anhydrous methanol. The two solutions were combined and stirred at room temperature for 2 h, after which the methanol was removed via rotary evaporator to yield a mixture of the desired liquid salt and NaCl(s). Chloroform (50 mL) was added to precipitate the solid NaCl, which was removed by vacuum filtration. The amount of NaCl impurity was monitored by silver nitrate test, and once the IL solution tested negative, the chloroform was removed by rotary evaporator to yield the IL.

Standard flash column chromatography (column diameter 1 inch) was performed to purify lidocaine docusate. The viscosity was reduced by adding a small amount of dichloromethane. Then the solution was passed through a 4 inch plug of silica (SiliCycle 230-400 mesh, pore diameter 60 Å, surface area 500 m^2/g) using an elution gradient of dichloromethane:methanol (98:2 to 90:10), in which the polarity was increase approximately 2% for every 100 mL until the final solvent system was reached. The purification progress was followed by TLC, where $KMnO_4$ stain was used for visualization. Once all of the compounds were eluted from the column, it was determined which aliquots contained the same compound by using TLC, then the corresponding fractions were combined. These combined fractions were placed into a large round-bottomed flask, and the solvent was removed by rotary evaporator.

The isolated IL was then analyzed by ^{1}H NMR to confirm the 1:1 ratio of cation to anion. Then, the ILs were subjected high vacuum for at least 12 hours with gentle heating to remove all residual solvent or water. Lidocaine docusate was obtained in a yield of 91% as clear, colorless syrup. ^{1}H NMR 500MHz (DMSO-d_6): 0.86 (12H, m), 1.25 (22H, m), 1.48 (2H, m), 2.17 (6H, s), 2.88 (1H, dd), 2.93 (1H, d), 3.22 (4H, m), 3.63 (1H, dd), 3.88 (4H, m), 4.17 (2H, s), 7.10 (3H, m); ^{13}C NMR 500MHz (DMSO-d_6): 8.7, 10.6, 10.7, 13.8, 17.9, 19.0, 22.3, 22.8, 22.9, 23.0, 23.1, 24.2, 24.5, 28.2, 29.4, 29.5, 29.6, 34.0, 38.0, 48.3, 59.9, 61.3, 65.9, 73.6, 126.5, 126.9, 127.8, 133.5, 134.8, 168.2, 170.9.

5.2.3 Solubility

The solubility of LD in water was experimentally determined at room temperature (22 ± 2 ºC). An amount of LD, exceeding the solubility limit, was added to deionized water (with a resistivity of 17.4 MΩ·cm) in 20-mL glass vials. The mixture was thoroughly shaken overnight, resulting in a stably cloudy solution. Phase separation could not be reached either by letting the solution stand for several days or by centrifuging. Therefore, drops of water were gradually added until miscibility was reached. After each drop addition, the mixture was shaken again, allowing for the system to equilibrate. When the solution turned completely miscible, an aliquot was taken with a pipette, and it was diluted with fresh water for subsequent analysis by ultraviolet (UV) spectroscopy.

A series of diluted solutions of LD in water were prepared by weight with a Denver Instrument M-220D analytical balance, precise to within $\pm 1 \times 10^{-4}$ g. The absorbances were measured at a wavelength of 192 nm with a Cary 3C UV-Vis spectrophotometer, by Varian Instruments, providing a calibration line relating absorbance and concentration. The absorbance

of the diluted aliquot of the saturated solution was measured, and its concentration calculated. The solubility test was run in triplicate, and the results were found to be in reasonable agreement within the expectable uncertainty of the method.

5.2.4 Mouse Antinociception Tests

The warm water tail withdrawal assays were conducted with mice at Furman University in accordance with guidelines from the Institutional Animal Care and Use Committee. Relative differences in the potency of LHCl and LD were assessed in adult naïve Swiss Webster mice of both sexes obtained from Taconic Laboratories (Germantown, NY). The mice were housed 4-5 per Plexiglas cage and maintained on a reverse 12:12 light/dark cycle (lights off 0700) at an ambient temperature of 22 ± 2 ^{o}C. Water and food (Harlan Mouse Chow) were provided ad libitum.

Antinociception was assessed using a modification of the tail-flick procedure established by D'Amour and Smith.[134] Two antinociceptive models were used: warm water tail withdrawal from 49 ˚C water in intact mice, and warm water tail withdrawal from a 47 ˚C bath, following tail injury. A 12 second cut-off latency was used for intact mice to prevent tissue damage, and due to the reduced intensity of the heat stimulus used for mice with tail injury, cutoff time was set at 20 s. The hyperalgesia produced by the tail injury approximates the physical conditions under which lidocaine have been widely used for regional analgesic therapy. For the tail injury, a hyperalgesic state was induced by lightly taping the distal 3 cm of the tail to a 52 ˚C hotplate for 60 s. In both cases, pre-drug baselines and all subsequent nociceptive measures, were determined by averaging two tail withdrawal latencies (separated by ca. 60 second) taken while

the mouse was lightly restrained in a cloth pouch with the distal third of its tail protruding into the water bath.

LHCl or LD were dissolved in 90% DMSO: 10% H_2O for transdermal administration according to the method of Kolesnikov, et al.[135] The animals were restrained in these same cloth pouches by submerging the distal half of the tail for 60 second in the drug solution. In order to minimize possible stress resulting from this restraint (unlike the tail withdrawal testing, which takes only a few seconds and the mice are free to squirm, tails need to be more or less stationary for 60 second) mice were habituated to this procedure on three separate occasions during the week before testing.

In the thermal injury test, mice were assessed for baseline sensitivity and then immediately exposed to the heat stimulus. Twenty minutes after injury, a second (post-injury) nociceptive baseline was determined, immediately followed by transdermal drug administration. Tail withdrawal latency was subsequently measured at 15, 30, 60, 90, 120, 150, and 180 min. Between the procedures, the mice were returned to their home cages. Three concentrations of each drug (1.0, 10.0, or 100.0 mM) were used to generate dose-response curves in the initial characterization and the higher dose (100 mM) was evaluated following injury.

An ANOVA was used to assess group differences in tail withdrawal latencies at each drug concentration (by repeated measures analysis across time; post-hoc analysis was determined by Fischer's LSD method). In all cases, the criterion for significance was set at $p \leq 0.05$.

5.2.5 Suppression of PC12 Neuritic Outgrowth.

Data collection was done using methods consistent with Takatori *et al.*,[136] who report ~5% neurite-bearing cells at day 3 and ~20% at day 4 using 100 ng/mL NGF and counting

process ≥ 1 times cell body diameter. PC12 cells were purchased from American Type Culture Collection (Manassas, VA). Cells were grown in RPMI medium supplemented with 15% normal horse serum and 5% fetal bovine serum and 25 U of penicillin and streptomycin in 1 cm diameter wells for 3 days at 37 ˚C under 5% CO_2 and 95% air. Cells were exposed to either no treatment (0), nerve growth factor (NGF) (50 μg/mL) alone, 400 and 4000 μM LHCl, or 400 and 4000 μM LD in the presence of NGF. Most cells were killed at 4000 μM LHCl and all cells were killed at 4000 μM LD. There were a total of 6 counts per bar/well. Cells were stained with nuclear red, and cell processes were counted using phase-contrast microscopy. A minimum cluster range of 80-100 cells were selected mid-center of the well-floor that were examined per data point. Only processes with lengths equivalent to 3-4 times the diameter of cell body were counted.

5.3. Results and Discussion

5.3.1. Synthesis and Purification.

Since both the cation and anion precursors were commercially available as salts, a metathesis route allowed for the direct use of the precursors without previous modification or purification. As previously stated in Chapters 3 and 4, the reaction combines equimolar methanol solutions of LHCl and sodium docusate, in which the evaporation of methanol yielded the IL and solid NaCl. The solid NaCl was removed by washing the IL with chloroform, thereby precipitating the NaCl, which was removed by vacuum filtration. Although silver nitrate $AgNO_3$ tests did not indicate NaCl contamination, NMR spectroscopy revealed that the cation:anion ratio was not 1:1, indicating presence of unreacted sodium docusate. It is thought that the sodium

docusate can become dissolved within the formed IL, which will show an increase in the docusate but not the lidocaine.

Flash column chromatography on Silicycle™ 230-400 mesh silica gel as stationary phase was employed to remove unreacted sodium with gradient elution solvent of dichloromethane:methanol (98:2 to 90:10). LD was diluted with a small amount of dichloromethane to reduce the viscosity and allow for easier loading onto the column. The purification progression was followed by TLC, where a $KMnO_4$ stain was used for visualization. The $KMnO_4$ stain is useful for detection of amines (specifically tertiary), alcohols, sulfides, and other oxidizable groups. The TLC analysis gave interesting spotting patterns depending on the composition of the ILs (Figure 5.2). When LD was present in a 1:1 ratio, two strong spots were seen, which were completely separated (spot B, Figure 5.1). However, if the IL contained excess anion, upper spot would exhibit lower intensity than the bottom spot (spot A, Figure 5.1).

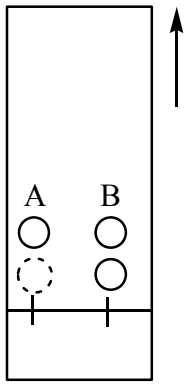


Figure 5.2. General TLC spotting during the purification of LD

This would seem to indicate an unequal ratio of ions, in which the cation, in deficit, possessed less chromatographic intensity. LD was isolated in 91% yield with the excess sodium docusate obtained in the low milligram quantity (~130 mg), as a white solid. NMR analysis of

the impurity showed almost 100% pure sodium docusate with only trace amount of lidocaine present. ^{1}H NMR was utilized to indicate the correct 1:1 cation:anion ratio (within the limits of NMR detection, ca, 5%). Water content of the synthesized IL via Karl-Fisher titration was found to be approximately 3 wt% after drying under high vacuum at 80 ^{o}C for 100 hours.

5.3.2. Solubility and Thermal Stability.

Transdermal preparations of local anesthetics or analgesics depend on drug-retaining layers that typically contain a water-soluble gel base that can release an active substance.[137] The IL-API strategy brings together the relatively hydrophobic lidocaine cation with a hydrophobic anion, docusate (an emollient) to produce a hydrophobic IL salt, which exhibits reduced or controlled water solubility and, thus should exhibit extended residence time on the skin. DSC analysis of LD (Table 5.1) indicates no melting point, but a glass transition (T_g) at -29 °C. TGA data suggest a one-step decomposition temperature ($T_{5\%onset}$) of 222 ^{o}C, which is significantly higher than that of LHCl. The decomposition temperature for LD is similar to that observed for sodium docusate.

Table 5.1. Comparison of IL properties with precursor anion and cation*

Ionic Liquid	**T_g (°C)**	**T_m (°C)**	**$T_{onset5\%}$ (°C)**	**Solubility (mM)**
Lidociane Docusate	-29	--	222	1.24
Precursor Salts				
LHCl	--	76-79	155	2488[138]
Na Docusate	--	153-157	219	33.7[109]

T_g - glass transition temperature; T_m - melting point on heating. Decomposition temperatures were determined by TGA, heating at 5 °C min^{-1} under air atmosphere and are reported as ($T_{onset\ 5\%}$) onset to 5 wt% mass loss.

LD is both hygroscopic (in common with many ILs)[50] and hydrophobic. The maximum water solubility of LD (neutral free-drift pH) is 1.24 mM, which is substantially lower than either starting material, LHCl or sodium docusate. This behavior is common with many hydrophobic ILs, where the water solubility is a tunable property.[139] When saturated, the water content of LD is 9.6 wt%. Yet, the water content can be reduced to 3 wt% after drying under high vacuum with the expected increase in viscosity. All of the observed behavior for LD is consistent with the behavior of hydrophobic ILs reported in the literature,[139] where a growing body of knowledge allows one to choose appropriate techniques for complete drying and control of water content.[140]

5.4. Biological Properties.

5.4.1 Antinociception Tests.

LD produced a longer duration of antinociceptive effect than LHCl as indicated by an overall analysis of the efficacy of the drugs administered at different concentrations in 90% DMSO/10% H_2O (Figure 5.3). For the intact mouse model, statistically-significant group differences in the antinociceptive area under the analgesic curve (AUC) were found: $F_{(5,66)} = 3.177$, $p < 0.05$ (analysis not shown). The effect of the 100 mM dose reflects greater antinociception in mice exposed to LD as indicated by Fisher's LSD post hoc analysis (Figure 5.2a). Repeated measure ANOVA (analysis of variance) demonstrated group differences for the 100 mM dose: $F_{(5,66)} = 3.0967$, $p < 0.05$, as well as a significant effect of time ($F_{(4,264)} = 20.486$, $p < 0.05$; Figure 2b). Notably there was also a significant group by time interaction ($F_{(20,264)} = 2.226$, $p < 0.05$) indicative of prolonged antinociception.

Enhanced antinociception produced by 100 mM LD over 100 mM LHCl was also found for the thermal injury, but over a longer time interval (Figure 5.4). Significant group differences

in AUC reflect enhanced efficacy and duration of the LD formulation: $F_{(1,47)} = 8.991$, $p < 0.01$. An ANOVA repeated measure analysis which captured the tail withdrawal responses 20 min after injury, and for 180 min following lidocaine exposure, demonstrates that LD produces greater antinociception. This was supported by the repeated measures analysis, with a main effect of group $F_{(1,47)} = 6.515$, $p < 0.05$, of time ($F_{(8,376)} = 7.377$, $p < 0.01$), and a significant interaction ($F_{(8,376)} = 2.317$, $p < 0.05$). These results indicate that LD, in comparison with LHCl, was more effectively absorbed through the skin of the mouse tail to reach afferent free-nerve endings for the production of antinociception.

Enhanced antinociception produced by 100 mM LD over 100 mM LHCl was also found for the thermal injury, but over a longer time interval (Figure 5.5). Significant group differences in AUC reflect enhanced efficacy and duration of the LD formulation: $F_{(1,47)} = 8.991$, $p < 0.01$. An ANOVA repeated measure analysis which captured the tail withdrawal responses 20 min after injury, and for 180 min following lidocaine exposure, demonstrates that LD produces greater antinociception. This was supported by the repeated measures analysis, with a main effect of group $F_{(1,47)} = 6.515$, $p < 0.05$, of time ($F_{(8,376)} = 7.377$, $p < 0.01$), and a significant interaction ($F_{(8,376)} = 2.317$, $p < 0.05$). These results indicate that LD, in comparison with LHCl, was more effectively absorbed through the skin of the mouse tail to reach afferent free-nerve endings for the production of antinociception.

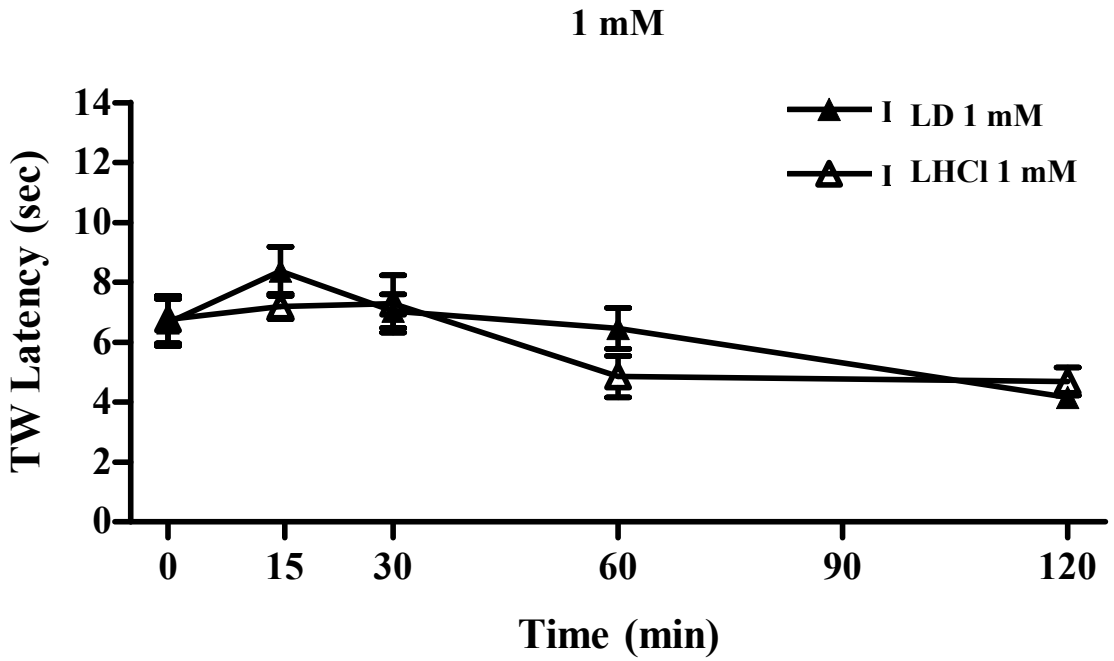


Figure 5.3. Effects of topical LD and LHCl on the warm-water mouse tail withdrawal (TW) latency response a 1mM**.** The * indicates statistical differences between LHCl and LD

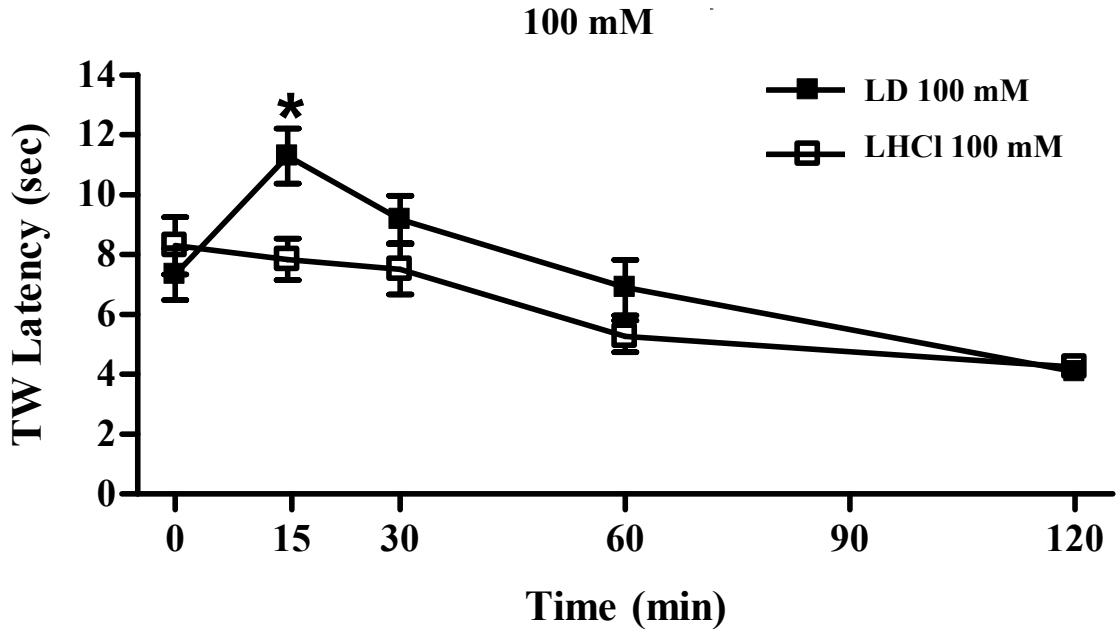


Figure 5.4. Effects of topical LD and LHCl on the warm-water mouse tail withdrawal (TW) latency response at 100 mM**.** The* indicates statistical differences between LHCl and LD.

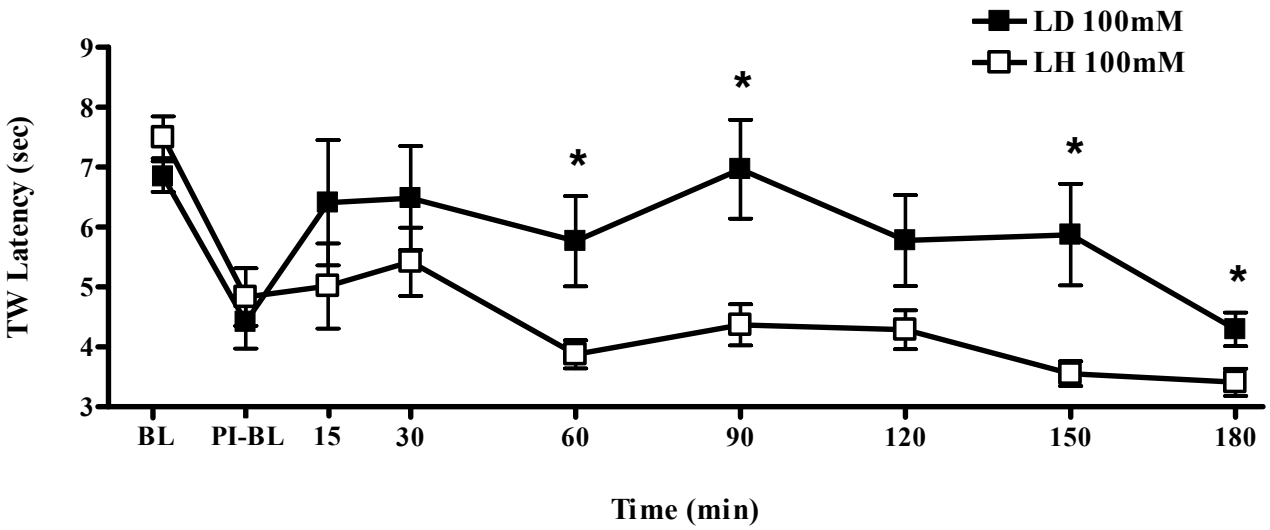


Figure 5.5. Effects of topical LD and LHCl on the warm-water tail-flick latency response of mice bearing a tail wound about a 1 cm^2 was placed in a 49 ^oC water bath (BL = baseline, PI-BL = post injury baseline). Drugs were administered 20 min after PI-BL. The * indicates statistical differences between LHCl and LD.

5.4.2 Suppression of PC12 Neuritic Outgrowth.

Local anesthetics suppress NGF mediated neuronal differentiation in rat pheochromocytoma (PC12) cells. This was used as a bioassay for detecting potential differences between PC12 cells treated with LD or LHCl. About 600 μM lidocaine is required to block afferent nociceptive fibers that include Aδ and C fibers in the rat sciatic nerve.[141] Therefore 4000 or 400 μM corresponds to the concentrations necessary for regional anesthesia. Typically, dosages for lidocaine-containing products vary from 0.5%-2.0% (20-80 mM), as lidocaine is diluted approximately one hundredth or less before it reaches the peripheral nerve.[142]

Figure 5.6 shows the effects of LHCl and LD on neuritic outgrowth in PC12 cells exposed to NGF. No neurites were found without NGF added to the media, while in the presence of NGF alone, about 30 neurites were counted. Most cells were killed at 4000 μM LHCl and all cells were killed at the same concentration with LD. At 400 μM LHCl an average of about 10 neurites were found, where with 400 μM LD, about twice as many neurites were found. There was a statistically-significant difference between the number of neurites produced with 50 mg/mL NGF alone and 50 mg/mL NGF in the presence of either LHCl or LD ($p < 0.05$). There was also a significant difference between the number of neurites found with 400 μM LD and LHCl ($p < 0.05$). Overall, even though cell death was complete at 4000 μM LD, at the lower 400 μM LD, cells appeared healthy and well organized. The obtained results were in good correlation with literature values for LHCl, as Takatori *et al.*[142] also observed no neurite growth at 4000 μM and depressed growth at 400 μM.

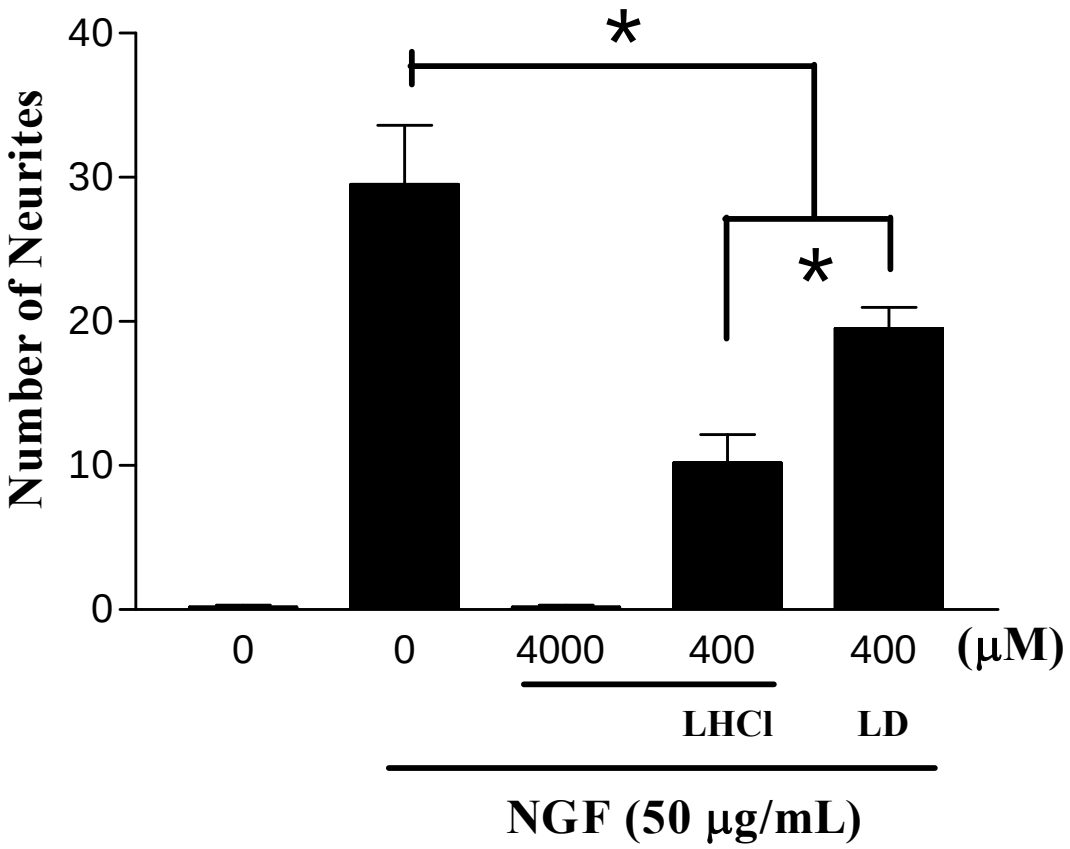


Figure 5.6. LHCl or LD suppression of neuritic outgrowth in PC12 cells treated with 50 ng/mL NGF. The * indicates statistical differences between LHCl and LD.

The PC12-NGF data suggests potential differences between LD and LHCl at the cellular level and indicate a mechanism of action entirely different for LD than that for LHCl. Docusate may enhance membrane permeability as has been shown in bacteria,[143] which may suggest at least one mechanism associated with the apparent increase in LD efficacy in vivo. However, while an increase in permeability may enhance transdermal transport and account for the longer duration and greater efficacy of LD in vivo, the longer duration of LD on the mouse tail-flick indicates an alternative slow release mechanism.

The PC12 data also suggest that the kinetics for LD are unique. One possible scenario is for lidocaine to be released from a hydrophobic-induced pairing of the lidocaine and docusate molecules, for example a 'leaching' of the component molecules into solution as observed for many hydrophobic ILs.[139] A slow release of lidocaine from the hydrophobic docusate interaction would account for the PC12 data if relatively small, and thus ineffective concentrations of lidocaine were available to the cell surface over time. This would allow for the formation of neuritic processes even in the presence of lidocaine in the media.

Bramer *et al.*,[144] have shown that mixtures of lidocaine hydrochloride and sodium docusate in physiological saline form micelles and/or vesicles which exhibited slow-release kinetics in gels. The hydrophobicity we observe for LD would also account for the increased duration of LD over LHCl as observed in our *in vivo* models and this may constitute a slow-release mechanism unique to any hydrophobic IL.

5.5. Conclusions

The synthesis of lidocaine docusate yielded interesting biological results that were not predicted. Although a typical metathesis reaction was employed, a small amount of the anion precursor was dissolved within the formed IL. Column chromatography was successful in the removal of this impurity and a 1:1 IL was isolated. Thermal stability was finally increased over both precursor salts, which was not obtained for the ILs discussed in Chapter 3 and 4. Although the biological testing conducted in this research only addressed transdermal administration, the results suggest unique physicochemical properties of LD that could be advantageous for topical application. At the highest tested concentration (100 mM) in the non-injury test, LD displayed increased anesthetic power over LHCl. LD exhibited both higher anesthetic power and duration than LHCl in the injury test, which we hypothesize, is due to the decreased water solubility of LD. Thus, the LD has longer resident time on the skin; therefore, more lidocaine can be absorbed.

Interestingly, the cellular test revealed that LD produces anesthetic power through an alternative mechanism than LHCl. This could indicate that LD exists as ion pairs, in which the docusate pulls the lidocaine molecule into the cellular membrane. Overall, the enhancement of the API bioactivity appears to occur in addition to, or apart from, the enhancing effects of the surfactant anion on membrane permeability. Unfortunately, with these exciting results comes a multitude of questions regarding the mechanism of action and how LD behaves in the aqueous phase. However, the formation of ILs can have negative synergistic effects, which was displayed previously in Chapter 3. Thus, synergistic effects are not predictable, thus further research regarding ILs behavior in solution and how this can affect the overall pharmaceutically activity is still needed.

CHAPTER 6

CONTROL OF LIDOCAINE AND PROCAINE SOLUBILITY WITH AN IONIC LIQUID APPROACH

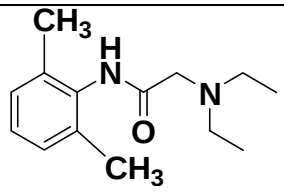
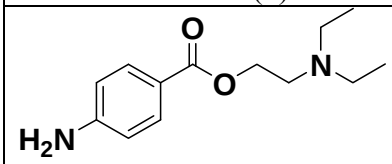
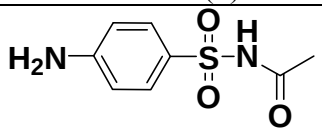
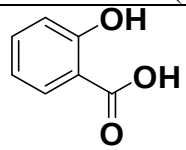
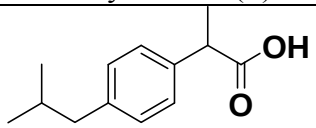
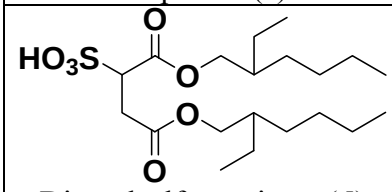
6.1. Introduction

In Chapter 5, interesting biological activity was demonstrated by LD, however, the underlying information regarding why the activity was substantially increased is not known. The knowledge base regarding the use of ILs as pharmaceuticals has grown substantially throughout this dissertation; however, more information regarding IL-APIs solution behavior is needed. It is hypothesized that the advantageous properties exhibited in LD could be due to hydrophobicity and/or ion pairing within solution. Thus, we will attempt control the physical properties of IL containing 'caine' drugs (as previously described in Chapter 5) by pairing with FDA-approved anions of varying hydrophobicity

Procaine, also commercially known as Novocain,[145] (Table 1, compound **2**) is a caine drug with relatively few side effects compared to cocaine. However, procaine suffers from a slow onset of anesthetic power, limited ability to penetrate the tissue, and the lowest potency is of all caine drugs, in addition to an anesthetic duration of only 30 minutes.[121,129] This hinders its use as a topical anesthetic, yet it has found application in infiltration anesthesia, peripheral nerve blockage, and spinal anesthesia.[146]

To broaden the knowledge base on the how the biological properties are controlled in ILs, we combined two widely known caines, lidocaine (previously discussed in Chapter 5) (**1**) and procaine (**2**), with a complementary biologically active counter ion, thereby, producing dual-functional IL analgesics. Thus, the overall properties of the caine drug could be adjusted without structural modification of the caine moiety itself. The selection of appropriate active anions in addition to the caine analgesics might allow for the introduction of new therapeutic properties not inherent in the pure caine drug, thus expanding the range of application. The chosen anions (Table 1, **a**-**d**) include a sulfacetamide (previously discussed in Chapter 4) (**a**), a metabolite of aspirin and NSAID drug, salicylic acid (**b**) and ibuprofen (previously discussed in Chapter 4) (**c**), and transdermal enhancer, dioctylsulfosuccinate (previously discussed in Chapter 5) (**d**). Salicylate has been combined with choline to produce a low melting, although not liquid, salt primarily utilized in infants and children for pain associated with teething and mouth ulcers.[147,148] In addition to biological function, the counter ions were also selected for their physical properties, namely hydrophobicity.

Table 6.1. Structures and functions of the utilized pharmaceuticals

Compound	Ion Function	Type of Biological Action	pK_a	Hydrophobicity
 Lidocaine (**1**)	Cation	All types of anesthesia (potency scale 4)	7.80	2.26
 Procaine (**2**)	Cation	Mostly dental anesthesia (potency scale 1)	9.00	2.10
 Sulfacetamide (**a**)	Anion	Sulfonamide class of antibiotics (skin and ophthalmic applications)	1.87	-0.96
 Salicylic Acid (**b**)	Anion	Metabolite of Non-Steroid Anti-Inflammatory (NSAID) drug	3.00	2.26
 Ibuprofen (**c**)	Anion	Non-Steroid Anti-Inflammatory (NSAID) drug	4.40	3.97
 Dioctylsulfosuccinate (**d**)	Anion	Agent that decreases the surface tension in the intestinal tract	0.08	5.96

As previously discussed in Chapter 5, we have hypothesized that the hydrophobicity of the counter ion could play a significant role in the anesthetic power of LD. Therefore by changing the hydrophobicity of the counter ion, it may be possible to understand its role in the biological activity of ILs. Our strategy for enhancing the transdermal penetration of a pharmaceuticals through was a formation of hydrophobic ILs containing caines, which can render a previously water soluble drug more hydrophobic. This change in hydrophobicity can potentially improve the transport to the site of action and increase the bioavailability and efficacy of the caine drug. Thus, we will describe controlling the solubility of two caine drugs, lidocaine and procaine by the appropriate choice of counter ion to form an IL.[149,150]

Here we present the synthesis of six new ILs, **1a**, **1c**, **2a**, **2b**, **2c**, and **2d.** Water solubility at neutral pH of the ILs, together with two previously reported **1b**[64] and **1d** (Chapter 5) was established and compared with literature values of the salt precursors. Finally, by utilizing electrospray mass spectrometry (ES-MS), it is possible to capture a "snapshot" of ion pairing in aqueous solution, thereby provide valuable information regarding potential mechanisms of action for the increase anesthetic power previously seen in lidocaine docusate.

6.2. Experimental

6.2.1 Chemicals

Lidocaine hydrochloride, procaine hydrochloride, sodium sulfacetamide, sodium salicylate, and sodium ibuprofen were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dioctylsulfosuccinate (sodium docusate) was donated by Cytec Industries (Woodland Park, NJ). All chemicals were used as received unless otherwise noted. Deionized water was obtained from

a commercial deionizer by Culligan (Northbrook, IL) with specific resistivity of 17.25 MΩ·cm at 25 C. Anhydrous methanol, acetonitrile, hexane, and ethyl acetate was purchased from Sigma-Aldrich (St. Louis, MO), and laboratory grade chloroform was used as received from Fisher Scientific without additional purification.

6.2.2 Synthesis and Purification

Each salt was prepared following the same general synthetic methodology. The caine HCl salt (1 mmol) and the sodium salt of the chosen anion (1 mmol) were each dissolved in 50 mL of anhydrous methanol. The two solutions were combined and stirred at room temperature for 2 h, after which the methanol was removed via rotary evaporator to yield a mixture of the desired liquid salt (except **2a)** and NaCl(s). For all non-sulfacetamide salts, chloroform (50 mL) was added to precipitate the solid NaCl, which was filtered out by vacuum filtration. Sulfacetamide salts were not soluble in chlorocarbons, therefore acetonitrile was substituted during this step. The amount of NaCl impurity was monitored by silver nitrate test and once the IL solution tested negative, the chloroform or acetonitrile was removed by rotary evaporator to yield the IL.

Standard flash column chromatography (column diameter 1 inch) was performed to purify each synthesized caine ILs. The viscosity of each IL was reduced by adding a small amount (1-5 mL) of dichloromethane or acetonitrile:methanol (10:1) for sulfacetamide ILs. Then each solution was then passed through a 4 inch plug of silica (SiliCycle 230-400 mesh, pore diameter 60 Å, surface area 500 m^2/g) using an elution gradient, in which the polarity was increased approximately 2% for every 100 mL until the final solvent composition was reached (Table 2). The purification progress was followed by TLC, where $KMnO_4$ stain was used for

visualization. Once all of the compounds were eluted from the column, it was determined which aliquots contained the same compound by using TLC, then the corresponding fractions were combined. These combined fractions were placed into large round-bottomed flasks, and the solvent was removed by rotary evaporator. The isolated ILs were then analyzed by ^{1}H NMR to confirm the 1:1 ratio of cation to anion. Then, the ILs were subjected high vacuum for at least 12 hours with gentle heating to remove all residual solvent or water.

Lidocainium Sulfacetamide (1a). Isolated in 95% yield (gel) with water content via Karl-Fischer titration of 450 ppm. ^{1}H NMR 500MHz (DMSO-d_6): 1.07 (t, 6H), 1.86 (s, 3H), 2.13 (s, 6H), 2.63 (quart, 4H), 3.15 (s, 2H), 6.11 (s, 2H), 6.58 (d, 2H), 7;06 (s, 3H), 7.53 (s, 2H), 9.17 (s, 1H). ^{13}C NMR 125 MHz (DMSO-d_6): 11.9, 13.9, 18.0, 23.1, 47.9, 56.6, 112.1, 123.9, 126.2, 127.5, 129.5, 135.0, 153.4.9, 168.2, 169.2.; T_g = 10 ^{o}C; $T_{5\%onset}$ = 118 °C.

Lidocainium Ibuprofenate (1c). Isolated in 85% yield (gel) with water content via Karl-Fischer titration of 220 ppm. ^{1}H NMR 500MHz (DMSO-d_6): 0.85 (d, 6H), 1.07 (t, 6H), 1.33 (d, 3H), 1.80 (sept, 1H), 2.13 (s, 6H), 2.40 (d, 2H), 2.61 (quart, 4H), 3.13 (s, 2H), 3.61 (quart, 1H), 7.06 (s, 3H), 7.10 (d, 2H); 7.17 (d, 2H), 9.16 (s, 1H) ^{13}C NMR 125MHz (DMSO-d_6): 11.6, 18.0, 18.4, 22.1, 29.4, 44.1, 47.9, 56.2, 79.0, 126.2, 126.9, 127.5, 128.8, 134.8, 134.9, 175.3.; T_g= -32 ^{o}C; $T_{5\%onset}$ = 145 °C.

Procainium Sulfacetamide (2a). Isolated in 80% yield (white solid) with water content via Karl-Fisher titration of 113 ppm. ^{1}H NMR 500MHz (DMSO-d_6): 0.97 (t, 6H), 1.85 (s, 3H), 2.56 (quart, 4H), 2.74 (t, 2H), 4.19 (t, 2H), 5.97 (s, 2H), 6.11 (2, 2H), 6.57 (dd, 4H), 7.51 (d, 2H), 7.64 (d, 2H). ^{13}C NMR 125 MHz (DMSO-d_6): 177.935, 165.596, 165.556, 143.173, 141.779,

136.411, 128.031, 124.742, 124.341, 74.028, 62.944, 59.199, 35.440, 24.015, 12.232, mp = 115 °C; $T_{5\%onset}$ = 168 °C.

Procainium Salicylate (2b). Isolated in 80% yield (orange gel) with water content via Karl-Fisher titration of 167 ppm. ^{1}H NMR 500MHz (DMSO-d_6): 1.19 (t, 6H), 3.12 (d, 4H), 3.36 (s, 2H), 4.44 (t, 2H), 6.55 (d, 2H), 6.68 (m, 2H), 7.19 (t, 1H), 7.67 (t, 3H). ^{13}C NMR 125 MHz (DMSO): 12.2, 21.2, 59.0, 61.8, 124.7, 128.1, 128.7, 131.1, 142.2, 143.4, 144.2, 165.9, 174.4, 177.6, 184.2.; Tg = 5.8 °C; $T_{5\%onset}$ = 166 °C.

Procainium Ibuprofen (2c). Isolated in 79% yield (yellow gel) with a water content via Karl-Fisher titration of 188 pm. ^{1}H NMR 500MHz (DMSO-d_6): 0.85 (d, 6H), 0.96 (t, 6H), 1.33 (d, 3H), 1.75 (s, 1H), 2.40 (d, 2H), 2.54 (quart, 4H), 2.72 (t, 2H), 3.59 (quart, 1H), 4.19 (t, 2H), 5.95 (s, 2H), 6.56 (d, 2H), 7.09 (d, 2H), 7.17 (d, 2H), 7.63 (d, 2H) ^{13}C NMR 125 MHz (DMSO): 11.7, 18.4, 22.0, 29.4, 44.1, 44.3, 46.9, 50.7, 61.8, 66.8, 112.5, 115.8, 126.9, 128.7, 130.9, 138.5, 139.3, 153.3, 165.6, 175.3. T_g = -15 °C; $T_{5\%onset}$ = 163 °C

Procainium Docusate (2d). Isolated in 90% yield (yellowish viscous liquid) with a water content via Karl-Fisher titration of 263 ppm. ^{1}H NMR 500MHz (DMSO-d_6): 0.89 (m, 12H), 1.23 (m, 22H), 1.48 (s, 2H), 2.81 (dd, 1H), 2.90 (dd, 1H), 3.22 (s, 4H), 3.49 (s, 2H), 3.63 (dd, 1H), 3.89 (m, 4H), 4.45 (t, 2H), 6.05 (s, 2H), 6.58 (d, 2H), 7.61 (d, 2H), 9.12 (s, 1H). ^{13}C NMR 125 MHz (DMSO): 8.49, 10.6, 10.6, 10.7, 13.7, 13.8, 22.2, 22.3, 22.8, 22.9, 23.0, 23.1, 28.2, 28.3, 29.4, 29.6, 33.9, 37.9, 38.0, 38.0, 38.1, 47.2, 49.8, 58.1, 61.3, 65.9, 65.9, 66.0.; T_g = -23 °C; $T_{5\%onset}$ = 118 °C.

6.2.3 Solubility

Solubility of the synthesized ILs in water was experimentally determined at room temperature (22 ± 2 ºC). A small amount of each liquid salt was placed into a clean 20-mL glass vial, and deionized water (Culligan (Northbrook, IL), resistivity of 17.4 MΩ·cm) was added dropwise with a pipette. After the dropwise addition of the DI water, the vial was placed on a Fisher Scientific (Pittsburg, PA) FS30H sonicator for 30 minutes at room temperature to ensure property mixing of the IL and water. The solution was then allowed to equilibrate at room temperature for 12 hours before drops of water were again added, and the solution was mixed. This process occurred until the solution become clear, indicating complete dissolution. To determine the concentration of this solution, a 5-microliter aliquot was taken with a pipette and diluted in a volumetric flask to either 10 mL or 25 mL with fresh DI water for subsequent analysis by UV spectroscopy. Each IL was subjected to this solubility procedure three times.

A series of diluted solutions of each salt in water were prepared by weight and their absorbance was measured with a Cary 3C UV-Vis spectrophotometer, by Varian Instruments (Palo Alto, CA) providing a calibration line relating absorbance and concentration. The wavelength was chosen based on spectra obtained for each IL, as the anion had a substantial effect on the molar absorptivities of the cation. Therefore, the selected wavelength is as follows: **1a** 258 nm, **1b** 296 nm, **1c** 190 nm, **2a** 266 nm, **2b** & **2c** 291 nm, and **2d** 290 nm. The absorbance of the diluted aliquot of the saturated solution was measured, and its concentration calculated.

6.2.4 Mass Spectrometry

Mass spectra was measured on a Bruker HCTultra PTM Discovery System mass spectrometer (MS) with an electrospray ionization source. 1mM aqueous solutions of each IL were prepared, which were diluted to 0.5, 5, and 50 μM solutions for MS analysis. Infusion was utilized as the introduce method with a Kd Scientific (200 Series, Holliston, MA) syringe pump at a flow rate (200 μL/min). The cone voltage was lowered to 2500 V with a source temperature of 250 °C to vaporize the water. No further ionization was needed, as ILs are composed of ions. Both positive and negative mode was utilized, although negative mode is less sensitive than positive mode, which results in a lower intensity of peaks. The m/z range of 50-2500 was scanned during the data collected with approximately 3 scans a second. Data was collected for 1 minute for each mode (positive and negative) and the spectra was then averaged.

6.3. Results and Discussion

6.3.1. Synthesis and Purification

Typically, ILs can be prepared by one of the following methods: (1) reaction directly with acid (anion) and base (cation), (2) metathesis reaction from inorganic salts forms of the cation and anion, or (3) alkylation. Since both the cation and anion precursors were commercially available as salts, a metathesis route allowed for the direct use of the precursors without previous modification or purification. Thus, six new salts forms were prepared from the combinations of lidocaine (**1**) and procaine (**2**) as their HCl salts with sodium salts of sulfacetamide (**a**), salicylic acid (**b**), ibuprofen (**c**), and dioctylsulfosuccinate (**d**) (Table 5.1). The general reaction procedure employed has previously been described in Chapter 5, in which, equimolar methanolic solutions of the caine HCl salt and the sodium salt of the chosen counter ion are combined. However,

sulfacetamide containing salts were found to not be soluble in chlorinated solvents, thus acetonitrile was employed to during the purification step. As previously seen (Chapter 5), $AgNO_3$ tests did not indicate NaCl contamination, yet NMR spectroscopy revealed that the cation:anion ratio was not 1:1, indicating presence of unreacted anion precursor.

Typically, flash column chromatography is not utilized during IL purification for two reasons: (1) the intense dissolving power of some ILs and (2) the binding strength of the IL with the stationary phase i.e., strength of the ion-dipole interactions. Evidence of dissolved stationary phase as a trace level contaminants in certain IL, has recently appeared in the literature;[151-153] however, these examples have *all* dealt with imidazolium-based ILs. Moreover, it has been determined that the dissolution power is primarily influenced by the anion structure. Thus, the same cation when paired with various anions will have considerably different; for example, 1-butyl-3-methylimidazolium chloride dissolves twice the amount of cellulose when compared to the bromide or thiocyanate versions. Additionally, the replacement of chloride anion by iodide or tetrafluoroborate results in complete absence of dissolution of cellulose.[154] As a result, we theorize that pharmaceutical based ILs will not exhibit little, if any dissolution power due to the organic nature of both ions. Another potential difficulty when utilizing column chromatography arises from the polarity of ILs, as highly polar molecules will interact strongly with the polar Si—O bonds of the stationary phase, thereby, the ILs will have a tendency to bind to the stationary phase. However, fairly polar solvents such as methanol or ethyl acetate typically allow for elution of polar materials.

Here flash column chromatography on Silicycle™ 230-400 mesh silica gel as stationary phase was used to remove 18-97% excess unreacted anion precursor with gradient elution solvent of dichloromethane:methanol (98:2 to 90:10) for all except those containing

sulfacetamide, in which ethyl acetate:hexane (1:9 to 7:3) was employed. Each salt was diluted with a small solvent to reduce the viscosity and allow for easier loading onto the column, dichloromethane (pure) for all salts except sulfacetamide containing ILs, where a mixture of acetonitrile and methanol (10:1) was employed. Gradient elution, e.g. increasing polarity of the eluent solvent was utilized with a polarity increase of 2% for every 100 mL until the final solvent system was reached (Table 6.2). The purification progression was followed by TLC, where a $KMnO_4$ stain was used for visualization. The TLC analysis gave the same spotting patterns as discussed in Chapter 5. The purified ILs were isolated in gram quantities with from 79% (**1c**) to 95% (**1a**) yields, in which ^1H NMR indicated the correct 1:1 cation:anion ratio (within the limits of NMR detection, ca, 5%). Water content via Karl-Fisher titration of each salt was found to be less than 500 ppm.

Table 6.2. Column chromatography solvent elution gradients and resulting purified ILs

Compound	**Starting Eluent**	**Final Eluent**	**Results**
1a	ACN:EtOAc (10:1)	EtOAc:Hexane (7:3)	Fraction A: 51 mg, contains 24% excess Na sulfacetamide. Fraction B: 1.5 g, 1:1 ratio
1c	CH_2Cl_2 (pure)	CH_2Cl_2 to CH_3OH (98:2)	Fraction A: 125 mg, 97% excess Na ibuprofen. Fraction B: 1.3g, 1:1 ratio
2a	ACN:EtOAc (10:1)	EtOAc:Hexane (7:3)	Fraction A: 95 mg , 92% contains excess Na sulfacetamide. Fraction B: 2.4 g, 1:1 ratio
2b	CH_2Cl_2 (pure)	CH_2Cl_2 to CH_3OH (94:6)	Fraction A: 13 mg, 72% excess Na salicylate. Fraction B: 1.3g, 1:1 ratio
2c	CH_2Cl_2 (pure)	CH_2Cl_2 to CH_3OH (90:10)	Fraction A: 70 mg, 67% excess Na ibuprofen. Fraction B: 1.1g, 1:1 ratio
2d	CH_2Cl_2 (pure)	CH_2Cl_2 to CH_3OH (90:10)	Fraction A: 800 mg, 18% excess Na docusate. Fraction B: 2.3 g, 1:1 ratio

6.3.2. Thermal Behavior

Five of the newly formed liquid salts exhibited only glass transitions, thereby classifying them as ILs (Table 6.3). Although LHCl itself has a lower melting point, 76-79 ^{o}C, when it is combined with various anions, the melting point was depressed for *all* resulting ILs, which exhibited no melting but only glass transition behavior. A similar trend of melting point depression was also found for procaine ILs, with the exception of **2a**, which was obtained as a white solid with a distinct melting point at 115 °C. However, even though **2a** did not melt below 100 °C, the melting point was depressed approximately 40 °C from the procaine hydrochloride (PHCl) precursor. We hypothesize that structural similarities of the procaine and sulfacetamide ions, a primary amine connected to an aromatic ring, increased the number of interactions via amine hydrogen bonding and π-π stacking of aromatic rings. Therefore stabilizing interactions could increase the lattice energy, resulting in the solid form. The glass transition temperature of the synthesized ILs ranged from 19 °C (**1b**) to -32 (**1c**) for lidocainium salts, and from 6 °C (**2b**) to -32 (**2c**) for procainium salts, which resulted in a substantial decrease of melting point from the precursors (76-79 °C (LHCl) and 200 °C (Na salicylate and Na ibuprofen)) (Table 6.3). Lidocaine containing ILs exhibited a smaller ΔT, 66 °C (**1a**) and 108 °C (**1c**), respectively, when compared with the ΔT of procaine based ILs, 148 °C (**2b**) to 176 °C (**2d**). This considerable difference in ΔT can be attributed to the low melting point of LHCl, 76-79 °C, compared to the melting point of PHCl, 154-158 °C.

The onset of decomposition to 5% mass loss ($T_{onset5\%}$) temperatures of the synthesized ILs ranged from 161 ^{o}C (**1a**) to 251 ^{o}C (**1d**) with the remaining ILs $T_{onset5\%}$ values within 8 °C of each other. Both lidocaine containing ILs, **1a (**161 °C) and **1c** (185 °C) exhibited an increased thermal stability over LHCl (155 °C), but decreased thermal stability compared to sodium salt

precursors. Interestingly, the procaine based ILs **1a**, **1b**, and **1c** were found to be less thermally stable than either of the precursor salts. Contrarily, only one IL, **2d**, possessed a higher thermal stability, approximately 32 °C, than both precursor salts. A similar increase had been previously noted for in lidocaine docusate, which indicates the docusate anion has a larger stabilizing effect than the other anions.

One-step decomposition was found for **4d**, **1c**, and **2c** (Figure 6.1). However, multi-step decompositions, two and four-steps, were seen for **2a** and **1a**, respectively. Interestingly, the precursor cations and the precursor anion, sulfacetamide, typically display one-step decomposition; therefore, the multi-step decomposition steps exhibited by the ILs may suggest the possible formation of alternative compounds during the heating process.

Table 6.3 Physical Properties of Target and Starting Salts*

Salt	T_g (°C)	T_m (°C)	$T_{onset5\%}$ (°C)	Solubility (mM)
1a	10	--	161	5.08
1b	19[64]		158[64]	7.02
1c	-32	--	185	13.0
1d	-29		219	1.24
2a	--	115	188	1.72
2b	6	--	180	7.90
2c	-15	--	183	0.497
2d	-23	--	251	0.19
Precursor Salts				
LHCl	--	76-79	155	2488[138]
PHCl	--	154-158	219	>120[155]
Na Sulfacetamide	--	183	323	2199[138]
Na Salicylate	--	200	259	687[156]
Na Ibuprofen	--	200	222	438[157]
Na Docusate	--	153-157	219	33.7[109]

*T_g - glass transition temperature; T_m - melting point on heating. Decomposition temperatures were determined by TGA, heating at 5 °C min^{-1} under air atmosphere and are reported as ($T_{onset\ 5\%}$) onset to 5 wt% mass loss.

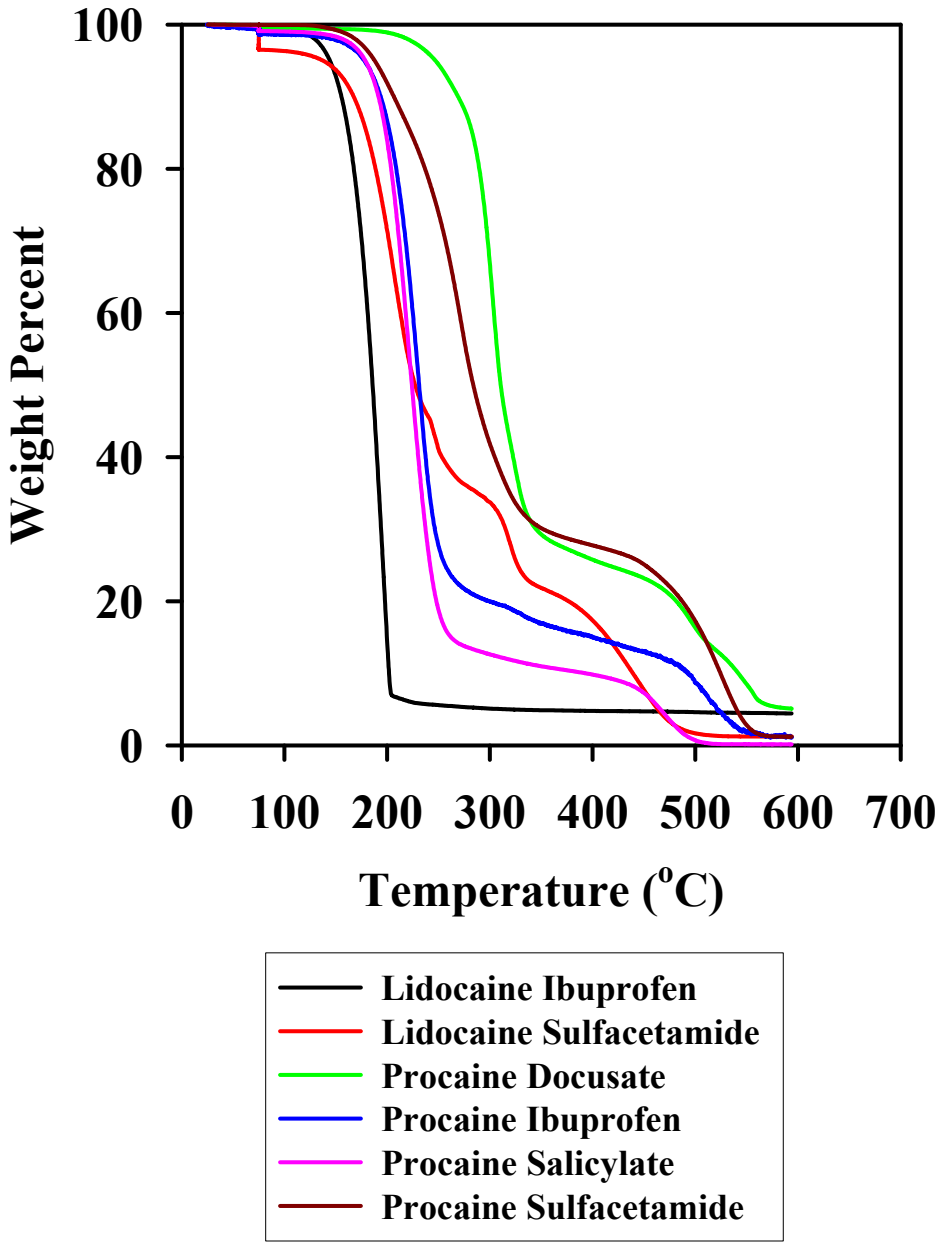


Figure 6.1. TGA analysis of synthesized caine ILs

6.3.3. Solubility

Unbuffered aqueous solubilities were measured for each salt in deionized water and compared to those of the precursor salts. As previously discussed, the sodium salts of the anions were selected based on the aqueous solubility, where hydrophilicity increases in the following order: sodium docusate < sodium ibuprofenate < sodium salicylate < sodium sulfacetamide. Interestingly, even though PHCl and LHCl are structurally similar, LHCl is approximately 20 times more soluble than PHCl. We hypothesized that aqueous solubility would decrease as the hypdrophobicity of the anion increased; however, it was unexpected that all prepared ILs would display dramatically lower solubilities than the starting salts, even when both the cation precursor and anion precursor had high solubilities.

Unfortunately, the typical procedure to determine solubility i.e., biphasic system of excess IL and saturated aqueous solution, was not applicable with these caine ILs. In the previous case of lidocaine docusate (**1d**), it was found that the IL formed a stable emulsion in water that could not be separated by centrifugation or by letting the solution stand for several days.[94] Thus, we have employed the same alternative method formerly employed for **1d**, in which drops of water were gradually added to a small amount of IL. After each drop addition, the mixture was shaken vigorously and then allowed to equilibrate at room temperature before the next water addition. These steps were repeated, addition of water then equilibrate, until miscibility of the system was reached, i.e., clear solution. An aliquot of the concentrated solution was diluted with fresh water for analysis by UV spectroscopy. Although many researchers would view formation of emulsion as a hindrance, we feel that could be an asset to pharmaceutically based ILs as a potential delivery vehicle.

It was found that the combination of LHCl (solubility = 2488 mM) and sodium sulfacetamide (2199 mM) to form **1a**, results in a salt with a solubility of only 5.08 mM (Table 3). Although the combination of docusate with procaine and lidocaine yielded the most hydrophobic ILs within each set, the remaining ILs did not show any discernable trends regarding the hydrophobicity of the precursor anion. The low solubility of docusate containing ILs can be attributed to the surfactant behavior of docusate, which when exposed to water will form micelles.[94] Recently, researchers have determined that aqueous solubility of ILs can be greatly influenced by the size of the ion, where larger ions are less soluble,[158] and amount of partial charges on the ions,[150] the lower amount of partial charges the less 'like' water. Therefore, the bulky sulfacetamide and ibuprofen anions would result in ILs with lower solubilities, which were displayed, regardless of the hydrophobicity of the precursor anion (Table 6.3). This also resulted in ILs containing salicylate to possess higher water solubility due to the small anion structure. However, **1c** did not coincide with these solubility parameters, instead, displaying the highest water solubility of all ILs. It was observed that the cation structure had almost no influence on the solubility when combined with salicylate, as these ILs yielded roughly the same solubility, 7.02 (**1b**) and 7.90 (**2b**), respectively. Nonetheless, this obtained solubility data could have important implications in the use of the IL strategy to <u>increase</u> solubility, which is typically why pharmaceuticals are delivered in a crystalline form.[159]

6.3.4. Ion Pairing

One explanation for the lower solubilities observed in these ILs could be unique interactions between the cations and anions, resulting in the formation of ion pairing or clustering in solution. These types of interactions could also have major implications in the

bioavailability of these liquid drug forms, specifically in their ability to pass through lipophilic membranes.[121] Thus to understand, at least somewhat, the ion pairing of ILs in dilute aqueous solution, we utilized electrospray mass spectrometry (ES-MS) to detect both cations (C^+), anions (A^-), and combinations of ions that yield a charged species.[160] ES-MS has been previously employed by other researchers to understand the clustering behavior of ILs containing imadizolium and pyridinium cations.[160,161] ES-MS has proven to be a powerful technique in determining ion pairing/clustering in solution, as it is able to produce intact molecular ions directly from the solution phase.[160-163] In this study, the caine ILs were diluted with DI water to 0.5, 5, and 50 μM, as we were interested in ion pairing/clustering at highly dilute concentrations. The IL solution was introduced into the MS via infusion with a syringe pump with a constant flow of 200 μL/min. The cone voltage was reduced to 2500 V with a source temperature of 250 °C to only vaporize the water with no fragmentation of the ILs themselves.

In addition to the major peak of the C^+ (*m/z* 235.1), lidocaine based ILs exhibited another species in the positive mode, as seen for **1d** (Figure 6.2). The species at *m/z* 469.1 ($[CL]^+$) was found to consist of a C^+ and a neutral lidocaine molecule (Figure 6.2), in which the two molecules could be connected via hydrogen bonding. This behavior has been previously observed for other ILs, in which either cations or anions can become hydrogen-bonded to neutral versions of .[64] The amount of $[CL]^+$ was found to increase with increasing concentration for all lidocaine ILs. **1a**, **1c**, and **1d** exhibited the highest percentage of this species, as compared to the C+ (taken as 100%) at 28%, 27%, and 27% at 50 μM, respectively. However, only **1c** displayed the $[CL]^+$ species at all concentrations. The anion mode, as previously mentioned, has less sensitivity than the cation mode; therefore no ion pairing/clustering was seen.

Interestingly, procaine (C^+, *m/z* 237.1, Figure 6.3)) ILs displayed different ionic species than the lidocaine ILs, which could be influenced by the additional hydrogen-bond donor/acceptor (primary amine attached to aromatic ring) that is not present on lidocaine. Thus, in solution, te primary amine could accept an hydrogen to become doubly protonated. The measurement pH of the utilized solutions was found to vary from 6.16 (**2b**) to 6.52 (**2a**). Since these solutions are slightly acidic, thus it is probable that the primary amine is protonated. Yet, only a small peak at *m/z* 119 was seen, therefore, the vast majority of procaine is present only as the singly protonated ion. Unfortunately, only **2d** displayed clustering at any concentration, in which two C^+ are coordinated to one A^- (Table 6.5). Clusters of this type have previously been isolated; however, imidazolium and pyridinium based ILs displayed larger clusters than seen here with a maximum of eleven C^+ with ten A^-.[161] This cluster type could potentially lead to increased analgesic power as the transdermal enhancer docusate could drag two lidocaine cations in the cell, therefore delivering a double dose of lidocaine per anion.

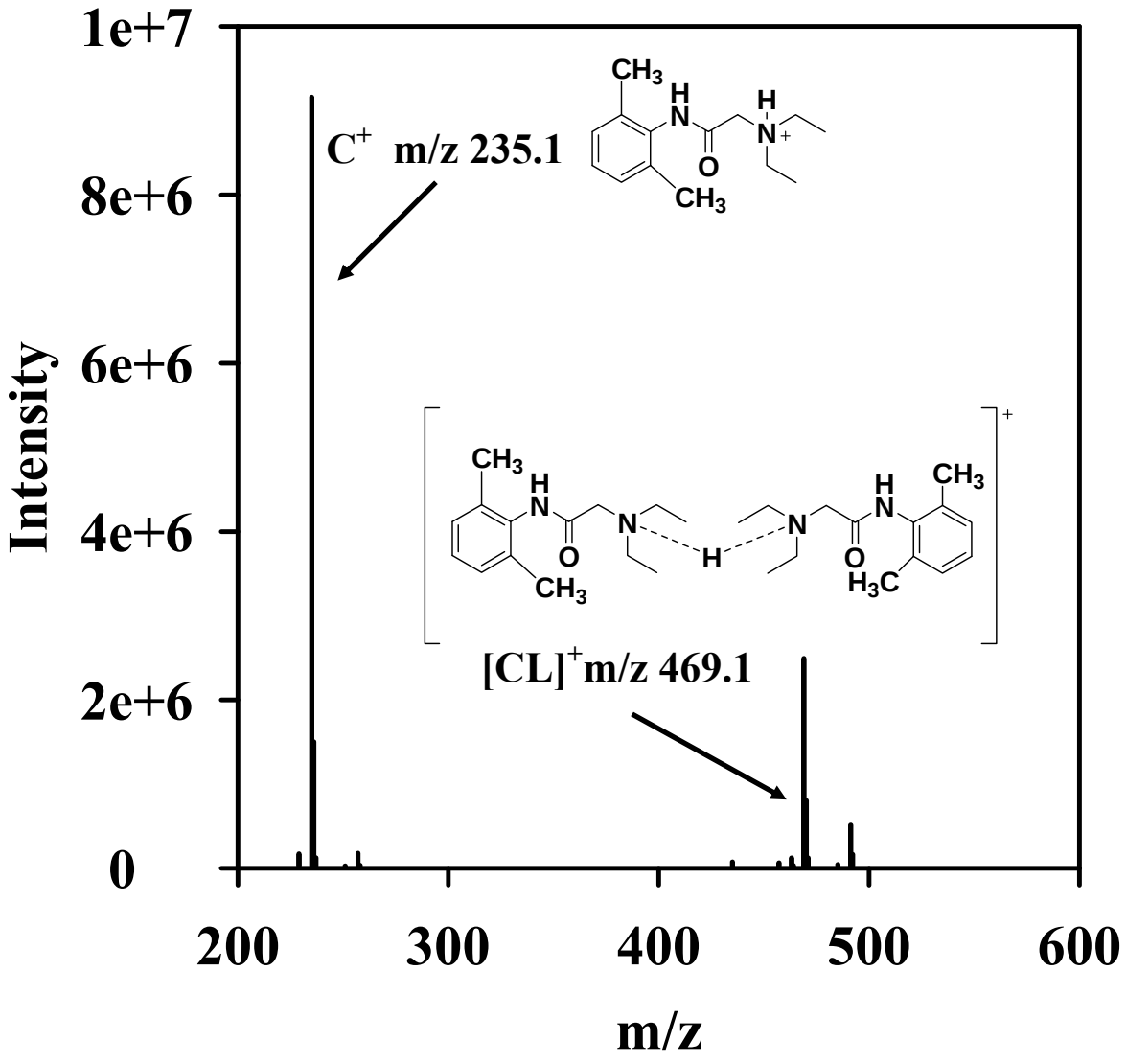


Figure 6.2. ES-MS spectra of **1d** (50 μM) with labeled species in this positive mode.

Table 6.4. Comparison of ion pairs/clusters in the positive mode within aqueous solutions of lidocaine ILs, as determined by ES-MS. Ratios were calculated from the intensity of the C^+ and the intensity of the ionic species.

Salt	**Concentration (μM)**	**I(m/z 469.1) I(m/z 235.1)**
1a	0.5	--
	5.0	0.165
	50	0.286
1b	0.5	--
	5.0	0.055
	50	0.123
1c	0.5	0.125
	5.0	0.151
	50	0.272
1d	0.5	--
	5.0	--
	50	0.272

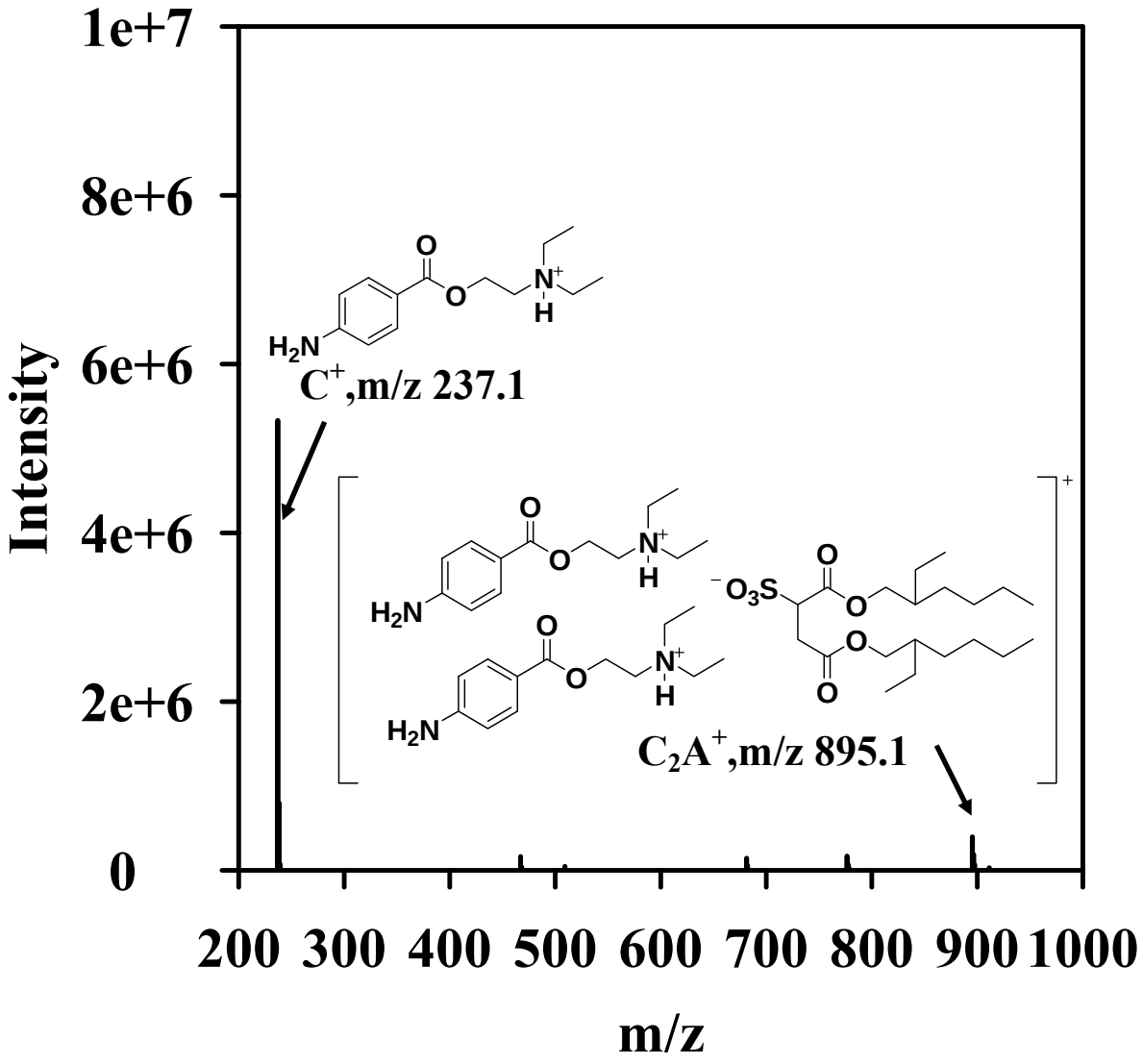


Figure 6.3. ES-MS spectra of **2d** (50 μM) with labeled species in the positive mode.

Table 6.5. Comparison of ion pairs/clusters in the positive mode within aqueous solutions of procaine ILs, as determined by ES-MS. Ratios were calculated from the intensity of the C^+ and the intensity of the ionic species.

Salt	**Concentration (μM)**	**I(m/z 895.5) I(m/z 237.1)**
2a	0.5	--
	5	--
	50	--
2b	0.5	--
	5	--
	50	--
2c	0.5	--
	5	--
	50	--
2d	0.5	--
	5	--
	50	0.074

6.4. Conclusions

We have been able to produce five liquid salts from solid precursors, although it is difficult to predict the exact melting point of the desired ILs, as illustrated in **2b**. During the synthesis, we encountered difficulties with the purification of the anion precursor salt from the desired ILs, yet we were able to use this problem to develop a relatively simple and straight forward purification method. This newly developed method allows for the isolation of ILs in high purity, which is especially useful given the limited purification techniques that can be utilized within the IL field. As previously demonstrated in Chapter 5, it is possible to substantially modify the thermal stability and solubility of ILs by changing the anion structure. This allows for not only the addition of another biological function, but also provides a mechanism to tune the physical, chemical, and biological properties of the resulting ILs. ES-MS offered preliminary insight into the solution behavior of these ILs as dilute concentrations. Interestingly, little ion pairing was exhibited; therefore it is difficult to draw conclusions regarding the unknown biological mechanism of lidocaine docusate. Additional biological testing is needed to understand how these pharmaceutically based ILs behave in the body.

CHAPTER 7

CONCLUSIONS

As ILs transition from solvent replacements for VOCs and energetic materials to pharmaceuticals, much knowledge is needed to determine how the combination ions along with ion structure affect not only the physical properties, but also the biological activity. As previously discussed, the API solid form can produce many complications during the synthesis, isolation, manufacture, and transport of drug products. However, a liquid form could overcome these problems along with potentially increasing the bioavailability and efficacy. Unfortunately, research in this field has been limited, as many researchers continue to focus of on the anti-bacterial activity of the heavily characterized imidazolium cations paired with simple halide counter ions. Yet, if biological activity was inherent in both ions, then it is possible to form ILs that possesses two uniquely different activities.

It was demonstrated in Chapter 3 that ILs with anti-microbial QACs cations could be successfully paired with sweetener anions to produce ILs with biological activity. Of the synthesized ILs, [HEX][Ace] was found to crystallize, although it was a low melting wax. This compound exhibited interesting crystalline orientation, in which the cation could form either π stacked dimers or polymers. In this project, only two ILs, both possessing [DDA] cations, were found to be liquids at room temperature. This depressed melting points was mostly likely due to the lack of π stacking. Biological testing of these liquid salts found the [DDA]-containing ILs to

be both toxic and irritating to the skin. Anti-bacterial testing of both [BA] and [DDA] containing ILs found negative synergistic effects, as these compounds possessed greater MIC and MBC values than the commonly used [BA][Cl] and [DDA][Br]. Therefore, the anion structure could hinder the mechanism of the QAC cations. Although the anti-bacterial activity was decreased, these ILs were found to exhibit promising insect deterrent ability, as the tested ILs were comparable to a known natural deterrent. This initial study of pharmaceutically active ILs yielded mixed results as the anti-bacterial activity of the QACs cations as decreased by the addition of the sweetener anion, however, these ILs were found to have good utility as an insect deterrent.

To increase the knowledge base regarding the combination of two APIs, the liquid salt formation of two commonly used drugs, ibuprofen and sulfacetamide, was analyzed in Chapter 4. For ibuprofen, the liquid salt can provide a way to bypass the severe GIT side effects associated with the oral delivery. Sulfacetamide has a tendency to form crystalline structures due to the number of hydrogen bond donors/acceptors, thus undesired crystallization is a constant issue. To alleviate these problems, the anions were paired with long chain QACs. This strategy successfully depressed the meting point, as all synthesized compounds were obtained as low melting salts. Now, sulfacetamide is no longer hindered by the crystalline form and the problems associated with polymorphism. Unfortunately, thermal stability still remains an issue with pharmaceutically based ILs, as only one synthesized ILs displayed an increased thermal stability over either of the precursor cation and anion salts.

Although, determination of the physical properties is needed, the understanding how the combination of the ions effects the overall pharmaceutical activity of the IL is essential. Thus, Chapter 5 focused on the synthesis and biological assessment of a topical analgesic based IL,

lidocaine docusate. Although a typical metathesis reaction was employed, a small amount of the anion precursor was found to be dissolved within the formed IL. A new IL purification method utilizing column chromatography was developed to remove the impurity, which resulted in the isolation of the pure IL. Interestingly, the different ratios of the ILs could be separated, as the cation and anion spotting intensity was directly proportional to the ratio, thereby indicating the ions are associated in solution. Thermal stability was found to be increased over both precursor salts, which was not obtained for the ILs discussed in Chapter 3 and 4. Contrary to Chapter 3 results, advantageous biological results were obtained, in which LD exhibited increased analgesic power and duration over the simple halide version, LHCl, in the injury based model. It was hypothesized that due to the decreased water solubility of LD, this compound has a longer resident time on the skin; therefore, more lidocaine can be absorbed.

However, the cellular test revealed that LD produces anesthetic power through an alternative mechanism than LHCl. This could indicate that LD exists as ion pairs, in which the docusate pulls the lidocaine molecule into the cellular membrane. Overall, the enhancement of the API bioactivity appears to occur in addition to, or apart from, the enhancing effects of the surfactant anion on membrane permeability. Unfortunately, with these exciting results comes a multitude of questions regarding the mechanism of action and how LD behaves in the aqueous phase.

The knowledge base regarding the use of ILs as pharmaceuticals has grown substantially throughout this dissertation; yet, more information regarding IL-APIs solution behavior is needed. As hypothesized from Chapter 5 results, the increased anesthetic power and duration was thought to be attributed to the increased hydrophobicity of the IL. However, the cellular assay indicated a totally different mechanism of action from LHCl. Thus in Chapter 6, we

attempted to control the ILs' properties by pairing 'caine' drugs with FDA-approved anions of varying hydrophobicity. Again during the synthesis, difficulties with the purification were encountered, as excess anion precursor salt was found in the synthesized ILs. As previously demonstrated in Chapter 5, it is possible to substantially modify the thermal stability and solubility of ILs by changing the anion structure. This allows for not only the addition of another biological function, but also provides a mechanism to tune the physical, chemical, and biological properties of the resulting ILs.

To study the solution behavior of IL-API, ES-MS was utilized to provide preliminary insight at dilute concentrations, which would be encountered in the body. Interestingly, a minimal amount of ion pairing was displayed for either procaine or lidocaine based ILs. Lidocaine ILs preferred to hydrogen bond between the tertiary amine of a lidocaine molecule and the protonated quaternary ammonium of another lidocaine molecule. Since this species is charged, it can easily pass through the lipid bilayer, thereby delivering two lidocaine molecules. This scenario would account for the increased anesthetic power and duration observed in the rat tail flick test, as more lidocaine molecules would be available in the cell vs. LHCl. As two lidocaine molecules diffuse across the lipid bilayer, insufficient concentrations of lidocaine would be found on the surface of the cell, therefore neurite growth would be possible as displayed in the cellular assay. Additionally, the hydrophobicity of the IL allows for longer retention time, which permits the lidocaine molecules to diffuse into the skin since the lipid bilayer is also hydrophobic. Although, some conclusions and hypotheses have been made regarding the biological mechanism of lidocaine based ILs, no information on procaine based ILs biological activity has been determined. Since procaine ILs did not exhibit hydrogen bonding between two procaine molecules, it can be hypothesized that these ILs would not show an increased anesthetic

power or duration. However, procaine docusate did possess a cluster of the type C_2A^+, which could deliver two procaine molecules. Yet, as seen in Chapter 3, the addition on the anion had a negative effect on the biological activity of the QACs. Thus, additional biological testing of procaine ILs is needed to compare with the results obtained for lidocaine docusate.

Overall, this dissertation demonstrated that a modular IL strategy could potentially provide a new strategy for the pharmaceutical industry, imparting a tool to customize the physical, chemical, and biological properties, which could result in improved pharmaceuticals and new treatment options. The IL-API strategy has been shown (Chapter 3-6) to successfully overcome problems such as polymorphism, solubility, bioavailability and adverse side effects that have hindered the commonly use pharmaceuticals. However, the knowledge base regarding the correct combination and type of ions already identified as pharmaceuticals to produce ILs is still relative small. This factor, along with ILs generally unique properties, has made predictions regarding the resulting IL-API properties quite difficult.

Although this work has supplied initially positive results of IL-APIs, much more R&D is needed to convince pharmaceutical companies and the FDA that these liquid salts are safe for human consumption. This task will be difficult as ILs do not behave as molecular liquids; therefore new synthesis, purification, and isolation methods are needed to ensure complete removal of all by-products, impurities, and solvent. Additionally, the fundamental knowledge base regarding the solution behavior of ILs is sparse. Substantial research in this area is needed to understand the ion association, as it has been demonstrated in Chapters 5-6 that these IL-APIs do not dissociate in solution like typical salts. Ion pairing/clustering seems to be the likely explanation for the increased anesthetic power and duration as exhibited by lidocaine docusate, however, it is unknown what structural features of the ions contributes to this behavior.

Additionally, experiments such as conductivity, membrane transport, cellular assays, and further ES-MS would help to provide a more complete picture of the solution behavior of IL-APIs in the body. Overall, this preliminary work has proven that liquid salts can be formed from solid APIs and can possess unique physical and biological properties, which can overcome problems associated with the solid form.

REFERENCES

(1) www.merriam-webster.com/dictionary/pharmaceutical last accessed March 17, 2010.

(2) Gassmann, O.; Reepmeyer, G.; Von Zebtwitz, M., *Leading Pharmaceutical Innovation Trends and Drivers for Growth in the Pharmaceutical Industry*, Springer, Berlin, 2009.

(3) Gordian, M.; Singh, N.; Zemmel, R.; Elias, T. *In vivo* **2006**, *Apr*.,1-8.

(4) Shrama, A.; Jain, C. P., *J. Glo. Pharma Tech*. **2010**, *2*, 18-28.

(5) Yu, L. X.; Gatlin, L.; Amidon, G. L. Predicting oral drug absorption. In: *Transport Processes in Pharmaceutical Systems.* Amidon, G. L.; Lee P. I., & Topp E. M., Eds. Marcel Dekker, Inc, New York, 2001.

(6) *Drug Bioavailability, Estimation of Solubitity, Permeability, Absorption, and Bioavailability*, van de Waterbeemd, H.; Testa, B, Wiley-VCH, Weinheim, 2nd edn. 2009, 11-28.

(7) *Water-Insoluble Drug Formulation*, Liu, R. Ed., CRC Press, New York, 2nd edn, 2006.

(8) Danielsson, I., Lindman, B. *Coll. Surf*. **1981**, *3*, 391-392.

(9) Lieberman, H. A.; Rieger, M. M., Banker, G. S., *Pharmaceutical dosage forms*, Marcel Dekker, Inc., New York, 1998.

(10) Patel, D.; Sawant, K. K. *Curr. Drug. Del.* **2009**, *6*, 419-424.

(11) Gershanik, T.; Benzeno, S.; Benita, S. *Pharm. Res*. **1998**, *15*, 863-869.

(12) Gershanik, T.; Benita, S. *Eur. J. Pharm. Biopharm*. **2000**, *50*, 179-188.

(13) Strickley, R. G. *Pharm. Res.* **2004**, *21*, 201–230.

(14) Jouyban, A. *J. Pharm. Pharma. Sci*. **2008**; *11*, 32-58.

(15) Chaumeil, J. C. *Meth. Find. Exp. Clinc. Pharma*. **1998**, *20*, 211-215.

(16) Chow, K.; Tong, H. H. Y.; Lum, S.; Chow, A. H. L. *J. Pharm. Sci*. **2008**, *97*,2855-2877.

(17) Byrn, S. S. R.; Pfeiffer, R. R.; Stowell, J. G. *Solid-State Chemistry of Drugs*, 2nd Edition, 1999.

(18) Sands, D. E., *Introduction to crystallography*. W.A. Benjamin, New York, 1969.

(19) Winn, D.; Doherty M. F. *AIChE J.* **2000**, *46*, 1348–1367.

(20) Price S.L. *Adv. Drug Deliv. Rev.* **2004**, *56*, 301–319.

(21) Van Arnum, P. *Pharm. Techn*. **2008**, *Nov*., 58-64.

(22) Yin, S. X.; Grosso, J. A. *Curr. Opin. Drug Discov. Dev*. **2008**, *11*, 771-777.

(23) Erdemir, D.; Lee, A. Y.; Myerson, A. S. *Curr. Opin. Drug Discov. Dev*. **2007**, *10*, 746-755.

(24) Lu, J.; Rohani, S. *Curr. Med. Chem.* **2009**, *16*, 884-905.

(25) Kitamura, M. *J. Cryst. Growth* **2004**, *4*, 1153-1159.

(26) Shekunov, B. Yu.; York, P. *J. Cryst. Growth* **2000**, *211*, 122-136.

(27) Weissbuch, I.; Lahav, M.; Leisevowitz, L. *J. Cryst. Growth* **2003**, *3*, 125-150.

(28) Tanaka, S.; Ataka, M.; Kubota, T.; Soga, T.; Homma, K.; Lee, W. C. Tanokura, M. *J. Cryst. Growth* **2002**, *234*, 247-254.

(29) Saleki-Gerhardt, A.; Zografi, G. *Pharma. Res*. **1994**, *11*, 1166-1173.

(30) Aso, Y.; Yoshioka, S.; Kojima, S. *J. Pharm. Sci*. **2004**, *93*, 384-391.

(31) Serajuddin, A. T. M. *J. Pharm. Sci*. **1999**, *88*, 1058-1066.

(32) Amidon, G.; Lennernäs, H.; Shah, V.; Crison, J. R. *Pharm. Res*. **1995**, *12*, 413-420.

(33) Lipinski, C. A.; Lombarbo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev*. **1997**, *23*, 3-25.

(34) Walden, P. *Bull. Acd. Imper. Sci. (St Petersburg)* **1914**, 1800.

(35) *An Introduction to Ionic Liquids*, Freemantle, M., Ed. RSC Publishing, 2010, 1-62.

(36) Wilkes, J. S.; Levisky, J. A.; Wilson, R. A.; Hussey, C. L. *Inorg. Chem*. **1982**, *21*, 1263.

(37) Wilkes, J. S.; Zaworotko, M. J. *J. Chem. Soc*. **1992**, 965-967.

(38) Murphy, D. W.; Broadhead, J.; Steele, B. C. H. (Eds), Plenum Press, New York, 1980, pp 111-122

(39) Gale, R. J.; Gilbert, B; Osteryoung, R. A. *Inorg. Chem*. **1978**, *17*, 2728-2729.

(40) Gordon, C. M.; Muldoon, M. J. Synthesis of Ionic Liquids, In: *Ionic Liquids in Synthesis* Wasserscheid, P., & Welton, T. Eds., 2nd ed. Volume 1. 2008.

(41) Fuller, J.; Carlin, R. T.; DeLong, H. C.; Haworth, D. *Chem. Commun*. **1994**, 299-300.

(42) *Purification of Laboratory Chemicals*, Armarego, W. L. F.; Perrin, D. D., 4th, Edn. Butterworth-Heinemann: London 1997.

(43) *A Textbook of Quantitative Inorganic Analysis*, Vogel, A. I. 3rd Edn., Longmans, Green and Co., London, 1961.

(44) Holbrey, J. D.; Rogers, R. D., Physicochemcical Properties of Ionic Liquids: Melting Points and Phase Diagrams, In: *Ionic Liquids in Synthesis*, Wasserscheid, P., & Welton, T., Eds., 2nd edition, vol. 1, 2008.

(45) Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T. *Phys. Chem. Chem. Phys.* **2003**, *5*, 2790-2794.

(46) Muldoon, M. J.; Gordon, C. M.; Dunkin, I. R. *J. Chem. Soc., Perkin Trans.* **2001**, *2*, 433-435.

(47) Earle, M. J.; Seddon, K. R. *Pure. Appl. Chem*. **2000**, *72*, 1391-1398.

(48) Wheeler, C.; West, K. M.; Liotta, C. L.; Eckert, C. A. *Chem. Commun.* **2001**, 887-888.

(49) Holbrey, J. D.; Seddon, K. R. *Clean Prod. Proc*. **1999**, *1*, 223-236.

(50) Welton, T. *Chem. Rev*. **1999**, *99*, 2071-2083.

(51) Wilkes, J. S. *J. Mol. Chem. A*. **2004**, *214*, 11-17.

(52) Welton, T. *Coord. Chem Rev*. **2004**, *248*, 2459-2477.

(53) *Solvents and Solvent Effects in Organic Chemistry*, Reichard, C., Ed., VCH, Weinheim, 3rd edn 2003.

(54) Anderson, J. L.; Ding, J.; Welton, T.; Armstrong, D. W. *J. Am. Chem. Soc*. **2002**, *124*, 14247-14254.

(55) Gozzo, F. C.; Santos, L. S.; Augusti, R.; Consorti, C. S.; Dupont, J.; Eberlin, M. N. *Chem. Eur. J*. **2004**, *10*, 6187-6193.

(56) Suarez, P. A. Z.; Einloft, S.; Dullius, J. E. L.; De Souza, R. F.; Dupont, J. *J. Chim. Phys. Phys. Chim. Biol.* **1998**, *95*, 1626-1639.

(57) Singh, R. J.; Verman, R. D.; Meshir, D. T.; Shreeve, J. M.; *Angew. Chem. Int. Ed.* **2006**, *45*, 3584-3601.

(58) Gao, H.; Ye, C.; Winter, R. W.; Gard, G. L.; Sitzmann, M. E.; Shreeve, J. M. *Eur. J. Inorg. Chem.* **2006**, 3221-3226.

(59) Katritzky, A. R.; Singh, S.; Kirichenko, K.; Smiglak, M.; Holbrey, J. D.; Reichert, W. M.; Spear, S. K.; Rogers, R. D. *Chem. Eur. J*. **2006**, *12*, 4630-4641.

(60) Pernak, J.; Sobaskiewicz, K.; Mirska, I. *Green Chem*. **2003**, *5*, 52-56.

(61) Docherty, K. M.; Kulpa, Jr., C. F. *Green Chem.* **2005**, *7*, 185-189.

(62) Pernak, J.; Feder-Kubis, J. *Chem. Eur. J*. **2005**, *11*, 4441-4449.

(63) Dean, P. M.; Turanjamin, J.; Yoshizawa-Fujita, M.; Macfarlane, D. R. *Cryst. Growth Des.* **2009**, *9*, 1137-1145

(64) Bica, K.; Rijksen, C.; Nieuwenhuyzen, M.; Rogers, R. D. *Phys. Chem. Chem. Phys*. **2010**, *12*, 2011-2017.

(65) Sallee, F. G.; Pollock, B. G. *Clin*. *Pharmacokinet* **1990**, *18*, 346-364.

(66) Gould, P. L. *Int. J. Pharmaceutics* **1986**, *33*, 201-217.

(67) *The Extra Pharmacopoeia*, Martindale, 30th edn., 1993.

(68) Aungst, B. J.; Hussain, M. A. *Pharm. Res.* **1992**, *9*, 1507-1509.

(69) Green, P. G. *Diss. Abstr. Int. B.* **1989**, *49*, 4247-4254.

(70) *Handbook of Pharmaceutical Salts Properties, Selection, and Use*, Stahl., P. H., & Wermuth, C. G., Eds. Wiley-VCH, New York, 2002.

(71) Seddon, K. R.; Stark, A.; Torres, M.-J. *Pure Appl. Chem*. **2000**, *75*, 2275-2287.

(72) Klingele, M. *Ionic Liquids Today* **2008**, *1*, 3.

(73) Sheldrick, G. M. Program for Semiempirical Absorption Correction of Area Detector Data, University of Göttingen, Germany, 1996.

(74) Sheldrick, G. M. SHELXTL, version 5.05, Siemens Analytical X-ray Instruments Inc., 1996.

(75) Shelton, R. S.; Van Campen, M. G.; Tilford, C. H.; Lang, H.C.; Nisonger, L.; Bandelin, F. J.; Rubenkoenig, H. L. *J. Am. Chem. Soc.* **1948**, *69*, 753-755.

(76) Jacobs , W. A.; Heidelberger, M. *Proc. Nat. Acad. Sci. U. S. A* **1915**, *1*, 226-228.

(77) Jacobs, W. A. *J. Exp. Med.* **1916**, *23*, 563-568.

(78) Jacobs, W. A.; Heidelberger, M.; Amoss, H. L. *J. Exp. Med.* **1916**, *23*, 569-576.

(79) Jacobs, W.A.; Heidelberger, M.; Bull, C. G. *J. Exp. Med.* **1916**, *23*, 577-601.

(80) Domagk, G. *Deut. Med. Wochenschr.* **1935**, *61*, 829-832.

(81) *Cationic Surfactants,* Richmond J. M., Ed.; Marcel Dekker, 1990.

(82) *Cationic Surfactants: Physical Chemistry*, Rubingh, D. N.; Holland, P. M., Eds.; Marcel Dekker, 1991.

(83) *Eur. Pat*., 1 182 669, 1986.

(84) *US Pat.,* 1 012 077, 2005.

(85) Petrocci, A.N. in *Disinfection, Sterilization and Preservation,* Block, S. S., Ed.; Lea & Febiger, 1983.

(86) Makosza, M. *Pure Appl. Chem.* **2000**, *72*, 1399-1403.

(87) *US Pat.,* 1 025 458, 2006.

(88) Oertel, J. *Holztechnologie* **1965**, *6*, 243-247.

(89) Butcher, J. A.; Preston, A. F.; Drysdale, J. *For. Prod. J.* **1977**, *27*, 19-22.

(90) Butcher, J. A.; Drysdale, J. *N.Z.J. For. Sci.* **1978**, *8*, 403-409.

(91) Cross, J. In *Cationic Surfactants, Analytical and Biological Evaluation Surfactant Science Series.* Cross, J. & Singer, E. J., Eds.; Marcel Dekker: New York, 1994; Vol. 53.

(92) Klinguer, C.; Beck, A.; De-Lys, P.; Bussat, M. C.; Blaecke, A.; Derouet, F.; Bonnefoy, J. Y.; Nguyen, T. N.; Corvaia, N.; Velin, D. *Vaccine* **2001**, *19*, 4236-4244.

(93) Liu, F.; Huang, L. *J. Controlled Release* **2002**, *78*, 259-266.

(94) Hough, W. L.; Smiglak, M.; Rodríguez, H.; Swatloski, R. P.; Spear, S. K.; Daly, D. T.; Pernak, J.; Grisel, J. E.; Carliss, R. D.; Soutullo, M. D.; Davis, Jr., J. H.; Rogers, R. D. *New J. Chem.* **2007**, *31*, 1429-1436

(95) Carter, E. B.; Culver, S. L.; Fox, P. A.; Goode, R. D.; Ntai, I. Tickell, M. D.; Traylor, R. K.; Hoffman, N. K.; Davis Jr., J. H. *Chem. Commun*. **2004**, 630-631.

(96) Pernak, J.; Stefaniak, F.; Weglewski, J. *Eur. J. Org. Chem.* **2005**, 650-652;

(97) Noble, A. C. *Trends Food Sci. Technol*. **1996**, *7*, 439-444.

(98) de Carvalho, L. C.; Segato, M. P.; Nunes, R. S.; Novak, C.; Cavalheivo, E. T. G. *J. Therm. Anal. Calorim.* **2009**, *97*, 359-365.

(99) Burgard, A. *US Pat*. 2003023084 A1, 2003.

(100) OECD Guideline for Testing of Chemicals No. 434: Acute Oral Toxicity – Acute Toxic Class Method, **2001**; 1-14

(101) OECD Guideline for Testing of Chemicals No. 404: Acute Dermal Irritation/Corrosion, **2002**, 1-13

(102) Nawrot, E.; Bloszyk, J.; Harmatha, L.; Nowotny; Drożdż, B. *Acta Entomol. Bohemosl.* **1986**, *83*, 327-335

(103) [HEX][Ace]: $C_{25}H_{42}N_2O_4S$; FW 466.67; Triclinic P-1; T 173 K; a 7.921(3), b 13.374(5), c 25.689(10) Å, α 76.755(7), β 82.225(7), γ 89.260(7)^o; Z 4; Vol. 2624.2(17) Å^3; r 1.181 g/cm^3; 7459 independent (R_{int}= 0.0249) and 5755 observed ([I > 2σ(I)]) reflections; GooF 1.070; R_1, wR_2 [I > 2σ(I)] 0.0462, 0.1208; R_1, wR_2 (all data) 0.0641, 0.1411.

(104) Ito, T.; Yamase, T. *Chem. Letters* **2009**, *38*, 370-371.

(105) Cybulski, J.; Wisniewska, A.; Kulig-Adamiak, A.; Lewicka, L.; Cieniecka-Roslonkiewicz, A.; Kita, K.; Fojutowski, A.; Nawrot, J.; Materna, K.; Pernak, J. *Chem.-Eur. J*. **2008**, *14*, 9305-9311.

(106) Zhou, Z. B.; Matsumoto H.; Tatsumi, K. *Chem. Eur. J.* **2005**, *11*, 752-766.

(107) Weeks, M. H.; Steinberg, M.; Rowe, S.; Boldt, R. E., *U.S. Clearinghouse Fed. Sci. Tech. Inform. A.D.*, **1970**; *867663*, pp 66.

(109) *The Merck Index,* 13th ed., ed. by M. J. O'Neil, A. Smith, P. E. Heckelman, S. Budavari, Merck & Co., Inc., Whitehouse Station, NJ, **2001**, 4906.

(110) Cheng, H.; Rogers, J. D.; Dermetriades, J. L.; Holland, S. D.; Seibold, J. R.; Depuy, E. *Pharm. Res*. **1994**, *11*, 824-830.

(111) Hawkey, C. J. *Best Prat. Res. Cli. Gas*. **2001**, *15*, 801-820.

(112) Hawkey, C. *J. Gas*. **2000**, *119*, 521-535.

(113) Tegeder, I.; Muth-Selbach, U.; Lotsch, J.; Rusing, G.; Oelkers, R.; Brune, K.; Meller, S.; Kelm, G. R.; Sorgel, F.; Geisslinger, G. *Clin. Pharmacol. Ther*. **1999**, *65*, 357-368.

(114) Magolis, D. J. *Cutis* **2005**, *75*, 8.

(115) Sridhar, M. S.; Gopinathan, U.; Garg, P.; Sharma, S.; Rao, G. N. *Surv. Opthalmol*. **2001**, *45*, 361.

(116) Maren, T. H. *Ann. Rev. Pharmacol. Toxicol*. **1976**, *16*, 309-327.

(117) Adsmond, D. A.; Grant, D. J. W. *J. Pharma. Sci*. **2001**, *90*, 2058-2077.

(118) Mesley, R. J.; Houghton, E. E. *J. Pharm. Pharmacol*. **1967**, *19*, 295-304.

(119) Belieres J. P.; Angell C. A. *J. Phys. Chem. B* **2007**, *111*, 4926-4937.

(120) MacFarlane, D. R.; Seddon, K. R. *Aust. J. Chem.* **2007**, *60*, 3-5.

(121) Ruetsch, Y. A.; Böni, T.; Borgeat, A. *Curr. Topics Med. Chem*. **2001**, *1*, 175-182.

(122) Mayer, E. *JAMA* **1928**, *82*, 876-884.

(123) Strichartz, G. R. Neural Physiology and Local Anesthetic action In: Cousins, M. J.; Bridenbaugh, P. O. eds. *Neural Blockade in Clinical Anesthesia and Management of Pain.* Philadelphia; J. B. Lippincott, 1988: 25-45.

(124) Grecken, R. C.; Auletta, M. J. *J. Am. Acad. Dermatol.* **1988**, *19*, 599-614.

(125) Arpey, C. J.; Lynch, W. S. *Clin. Dermatol.* **1992**, *10*, 275-283.

(126) Auletta, M. J.; Grecken, R. C. eds, *Local Anesthsia for Dermatologic Surgery*, New York: Churchill Livingstone, 1991, 1-3.

(127) Skidmore, R. A.; Patterson, J. D.; Tomsick, R. S. *Dermatol. Surg*. **1996**, *22*, 511-522.

(128) Ward, P. D.; Tippin, T. K.; Thakker, D. R. *Pharm. Sci. Technol. Today* **2000**, *3*, 346–358

(129) Covino, B. G. Clinical Pharmacology of local anesthetic agents. In: Cousins, M. J.; Bridenbaugh, P. O. eds. *Neural Blockade in Clinical Anesthesia and Management of Pain.* Philadelphia: J. B. Lippincott, 1988, 111-144.

(130) Jamakandi, V. G.; Ghosh, B.; Desai, B. G.; Khanam, J. *Ind. J. Pharma. Sci*. **2006**, *68*, 556-561.

(131) Ghosh, B.; Reddy, L. H.; Kulkarn, R. V.; Khanam, J. *Indian J. Exp. Biol*. **2000**, *36*, 42-49.

(132) Davis, Jr., J. H.; Fox, P. A. *Chem. Commun.* **2003**, 1209-1212.

(133) Legen, I.; Salobir, M.; Kerc, J. *Int. J. Pharm.* **2005**, *291*, 183-188.

(134) D'Amour, F. E.; Smith, D. L. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74-79.

(135) Kolesnikov, Y. A.; Chereshnev, I.; Pasternake, G.W. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 546-551.

(136) Takatori, M.; Kuroda, Y.; Hirose M. *Anesth Analg.* **2006**, *102*, 462-467.

(137) Joshi, R.; Arora, V.; Desjardins, J. P.; Robinson, D.; Himmelstein, K. J.; Iversen, P. L. *Pharm. Res.* **1998**, *15*, 1189-1195.

(138) Labastidas, I.; Martínez, F. *Acta Farm. Bonaerense* **2006**, *25*, 55-63.

(139) Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D. *Green Chem*. **2001**, *3*, 156-164.

(140) Seddon, K. R.; Stark, A.; Torres, M.-J. *Pure Appl. Chem.* **2000**, *72*, 2275-2287.

(141) Huang, J. H.; Thalhammer, J. G.; Raymond, S. A.; Strichartz, G.R. *J. Pharmacol. Exp Ther.* **1997**, *292*, 802-811.

(142) Takatori, M.; Kuroda, Y.; Hirose, M. *Anesth. Analg*. **2006**, *10*, 462-467.

(143) Simonetti, G.; Simonetti, N.; Villa A. *J. Chemother.* **2004**, *16*, 38-44.

(144) Bramer, T.; Dew, N.; Edsman K. *J. Pharm. Sci*. **2006**, *95*, 769-780.

(145) Becker, D. E.; Reed, K. L. *Anesth. Prog.* **2006**, *53*, 98-109.

(146) Tuckers, G. T.; Mather, L. E. Properties, Absorption, and Deposition of Local Anesthetic Agents. In: Cousins, M. J.; Bridenbaugh, P. O. (Eds.) *Neural Blockage in Clinical Anesthesia and Management of Pain*, (J. B. Lippincott, Philadelphia, 1988)

(147) Wolf, J.; Aboody, R. *Int. Rec. Med*. **1960**, *173*, 234-241.

(148) Oshlack, B.; Pedi, F.; Zirlis, J. *Eur. Pat.* 519371 A1 19921223, 1992.

(149) Freire, M. O.; Santos, L. M. N. B. F.; Fernandes, A. M.; Coutinho, J. A. P.; Marrucho, I. M. *Fluid Phase Equilib*. **2007**, *261*, 449-454.

(150) Klähn, M.; Stüber, C.; Seduraman, A.; Wu, P. *J. Phys. Chem. B*. **2010**, *114*, 2856-2868.

(151) Endres, F.; Zein El Abedin, S.; Borissenko, N. *Phys. Chem*. **2006**, *220*, 1377-1394.

(152) Clare, B R.; Bayley, P. M.; Best, A. S.; Forsyth, M.; MacFarlane, D. R. *Chem. Commun*., **2008**, 2689–2691.

(153) Stark, A.; Behrend, P.; Braun, O.; Mueller, A.; Ranke, J.; Ondruschka, B.; Jastorff, B. *Green Chem.* **2008**, *10*, 1152–1161.

(154) Sashina, E. S.; Novoselov, N. P. *Russ. J. Gen. Chem.* **2009**, *79*, 1057-1062.

(155) Hays, P. A. *J. Forensic Sci*. **2005**, *50*, 1342-1360.

(156) www.chemicalland21.com/arokorhi/lifescience/phar/SODIUM%20SALICYLATE.html last accessed November 3rd, 2009.

(157) www.sigmaaldrich.com/catalog/ProductDetail.do?N4-I1892FLUKA&N5=SEARCH_CONCAT-OBRAND_KEY &F=SPEC last accessed November 3rd, 2009.

(158) Ranke, J.; Othman, A.; Fan, P.; Muller, A. *Int. J. Mol. Sci*. **2009**, *10*, 1271-1289.

(159) Stahl, P. H.; Wermuth C. G. (eds) *Handbook of Pharmaceutical Salts Properties, Selection, and Use*, (Wiley-VCH, New York 2002)

(160) Alfassi, Z. B.; Huie, R. E.; Milman, B. L.; Neta**.** P. *Anal Bioanal Chem*. **2003**, *377*, 159–164.

(161) Fernandes, A. M.; Coutinho, J. A. P.; Marrucho, I. M. *J. Mass Spec*. **2008**, *44*, 144-150.

(162) Gozzo, F. C.; Santos, L. S.; August, R.; Consorti, C. S.; Dupont, J.; Eberlin, M. N. *Chem.-Eur. J.* **2004**, *10*, 6187-6193.

(163) Bini, R.; Bortolini, O.; Chiappe, C.; Pieraccini, D.; Siciliano, T. *J. Phys. Chem. B* **2007**, *111*, 598-604.