2023

Understanding the Role of HBA/HBD Variations on Deep Eutectic Solvent Properties and Activity Using Acetylsalicylic Acid as a Model Drug

Michael Frimpong
South Dakota State University, mrxmf6@gmail.com

UNDERSTANDING THE ROLE OF HBA/HBD VARIATIONS ON DEEP EUTECTIC SOLVENT PROPERTIES AND ACTIVITY USING ACETYLSALICYLIC ACID AS A MODEL DRUG

BY

MICHAEL FRIMPONG

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Chemistry

South Dakota State University

2023

DISSERTATION ACCEPTANCE PAGE
Michael Frimpong

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Douglas Raynie
Advisor Date

Matthew Miller
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

ACKNOWLEDGEMENTS

I extend my profound gratitude to the Lord for guiding me through this journey. Despite the myriad challenges encountered, His unwavering grace and favor paved the way for the successful completion of my doctoral studies. My heartfelt appreciation goes to Dr. Douglas Raynie, who not only accepted me as his student but also mentored and supervised with unparalleled dedication. Acknowledgment is due to my esteemed committee members, Dr. Cliff Hall, Dr. Brian Logue, and Dr. Halaweish Fathi, for their invaluable insights and consistent support. Gratitude extends to my lab mates and friends whose camaraderie bolstered my spirit. Lastly, a special note of thanks to my parents, whose relentless support and prayers were the bedrock of this achievement.

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LIST OF ABBREVIATIONS

α.....Hydrogen bond donor acidity

β.....Hydrogen bond donor basicity

π^*..... Dipolarity/polarizability parameter

FTIR..... Fourier Transformed Infra-Red

ChCl… .....Choline Chloride

ChBr..... Choline Bromide

ChI….....Choline Iodide

HBA… .....Hydrogen Bond Acceptor

HBD… .....Hydrogen Bond Donor

UV-Vis….....Ultraviolet Visible

DMSO .....Dimethyl Sulfoxide

D_2O.....Deuterated water

DES .....Deep eutectic solvent

ASA…..... Acetyl Salicylic Acid

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ABSTRACT

UNDERSTANDING THE ROLE OF HBA/HBD VARIATIONS ON DES PROPERTIES AND ACTIVITY USING ACETYL SALICYLIC ACID AS A MODEL DRUG

MICHAEL FRIMPONG

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Deep eutectic solvents (DES) are emerging as a versatile class of solvents, attracting considerable attention for their unique solvent properties and potential applications. This study examines the effects of variations in combinations as well as molar ratios of hydrogen-bond acceptor (HBA) and hydrogen-bond donor (HBD) on the structure of DES. It further explores the structure-activity relationship, measured using the rate of acetylsalicylic acid (ASA) degradation in the solvent. The project is based on the model that variations in HBA/HBD combinations and their relative molar ratios impact the solvent structure (hydrogen bonding), observable in physicochemical measurements, infrared (IR), and nuclear magnetic resonance (NMR) spectra. It is posited that the structure of the solvent influences its activity, as measured using the rate of degradation of ASA in the solvent.

Physicochemical measurements, IR and NMR spectra were employed to elucidate the structural changes in the solvents. Clustering analysis was employed to visualize the structural relationship of the solvent studied via systematic variations in HBA and HBD.

Correlation analysis was also employed to reveal the association between the measured physicochemical parameters and activity.

For studies involving the impact of mole ratio variation on the structure and activity of the solvent, choline chloride (ChCl)-water in the ratios 1:2, 1:5 and 1:10 was used. Both physicochemical measurements as well as spectroscopic measurements revealed differences in the structure of the solvent with ChCl-water 1:2 being structurally distinct from ChCl-water 1:5 and 1:10. Activity studies revealed that acetylsalicylic acid (ASA) was most stable in ChCl-water 1:2 and activity negatively correlated with ChCl fraction, density, basicity, and viscosity.

Distinct structural differences were also observed when different HBD and HBA were paired. Activity was observed to be lowest in solvents where water was used as HBD as compared to ethanol and methanol. When either water or ethanol was used as HBD, activity was lowest in ChI as compared to ChCl and ChBr. Again, activity negatively correlated with density and positively correlated with basicity.

Overall, the data supports the pivotal role of HBA/HBD combinations in shaping DES structure and its subsequent activity. The insights gained are crucial for optimizing solvent selection, especially in applications like enhancing drug stability, using aspirin as a benchmark.

CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

In the quest for environmentally friendly and sustainable chemistry, the concept of "green solvents" has gained immense importance in recent decades [1]. Chemical processes often rely on conventional solvents, which can pose significant environmental, health and safety risks. As a result, there has been interest in seeking alternatives to mitigate these impacts and advance towards a greener future[1-3]. In response to this challenge, some approaches such as the use of "biosolvents" derived from renewable resources, supercritical CO_2 as a replacement for volatile organic solvents, and ionic liquids (ILs) with low vapor pressure and no emissions have been explored. Another innovative solution that has emerged as a promising candidate is DES[1, 4].

DES are defined as solvents composed of at least two components: a HBA and HBD with the resulting solvent exhibiting a lower melting point compared to the individual melting points of its constituent components (Figure 1.1)[1].

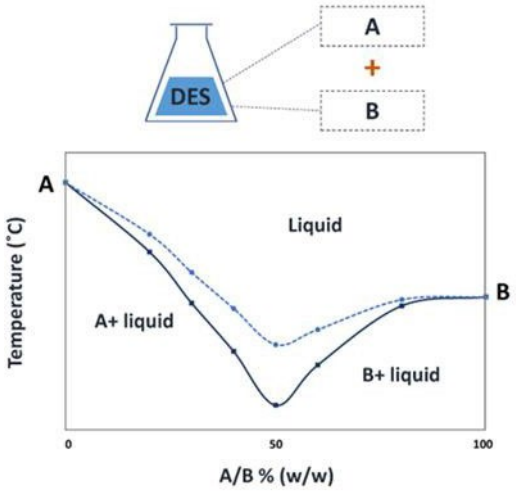


Figure 1.1. Synthesis of DES from the combination of two components, A and B, resulting in a significant depression of the melting point, indicative of DES formation1.

Research in DES gained prominence after studies by Abbott and co-workers in which they formulated a solvent by mixing urea and ChCl[1, 5]. They discovered that, the resulting mixtures exhibited a eutectic behavior and intriguing properties, making them suitable as alternative solvents for chemical processes[5]. Up until now, the research on DES have continuously expanded with novel formulations and diverse applications (Figure 1.2).

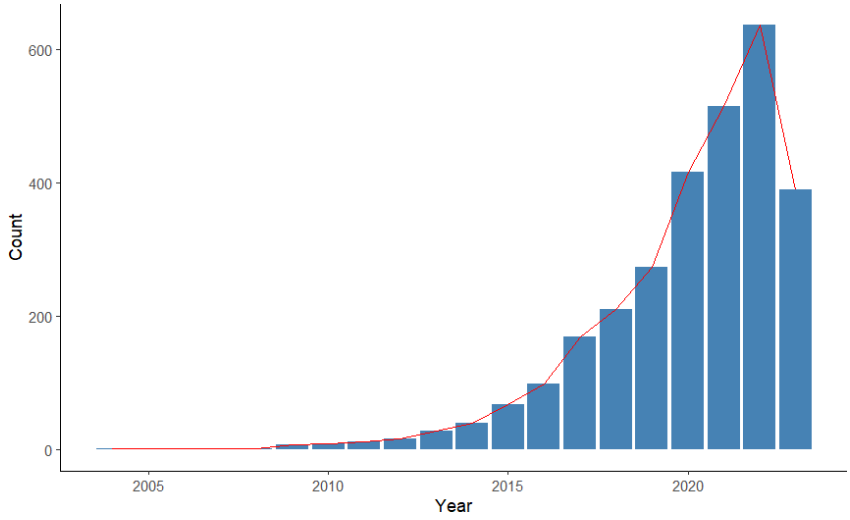


Figure 1.2. A plot on the number of publications on the subject DES by year. The plot was made from PubMed after the search word "DES" was used.

DES can be categorized into four distinct types, depending on their fundamental composition, as given by the formula Cat+ X− zY. Within this formula:

- **Cat+** stands for a cation, which can be ammonium, phosphonium, or sulfonium.
- **X** symbolizes a Lewis base, predominantly observed to be a halide anion.
- **Y** can be either a Lewis or a Brønsted acid, signifying the nature of acidic components involved.
- **z** illustrates the number of Y molecules interacting with the associated anion, thus reflecting the stoichiometry of the mixture[6].

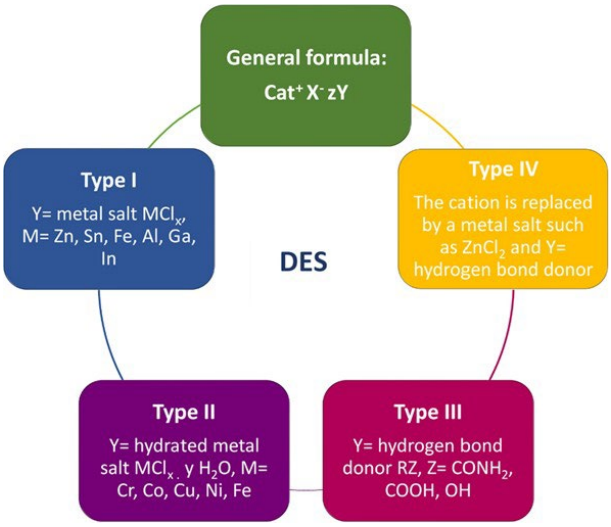


Figure 1.3 Four types of DES based on the general formula Cat+ X− zY, where Cat+ (cation) is generally an ammonium, phosphonium, or sulfonium, while X is a Lewis base, usually a halide anion. Y represents a Lewis or Brønsted acid and z is the number of Y molecules[6]

Type III eutectics are the most studied and have gained significant attention in scientific literature[6]. Type III eutectics are often based on ChCl as HBA paired with various HBD. ChCl widespread adoption can be attributed to its affordability, non-toxicity, and biodegradability. Its credentials as a safe compound are further bolstered, given its approval as a natural additive across multiple animal species. Interestingly, the pioneering DES Type III DES had ChCl as its foundation.

Prominent among the HBA are quaternary ammonium or phosphonium salts. In contrast, amides, alcohols, and carboxylic acids frequently emerge as the preferred HBD[6].

Research based on DES continues to increase, with natural DES increasingly incorporating components like sugars, sugar alcohols, and amino acids[6, 7]. A subsequent advancement in DES research is the development of hydrophobic DES. These solvents integrate hydrophobic components such as tetrabutylammonium bromide, menthol, thymol, and

fatty acids as their HBA. Alongside, long alkyl chain alcohols and carboxylic acids typically function as their HBD[8, 9].

Adding another dimension to the versatility of DES, researchers have now incorporated active pharmaceutical ingredients into these solvents[6]. Examples include ibuprofen, lidocaine, and phenylacetic acid. When these medicinal components form the solvents primary composition, the resultant mixtures are termed therapeutic DES[10] .

The diverse classifications and evolutions of DES underscore their multifaceted properties and the vast potential they hold across different applications. Each category of DES, with its distinct components and functionalities, offers unique insights and possibilities for exploration.

The driving force for the formation of DES has primarily been attributed to hydrogen bonding interactions between the HBA and HBD (Figure 1.3)[11]. However electrostatic forces and van der Waals interactions may also play a role in the solvent formation. [11, 12].

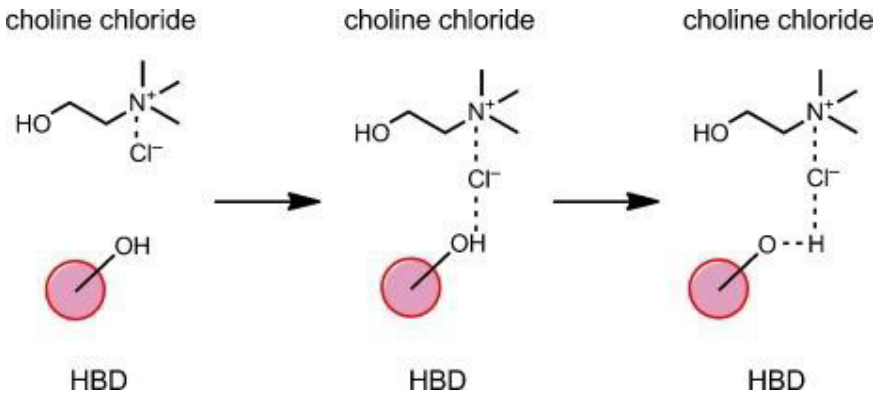


Figure 1.4. Formation of DES driven by hydrogen bonding interaction between components[1]

Potentially, the field of DES has gained attention with continual research expansion due to the promising tuneability of the solvent properties allowing for physicochemical tuning for diverse applications and their potential as safe and inexpensive alternatives to replace conventional solvents in the future.

1.2 DES: Potential as Safe and Inexpensive Alternatives to Replace Conventional Solvents in the Future

Among the reasons for the rising interest in DES is their ease of use, cost-effectiveness, and safety[1, 13]. Conventional solvents are important in industries including chemicals, pharmaceuticals, and coatings, supporting various chemical reactions and processes. They speed up reactions, aid in separating substances, and assist in drug and coating creation.[14]. While these solvents have desirable qualities, they pose safety, health and environmental risks[1, 15]. For instance, the inherent volatility of some solvents allows for their recovery and purification through distillation. However, this same characteristic can lead to undesirable air emissions and potential risks to workers[15]. Again, while the high polarity of amide solvents is desirable for dissolving a wide range of substrates and speeding up reactions, this functionality is associated with, and can contribute to the risk of reproductive toxicity[15]. Hydrocarbon solvents on the other end of the polarity spectrum have the capacity to dissolve oils and other nonpolar compounds making them useful and desirable in extractions and separations. However, these solvents are flammable posing safety concerns. Additionally, their low solubility in water as reflected by their high logP value, can contribute to bioaccumulation and toxicity to aquatic life[15]. The importance of solvents despite these risks has led to the development of specific criteria and principles for solvent

selection, with a particular emphasis on sustainable chemistry. The principles of sustainable green chemistry (Figure 1.4) have been introduced as a way to guide the development of eco-friendly chemical processes and products [14].

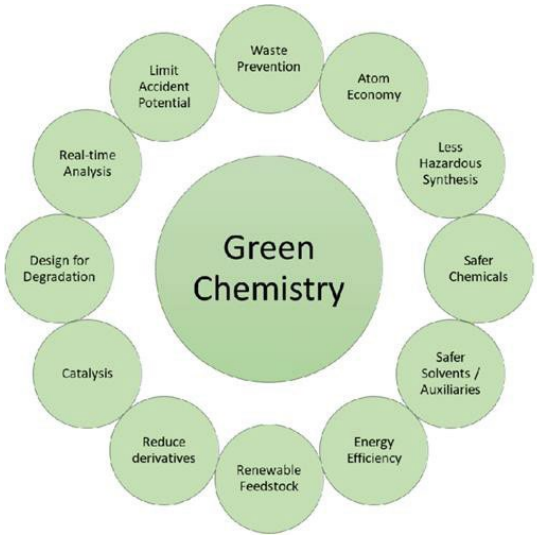


Figure 1.5. An Overview of the fundamental principles of green chemistry

These principles emphasize waste prevention, resource efficiency, and the use of greener alternatives to minimize environmental impacts and enhance human well-being. They also focus on maximizing material incorporation, reducing toxic substances, and employing renewable resources to develop safer and more sustainable chemical products and processes. Additionally, the principles encourage energy-efficient reactions and real-time monitoring to prevent pollution[16]. Research in DES reveals that it aligns with the principles of sustainable chemistry making it attractive for providing a sustainable solution to the toxicity concerns associated with conventional solvents[1, 16].

1.3 Promising Tuneability and Fascinating Properties of DES for Diverse Applications

Another reason contributing to the interest in DES is their remarkable tunability, achieved by carefully selecting and combining different components in specific ratios[13]. This enables the manipulation of physical-chemical properties to suit specific applications. By customizing DES in this manner, they become highly adaptable and versatile, making them promising candidates for a wide range of uses (Figure 1.5)[13].

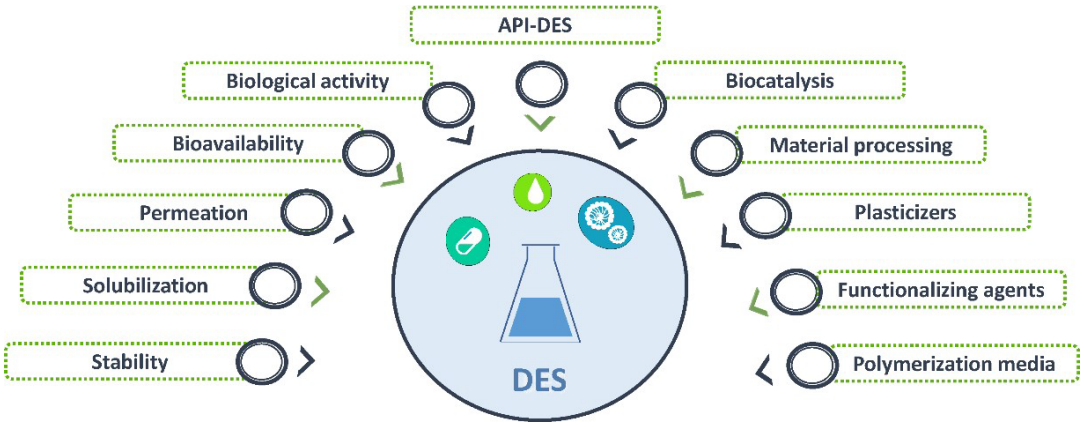


Figure 1.6. Diverse application of DES[13]

The tunability and versatility of DES has practically been demonstrated in the field of biocatalysis[13]. It has been reported that, DES can serve as solvents and co-solvents in biocatalytic reactions for the sustainable synthesis of active pharmaceutical ingredients (APIs) and their precursors, presenting promising opportunities within the pharmaceutical sector[17]. Generally, organic solvents are commonly used in these reactions, however, these solvents can impact enzymatic activity including denaturation. DES has emerged as a viable alternative due to its ability to enhance enzyme activity and selectivity, leading to

favorable outcomes[13]. For instance, in an enzyme-catalyzed reaction where DES such as ChCl–urea and ChCl–glycerol was studied as an alternative co-solvent for API precursor production, it was found that DES enhanced enzyme activity and contributed to structural and thermal stability of the enzyme[18]. Additionally, DES enhanced the activity and stability of lipases in aqueous environments improving their enantioselectivity[19, 20]. Studies were performed using Candida rugosa lipase's in synthesizing a model pharmaceutical intermediate. The authors found that the enzyme activity was significantly enhanced when DES was used as a co-solvent. The observed enzymatic activity enhancement by the DES surpassed the efficiency of traditional co-solvents and ionic liquids in aqueous solutions[20]. Other enzymatic stability enhancement by DES has also been studied using glycerol-based deep eutectics. The enzymatic stabilizing effect of DES was due to the strong hydrogen bond donating power of DES that resulted in their improved performance in biocatalysis[20]. The stabilizing effect from DES has also been observed in APIs or drugs. The stability of drugs is important for the efficacy and safety of drugs. Drug stability is influenced by a multitude of factors including exposure to light, variations in temperature, pH levels, oxidation, and enzymatic reactions[13]. Drugs can occasionally contain specific functional groups, such as esters, which are particularly susceptible to hydrolysis. When these drugs such as aspirin are stored in water for prolonged periods, the likelihood of hydrolysis increases significantly. This chemical reaction can alter the structure of drugs and, consequently, its therapeutic properties[13]. While organic solvents can mitigate water-mediated decomposition, they may not be ideal alternatives due to safety and health considerations. Researchers have also reported that, porphyrins which hold promise in photodynamic therapy but suffer from poor photostability showed enhanced photostability

in DES when compared to methanol[13]. Additionally, it has also been shown that curcumin, another valuable API, experiences increased stability when stored in DES, such as ChCl–glycerol, even under sunlight exposure[13].

Besides stability enhancement in both proteins and drugs, DES have also proven effective in solubilizing APIs[13]. Water, due to its safety and universal solvent properties, is the preferred choice in pharmaceutical formulations. However, the challenge arises when dealing with APIs that exhibit low solubility in water. Identifying suitable alternative solvents that can effectively dissolve these APIs, while maintaining safety and efficacy, becomes a critical aspect of pharmaceutical development. DES have emerged as a promising solution, outperforming other co-solvents like glycerol, propylene glycol, and polyethylene glycol, by significantly enhancing the solubility of various APIs, including anti-inflammatory and antifungal drugs. For example, the DES ChCl–levulinic acid increased the solubility of ibuprofen by over 5400-fold, surpassing the solubility enhancement achieved with the mixture menthol–camphor (1:1)[13, 21]. Enhancements in solubility have been observed not only with ibuprofen but also with other anti-inflammatory drugs such as naproxen and ketoprofen. Studies indicate that when utilizing DES, these drugs exhibit substantial improvements in solubility, achieving up to 740- and 960-fold enhancements, respectively[13].

In summary, the widespread interest in DES stems from its alignment with the principles of sustainable chemistry, providing a greener and safer alternative to conventional solvents. Furthermore, their tunability makes them adaptable for a wide range of applications, particularly in biocatalysis, APIs solubilization, and APIs stability enhancement. The careful selection of HBD and HBA components allows researchers to tailor DES properties,

rendering them highly versatile and attractive for various industrial processes and pharmaceutical applications. As a result, DES holds significant potential to revolutionize the field of chemistry and contribute to more sustainable and efficient processes.

CHAPTER 2. PURPOSE AND RATIONALE OF THE STUDY

2.1 Background and Motivation

DES have emerged as a promising class of solvents in recent years, exhibiting a range of unique properties and applications in various fields, including the pharmaceutical sector[22, 23]. DES are formed by combining an HBA, such as ChCl, with an HBD, such as urea or water. The resulting mixture exhibits a eutectic point where the melting point is significantly lower than that of the individual components[1].

The formulation of DES presents a versatile approach, as the ratio of both the HBA and HBD compounds can be adjusted to tune their physicochemical properties to suit specific applications. This inherent tunability of DES holds significant implications for enhancing drug solubility, delivery, and optimization in therapeutic formulations[1, 24].

In addition to these benefits, DES have lower toxicity and are known to be eco-friendly, aligning with the growing demand for sustainable practices in the pharmaceutical industry[11]. Considering their tunability, versatility, safety, eco-friendliness, and potential applications in various industries, there is a pressing need for the formulation and characterization of novel DES. This is crucial to deepen the understanding of the solvent system and to provide alternative solutions to the current solvents in various industries.

The primary objective of this research is to characterize DES and elucidate their potential applications in therapeutics. This research is centered on examining the effects of varying the ratio of HBD or HBA as well as the effect of changing the HBA and HBD components on the structural and physicochemical properties of DES. The insights derived from this research are expected to significantly contribute to the existing body of knowledge on DES.

The findings could potentially lead to the development of innovative DES formulations, thereby enhancing the efficacy of drug delivery systems and therapeutics.

2.2 Problem Statement

Despite the increasing interest in DES, there remains a significant need for further exploration and understanding of their properties, particularly in relation to specific formulations. The choice of salt and HBD and variations in water ratios can significantly influence the properties of the resulting DES, including their melting points, viscosity, conductivity, and solvation abilities[1]. Thus, to fully exploit the potential of DES, it is essential to systematically investigate the effects of different salt and HBD combinations and variations of water ratios on their physicochemical properties.

Furthermore, the stability of pharmaceutical drugs is paramount for their effectiveness and safety during manufacturing, storage, and administration. However, drugs with poor stability profiles present significant challenges in terms of formulation and preservation[25]. Conventional stabilizers or adjuvants may fall short of providing adequate protection against degradation, potentially compromising drug potency and causing adverse effects[13]. Therefore, a need to explore alternative approaches to enhance drug stability exists.

DES can be promising candidates for drug stabilization due to their unique physicochemical properties[13]. By examining the effects of combination of different ratios of HBD and HBA as well as the effects of changing HBD and HBA in DES constituents, we can potentially discover novel solvents that are adept at promoting drug stability. This exploration could pave the way for advancements in pharmaceutical formulations including enhancing drug stability and efficacy.

2.3 Objectives of The Study

This study is designed with the primary objective of conducting an in-depth characterization and formulation of DES for potential therapeutic applications. This will be accomplished through a comprehensive approach that encompasses structural analysis and physicochemical characterization of DES. The first goal is to investigate the influence of the ChCl: water molar ratio on the properties of DES. This will be achieved by formulating DES with varying ratios of ChCl to water (1:2, 1:5, and 1:10) and subsequently assessing their physicochemical properties. The second goal is to examine the effects of different halogen salt/HBD combinations on the properties of DES. This will involve the formulation of DES using combinations of ChBr, ChI, and ChCl with water, ethanol, and methanol, maintaining a molar ratio of 1:4 salt to HBD. Structural analysis of the formulated DES will be conducted using Nuclear Magnetic Resonance (NMR) and Infrared (IR) spectroscopy to gain insights into the molecular structure and interactions within the DES, including hydrogen bonding.

Key properties such as viscosity, which can influence solute solubility and diffusivity, will be measured and analyzed. The solvatochromic parameters of DES will be assessed using Kamlet-Taft parameters to provide information on their polarity, solvation capability, and potential to enhance the solubility of hydrophobic drugs.

The pH of DES formulations will be determined, as the acidity or basicity of a solvent can influence drug stability and compatibility. The specific heat capacity of DES will be investigated to understand their thermal behavior and heat transfer capabilities, crucial for designing temperature-sensitive drug delivery systems.

The stability of acetylsalicylic acid (ASA), a model drug, in DES formulations will be assessed to determine the compatibility and long-term stability of DES with active pharmaceutical ingredients. Finally, the rate order of reactions involving DES will be determined, contributing to the optimization of reaction conditions and the design of DES-based synthetic routes for therapeutic agents.

By achieving these objectives, our understanding of DES for therapeutic applications will be advanced. It is anticipated that this research will contribute significantly to the development of innovative DES-based formulations with enhanced solubility, stability, and drug delivery capabilities.

2.4 Scope and Limitations

A comprehensive characterization and formulation of DES will focus on the use of ChCl and water at varying molar ratios. The effects of different combinations of halogen salts as HBA and HBD on the properties of DES will be explored.

The research involves formulating DES by combining ChCl as the HBA with water as the HBD at molar ratios of 1:2, 1:5, and 1:10. The aim is to elucidate the influence of the HBA-HBD ratio on the properties of DES. Further, the study formulates DES using different combinations of halogen salts (chloride, bromide, and iodide) and HBD (water, ethanol, and methanol) to identify optimal combinations for specific applications and understand their impact on DES properties.

The study characterizes various properties of DES formulations, including viscosity, solvatochromic parameters, pH, specific heat capacity, and the stability of ASA. It employs

a range of experimental techniques such as rheological measurements, spectrophotometric analysis, pH measurements, calorimetry, stability studies of ASA, and kinetic analysis.

The stability of ASA within DES systems is evaluated as a model drug, providing insights into the potential use of DES as solubilizing and stabilizing agents for therapeutic compounds.

However, the study has its limitations. The research primarily focuses on the ChCl: water system, and the findings may not directly apply to other DES systems with different HBAs and HBDs. While various properties of DES formulations are covered, an exhaustive analysis of all possible properties or interactions are not provided. The experiments and measurements conducted under specific laboratory conditions may not fully represent real-world scenarios or in vivo conditions. Lastly, the stability analysis primarily focuses on ASA as a model drug, and the results may not directly translate to other drugs or compounds due to their unique chemical characteristics.

Despite these limitations, this research offers valuable information into the formulation and characterization of DES for therapeutic applications. The data obtained contributes to the existing body of knowledge and paves the way for further research and development in the use of DES as innovative solvents in the pharmaceutical industry.

2.5 Significance of The Study

This research has significant potential to advance our understanding and application of DES in therapeutics. The potential of DES as an innovative solvent system will be explored, thereby expanding our knowledge base and providing insights into the development of novel drug delivery systems. Enhanced by the unique properties of DES, these systems

could improve the solubility, stability, and bioavailability of therapeutic compounds, leading to more effective treatments.

Moreover, the study aligns with the growing demand for environmentally friendly technologies in the pharmaceutical industry. DES, derived from biocompatible and renewable components, offer greener alternatives to conventional solvents. Thus, this research contributes to the development of sustainable practices in pharmaceutical manufacturing.

The outcome of the research also contribute to our knowledge base by investigating various properties of DES formulations. These findings serve as a valuable reference for researchers and practitioners in the field, inspiring further studies in this area.

Finally, the practical implications of this study extend to the pharmaceutical sector. The optimized DES formulations and knowledge gained from this research can be applied in the development of new products, processes, and technologies.

CHAPTER 3. CHARACTERIZATION OF CHCL-BASED DES WITH VARYING WATER RATIOS

Abstract

DES have garnered significant attention as eco-friendly and sustainable alternatives to traditional solvents in various chemical processes. In this study the structural characteristics and physicochemical properties of DES samples with different ChCl: water ratios (1:2, 1:5, and 1:10) were investigated. Comprehensive approach of combining physicochemical measurements, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and chemometric analysis using the ChemoSpec package in R, provided valuable insights into the behavior of these DES samples. Physicochemical measurements revealed that viscosity varied significantly depending on the ChCl: water ratio, with the 1:2 ratio having the highest viscosity due to stronger hydrogen bonding interactions. Furthermore, the Kamlet-Taft solvatochromic parameters provided quantitative representations of the solvent polarity, indicating that the DES samples exhibited a higher polarity compared to methanol. The IR spectra exhibited a broad and intense peak in the region associated with O-H stretching vibrations, indicative of strong hydrogen bonding in the DES samples. Chemometric analysis highlighted the specific frequencies that contributed most to the differences between the samples. In the NMR spectra, we observed slight shifts in chemical shift values for protons involved in hydrogen bonding interactions, providing evidence of hydrogen bonding between water and ChCl. Clustering analysis revealed that DES samples with higher water content (1:5 and 1:10) clustered together, suggesting similar physicochemical properties due to the dominance of water in these samples. Conversely, the 1:2 ratio sample formed a separate cluster,

indicating distinct properties arising from its higher proportion of ChCl. In conclusion, the combination of physicochemical measurements, IR, NMR spectroscopy, and chemometric analysis allowed for a comprehensive understanding of the structural characteristics and properties of the DES samples. This information is essential for optimizing the use of DES in various chemical processes, offering sustainable and efficient alternatives to traditional solvents.

3.1 Introduction

The driving force for the formation of DES is the formation of hydrogen bonding[1]. The hydrogen bonding within the solvent structure can be affected by factors including the components of the solvent and the precise mole ratios of the components (HBA-HBD). Hydrogen bonding is significant as it impacts the phase behavior and transport properties of the solvent[26], the physicochemical properties[27] of the solvent and thus the utility and application of the solvent for a particular process[28]. Thus, by carefully selecting the HBA and HBD of the components, and by varying the molar ratios of the component, the degree of hydrogen bonding can be altered, and this alteration can have profound effect on the activity of the solvent.

The focus for this part of the project is to investigate the effects of varying water ratios (1:2, 1:5, and 1:10) in ChCl-water DES systems. We hypothesize that the variation in water ratios will lead to changes in solvent structure (hydrogen bonding interactions) as reflected in physicochemical measurement, IR and NMR spectra, and the structure of the solvent influences the activity, or the utility of the solvent as measured by drug stability using acetyl salicylic acid as a drug model (Figure 3.1).

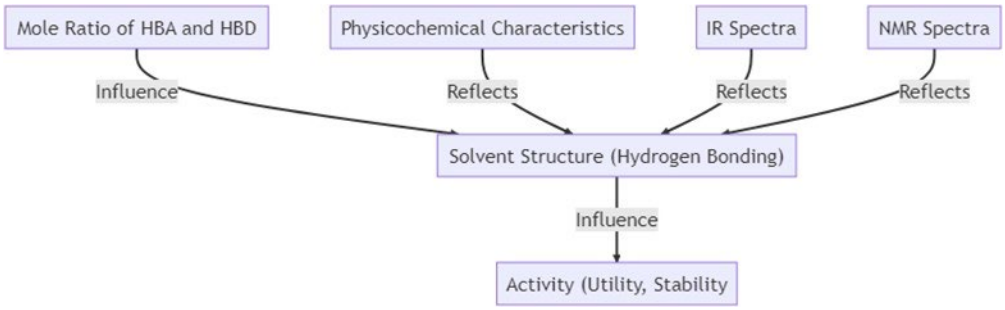


Figure 3.1. Model for project work. This research is built on the model that, varying the ratios of HBA and HBD affect solvent structure which is revealed in its physicochemical characteristics, IR and NMR spectra. The solvent structure concomitantly has influence on the activity (utility) of the solvent.

ChCl is a quaternary ammonium compound with the chemical formula $(CH_3)_3N^+CH_2CH_2OHCl^-$. ChCl possesses a unique structure that allows it to act as an effective HBA in DES[1]. As an HBA, ChCl can form hydrogen bonds with HBD to create a network of intermolecular interactions within the solvent system. These hydrogen bonds are typically formed between the chloride ion (Cl^-) and the hydrogen atoms of the HBD[1]. The presence of the hydroxyl group (-OH) in ChCl can also play a crucial role in its hydrogen bonding capability[29].

Water on the other hand is a ubiquitous and versatile solvent known for its ability to act as both a HBD and HBA in chemical interactions. Water has the capacity to donate its hydrogen bonded oxygen to a nearby HBA and water can use its oxygen to accept hydrogen to participate in hydrogen bonding. The existence of hydrogen bonding between ChCl and water has been demonstrated by Asare (2018) [30].

Thus, the proposed project aims to formulate DES composed of ChCl and water in ratios of 1:2, 1:5, and 1:10. The significance of this project lies in its comprehensive approach to understanding the physicochemical properties and solvatochromic parameters of these DES, and their implications for drug stability. The physicochemical properties that were measured include pH, density, viscosity, and specific heat capacity. These properties are crucial as they reflect the solvent structure and the extent of hydrogen bonding within the DES. Additionally, solvatochromic parameters such as acidity (α), basicity (β), and polarizability (π*) will be evaluated. These parameters provide insights into the behaviors of solvents in different chemical environments and its ability to stabilize or destabilize certain compounds. Hierarchical clustering, based on the physicochemical properties, was conducted to discern the similarities and differences among the solvents, shedding light on the relationships between various DES and their potential uses. Additionally, the study employed Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy to characterize the solvents. These methodologies elucidated the molecular structures and interactions within the DES. Moreover, the study evaluated the potential of DES in enhancing the stability of pharmaceuticals, using acetylsalicylic acid as a model compound. This exploration is pivotal as it underscores the prospective application of DES in pharmaceutical formulations.

By analyzing the correlation between structural descriptors and activity, the study elucidated the potential to establish tailored applications for solvents. This methodology is fundamental, as it offers a systematic approach for the precision-driven selection and adaptation of solvents in pharmaceutical formulations.

3.2 Physicochemical Properties Measurement: An Insight into the Solvent Structure

3.2.1 Density, Viscosity, pH and Specific Heat Capacity

The investigation of properties, including heat capacity, density, pH, and viscosity, in the ChCl-water DES system at different molar ratios (1:2, 1:5, and 1:10) is of great importance. As the formation of DES is driven by hydrogen bonding interactions[1], the extent and strength of these interactions are influenced by the molar ratios of the HBD and HBA components[13]. Understanding the properties of the DES system is crucial as they are directly affected by the hydrogen bonding interactions within the solvent. In addition, these properties play a significant role in determining the utility and performance of the solvent in various applications[31].

Density is a critical property that significantly influences the applications of DES particularly in liquid-liquid extraction processes as the density determines whether the extractant is collected from the upper or lower phase[32]. Research indicates that hydrophobic DES, which generally have a lower density than water, yield extract-rich phases from the upper liquid phase when used in extraction processes[32]. Density also provides important information about sample purity and molecular interactions in the liquid[32]. Density is affected by the nature of the HBD, the number of -OH functional groups in the HBD and the molar ratio of the HBA to the HBD. More -OH lead to a higher number of hydrogen bonds, which in turn reduces the available free spaces and increases the density[31].

Viscosity is a transport parameter which is defined as the internal resistance of DES to shear stress[32]. Viscosity of DES is influenced by factors such as the nature of the HBD, the molar ratio of the HBA to the HBD, and other factors like the alkyl chain length. For instance, it has been found that DES containing ethylene glycol, phenol, or levulinic acid

have relatively low viscosities, while those based on sugar, glycerol, and polycarboxylic acid exhibit higher viscosities. Again, changes in the molar ratio of the DES also correlate with their viscosity[32]. Stronger interactions have often been found to result in higher viscosity[32].

The measure of the acidity or the basicity of the solvent is pH[32]. Determining the pH (the acidity and the basicity) of the solvent is important in various processes (Figure 3.2). For instance, acidic DES have been found to be extensively used in biomass conversion, extraction and separation, drug solubilization, and catalytic reactions due to their excellent solubility[32]. The acidity of DES has also been associated with the solubility of certain substances like starch and metal oxides, and the dissolution of specific drugs. The pH of DES is also influenced by the nature of the HBD, the alkyl side chain of the compound temperature and water content.[32]

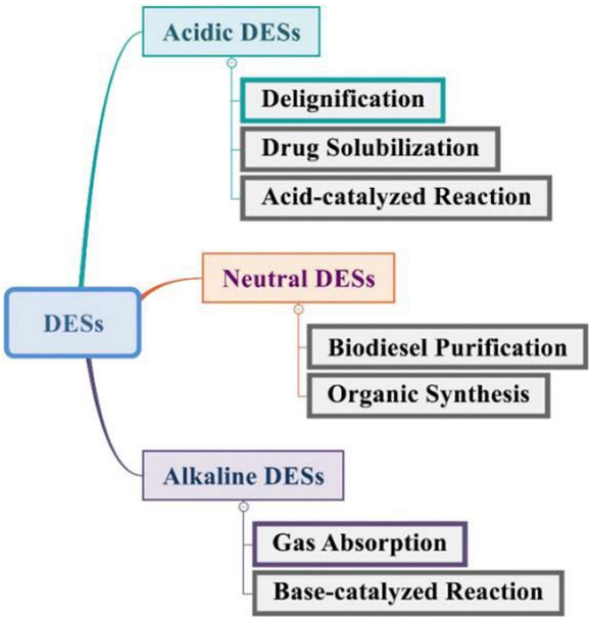


Figure 3.2. Significance of pH and its application[32]

Heat capacity, as a measure of the heat energy required to raise the temperature of the solvent, can be influenced by the extent and strength of hydrogen bonding. The heat capacity provides insights into the thermal behavior and energy storage capacity of the solvent system. Understanding the heat capacity characteristics is essential for designing and optimizing processes that involve heat transfer and energy storage[32].

3.2.2 Polarity Measurement Using Solvatochromism and Kamlet-Taft Solvatochromic Parameters

Determining the solvent polarity is of utmost importance as polarity plays a crucial role in various chemical processes, exerting significant influence over solubility, reaction kinetics, and selectivity. Solvent polarity reflects molecular structure and generally, polarity increases with increasing intermolecular attraction.[32]. DES polarity is also affected by the HBD-HBA combination and their ratios as well. [32]

Classically, polarity has been understood and determined using a qualitative approach of "like dissolves like" which seems to suggest that solvents with similar polarities exhibit favorable solubility, while solvents with differing polarities may lead to limited or no solubility[33]. While this approach has its merits, it is not without its challenges. One of the primary challenges of the qualitative approach lies in its subjective nature and reliance on human interpretation. Assigning a qualitative polarity descriptor to a solvent requires a subjective assessment of its behavior and interaction with solutes. This subjectivity can introduce inconsistencies and variations in the classification of solvents[33]. Consequently, the lack of standardization in the qualitative assessment of solvent polarity may hinder the reproducibility and comparability of results across different studies.

Another limitation of the qualitative approach is the absence of quantitative data. Polarity, being a continuous property, cannot be adequately captured using a categorical or qualitative scale alone. The "like dissolves like" concept provides a general framework for solvent selection based on similar polarities, but it does not provide precise information regarding the magnitude or degree of polarity difference between solvents and solutes[33]. Consequently, the qualitative approach may not provide sufficient granularity to differentiate solvents with subtle polarity differences or to discern their impact on solubility, reaction kinetics, or selectivity accurately.

Furthermore, the qualitative approach may lead to potential misclassification of solvents, especially in cases where solvents possess mixed or ambiguous polarity characteristics. Some solvents exhibit complex behavior due to the presence of multiple functional groups or the existence of hydrogen bonding capability[33]. These nuances may not be adequately captured by a qualitative approach, resulting in misclassification or oversimplification of solvent polarity characteristics. Consequently, this misclassification may lead to erroneous solvent selection, suboptimal reaction conditions, or unexpected outcomes in chemical processes.

An additional and quantitative approach for determining solvent polarity is through solvatochromism, a phenomenon where certain solutes or solvatochromic probes exhibit changes in wavelength when placed in solvents of varying polarity[33] (Figure 3.3). Solvatochromism relies on the principle that the polarity of the solvent environment influences the energy levels and electronic transitions of a solvatochromic dye, resulting in a shift in the absorption or emission wavelength (Figure 3.3B)[34]. These spectral shifts

depending on the dye can be used to both qualitatively and quantitatively measure polarity[33].

A

B

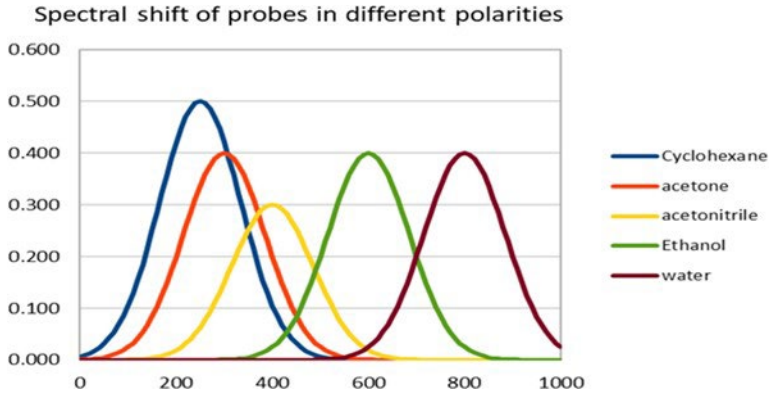


Figure 3.3. Solvatochromism, A) Same solute but different color in different polarity. B) Spectral shifts of solvatochromic dyes in different solvent. The more right-shifted (bathochromic shift) the spectra is, the more polar the solvent.

The sensitivity of the solvatochromic dyes to their environment (polar or nonpolar) is attributed to their structure. These dyes described as push-pull dyes are structured with both electron donor and acceptor groups incorporated into a π-conjugated system, such as polycyclic hydrocarbons and heterocyclic aromatic compounds[33]. Upon UV or light absorption, charge is transferred from the donor group to the acceptor, which creates a

highly dipolar excited state[34, 35] (Figure 3.4). The energy of this excited state is stabilized by interactions between the dye dipole and the surrounding solvent molecules. This stabilization effect is influenced by the polarity of the molecules around the dye, meaning a molecule with higher polarity leads to more stabilization and a larger bathochromic shift of the dye fluorescence (fluorescence solvatochromism). Additionally, this stabilization is dependent on the rate at which solvent molecules reorient around the excited dipole of the dye, a process limited by the lifetime of the dye[34, 35].

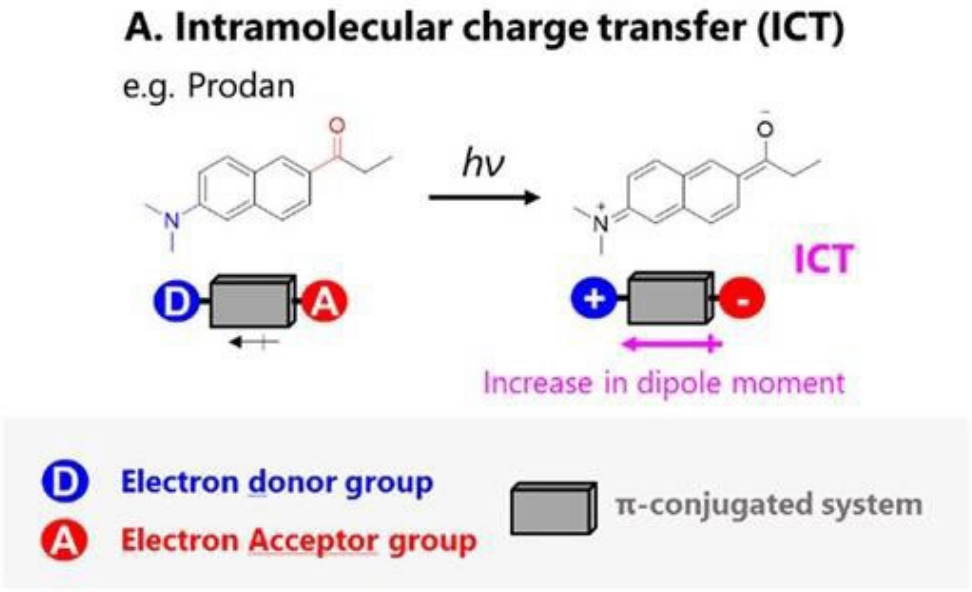


Figure 3.4. Mechanism responsible for spectral shift for solvatochromic dyes[34]

Besides using the spectral shifts to qualitatively determine the polarity of solvent, the solvent polarity can also quantitatively be represented using Kamlet-Taft Solvatochromic parameters (α, β, π^*). The α parameter represents the acidity of the solvent and it measures the ability of the solvent to act as a HBD, reflecting the capacity of the solvent to donate protons or form hydrogen bonds with solutes. The basicity parameter (β) assesses the ability of a solvent to act as a HBA, representing the solvent's capacity to accept protons

or form hydrogen bonds with solutes. The dipolarity-polarizability parameter (π*) is presumed to represent solute solvent interaction in the absence of strong forces such as hydrogen bonding or ion dipole interactions[33]. The solvatochromic parameters are determined using special solvatochromic dyes and equations[33].

The dipolarity-polarizability parameter (π*) is measured using nitroaniline dyes N,N-diethyl-4-nitroaniline. Alkyl substitution on the aniline nitrogen makes the dye exclusively sensitive to π*[33].

The measurement of solvent HBD acidity is achieved by analyzing the shifts of two distinct indicator dyes. One dye, a HBA, is believed to be responsive to HBD acidity, while the other is thought to be unaffected by it[33]. To establish the acidity scale, a dye sensitive to acidity and a π* dye are needed to offset the influence of the solvent's dipolarity-polarizability on the energy shift of the electronic transition of the acidity-sensitive dye. A commonly used pair of indicator dyes for this process is ET-30 and N,N-diethyl-4-nitroaniline.

The basicity is generated based on the indicator dye pair 4-nitroaniline and N,N-diethyl-4-nitroaniline[33].

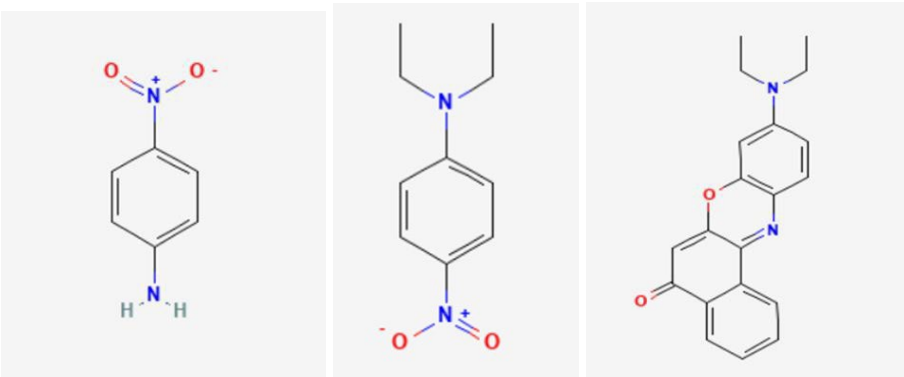


Figure 3.5. Structural representation of solvatochromic dyes. A) 4 – Nitroaniline B) N,N – diethyl nitroaniline C) Nile Red.

The solvatochromic parameters are estimated using the equations:

ET (NR) kcal.mol-1= 28591.44/ λmax (nm) Equation 3.1

$\alpha = 0.0649$ ET (NR) – 2.03 – 0.72 π^* Equation 3.2

$\beta = (1.035\ \upsilon DENA + 2.64 - \upsilon 4NA) / 2.80$ Equation 3.3

$\pi^* = 0.314\ (27.52 - \upsilon DENA)$. Equation 3.4

3.2.1 Experimental

3.2.1.1 Materials

Choline Chloride (99%) was purchased from Acros Organics (Fair lawn, NJ). Ultra-pure water was obtained in the lab with instrument Solution 2000 Water Purification. All the chemicals were used as supplied. Nile red, and 4-nitroaniline were all purchased from Acros Organics and had a purity of ≥ 99%. N, N-diethyl-4-nitroaniline (Frinton Laboratories, Inc.) were used.

3.2.1.2 Preparation of ChCl-water DES.

DES were prepared using ChCl as HBA and water as HBDs at a molar ratio of 1:2, 1:5, and 1:10. Prepared ChCl water mixtures were heated at 60˚C for about 15-45 minutes. The solution was stirred constantly at 600 rpm under atmospheric pressure until a clear homogenous solution was obtained. The prepared DES were cooled at room temperature and then kept in sealed laboratory vials and stored in a desiccator to prevent moisture absorption. High-precision analytical balance was used to weigh all the components, and the synthesis was done in a fume hood. The synthesized DES were used without further purification.

3.2.2 Approach

3.2.2.1 Density

The density of the DES was determined using the gravimetric approach. The mass of an empty 5 ml Erlenmeyer flask was recorded using Mettler Toledo analytical balance (Columbus, OH). The DES sample was then carefully added to the flask, and its combined mass (container + DES) was measured. From the mass and volume measurements, the density of the DES sample was calculated using the formula:

Density = Mass / Volume. Densities were determined in triplicate.

3.2.2.2 pH

pH measurement of the DES was performed using the Accumet AB 15 Plus pH meter. To ensure accurate pH measurements, the pH meter was calibrated before the analysis. Calibration was carried out using standard buffer solutions with known pH values (4, 7, and 10). Following calibration, a small volume of the DES sample was transferred into a clean glass container. The glass electrode of the Accumet AB 15 Plus pH meter was

carefully inserted into the DES sample, ensuring that the electrode was fully submerged and surrounded by the solvent. The pH meter was allowed to stabilize, and the pH value of the DES sample was recorded from the digital readout of the instrument.

3.2.2.3 Specific Heat Capacity

The specific heat capacity of DES was determined using styrofoam cups. The approach involved cup calibration and subsequent specific heat capacity measurements. To calibrate the styrofoam cups, a procedure was followed to determine their heat capacity. This step was important to account for any heat absorbed or released by the cups during subsequent measurements. To calibrate the Styrofoam cups, approximately 50 ml of cold water was added to the calorimeter, and its mass was recorded. Hot water, heated to a temperature of 60-70^{o}C, was prepared and its initial temperature was recorded. About 50 ml of the hot water was transferred into the calorimeter containing the cold water and the equilibrated temperature was measured. Temperature measurements were taken with a LabQuest device equipped with two temperature probes with one probe used for measuring the temperature of cold water and the other, the temperature of the hot water. The cup was covered with a lid to minimize heat loss. The calorimeter, along with the water, was weighed after removing the temperature probe.

The final temperature of the water in the cup was measured, and the heat absorbed by the cup was calculated using the mass of the water, the specific heat capacity of the water, and the temperature difference. The cup heat capacity was determined by dividing the calculated heat absorbed by the cup by the temperature difference between the water and the room temperature.

Total energy of the system = $Q_{hot} + Q_{cold} + Q_{cal} = 0$ Equation 3.5

$Q_{cal} = -Q_{hot} - Q_{cold}$ Equation 3.6

$Q_{hot,W} = M_{hotW} C_{p,W}(T_f - T_{hotW})$ Equation 3.7

$Q_{cold,w} = Mcold,wC_{p,w}(T_f - T_{cold})$ Equation 3.8

$C_{p,cal} = Q_{cal}/ (T_f - T_{cold})$ Equation 3.9

For the determination of the specific heat capacity of the DES, a clean and dry styrofoam cup was used. The DES was weighed and heated to a specific temperature. A styrofoam cup was used as the calorimeter, and its mass was recorded. Approximately 50 ml of water at 25ºC was added to the calorimeter, and its mass and initial temperature were recorded. The heated DES was quickly transferred to the calorimeter, and the final temperature change was measured. The calorimeter was covered with a lid to minimize heat loss. The calorimeter was gently swirled, and the temperature of the mixture was monitored until it reached a constant value. The final temperature was recorded.

The heat lost or released by the DES was calculated using the mass of the DES, the specific heat capacity of the DES, and the temperature difference. The net heat absorbed by the DES was obtained by subtracting the heat absorbed by the cup (previously determined cup heat capacity) from the total heat absorbed. Finally, the specific heat capacity of the DES was calculated by dividing the net heat absorbed by the DES by the product of the DES mass and the temperature difference.

Total energy of the system = $Q_{hot, DES} + Q_{cold, water} + Q_{cal} = 0$ Equation 3.10

$Q_{hot,DES} = M_{DES} C_{p,DES}(T_f - T_{hot,DES})$ Equation 3.11

$Q_{cold,w} = M_{cold,w}C_{p,w}(T_f - T_{cold,w})$ Equation 3.12

$Q_{cal} = C_{cal}(T_f - T_{cold})$ Equation 3.13

$Q_{DES} = -Q_{cold,\ water} - Q_{cal}$ Equation 3.14

$C_{p,\ DES} = Q_{DES}/M_{DES}*(T_f - T_c)$ Equation 3.15

Where Q_{cal} = Is the Energy (gained) by the calorimeter

$Q_{hot,w}$ = Energy of hot water (negative when energy is lost by hot water)

$M_{hot,w}$ = Mass of hot water

$C_{p,w}$ = Specific heat capacity of water

$M_{cold,w}$ = Mass of cold water

$Q_{cold,w}$ = Energy of cold water

$C_{p,cal}$ = Specific Heat capacity of the calorimeter

M_{DES} = Mass of DES

$Q_{hot,DES}$ = Energy of DES (hot)

$T_{hot,DES}$ = Temperature of hot DES

$M_{cold,w}$ = Mass of cold water

$C_{p,DES}$ = Specific heat capacity of DES

To ensure accuracy and reproducibility, the specific heat capacity measurement was performed in triplicate. The average value of the specific heat capacity was calculated from the triplicate measurements, and the standard deviation was determined to assess the precision of the measurements. The specific heat capacity of the DES was reported as the average value ± standard deviation.

3.2.2.4 Viscosity

The methodology involved measuring the viscosity of the DES using the StressTech CC 25 CCE instrument. Two control samples, vegetable oil, and orange juice, were also included for comparison. The instrument was set up and calibrated to ensure accurate

measurements. Using the instrument's software interface, specific settings were adjusted to accommodate the viscosity measurement process. This included the sample loading method, maximum loading force, and rotor release prompt. A single measurement was performed with a 20-second interval between measurements to collect viscosity data. The instrument was configured to apply shear rates ranging from 1 to 200 1/s, with appropriate delay and integration times to capture precise viscosity measurements. To ensure reliable results, the instrument waited for the samples to reach a steady state within a tolerance of ±0.010 before recording the viscosity measurements. The regulator strength was set at 100.0% to maintain consistent measurement conditions throughout the experiment. The DES, vegetable oil, and orange juice samples were individually loaded into the instrument, and the viscosity measurements were initiated. The instrument automatically recorded viscosity data at regular intervals. The collected data, including shear rates and corresponding viscosity measurements, were saved for further analysis and interpretation.

3.2.2.5 Polarity

The solvatochromic assay involved the use of a UV-Vis spectrophotometer (Synergy|H1 microplate reader, Biotek) to determine the maximum absorption wavelength (λmax) from the absorption spectra. The absorbance readings were collected by performing spectral scans of all probes in DES from 230nm to 700nm at 10-nm intervals. To minimize the influence of solute-solute interactions on the observed solvent effect, a low working concentration of 0.5×10^{-4} M was maintained for all the solvatochromic probes.

3.2.3 Results

3.2.3.1 Visual Observation, pH, Density, and Specific Heat Capacity of ChCl:Water DES Mixtures at Different Ratios

Table 1 provides information on the physical properties of ChCl-water DES mixtures at different ratios. The mixtures were evaluated for visual observation, pH, density, and specific heat capacity. All mixtures were observed to be colorless with ChCl-water 1:2 appearing to be viscous in comparison to ChCl-water 1:5 and ChCl-water 1:10. The pH values of the ChCl-water DES mixtures ranged from 7.091 to 7.485. These values indicate a slightly acidic to neutral nature of the mixtures. The density values of the mixtures varied slightly depending on the ratio of ChCl to Water. The 1:2 mixture had the highest density value of 1.09330 ± 0.00501 g/ml, followed by the 1:5 mixture with a density of 1.06700 ± 0.00550 g/ml. The 1:10 mixture had the lowest density value of 1.0581 ± 0.0105 g/ml. These variations in density can be attributed to the different ratios and the interactions between ChCl and water molecules within the mixtures. The specific heat capacity values of the ChCl-water DES mixtures also exhibited variations. The 1:10 mixture had the highest specific heat capacity value of 4.070 ± 0.153 J/g°C, followed by the 1:5 mixture with a value of 2.0415 ± 0.0801 J/g°C. The 1:2 mixture had a specific heat capacity of 2.016 ± 0.571 J/g°C. These variations indicate that the specific heat capacity is influenced by the ratio of ChCl to Water, with higher ratios resulting in increased specific heat capacity.

Table 3.1

Visual Observation, pH, Density, and Specific Heat Capacity of ChCl: water DES Mixtures at Different Ratios

DES	Visual Observation	pH	Density (g/ml)	Specific Heat Capacity (J/g^0C)
ChCl-water 1:2	Colorless, slightly viscous	7.135 – 7.485	1.09330±0.00501	2.016±0.571
ChCl-water 1:5	Colorless, not viscous	7.108 – 7.218	1.06700±0.00550	2.0415±0.0801
ChCl-water 1:10	Colorless, not viscous	7.091 – 7.180	1.0581±0.0105	4.070±0.153

3.2.3.2 Effects of varying water (HBD) ratios on the viscosity of the solvents

Viscosity indicates the resistance of the fluids to flow. A higher viscosity value indicates a thicker and more viscous fluid, while a lower viscosity value indicates a thinner and less viscous fluid[32]. From the results (Figure 3.6), it can be observed that the viscosity varies depending on the composition of the DES. The ChCl-water mixtures at different ratios show a significant difference in viscosity, with ChCl-water 1:2 having the highest viscosity (0.059 Pa S), followed by ChCl-water 1:5 (0.012 Pa S), and ChCl-water 1:10 (0.00175 Pa S). Interestingly, the viscosity of vegetable oil is the same as that of ChCl-water 1:2 (0.059 Pa S), suggesting similar viscosity between these two samples.

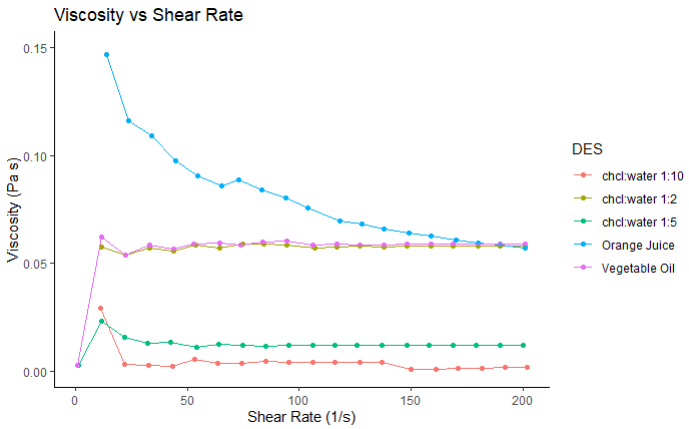


Figure 3.6. Effects of varying water ratios on the viscosity of the solvents

In DES, the formation of hydrogen bonds between the components of the solvent mixture influences their molecular interactions and, consequently, their viscosity. ChCl acts as a HBA, while water acts as a HBD. The presence of hydrogen bonding between ChCl and water molecules leads to increased intermolecular forces and stronger interactions, resulting in higher viscosities and is the reason why the ChCl-water 1:2 DES sample exhibits the highest viscosity among the tested ratios (0.059 Pa S). The larger proportion of ChCl in the mixture enhances the potential for hydrogen bonding, leading to increased viscosity.

On the other hand, as the ChCl-water ratio decreases (ChCl-water 1:5 and ChCl-water 1:10), the relative contribution of water as a HBD decrease. This reduction in the number of hydrogen bonding sites leads to weaker intermolecular forces and reduced viscosity. Hence, the ChCl-water 1:5 DES sample shows a lower viscosity of 0.012 Pa S, while the ChCl-water 1:10 DES sample had the lowest viscosity of 0.00175 Pa S.

Interestingly, the viscosity of vegetable oil is similar to that of ChCl-water 1:2 (0.059 Pa S). Vegetable oil and orange juice were included as control samples to investigate the flow

behavior of the DES. The inclusion of these controls allows for a comparison between Newtonian and non-Newtonian fluids. The behavior of orange juice, which generally exhibits non-Newtonian characteristics, was observed to follow a decrease in viscosity with an increasing shear rate. On the other hand, vegetable oil was found to exhibit Newtonian behavior, as its viscosity remained constant regardless of the shear rate. Newtonian fluids maintain a constant viscosity regardless of the applied shear rate, indicating a linear relationship between shear stress and shear rate. The categorization of fluids into Newtonian and non-Newtonian types is important as it has practical implications for the utility of the solvents. Understanding the flow behavior and viscosity characteristics of solvents is crucial in various applications, such as manufacturing processes, pharmaceutical formulations, and food processing. Newtonian fluids are often preferred in situations where a consistent viscosity is desired, while non-Newtonian fluids can be advantageous in applications where viscosity modulation and control are required.

Based on the results obtained, it can be concluded that the prepared DES in their current composition and molecular interactions possess a consistent viscosity profile across varying shear rates and thus, exhibits Newtonian flow behavior.

3.2.3.2 Polarity Determination and Quantification

The results indicated that the formulated DES exhibited a relatively higher polarity compared to methanol (Figure 3.7), as evidenced by the observed slight shift in the maximum wavelength towards longer wavelengths (rightward shift) when using N,N-diethyl-4-nitroaniline (DENA) as the solvatochromic probe.

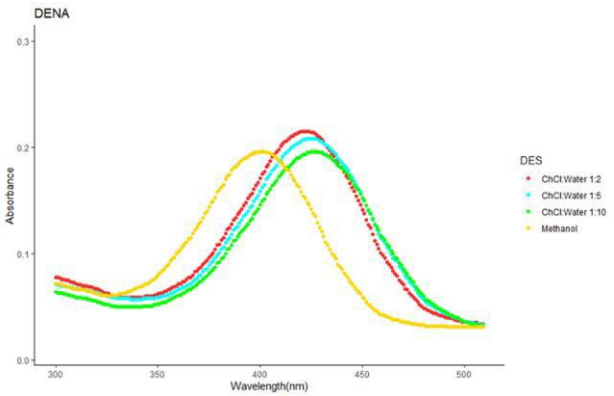


Figure 3.7. Spectra shift of DENA in DES

The solvatochromic parameters (Figure 3.8) for the solvent revealed that the acidity (α) for ChCl-water 1:2 value was found to be 0.78, indicating a moderate hydrogen bond donating ability of the solvent. The Beta (β) value was 0.46, indicating a relatively low hydrogen bond accepting ability. The Pi (π^*) value was determined as 1.20, representing the dipolarity/polarizability of the solvent. In the case of ChCl-water 1:5, the acidity (α) value was 0.89, suggesting a higher hydrogen bond donating ability compared ChCl-water 1:2. The basicity (β) value was 0.33, indicating a lower hydrogen bond accepting ability. The dipolarity/polarizability (π) value for ChCl-water 1:5 was measured as 1.25. Lastly, for ChCl-water 1:10, the acidity (α) value was determined to be 0.98, signifying a relatively high hydrogen bond donating ability. The basicity (β) value was 0.30, indicating a low hydrogen bond accepting ability. The dipolarity/polarizability (π) value was found to be 1.29.

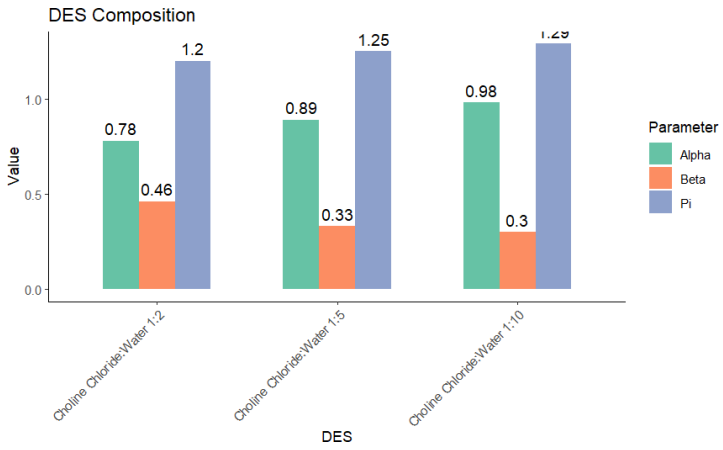


Figure 3.8. Estimation of solvatochromic parameters in DES samples.

The significance of Kamlet-Taft solvatochromic parameters lies in their ability to serve as descriptors for solvents which can be used in modeling to predict chemical reactions of unknown solvents and also in clustering analysis to reveal similarities of unknown solvents to known solvents with established parameters.

To reveal the similarities of the formulated solvent with conventional solvents, both ternary diagrams and clustering analysis were conducted using the solvatochromic parameters and known solvent data (Figure 3.9). The clustering analysis and ternary diagrams revealed that the DES solvent clustered closely with alcohols and water, implying a possible protic nature of the solvent. This finding suggests that the solvent might possess hydrogen bond donating and accepting capabilities, similar to protic solvents such as alcohols and water.

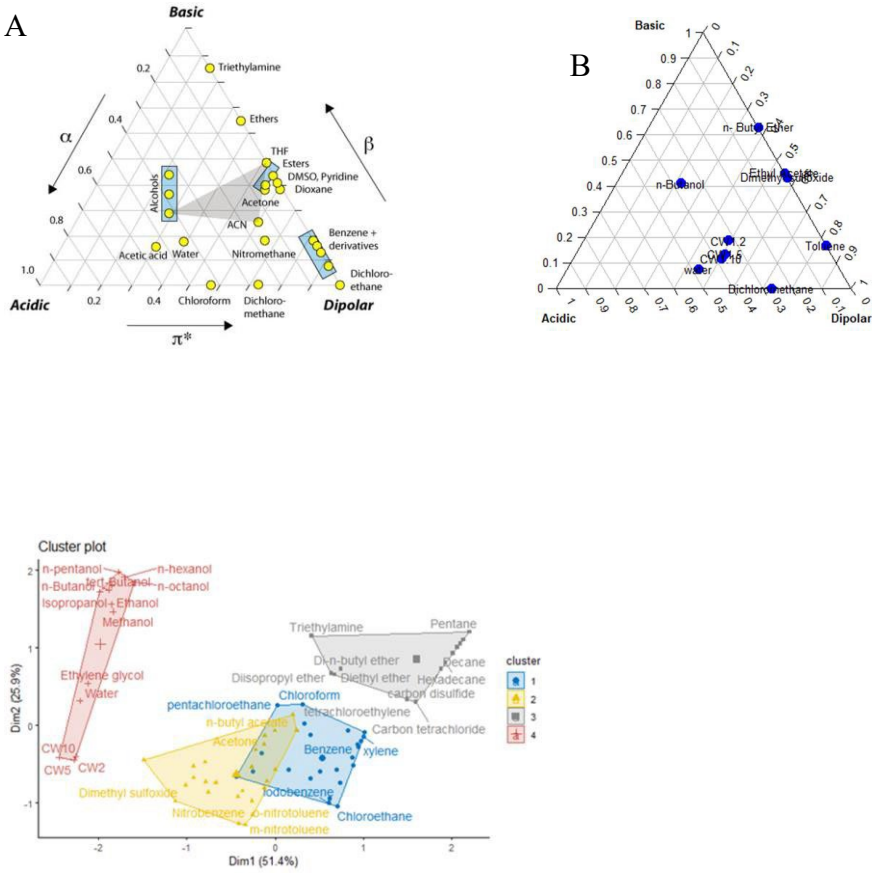


Figure 3.9. Clustering of DES with known solvent to reveal similarities based on solvatochromic parameters.

3.2.3.2 Correlation of Physicochemical Properties and Similarities (Dissimilarities) Between DES samples Based on Physicochemical Properties Using Clustering

A correlation plot was used to visually observe the relationship between the measured physicochemical properties (Figure 3.10).

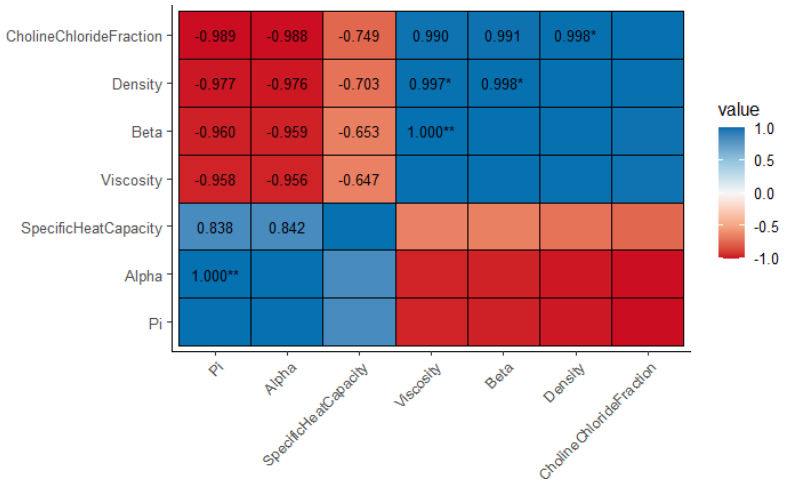


Figure 3.10. Correlation plot to reveal relationship between measured physicochemical properties.

The analysis revealed several significant relationships between the properties of the DES samples. The ChCl fraction acting as the HBA, shows a strong positive correlation with density, basicity and viscosity. This suggests that an increase in the proportion of ChCl in the mixture enhances the density, viscosity and basicity of the DES, likely due to the increased potential for hydrogen bonding.

Moreover, the strong positive correlation between basicity and viscosity indicates that the stronger the basicity of the DES, the higher its viscosity. This observation aligns with the fact that the DES sample with the highest ChCl-water ratio (1:2) exhibits the highest viscosity, likely due to the enhanced potential for hydrogen bonding in the presence of a larger proportion of ChCl.

The density of the DES also shows a strong positive correlation with viscosity, further supporting the idea that increased intermolecular forces resulting from hydrogen bonding lead to higher viscosities. However, while the ChCl fraction shows a strong positive correlation with viscosity and basicity, these correlations are not statistically significant.

This suggests that while the proportion of ChCl in the mixture may influence the viscosity and basicity of the DES, other factors may also be at play.

The results also indicate a strong negative correlation between polarizability/dipolarizability (π^*) and the ChCl fraction, density, viscosity, and basicity. This suggests that as these properties increase, the polarizability/dipolarizability of the DES decreases. Similarly, acidity also shows a strong negative correlation with the ChCl fraction, density, viscosity, and basicity, indicating that as these properties increase, the acidity of the DES decreases.

The acidity of a solution is determined by the concentration of hydrogen ions (H^+). In the case of DES, ChCl acts as a HBA, while water acts as a HBD. As the ChCl fraction increases, more hydrogen bonds are formed, which could reduce the availability of free hydrogen ions, thereby decreasing the acidity of the solution. Similarly, an increase in density and viscosity, which are indicative of stronger intermolecular forces and interactions, could also limit the mobility of hydrogen ions, leading to lower acidity.

Based on the physicochemical properties, clustering which is an unsupervised machine learning algorithm was performed to group the DES (Figure 3.11). Clustering was performed so as to cluster or group the DES based on their similarities on the measured physicochemical properties. The results revealed that ChCl-water in the ratio of 1:5 and 1:10 clustered together indicating the close similarity of these two samples.

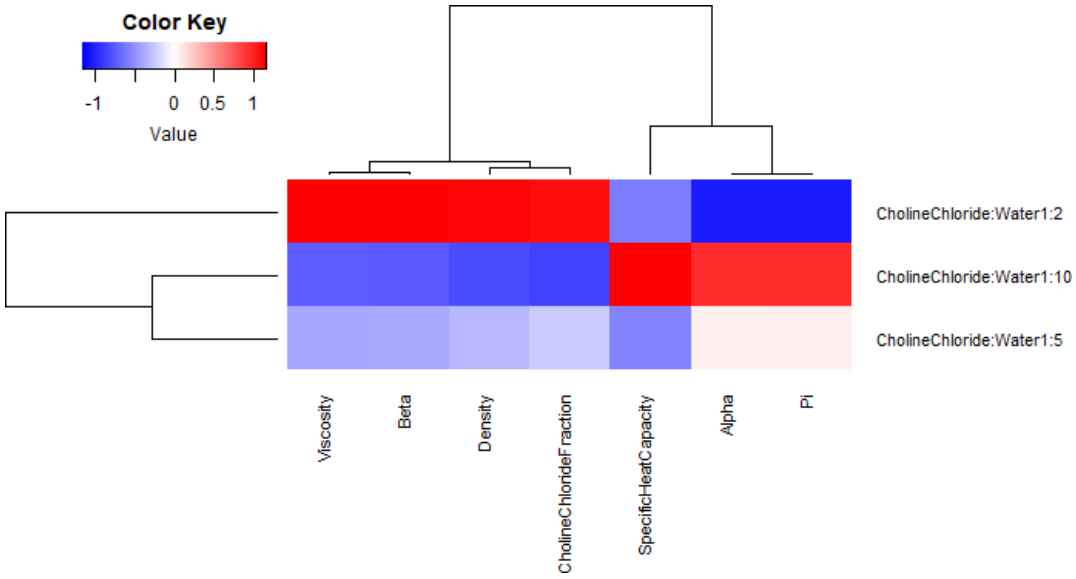


Figure 3.11. Clustering of DES to reveal sample similarities based on the physicochemical properties. Red indicates higher values; White indicates median values and Blue indicates low values.

The results from the physicochemical measurements reveal that, varying the HBD-HBA ratio of DES results in changes in the structure of the solvents as reflected in the measured physicochemical properties. ChCl-water in the ratios 1:5 and 1:10 were found to be structurally (based on the physicochemical measurement) more similar than the two samples to ChCl-water 1:2. With ChCl-water in the ratio 1:2 been the sample with the highest viscosity and with the strong positive correlation of viscosity to density, ChCl fraction and basicity, it can be implied that ChCl: water in the ratio of 1:2 probably has the strongest intermolecular hydrogen bonding.

3.3. Structural Exploration of DES Samples Using Spectroscopic Techniques

The project's general concept revolves around the idea that by altering the ratios of HBD and HBA components of DES affects the solvent structure (mainly driven by hydrogen bonding). The solvent structures reflect its physicochemical chemical properties, IR and NMR spectra. Based on the physicochemical measurements, it was revealed that ChCl-water in the ratios of 1:5 and 1:10 are more similar than they are to ChCl-water 1:2 (Figure 3.11). A profound difference between the samples is their viscosity where with ChCl-water 1:2 was the most viscous. From the correlation plot, viscosity was strongly positively correlated to ChCl fraction, basicity, density.

Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy are powerful analytical tools that can be employed to investigate the structural composition of samples. Samples are made up of molecules, which in turn consist of functional groups. These functional groups, characterized by specific arrangements of atoms, exhibit consistent physical and chemical properties, irrespective of the larger molecule they are part of [36]. Infrared (IR) spectroscopy capitalizes on the relationship between the vibrational frequencies of chemical bonds in these functional groups and the overall molecular structure to identify chemical compounds. When functional groups absorb IR light, they do so at resonant frequencies. These frequencies correspond to the vibrational frequency of a specific bond aligning with the frequency of the absorbed radiation.

The resulting IR spectra offer valuable structural insights by highlighting specific regions of interest. For instance, O-H bonds involved in hydrogen bonding typically exhibit a stretching frequency in the region of 3200-3550 cm^{-1}. The associated peak is often broad, reflecting the range of hydrogen bond strengths present in the sample[37].

NMR operates on the principle that samples are composed of molecules, which are further made up of various atoms. These atoms, particularly hydrogen atoms, have specific nuclear properties that are influenced by their chemical environment, making them unique identifiers of molecular structure[38, 39]. Proton NMR spectroscopy capitalizes on these properties to provide detailed information about the molecular structure of a compound.

In Proton NMR spectroscopy, the sample is exposed to a strong magnetic field, causing the hydrogen nuclei (protons) to align with the field. When subjected to a radio frequency pulse, these protons absorb energy and resonate at specific frequencies, which are dependent on their chemical environment[39]. This resonance is detected and used to generate an NMR spectrum. The resulting NMR spectrum provides valuable structural insights by highlighting specific regions of interest, known as chemical shifts[39].

Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopies are powerful techniques that offer visual insights into the structural characteristics of a sample. However, a visual interpretation of these spectra can sometimes lead to overlooked or misinterpreted information due to the complexity of the data. To enhance the accuracy and depth of spectral analysis, chemometrics was employed.

Chemometrics is a data-driven approach that applies mathematical and statistical methods to extract valuable information from complex chemical data[40]. It can help distinguish samples based on their spectral data, even when the differences are subtle and not easily discernible through visual inspection[40]. One of the tools available for chemometric analysis is the ChemoSpec package in R. ChemoSpec is designed specifically for the analysis of spectral data, including IR and NMR spectra. It provides a comprehensive suite of

functions for exploratory data analysis, clustering, and classification, making it a valuable tool for enhancing the interpretation of complex spectral data[40].

The workflow in ChemoSpec begins with data preprocessing, a crucial step that involves the removal of unwanted frequencies or samples, binning of the spectra, normalization, scaling, and baseline correction. These processes are essential to reduce noise, ensure all spectra are on a comparable scale, and remove any background signals that could interfere with the analysis (Figure 3.12)[40].

Exploratory data analysis is performed after data preprocessing. Here, visualization tools are employed to gain a comprehensive understanding of the data's structure and to identify any discernible patterns or anomalies. This is achieved through plotting the spectra for an overview of the spectral data, loadings to reveal contributing variables to this variation (Figure 3.13A), scores for variation in the data (Figure 3.13B), and a scree plot to assist in determining the number of components to retain in the analysis[40].

The final stage of the workflow is data analysis, where the preprocessed and explored, data is subjected to various statistical methods to extract meaningful insights. The working horse for the package is Principal Component Analysis (PCA) which is a statistical technique used for dimension reduction[41]. PCA is particularly useful in dealing with multicollinearity issues or when handling many predictors. It transforms the original variables into a new set of uncorrelated variables, known as principal components. These components are linear combinations of the original variables and are constructed in such a way that they capture the maximum variance in the data[41]. The first principal component is the linear combination of variables that explains the most overall variance in the data. The second principal component, orthogonal to the first, explains the second most variance

and is uncorrelated with the first component. This process continues for as many components as there are variables, each time maximizing the variance and ensuring no correlation with the preceding components[41]. The relationship between samples is revealed with principal components are plotted against each other. Samples that are closer to each other are deemed similar whereas those at a distance are considered outliers. The sample distances and similarities are observed using Hierarchical Cluster Analysis (HCA)[40].

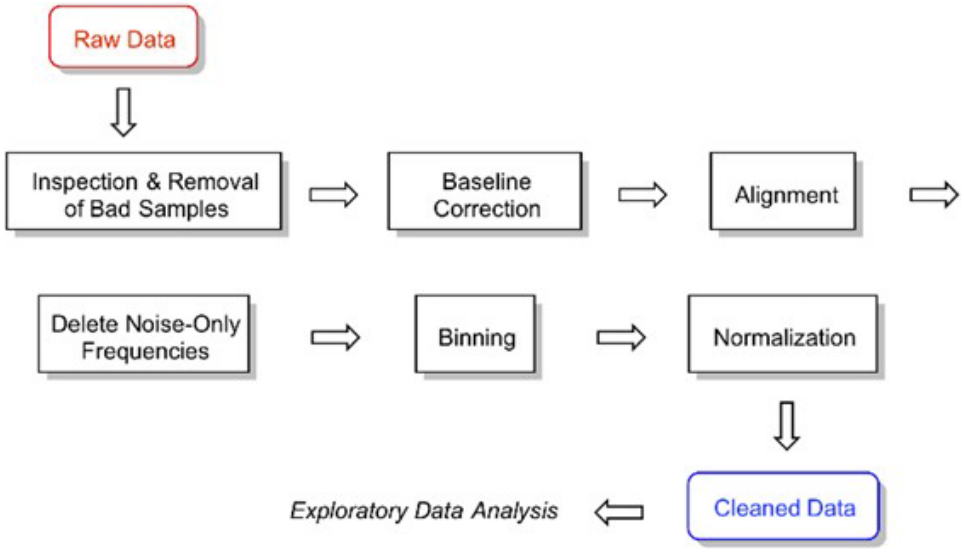
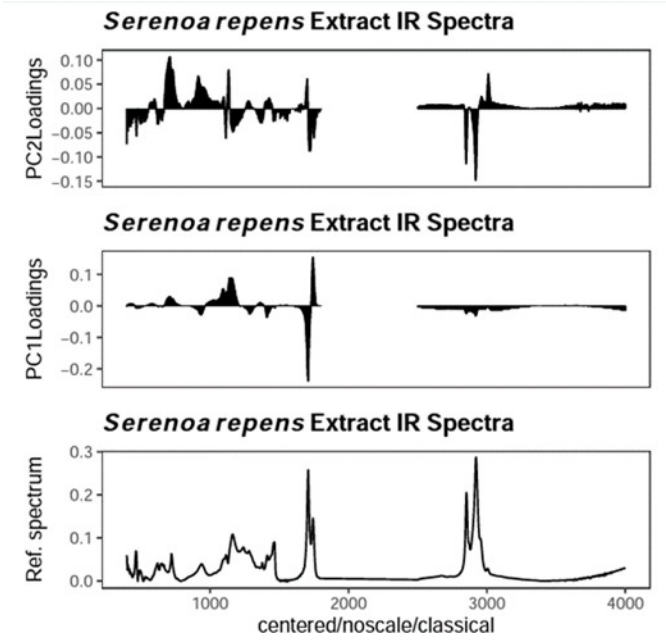


Figure 3.12. A typical workflow of spectra analysis using the ChemoSpec package in R.

A



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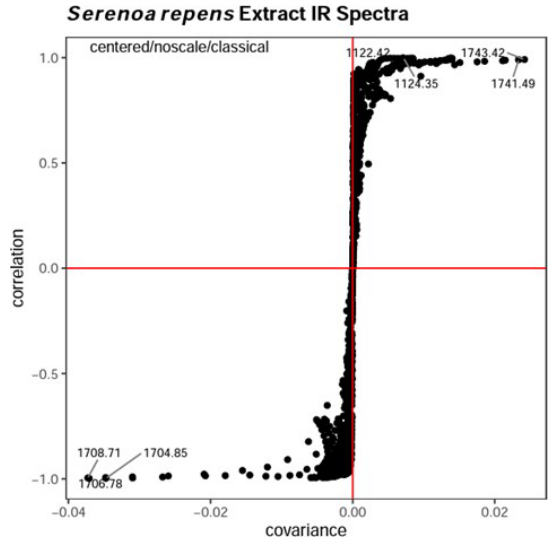


Figure 3.13. Example of A) Plot loadings which indicate how each variable (frequencies in spectral applications) impacts the scores. This can be important in understanding the results. The figure shows that different carbonyl peaks have a significant and opposing effect on PC 1. PC 2, on the other hand, is influenced by several peaks, with some intriguing opposing peaks in the hydrocarbon region. B) S-shaped plot which shows the most influential frequency variables located in the upper right-hand and lower left quadrants. In this, the influence of the carbonyl peaks is clearly visible. [40].

In this section, the objective is to leverage both Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy as analytical tools to uncover structural commonalities and differences among the DES samples. To achieve this, dual approach that combines visual examination of the spectra and chemometric analysis using the ChemoSpec package in R was utilized. This comprehensive methodology allows for a more comprehensive understanding of the structural characteristics of the DES samples.

3.4 Experimental

3.4.1 FTIR SPECTROSCOPY

Thermo-Fisher Scientific Nicolet 380 FTIR spectrometer (Waltham, MA) was used to obtain FTIR spectra of all the DES formulated. The resolution was set at 8 nm^{-1}. The measurement spectrum range was 4000-400cm^{-1}. The spectra were produced and processed with EZ OMNIC software by Thermo-Fisher Scientific. Further analysis was conducted using the R software and ChemoSpec package.

3.4.2 PROTON (H^1)-NMR

Proton NMR(H^1-NMR) spectra were obtained for all the DES and the constituent compounds. A Bruker 400 MHz spectrometer (Billerica, MA) equipped with QNP 5 mm probe at 400 MHz and

22^{oC} was used to obtain the 1H-NMR spectra. Deuterated DMSO (DMSO-d_6) and deuterated water (D2O) were used as solvents for the DES. The D_2O were used directly. In the case of DMSO-d_6, 5 µl of the DES prepared with deionized water were added to sufficient amounts of DMSO-d_6. The ^{1}H-NMR parameters were set as follows: 90^o pulse

angle, 25 pulse rate, and 512 scans. All DES were kept in a desiccator prior to the H^1-NMR scan.

3.4.3 Data Preprocessing

The preprocessing of the infrared (IR) spectral data for exploratory analysis was conducted using the ChemoSpec package in R (Figure 3.14).

The data was subjected to these processing steps. First, the baseline of the IR spectra was corrected to adjust for drift. Then, the spectra were normalized to make them comparable for meaningful comparisons. Exploratory data analysis was performed, including generating a scree plot to determine the number of principal components to retain using PCA. Loadings plots helped identify the most influential frequency variables for each component. A scores plot was used to identify variations in the data. Lastly, hierarchical cluster analysis (HCA) of the scores was conducted to group similar observations based on their scores for the first five principal components, providing insights into the data structure and patterns.

The preprocessing for NMR (Nuclear Magnetic Resonance) data followed a similar workflow as the IR spectra but included an additional step of peak alignment. Peak alignment corrects for any variations in peak positions that may arise due to factors like instrument drift or sample preparation differences[40].

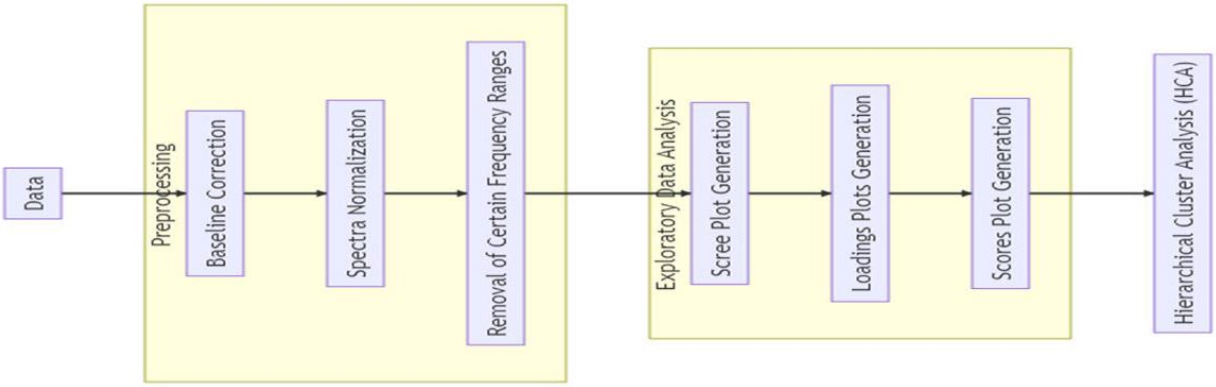


Figure 3.14. Workflow for the analysis of IR and NMR spectra using ChemoSpec package in R

3.5 Results and Discussion

3.5.1 IR Spectra

The infrared (IR) spectra of the DES samples exhibited a broad, intense peak between 3000 and 4000 cm^{-1} (Figure 3.15), a region typically associated with O-H stretching vibrations indicative of hydrogen bonding[42, 43]. The intensity and breadth of this peak suggest strong and extensive hydrogen bonding in the DES samples. In contrast, the ChCl control showed a less intense and narrower peak in this region, suggesting weaker or less extensive hydrogen bonding in the absence of water (Figure 3.15).

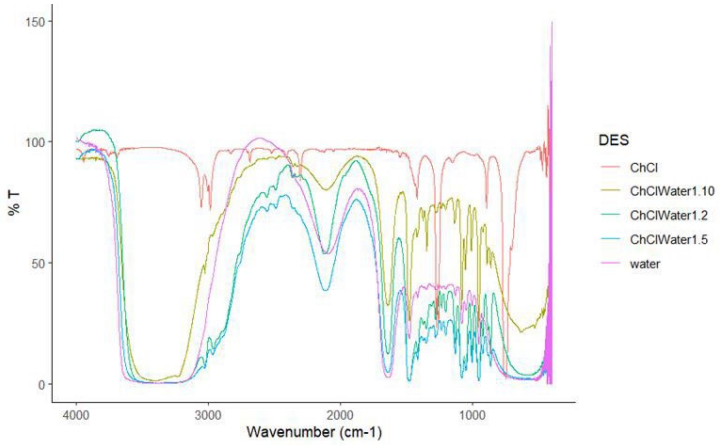
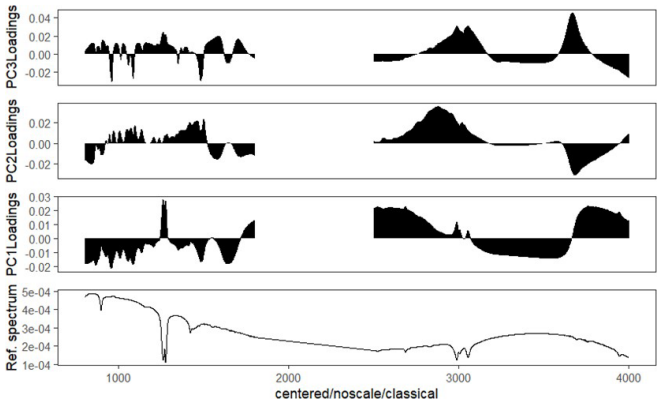


Figure 3.15. IR spectra results for DES and water samples

Analysis using the ChemoSpec package revealed that, the frequencies 1241, 3667, 957, 4000, and 3999 cm^{-1} had high absolute loadings (Figure 3.16A) and were shown at both the upper right and lower left of the S-plot indicating their importance (Figure 3.16B)[40]. Frequencies with high absolute loadings are crucial in explaining the differences between the samples[40]. This implies that, these specific molecular vibrations are instrumental in explaining the observed differences in the samples[40]. Frequencies at 3600 – 4000 cm^{-1} correspond to vibrations associated with the formation of hydrogen bonds. The analysis implies that samples with a high number of hydrogen bonds would exhibit a strong signal at this frequency range.

A



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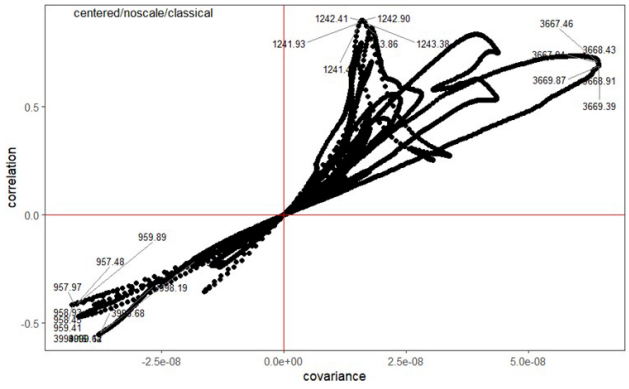


Figure 3.16. Plot to show variations in the sample using A) Loading plot and B) Score plot

The HCA, based on the scores for the first five PCs, revealed distinct clustering of the samples (Figure 3.17). The DES sample with the highest water content (ChCl: water 1:10) clustered with the water control, suggesting similar physicochemical properties due to the dominance of water in this sample. This could be due to the dilution effect, where the increasing water content diminishes the contribution of ChCl to the overall properties of the solvent. The DES samples with lower water content (ChCl: water 1:2 and 1:5) clustered together, indicating similar properties due to a more balanced ratio of ChCl to water. The ChCl control formed a separate cluster, reflecting its distinct properties in the absence of water. This is consistent with the idea that, in the absence of water, the hydrogen bonding capability and the physicochemical properties of ChCl are distinctly different from the DES mixtures containing water.

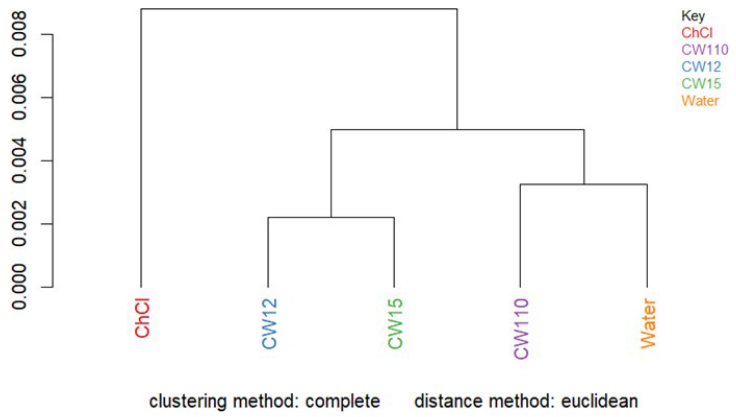


Figure 3.17. Clustering of samples using PCA-HCA to reveal similarities between samples based on the IR spectra

3.5.2 NMR spectra

Before delving into the results of the NMR spectra, it is important to discuss the significance and effects of hydrogen bonding on NMR signal. Hydrogen bonding significantly influences the resonance signals of protons in NMR spectroscopy[38]. Experimental evidence consistently supports the notion that hydrogen bonding shifts the resonance signal to a lower field, corresponding to a higher frequency[38]. For instance, in the case of alcohols, the chemical shift of the hydroxyl hydrogen has been found to vary with concentration. In very dilute solutions of 2-methyl-2-propanol, $(CH_3)_3COH$, dissolved in carbon tetrachloride, the hydroxyl resonance signal appears at a relatively high-field chemical shift, usually at less than 1.0 δ (parts per million). However, as the concentration of the alcohol increases, this signal shifts to a lower field, typically near 2.5 δ[38]. This concentration-dependent shift is a characteristic indication of hydrogen bonding occurring between the hydroxyl hydrogen and nearby solvent molecules. As the alcohol concentration increases, more hydrogen bonding interactions take place, resulting in a pronounced downfield shift of the resonance signal. Another example is the case of phenol, which possesses a more acidic hydroxyl group compared to alcohols, also demonstrates a concentration-dependent shift in its hydroxyl resonance signal. The phenol hydroxyl hydrogen generates a lower-field resonance signal that exhibits a similar concentration dependence to that observed in alcohols (Figure 3.18).

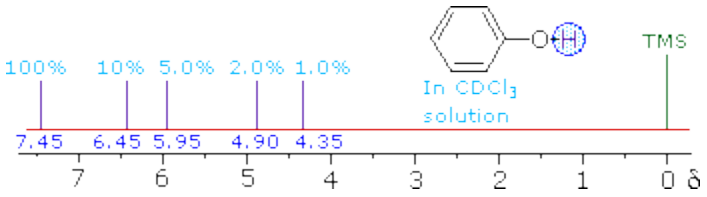


Figure 3.18. Impact of hydrogen bonding (concentration dependent shift) on NMR resonance signals for phenol

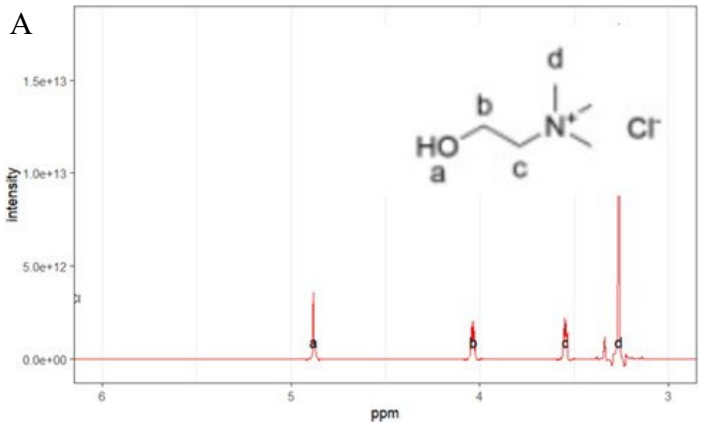
Thus, in the presence of hydrogen bonding the resonance signals of protons involved in hydrogen bonding are shifted to lower field (higher frequency).

When this concept is extended to the formulated DES, where research suggests the presence of hydrogen bonding between the hydrogen (H) of the HBD and the chloride ion (Cl^-) bonded to $N^+(CH_3)_3$ (Figure 1.4) , it can be theoretically anticipated that a downfield shift (higher frequency) for the protons involved in these hydrogen bonding interactions should be expected. Additionally, it should be expected that the hydroxyl (OH) protons in water also experience a downfield shift (higher frequency) as the concentration of water increases.

In the NMR spectrum of pure ChCl (Figure 3.19A), which serves as a reference for the unbound ChCl, specific chemical shifts were observed, providing essential information about its molecular structure. The spectrum displayed a singlet peak at approximately 3.26 ppm, corresponding to the presence of the trimethylammonium group ($N^+(CH_3)_3$). This singlet peak arises from the symmetric environment of the three methyl groups within the trimethylammonium moiety. Since all three methyl groups experience an identical chemical environment, they give rise to an identical NMR signal, resulting in a single peak without any splitting or multiplicity.

Furthermore, multiplet peaks were detected at approximately 3.544 ppm and 4.03997 ppm, signifying the presence of methylene groups (CH_2) within the compound. These multiplet peaks originate from the interactions with neighboring protons, leading to a splitting pattern in the NMR signal. Methylene protons typically exhibit multiplet patterns because they interact with adjacent protons, resulting in different splitting patterns depending on the number and type of neighboring protons. In addition to the peaks, another singlet peak was identified at around 4.88 ppm, indicating the presence of a hydroxyl group (-OH).

A



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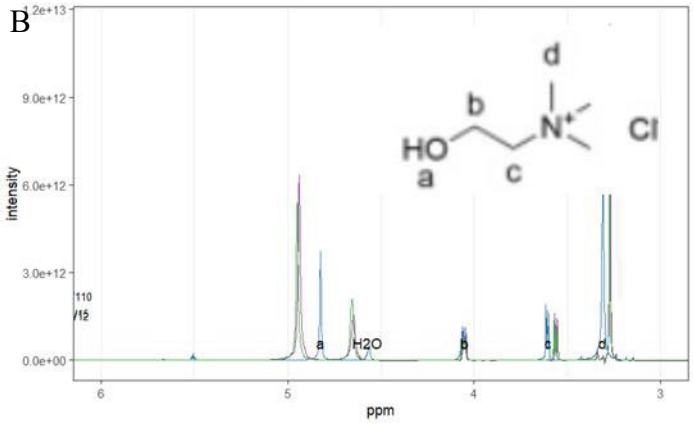


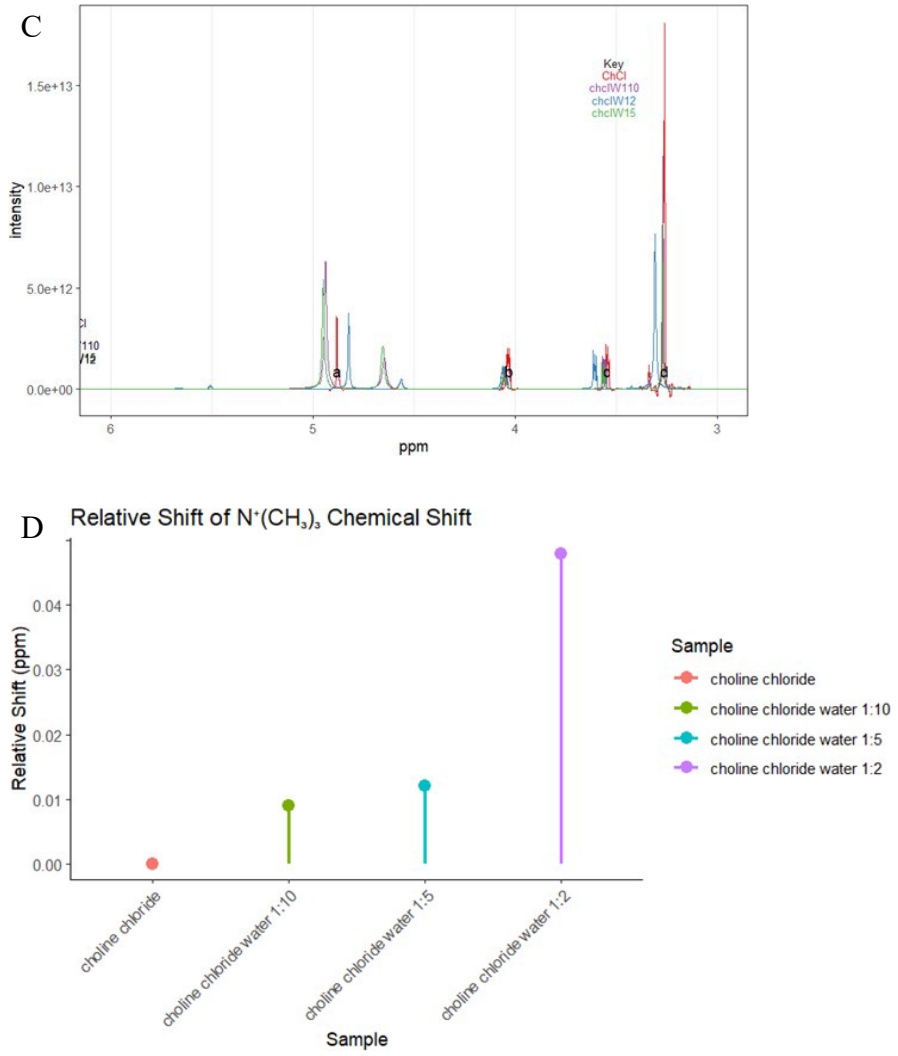


Figure 3.19. Determining the extent of hydrogen bonding in DES samples. A) NMR spectra for ChCl. B) NMR spectra for ChCl-water DES. C) Merged NMR spectra for samples. D) Measurement of relative shift of $N^+(CH_3)_3$ protons in samples indicative of extent of hydrogen bonding

The NMR spectra of the ChCl-water eutectics exhibited similar signals to those of pure ChCl, albeit with slight shifts in chemical shift values (Figure 3.19B). Additionally, a distinct water peak appeared around 4.6 ppm in the eutectics. Notably, the spectra showed variations at the ($N^+(CH_3)_3$) position, with ChCl-water 1:2 displaying a slightly downfield

shift compared to ChCl-water in the ratios 1:5 and 1:10. Similarly, the methylene group (b) in proximity to the trimethylammonium moiety also exhibited a downfield shift for ChCl-water 1:2 relative to ChCl-water in the ratios 1:5 and 1:10. These shifts suggest the presence of hydrogen bonding between the water protons and the Cl^- bonded to ($N^+(CH_3)_3$). The downfield shift of the methylene group likely arises from its interaction with the nearby hydrogen bonding.

Table 3.2.

Frequencies (ppm) of assigned peaks in samples

Sample	$N^+(CH_3)_3$ (d)	CH_2 (c)	CH_2 (b)	H_2O	OH (a)
ChCl-water 1:2	3.308	3.598	4.05	4.568	4.825
ChCl-water 1:5	3.272	3.563	4.056	4.65	4.94
ChCl-water 1:10	3.269	3.557	4.049	4.63	4.927
ChCl	3.26	3.544	4.03997		4.88

Moreover, two other frequencies showed variability: the "water" peak and the hydroxyl peak. Specifically, the water peak in the ChCl-water ratios of 1:5 and 1:10 appeared more downfield compared to the other peaks, indicating that increasing the concentration of water led to a downfield shift of the hydroxyl proton in water. Similarly, the OH peak displayed variability, with ChCl-water in the ratios 1:5 and 1:10 exhibiting a more downfield shift. This observation suggests that the excess water influences the chemical environment of the hydroxyl group (-OH) present in ChCl. Both the plot loadings and S plot showed variability at frequencies 4.88 ppm, 4.84 ppm and 3.27 ppm (Figure 3.20).

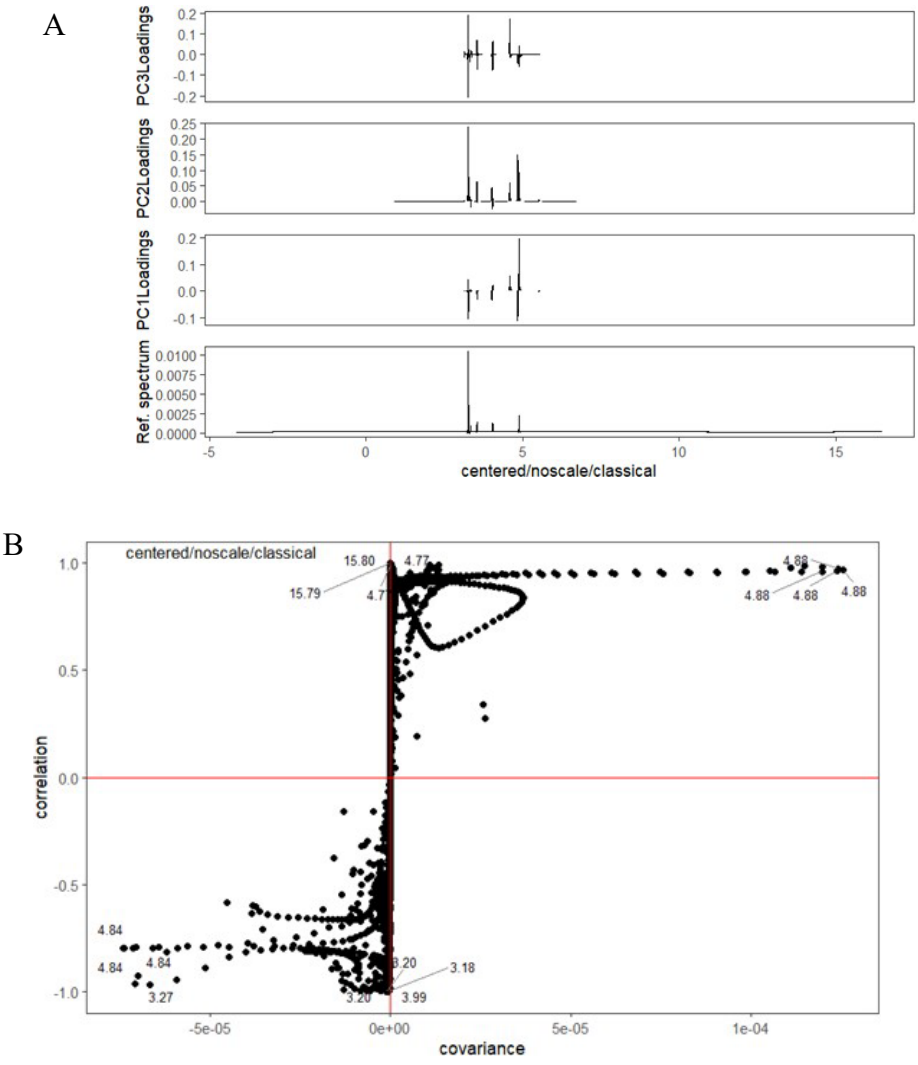


Figure 3.20. Identification of highly variable peaks using A) PC loadings and B) S plot

The clustering analysis revealed a distinct pattern in the results, grouping the ChCl: water ratios of 1:5 and 1:10 together indicating their similarity (Figure 3.21).

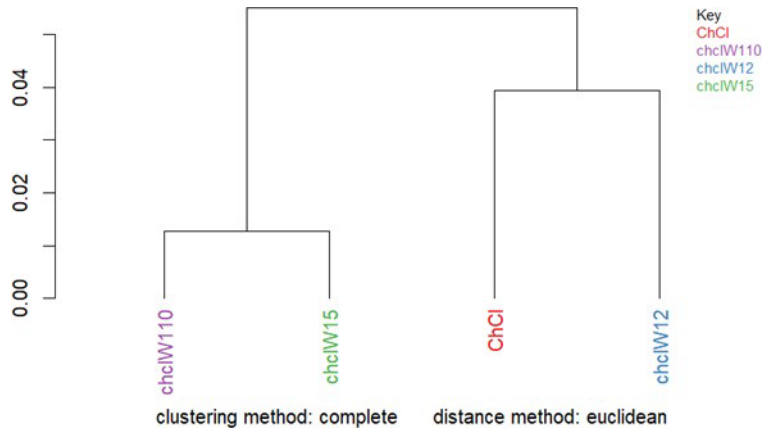


Figure 3.21. Clustering of samples using PCA-HCA to reveal similarities between samples based on the NMR spectra

3.4 Assessment of Drug Stabilizing Ability In Choline-Based DES

Structural exploration of the DES revealed that the solvents are structurally distinct. Physicochemical measurements revealed that DES ChCl-water in the ratios 1:5 and 1:10 were similar as compared to ChCl-water 1:2. A potential differentiating factor between the samples from the physicochemical measurement is viscosity which strongly positively correlated with ChCl fraction, basicity and density. Further structural exploration of the solvent with spectroscopic approach using IR revealed that ChCl-water in the ratio 1:2 and 1:5 were more similar when compared to ChCl-water 1:10. A visual observation of width of the OH vibration region revealed a broader width of ChCl-water in the ratios 1:2 and 1:5 than ChCl-water 1:10. This suggests a possible stronger hydrogen bonding in ChCl-water in the ratio 1:2 and 1:5 than ChCl-water 1:10. NMR studies confirmed the existence of hydrogen bonding in the solvent with ChCl-water 1:2 exhibiting a more downfield shift

of the trimethylammonium protons. Clustering from the NMR revealed that ChCl-water in the ratios 1:5 and 1:10 were more similar. Thus, from these, it can be inferred that the strength in hydrogen bonding is in the rank ChCl-water 1:2 > ChCl-water 1:5 > ChCl-water 1:10.

In line with the model of the project (Figure 3.1), the subsequent focus is on the structure-activity relationship, a concept that underscores the influence of a solvent's structure on its activities. Consequently, this segment of the project aims to evaluate the stability of drugs within the DES solvent, using acetyl salicylic acid, commonly known as aspirin, as a representative drug.

Hydrolytic degradation is a significant concern in the pharmaceutical industry, particularly in the stability of Active Pharmaceutical Ingredients (API)[42]. This process involves the splitting of a chemical bond in the presence of water, a reaction that is heavily influenced by factors such as atmospheric humidity, pH, and the reactivity of the functional group involved. For instance, esters are more prone to hydrolysis than amides[44].

Acetylsalicylic acid (ASA), widely recognized for its analgesic, antipyretic, and antiplatelet properties, is one such drug susceptible to hydrolytic breakdown. ASA, an esterified pro-drug of salicylic acid (SA), was developed to mitigate the adverse gastrointestinal effects associated with SA. However, ASA's stability is compromised when exposed to humidity, leading to hydrolysis and the formation of salicylic and acetic acids[44]. The kinetics of this reaction may increase significantly depending on the formulation type and processing conditions, thereby necessitating measures to prevent hydrolytic alterations that could jeopardize its safety.

Common strategies to reduce hydrolytic loss of drug activity include limiting humidity exposure during development and manufacturing, adding buffers to control pH, and using protective packaging. Moreover, polymeric coatings can also serve as moisture barriers for solid dosage forms. However, these coatings require solvents that must be subsequently evaporated. Hygroscopic or water-soluble polymers are not ideal for water-sensitive actives, while other polymers necessitate the use of inorganic solvents, which must be recovered due to health and environmental considerations[44].

In this context, DES emerges as a promising alternative to standard polymer coatings. These solvents offer a potential solution to the challenges of hydrolytic degradation, presenting a new avenue for enhancing the stability and safety of pharmaceutical products. Other research have also proven the capacity of DES in stabilizing proteins and drugs[13]. This study aims to delve into the drug-stabilizing potential of ChCl-water based DES, with a particular focus on the role of hydrogen bonding interactions in bolstering drug stability. Using ASA as a representative drug, we intend to shed light on how the distinctive chemistry of ChCl-water DES, underscored by its hydrogen bonding capabilities, can impact the drug's stability within the solvent matrix.

A comprehensive understanding of the intermolecular interactions and solvent properties that contribute to drug stabilization is paramount for the optimization of pharmaceutical formulations and the progression of environmentally friendly, efficient drug delivery systems. By drawing a connection between the observed hydrogen bonding interactions in the solvent and the enhanced stability of ASA, this study aims to provide valuable insights that could pave the way for the design of innovative and sustainable drug formulations.

3.4.2 Experimental

3.4.2.1 Approach

The experimental approach for this study is predicated on the understanding that ASA degrades into salicylic acid when exposed to various solvents. The resulting salicylic acid can then interact with a ferric chloride solution, producing a purple coloration. The absorbance of this color can be measured, providing a quantifiable means of tracking the rate of ASA degradation, which serves as an indicator of its stability.

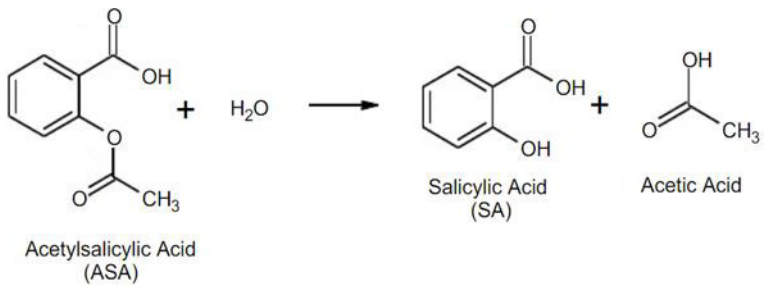


Figure 3.22. Reaction for the degradation of acetyl salicylic acid in water.

To ascertain the stability of ASA within the solvent, a calibration curve (Figure 3.23) was constructed using a range of known salicylic acid concentrations. The absorbance spectra derived from this calibration curve allowed for the identification of the maximum absorption point, which occurred at 540 nm. This wavelength was subsequently utilized as the endpoint for measuring the amount of salicylic acid produced from the ASA sample.

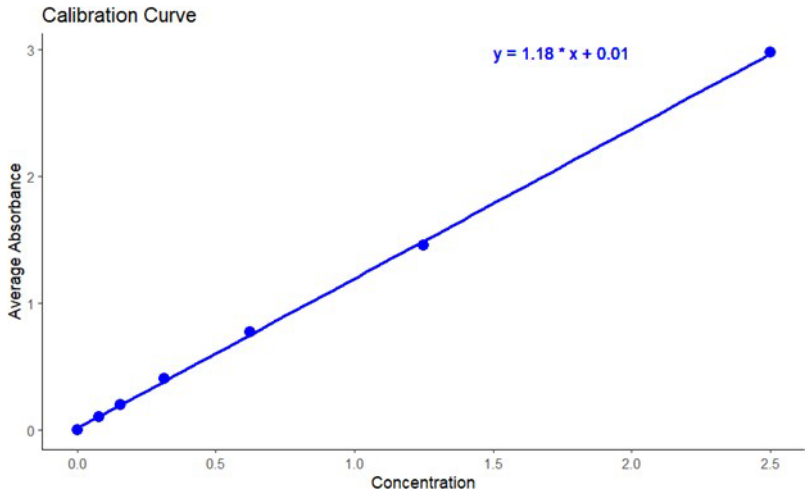


Figure 3.23. Standard calibration curve obtained by plotting concentration of salicylic acid with absorbance.

The stability assessment began with the dissolution of 500 mg of ASA in 50 ml of 95% ethanol. From this solution, 5 ml was extracted and combined with 20 ml of the solvent samples. At predetermined intervals, a 1 ml sample was taken to which three drops of ferric chloride were added. The absorbance of the resulting solution was measured at 540 nm, as determined from the standard solution. The recorded absorbance was then converted to a concentration value using the best-fit line derived from the calibration curve.

3.4.2.2 Data Analysis

The order of the reaction was determined using a 'best fit line' approach, applied to three different models. For the zero-order reaction, the concentration of salicylic acid against time (in days) was plotted. For the first-order reaction, the natural logarithm of the salicylic acid concentration was used, and for the second-order reaction, the inverse of the salicylic acid concentration against time was plotted. The model with the highest R-squared value, indicating the closest fit to the observed data, was selected to represent the order of the reaction.

3.4.3 Results for Drug Stabilization Studies

The results revealed that ASA exhibited the highest stability in a eutectic solvent composed of ChCl and water in a 1:2 ratio (Figure 3.24). This was evidenced by the relatively low degradation constant of 0.077473 per day, indicating a slower rate of ASA degradation in this solvent compared to others.

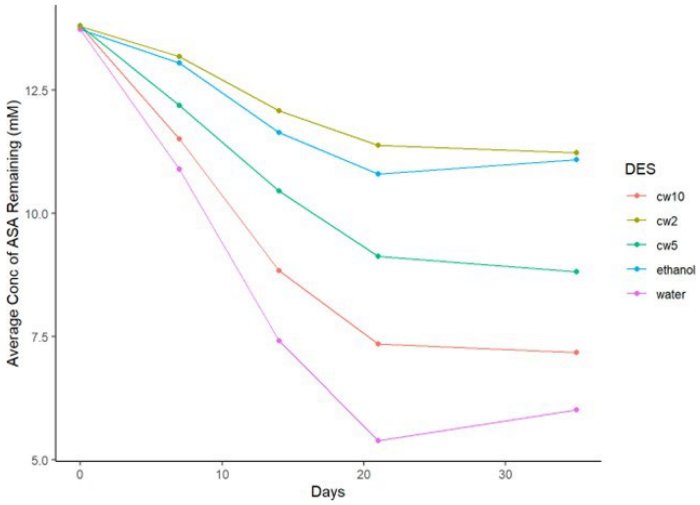


Figure 3.24. Kinetics of acetyl salicylic acid degradation in samples

The results also seem to suggest a correlation between the water content in the solvent and the rate of ASA degradation (Figure 3.25). Specifically, an increase in water content appeared to accelerate the degradation process. This observation may be attributed to the potential role of water in facilitating the hydrolysis of ASA, leading to a faster degradation rate.

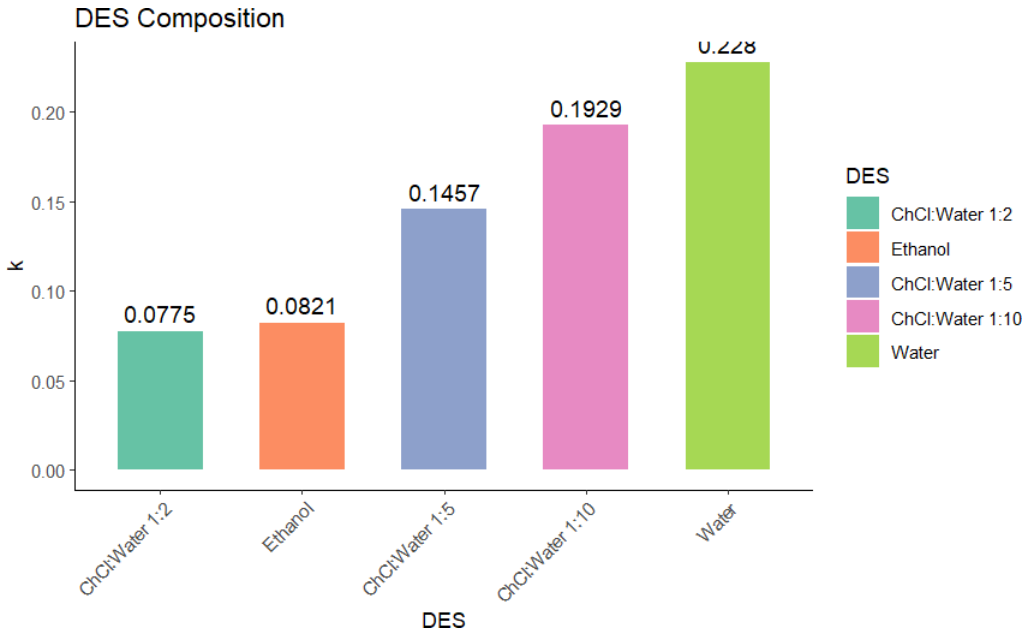


Figure 3.25. Comparison of rate of degradation (activity) (k) between samples

3.5 Chapter Summary. Structure Activity Relationship

The primary objective of this chapter was to investigate the impact of altering the molar ratios of HBD-HBA of DES on the structure and potential utility of the solvent in drug stability studies. Existing literature suggests that the formation of DES is significantly influenced by the creation of hydrogen bonds[1]. Consequently, it was hypothesized that altering the ratios of both the HBA and the HBD would affect the solvent structure (hydrogen bonding), which would, in turn, impact the physicochemical properties, structure, and utility of the solvent.

To test this hypothesis, physicochemical properties (structural descriptors), including density, specific heat capacity, pH, viscosity, and solvatochromic parameters (acidity, basicity, and dipolarity-polarizability) were measured. Structural analysis of the solvent

using both Infrared (IR) and NMR spectroscopy was also performed. Finally, we examined the solvent's ability to stabilize a drug, using acetyl salicylic acid as a model.

Our findings revealed a strong negative correlation (Figure 3.26A) between the rate of degradation (k) of acetyl salicylic acid and the ChCl fraction, density, basicity, and viscosity. The clustering analysis further differentiated the ChCl: water 1:2 ratio as structurally unique when compared to the 1:5 and 1:10 ratios (as shown in Figure 3.26B). The 1:2 ratio exhibited the lowest degradation rate (k), acidity, and dipolarity/polarizability. Interestingly, acidity and dipolarity-polarizability clustered together, indicating their similarities. Moreover, they were closely associated with the degradation rate (k), suggesting a shared influence on this parameter. These properties exhibited a negative correlation with the ChCl fraction, density, basicity, and viscosity. Notably, the ChCl: water 1:2 ratio demonstrated the highest values for these parameters. This observation could be attributed to the enhanced hydrogen bonding capacity of the solvent at this ratio. The increased hydrogen bonding could potentially stabilize the acetyl salicylic acid, thereby decelerating its degradation.

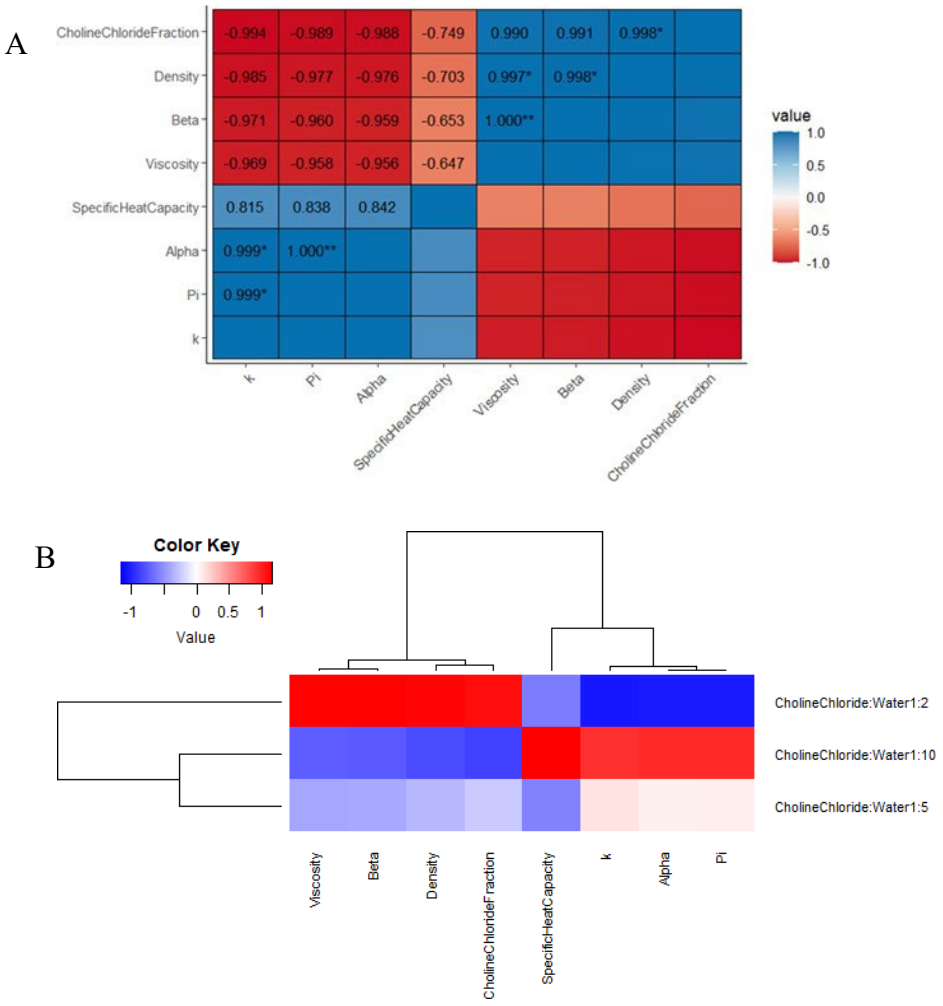


Figure 3.26. Correlation and Clustering plot to reveal association of physicochemical parameters with activity (k)

In conclusion, this study showed that the ratio of the HBD in DES significantly influences their structure and physicochemical properties, which in turn affects their utility in drug stability studies. The strong correlation between the physicochemical properties and the spectroscopic data underscores the importance of a holistic approach in solvent design. Future work should focus on leveraging this understanding to tailor the properties of DES for specific applications, such as drug stabilization.

CHAPTER 4. STRUCTURAL-ACTIVITY EFFECTS OF CHANGING EITHER HBA OR HBD

Abstract

In this study, the structural-activity relationships of DES with varying HBD and HBA was explored. The choice of HBD and HBA was found to significantly influence the structural characteristics of the solvents, as evidenced by the variations in the OH vibration peaks in the IR spectra. Stronger hydrogen bonding was observed in solvents where water served as the HBD, leading to broader OH vibration peaks. Conversely, solvents with a higher density, indicative of more extensive hydrogen bonding, were associated with decreased stability of acetyl salicylic acid. The study also revealed that the stability of acetyl salicylic acid was highest in DES, particularly those where water was the HBD. These findings highlight the critical role of HBD and HBA selection in the design of DES, offering valuable insights for the development of more effective and tailored solvents for various applications.

4.1 Introduction

DES formation is fundamentally driven by hydrogen bonding interactions. In the case of ChCl-based DES, the halide ion (Cl^-) plays a pivotal role by acting as a bridge in the formation of hydrogen bonds. Specifically, it connects the hydrogen from the HBD to the nitrogen atom of the ChCl, thereby establishing a robust hydrogen bonding network. The capacity of a solvent to form hydrogen bonds, dictated by its HBD and HBA, can profoundly influence its interactions within the DES system. This project based on the premise that variations in HBD and HBA can modulate the solvent structure, particularly the hydrogen bonding network. This modulation is subsequently mirrored in the solvent's physicochemical properties and its infrared (IR) spectra (Figure 4.1). Therefore, a comprehensive understanding of these interactions can provide valuable insights into the behavior and potential applications of DES.

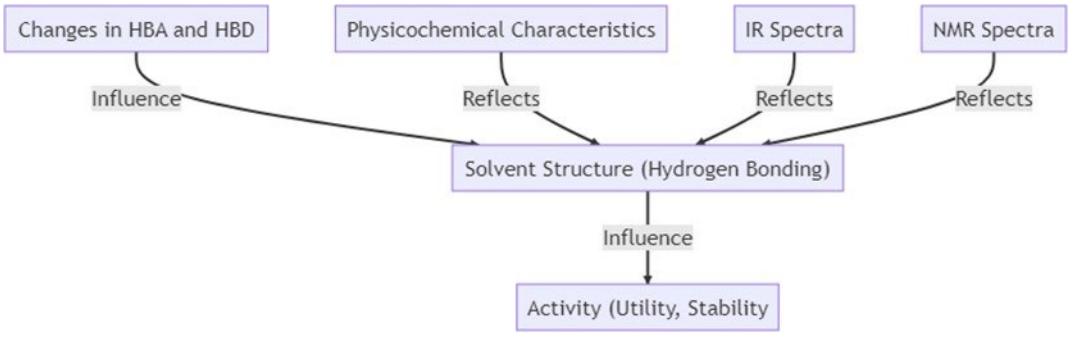


Figure 4.1. Model of Project

Water, methanol, and ethanol, common solvents in many chemical reactions, can act as both HBD and HBA due to the presence of hydroxyl (-OH) groups. These solvents can form hydrogen bonds with reactants, potentially stabilizing transition states and

influencing the reaction rate. In contrast, quaternary ammonium salts like ChCl, ChBr, and ChI are strong HBA. When combined with an HBD such as water, ethanol, or methanol, these salts can form unique solvent environments, leading to an altered hydrogen bonding network and a different microenvironment compared to the pure solvents.

The reactivity and ability to form hydrogen bonds of iodide, chloride, and bromide ions can be influenced by several factors, including their size, electronegativity, and polarizability. Larger ions are generally more polarizable, meaning their electron clouds can be more easily distorted to create temporary dipoles that can interact with other molecules. This can increase their reactivity and ability to participate in non-covalent interactions like hydrogen bonding.

In this study, the degradation rate of ASA in various solvent environments, including water, ethanol, methanol, and combinations of choline salts with these solvents were investigated. By comparing the degradation rates, represented by the degradation constant k, we aim to understand how the nature of the solvent, particularly its ability to form hydrogen bonds, influences the reaction rate. This investigation will provide valuable insights into the role of solvents in chemical reactions and could have implications for various fields, including pharmaceutical chemistry and materials science.

4.2 Experimental

4.2.1 Materials

The materials used in this study were procured from various sources. ChCl was purchased from Sigma-Aldrich, St. Louis, MO, USA. Similarly, Choline Bromide and Choline Iodide were procured from Alfa Aesar, Haverhill, MA, USA. Methanol and Ethanol were obtained

from Fisher Scientific, Hampton, NH, USA. In addition to these, high-purity water, sourced from a Milli-Q water purification system was used. Acetyl Salicylic Acid was purchased from Acros Organics, Geel, Belgium. Lastly, Ferric Chloride was procured from VWR International, Radnor, PA, USA. All materials were of analytical grade and were used without further purification. The selection and combination of these materials were instrumental to the study's aim of exploring the structural similarities and differences in DES and their impact on drug stability. In terms of hydrogen bonding, water is generally stronger than methanol and ethanol due to its ability to form more hydrogen bonds per molecule. This is reflected in properties such as boiling point, where water has a higher boiling point (100°C) compared to methanol (64.7°C) and ethanol (78.37°C).

4.2.2 Preparation of ChCl-water DES.

DES were prepared from combination of HBA (ChCl, ChBr, ChI) and HBD (water, methanol, ethanol) at a molar ratio of 1:4. The combined mixtures were heated at 60˚C for about 15-45 minutes. The solution was stirred constantly at 600 rpm under atmospheric pressure until a clear homogenous solution was obtained. The prepared DES were cooled at room temperature and then kept in sealed laboratory vials and stored in a desiccator to prevent moisture absorption. High-precision analytical balance was used to weigh all the components, and the synthesis was done in a fume hood. The synthesized DES were used without further purification.

4.2.2 Approach

4.2.2.1 Density

The density of the DES was determined using the gravimetric approach. The mass of an empty 5 ml Erlenmeyer flask was recorded using Mettler Toledo analytical balance

(Columbus, OH). The DES sample was then carefully added to the flask, and its combined mass (container + DES) was measured. From the mass and volume measurements, the density of the DES sample was calculated using the formula:

Density = Mass / Volume. Densities were determined in triplicate.

4.2.2.2 pH

pH measurement of the DES was performed using the Accumet AB 15 Plus pH meter. To ensure accurate pH measurements, the pH meter was calibrated before the analysis. Calibration was carried out using standard buffer solutions with known pH values (4, 7, and 10). Following calibration, a small volume of the DES sample was transferred into a clean glass container. The glass electrode of the Accumet AB 15 Plus pH meter was carefully inserted into the DES sample, ensuring that the electrode was fully submerged and surrounded by the solvent. The pH meter was allowed to stabilize, and the pH value of the DES sample was recorded from the digital readout of the instrument.

4.2.2.3 Degradation studies

In order to measure the activity of the solvent in stabilizing ASA, a calibration curve was constructed using known concentrations of salicylic acid. The curve generated absorbance spectra that facilitated the identification of the maximum absorption point. The wavelength was subsequently adopted as the reference point for measuring the amount of salicylic acid produced from the ASA sample.

The stability assessment was initiated by dissolving a predetermined amount of ASA in ethanol. A portion of this solution was then combined with the solvent samples. At specific intervals, a sample was extracted from this mixture, and ferric chloride was added. The absorbance of the resulting solution was measured at the reference wavelength. The

recorded absorbance was then translated into a concentration value using the best-fit line derived from the calibration curve.

4.3 Structural-Activity Effects of Changing HBD

The impact of varying the HBD on the structure and properties of DES was evaluated through a comprehensive analysis of physicochemical properties and infrared (IR) spectroscopy. This multi-faceted approach provides a holistic understanding of the influence of HBD variation on DES structure and functionality.

Physicochemical properties such as density, pH, and polarity (as measured using solvatochromic parameters) are critical descriptors of the solvent's structure. These properties are largely dictated by the nature of the HBD and its interaction with the HBA within the DES.

Infrared (IR) spectroscopy, on the other hand, offers a direct probe into the molecular structure of the DES. The IR spectra can reveal the presence of specific functional groups and their interactions, including hydrogen bonding. By comparing the IR spectra of the DES with different HBDs, subtle changes in the hydrogen bonding network and overall molecular structure can be discerned. For instance, shifts in the OH stretching frequencies can indicate changes in the strength and nature of the hydrogen bonds within the DES.

The combination of physicochemical property evaluation and infrared (IR) spectroscopy offers an extensive understanding of the influence exerted by the variation of HBD on the structure and properties of DES. This approach establishes a crucial link between the inherent activity of the solvent and the degradation kinetics of acetylsalicylic acid, as quantified by the degradation rate constant (k).

4.3.1 Solvents Structural Changes as A Result of HBD Changes Based on Physicochemical Measurements

Physicochemical measurements consisting of density, pH, solvatochromic parameters were used to measure the structural changes from HBD variation (Table 4.1 and 4.2). The results revealed that, ChCl-water DES exhibited the highest density among the group (Table 4.1). This could be attributed to the strong hydrogen bonding interactions between ChCl and water molecules, resulting in a more densely packed molecular structure. Water is a better HBD compared to methanol and ethanol and thus, it can form stronger hydrogen bonding interactions than methanol and ethanol. The pH for ChCl-water exhibited a neutral pH (Table 4.1).

Table 4.1

Results for solvent physicochemical properties measurement using ChCl as HBA

DES	Alpha	Beta	Pi	Density	pH
ChCl-water	0.952328	0.345381	0.863227	1.071167	7.015
ChCl-ethanol	0.900827	0.56004	0.847783	0.897667	6.886
ChCl-methanol	0.797992	0.56004	0.880529	0.9527	7.469
water	0.866596	0.66355	1.299685	0.997089	6.833
methanol	0.76947	0.531419	0.847783	0.785156	6.305
Ethanol	0.681381	0.56004	0.797163	0.767044	6.27

In contrast, both ChCl-ethanol and ChCl-methanol DES displayed lower densities compared to their water-based counterpart (Table 4.1). This could be due to the less extensive hydrogen bonding network in these DES, leading to a less densely packed structure.

The simple solvents - water, methanol, and ethanol - showed lower densities compared to the DES. This is expected as the DES are combinations of these HBDs with ChCl, which inherently has a higher molecular weight.

When ChBr was used as the HBA, similar trends were observed. The ChBr-water DES had the highest density value (1.2267 g/ml), indicating strong hydrogen bonding interactions (Table 4.2).

Table 4.2

Results for solvent physicochemical properties measurement using ChBr as HBA

DES	Alpha	Beta	Pi	Density	pH
ChBr-ethanol	0.767731	0.345381	1.056739	0.886067	6.656
ChBr-water	0.933131	0.345381	1.195228	1.2267	6.992
ChBr-methanol	0.754012	0.56004	1.181076	1.067967	6.122
water	0.866596	0.66355	1.299685	0.997089	6.833
methanol	0.76947	0.531419	0.847783	0.785156	6.305
Ethanol	0.681381	0.56004	0.797163	0.767044	6.27

For solvatochromic parameter measurement (Figure 4.2), the Alpha parameter, indicative of the hydrogen bond donating ability, was found to be highest for the ChCl-water DES (0.95232772), suggesting a strong hydrogen bond donating ability. This was followed by ChCl-ethanol (0.900827458), ChCl-methanol (0.797992154), water (0.866595874), methanol (0.769469696), and ethanol (0.681381364).

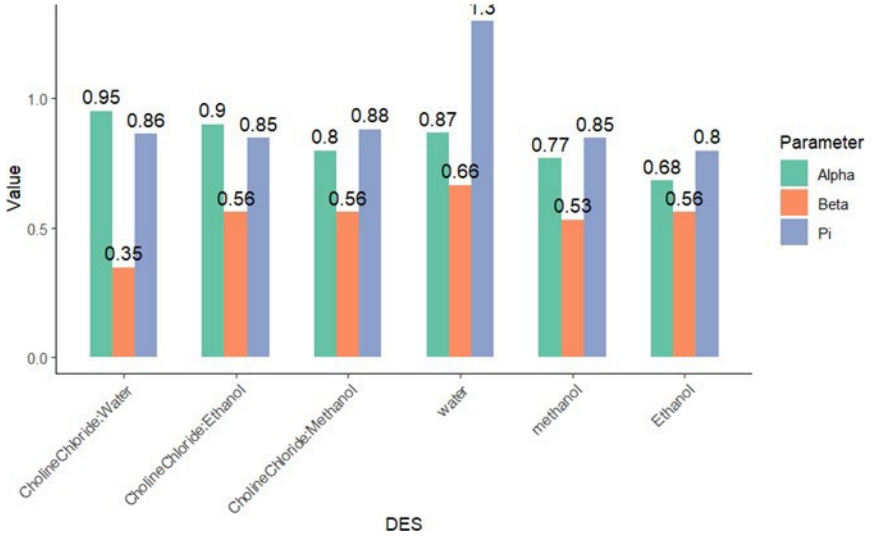


Figure 4.2. Solvent solvatochromic parameters measurement with ChCl as HBA indicative of solvent polarity.

The Beta parameter, indicative of the hydrogen bond accepting ability, was found to be similar for ChCl-ethanol and ChCl-methanol (both approximately 0.56) (Figure 4.2), suggesting comparable hydrogen bond accepting abilities. The similar Beta values for ChCl-ethanol and ChCl-methanol suggest that these DES have similar abilities to accept hydrogen bonds.

The Pi parameter, indicative of the dipolarity/polarizability, was found to be highest for water (1.299685424), followed by ChCl-methanol (0.880528641), ChCl-water (0.86322699), ChCl-ethanol (0.847782854), methanol (0.847782854), and ethanol (0.797163088).

A similar trend in solvatochromic parameters was again observed for ChBr-based DES (Figure 4.3). ChBr-water had the highest acidity value of 0.933130603, indicating a strong ability to donate hydrogen bonds. This can be attributed to the inherent polarity of water and its ability to form extensive hydrogen bonding networks. Both ChBr-ethanol and ChBr-

methanol DES exhibited lower alpha values, indicating a weaker hydrogen bond donating ability compared to the water-based DES. Interestingly, for the basicity parameter, ChBr-methanol DES showed a higher value. For the dipolarity/polarizability parameter, ChBr-water exhibited the highest value, indicating a high level of dipolarity/polarizability.

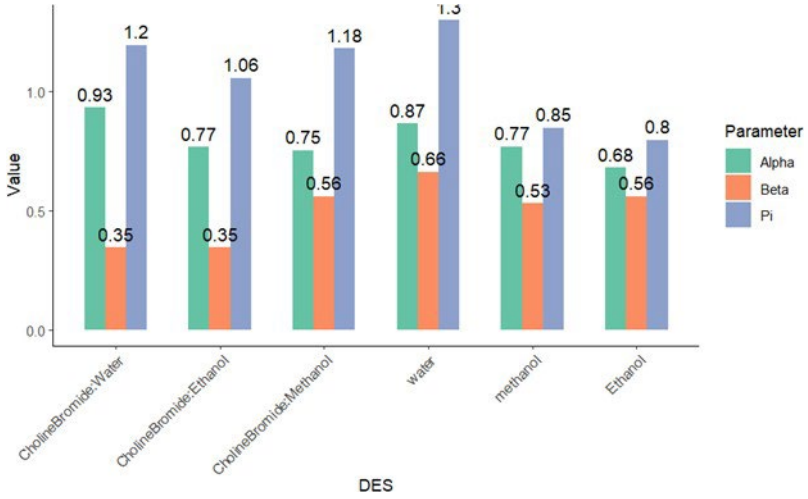


Figure 4.3. Solvent solvatochromic parameters measurement with ChBr as HBA indicative of solvent polarity.

In order to reveal similarities/dissimilarities of the solvent based on the physicochemical measurements, clustering analysis was used. The clustering results revealed methanol and ethanol clustered together (Figure 4.4), suggesting that these two solvents have similar physicochemical properties. This is not surprising given that both are alcohols and share similar molecular structures, with both having a polar hydroxyl (-OH) group that can form hydrogen bonds, and a nonpolar alkyl (R-) group. Furthermore, the DES ChCl-ethanol and ChCl-methanol were also clustered together. This suggests that the addition of ChCl to these alcohols results in DES with similar physicochemical properties. This could be attributed to the similar hydrogen bonding capabilities of ethanol and methanol, which

could result in similar interactions with ChCl. The clustering of ChCl-ethanol and ChCl-methanol together suggests that these DES may have similar solvent properties, potentially making them interchangeable in certain applications.

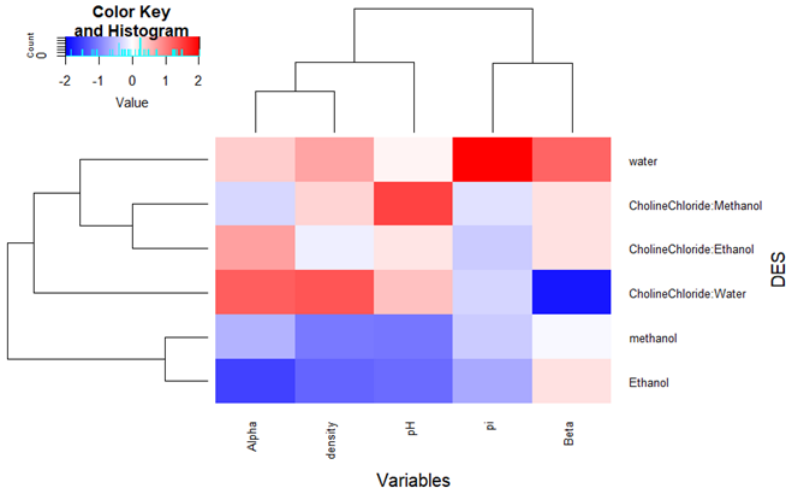


Figure 4.4. Solvent similarities/dissimilarities based on physicochemical parameters as a result of variations in HBD with ChCl as HBA. Red indicates higher values; white indicates median values and blue indicates lower values.

A similar clustering results was also observed when ChBr was used as HBA. ChBr-ethanol and ChBr-methanol clustered together indicating similar solvent properties. Whereas ChBr-water clustered together with water as well (Figure 4.5).

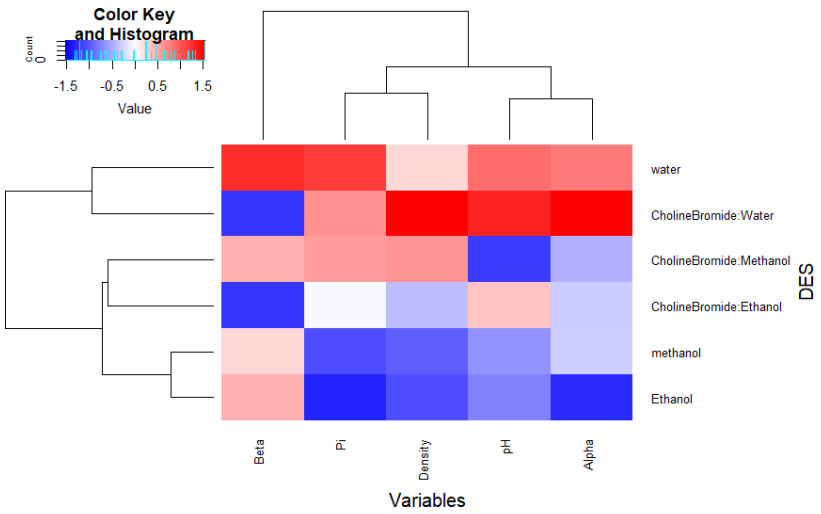


Figure 4.5. Solvent similarities/dissimilarities based on physicochemical parameters as a result of variations in HBD with ChCl as HBA. Red indicates higher values; white indicates median values and blue indicates lower values.

4.3.2 Solvents Structural Changes as a Result of HBD Changes as Reflected in IR Spectra

The structural changes in solvents as a result of variations in HBD were investigated using Infrared (IR) Spectroscopy, with ChCl and ChBr serving as the HBA.

When ChCl was the HBA and the HBD varied between water, methanol, and ethanol, distinct patterns were observed in the IR spectra. Both ChCl-water and ChCl-ethanol exhibited broader peaks in the OH vibration region, indicative of stronger hydrogen bonding due to the formation of a more extensive hydrogen bonding network (Figure 4.6). Conversely, ChCl-methanol, methanol, and ethanol displayed narrower peaks, suggesting weaker hydrogen bonding, possibly due to less extensive hydrogen bonding networks.

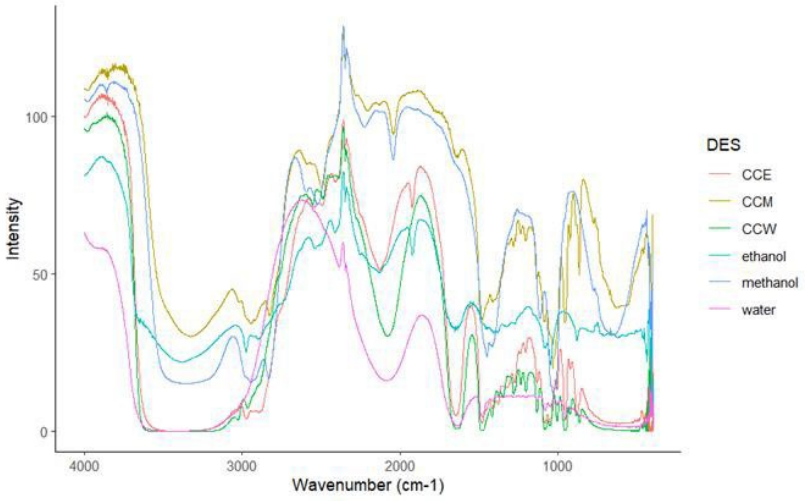


Figure 4.6. IR Spectra for solvents with ChCl as HBA

Clustering analysis of the IR spectra further corroborated these observations (Figure 4.7). ChCl-water and ChCl-ethanol formed a distinct cluster, suggesting structural similarities likely attributed to similar hydrogen bonding patterns. In contrast, ChCl-methanol clustered with methanol, indicating structural similarities and possibly suggesting the absence of significant interactions between ChCl and Methanol. This could imply that a eutectic solvent may not have been formed in this case.

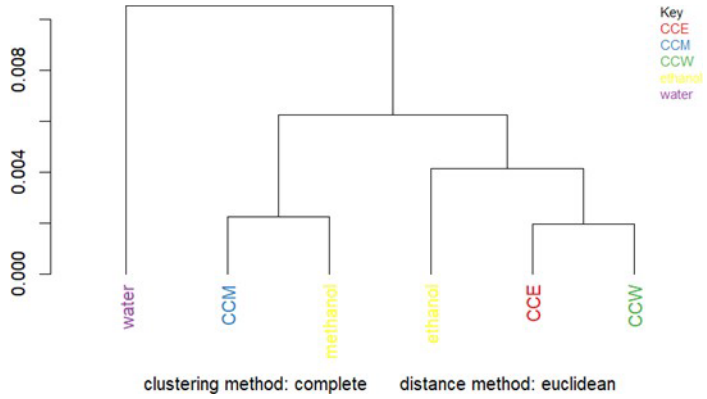


Figure 4.7. Clustering Analysis based on the IR spectra to reveal structural similarities/dissimilarities of the solvent with ChCl as HBA.

A similar trend was observed when ChBr was used as the HBA. Both methanol and ethanol displayed narrower peaks at the OH vibration region, mirroring the observations with ChCl (Figure 4.8).

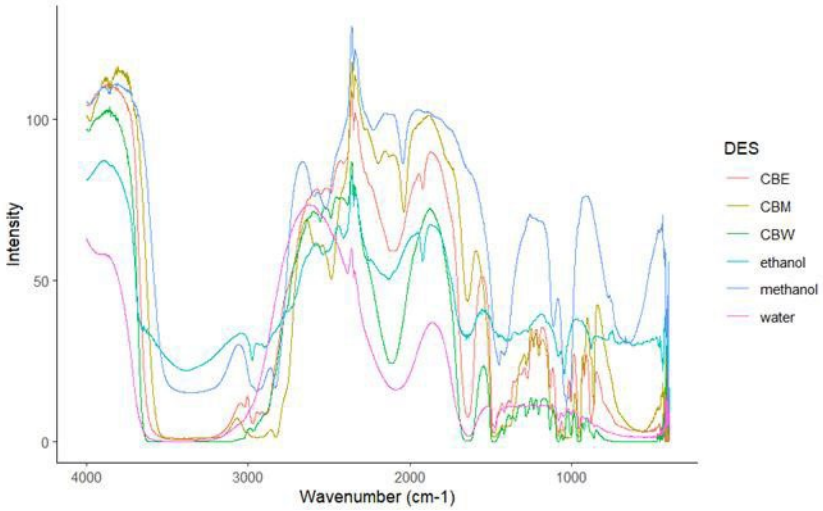


Figure 4.8. IR Spectra for solvents with ChBr as HBA

The clustering analysis also paralleled the previous trend (Figure 4.9). ChBr-water and ChBr-ethanol formed a cluster, indicating structural similarities. Meanwhile, ChBr-methanol clustered with methanol, again suggesting structural similarities and potentially the absence of a formed eutectic solvent.

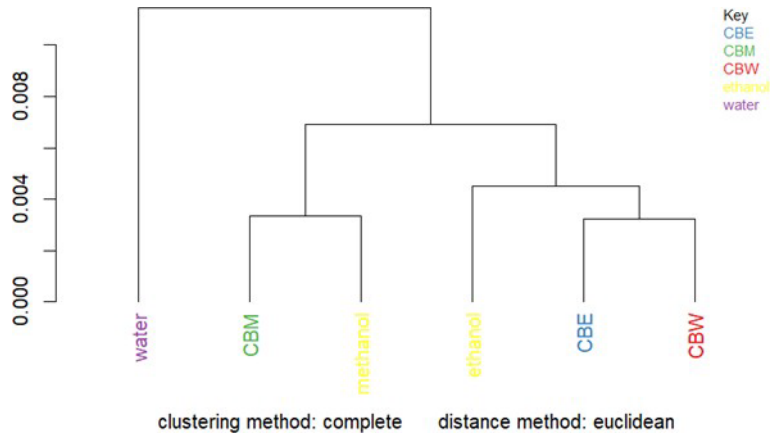


Figure 4.9. Clustering Analysis based on the IR spectra to reveal structural similarities/dissimilarities of the solvent with ChBr as HBA.

These findings highlight the significant role of the HBD in determining the structure and properties of DES, and the potential to tune these properties by careful selection of the HBD.

4.3.3 Influence of Solvent Structural Changes as A Result of HBD Changes On Activity

The influence of solvent structural changes, as a result of variations in the HBD, on the stability of acetyl salicylic acid was investigated. The findings revealed a significant correlation between HBD and the stability of acetyl salicylic acid. DES where water served as the HBD, acetyl salicylic acid demonstrated the highest stability This high stability effect

of water as HBD was demonstrated in solvents where ChCl and ChBr served as HBA (Figure 4.10, 4.11). This could be attributed to the strong hydrogen bonding network in these DES, which could potentially stabilize the acetyl salicylic acid molecule and reduce its degradation.

Conversely, the stability of acetyl salicylic acid was found to be the lowest in simpler solvents such as methanol, ethanol, and water alone (Figure 4.10A and B). These solvents, lacking the complex hydrogen bonding network present in DES, may not provide the same level of stabilization for acetyl salicylic acid, leading to increased degradation. These observations underscore the pivotal role of the HBA-HBD pairing in the stability of acetyl salicylic acid. The enhanced stability of acetyl salicylic acid in DES, particularly those with water as the HBD, suggests that the unique intermolecular interactions facilitated by the HBA-HBD pairing in DES could play a crucial role in enhancing the stability of pharmaceutical compounds.

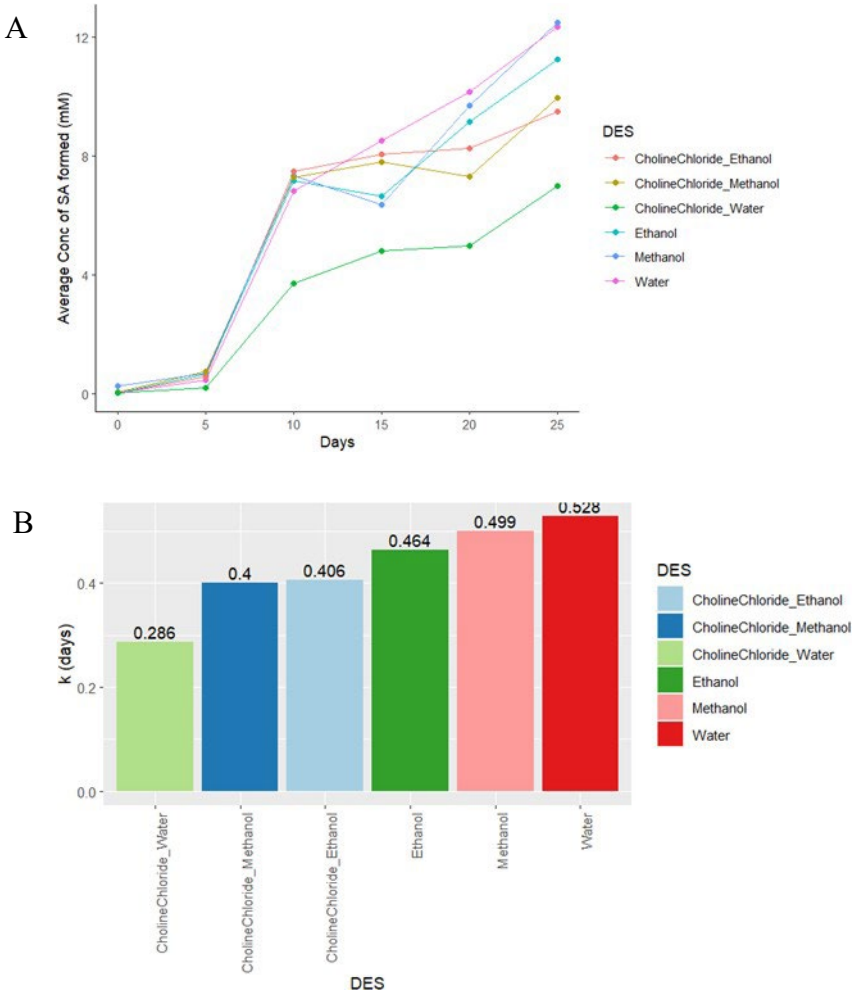


Figure 4.10. Stability of acetyl salicylic acid in solvents with ChCl as HBA. A) Measurement of salicylic acid formed overtime B) The rate constant, extrapolated from the zero order of the solvent measurement of the solvent. The rate constant is the index of activity.

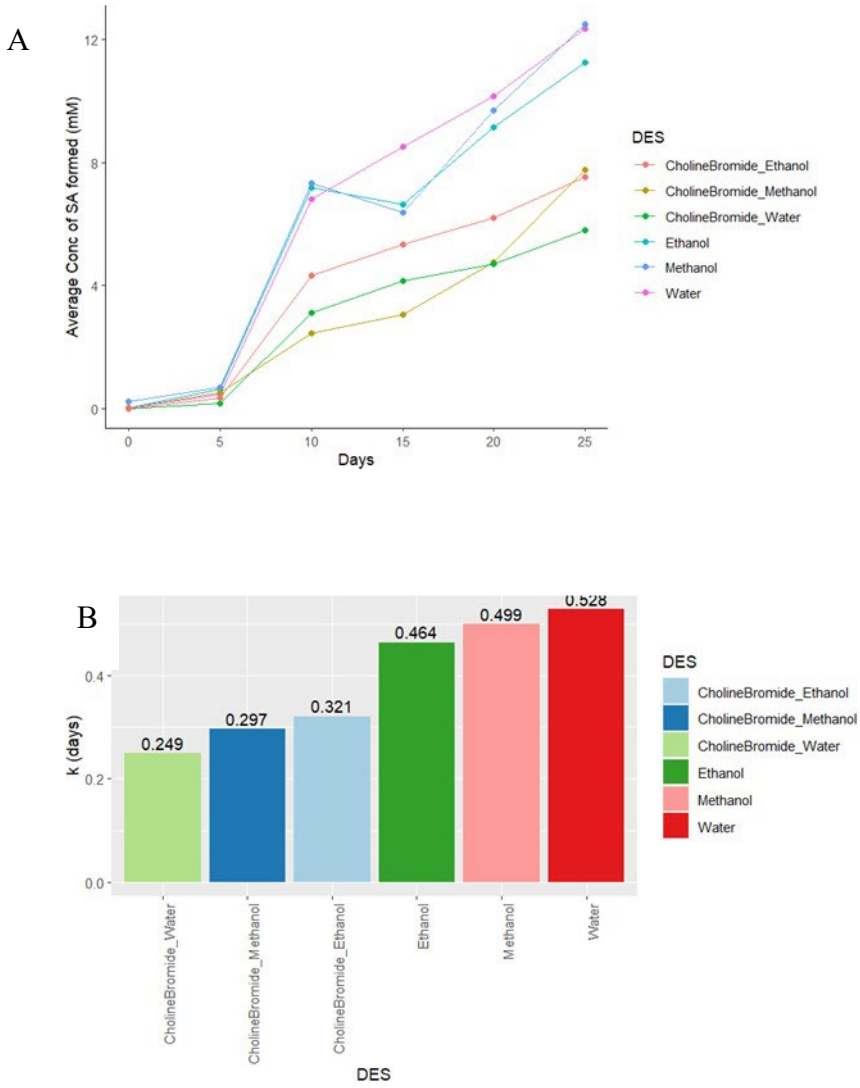


Figure 4.11. Stability of acetyl salicylic acid in solvents with ChBr as HBA. A) Measurement of salicylic acid formed overtime B) The rate constant, extrapolated from the zero order of the solvent measurement of the solvent. The rate constant is the index of activity.

The activity of the solvents, as measured by the degradation constant of acetyl salicylic acid, was found to have a significant correlation with both the basicity and density of the solvents (Figure 4.12). A positive correlation was observed between the activity and the basicity of the solvents. This suggests that solvents with a higher basicity, which indicates a stronger ability to accept hydrogen bonds, tend to enhance the stability of acetyl salicylic

acid. Acetyl salicylic acid (ASA) has both a carboxyl group (COOH) and an ester group (RCOOR). The carboxyl group can serve as both HBD and HBA. Thus, in the presence of a basic solvent, which can accept these hydrogen bonds, hydrogen bonding is established leading to ASA experiencing stability.

A negative correlation was observed between the activity and the density of the solvents. This suggests that solvents with a higher density, which is indicative of a more densely packed molecular structure, tend to decrease the stability of acetyl salicylic acid. Eutectics with water as the HBD had the highest density. This underscores the fact that water, being a better proton donor due to its high polarity and ability to form multiple hydrogen bonds, can facilitate the formation of a more extensive hydrogen bonding network in the DES. This leads to a higher density of the solvent, which could potentially limit the mobility of acetyl salicylic acid molecules and increase their susceptibility to degradation, as reflected in the lower degradation constant (k) values.

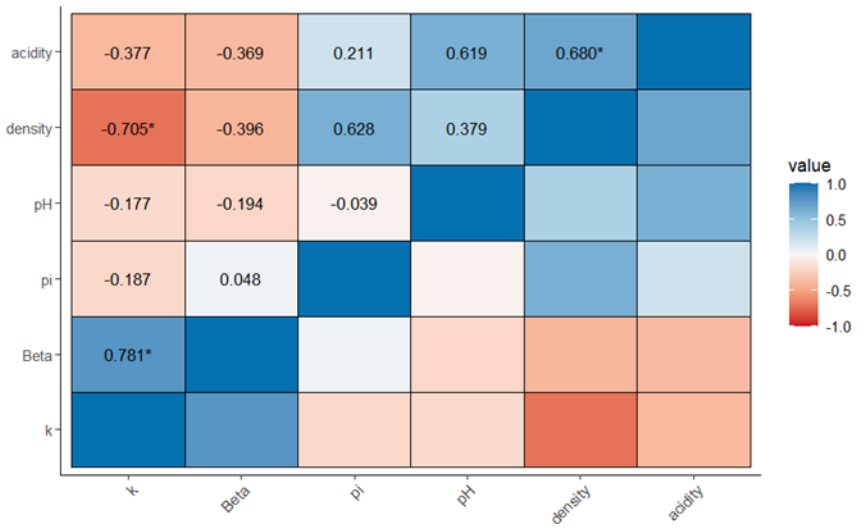


Figure 4.12. Correlation of rate constant, k (activity) with physicochemical parameters of the solvent

4.4 Structural-Activity Effects of Changing HBA

In DES, the choice of HBA can also influence the solvent structure. The HBA can influence a range of characteristics, including the solvent's density, pH, and solvatochromic parameters. These, in turn, can impact the solvent's activity, including its ability to stabilize or destabilize certain compounds. In this section, we delve into the structural-activity effects of changing the HBA. We explore how alterations in the HBA can lead to significant changes in solvent properties and discuss the implications of these changes for the solvent's activity. This analysis provides valuable insights into the role of the HBA in modulating solvent behavior and highlights the importance of careful HBA selection in achieving desired outcomes in various applications.

4.4.1 Solvents Structural Changes as A Results of HBA Changes Based On Physicochemical Measurements

The physicochemical measurements reveal interesting insights into the structural changes in DES as a result of varying the HBA (Table 4.3 and 4.4).

Table 4.3

Physicochemical property measurement of solvent with water as HBD

DES	Alpha	Beta	Pi	Density	pH
ChI-water	0.866596	0.738691	1.299685	1.3544	6.085
ChBr-water	0.933131	0.345381	1.195228	1.2267	6.992
ChCl-water	0.952328	0.345381	0.863227	1.071167	7.015
water	0.866596	0.66355	1.299685	0.997089	6.833

Table 4.4

Physicochemical property measurement of solvent with ethanol as HBD

DES	Alpha	Beta	Pi	Density	pH
ChI-ethanol	0.942145	0.765127	1.181076	1.150467	6.342
ChBr-ethanol	0.767731	0.345381	1.056739	0.886067	6.656
ChCl-ethanol	0.900827	0.56004	0.847783	0.897667	6.886
Ethanol	0.681381	0.56004	0.797163	0.767044	6.27

When water served as the HBD (Figure 4.13), the Alpha parameter, indicative of the hydrogen bond donating ability, was found to be highest for ChCl-water (0.952328), followed by ChBr-water (0.933131), and ChI-water (0.866596). This suggests that the hydrogen bond donating ability of the DES increases with the size of the halide ion in the HBA, with chloride > bromide > iodide (Figure 4.13). This could be attributed to the increasing polarizability of the larger halide ions, which enhances their ability to participate in hydrogen bonding. The Beta parameter, indicative of the hydrogen bond accepting ability, was highest for ChI-water (0.738691), suggesting a strong hydrogen bond accepting ability. This was followed by water (0.66355), ChCl-water (0.345381), and ChBr-water (0.345381). The high Beta value for ChI-water suggests that iodide, being a larger and more polarizable ion, is a better HBA. The Pi parameter, indicative of the dipolarity/polarizability, was found to be highest for water (1.299685), followed by ChI-water (1.299685), ChBr-water (1.195228), and ChCl-water (0.863227). The high Pi value for water suggests a high dipolarity/polarizability, which could be attributed to the polar nature of water.

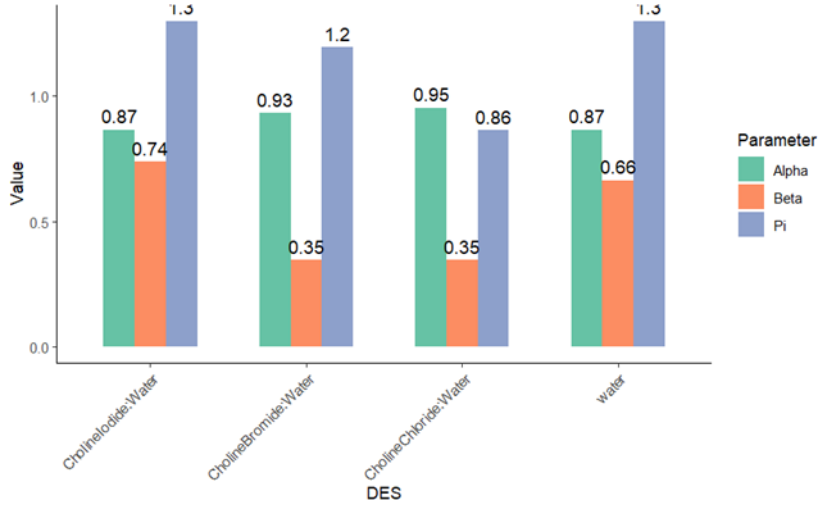


Figure 4.13. Solvatochromic parameters for solvents with Water as HBD

When ethanol served as the HBD (Figure 4.14) the Alpha parameter was highest for ChI-ethanol (0.942145), followed by ChCl-ethanol (0.900827), and ChBr-ethanol (0.767731). The Beta parameter was highest for ChI-ethanol (0.765127), followed by ChCl-ethanol (0.56004), and ChBr-ethanol (0.345381). The Pi parameter was highest for ChI-ethanol (1.181076), followed by ChBr-ethanol (1.056739), and ChCl-ethanol (0.847783).

In both cases, the density of the DES increased with the size of the halide ion in the HBA, while the pH was found to be slightly acidic for all DES. These findings provide a comprehensive understanding of the influence of HBA variation on the structure and properties of DES.

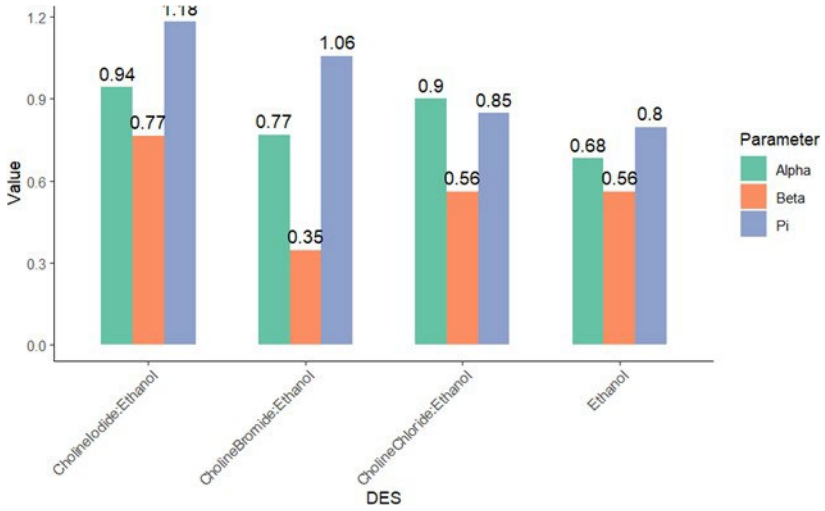


Figure 4.14. Solvatochromic parameters for solvents with ethanol as HBD

In clustering analysis where water was used as the HBD (Figure 415), a distinct clustering pattern was observed. Specifically, the DES ChCl-water and ChBr-water formed a distinct cluster (Figure 4.15). This suggests that these two DES share similar structural and physicochemical properties, likely due to the similar hydrogen bonding patterns facilitated by water as the HBD. On the other hand, ChI-water and water formed a separate cluster, indicating that these solvents exhibit different structural characteristics compared to the ChCl-water and ChBr-water cluster.

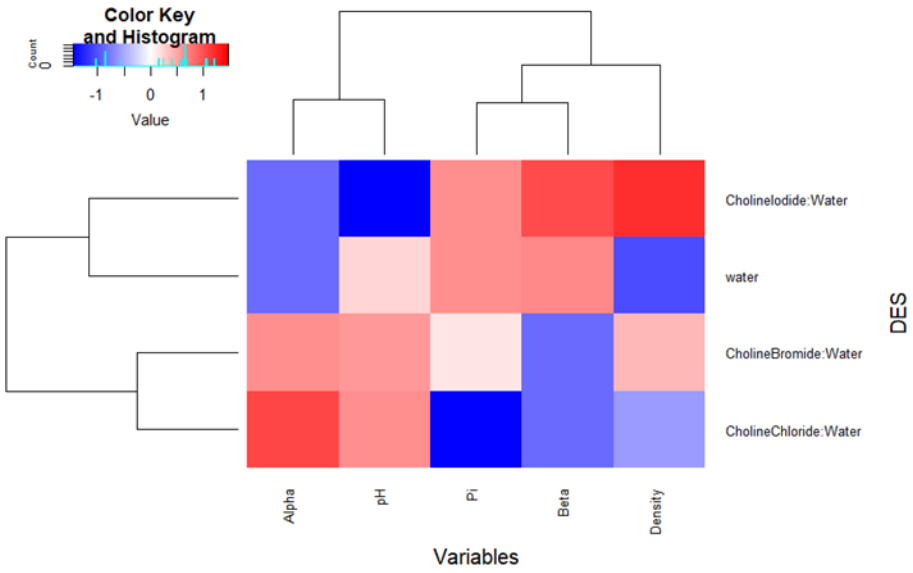


Figure 4.15. Clustering analysis to reveal the similarities in physicochemical properties of the solvent with water as HBD. Red indicates higher values; white indicates median values and blue indicates lower values.

Similarly, when ethanol was used as the HBD (Figure 4.16), ChCl-ethanol and ChBr-ethanol formed a distinct cluster, suggesting that these DES share similar structural and physicochemical properties. This clustering pattern underscores the influence of the choice of HBD on the properties and structure of the resulting DES. The observed clustering patterns provide valuable insights into the role of HBD in shaping the properties of DES.

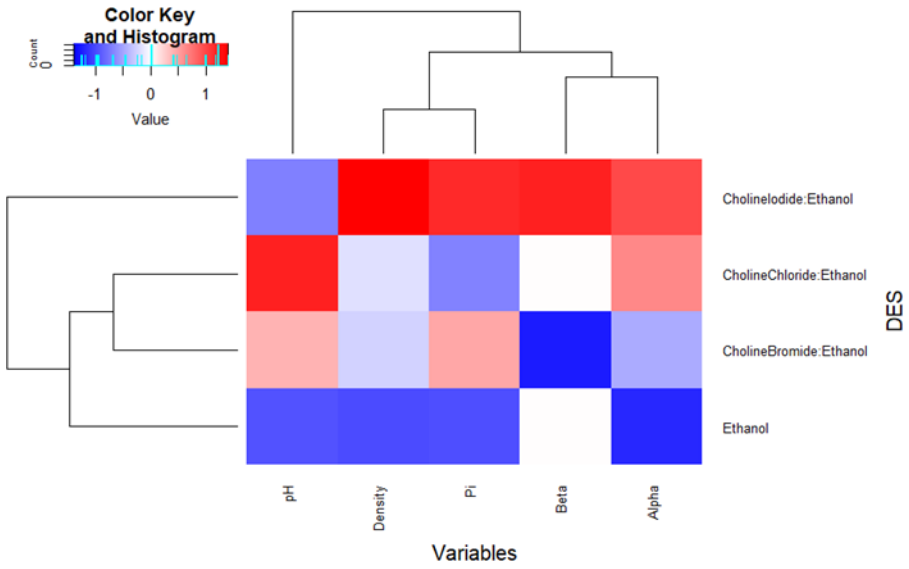


Figure 4.16. Clustering analysis to reveal the similarities in physicochemical properties of the solvent with ethanol as HBD. Red indicates higher values; white indicates median values and blue indicates lower values.

4.4.2 Solvents Structural Changes as a Result of HBA Changes as Reflected in IR Spectra

IR spectra revealed structural disparities between solvents. Using water as the HBD, all DES revealed a consistent breadth in the OH vibration domain, indicative of robust hydrogen bonding (Figure 4.17).

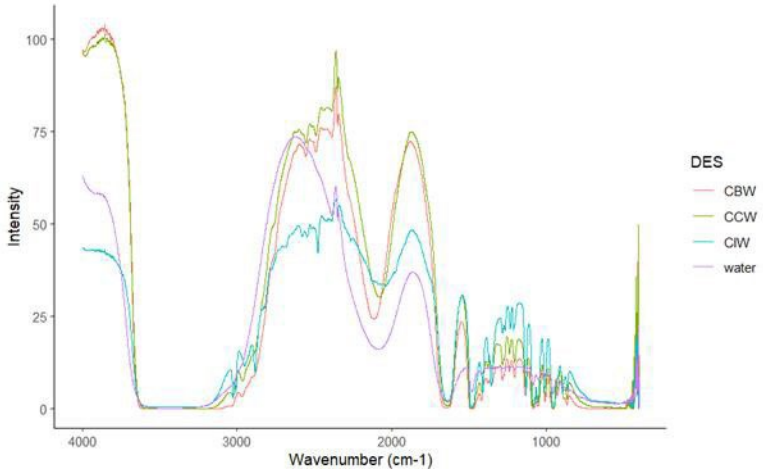


Figure 4.17. IR spectra of solvents with water as HBD.

A more detailed distinction emerged from the clustering analysis based on IR spectra. ChCl-water and ChBr-water formed a unique cluster, differentiating them from water (Figure 4.18). This hints at the intrinsic structural attributes of these DES, potentially shaped by the specific HBA-HBD interaction. This clustering pattern concurs with physicochemical analysis, where these DES exhibited similar grouping, emphasizing the impact of HBA variations on the DES's structural characteristics.

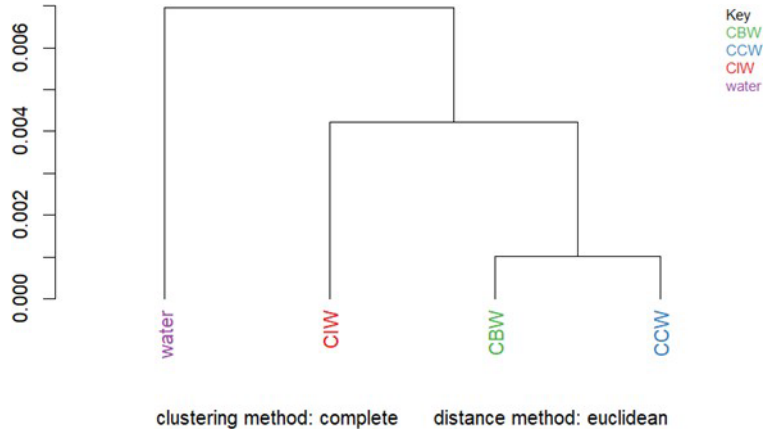


Figure 4.18. Clustering analysis of solvents with water as HBD based on the IR spectra

Conversely, with ethanol as the HBD (Figure 4.19), all DES also presented with an enlarged OH peak compared to pure ethanol, pointing to enhanced hydrogen bonding.

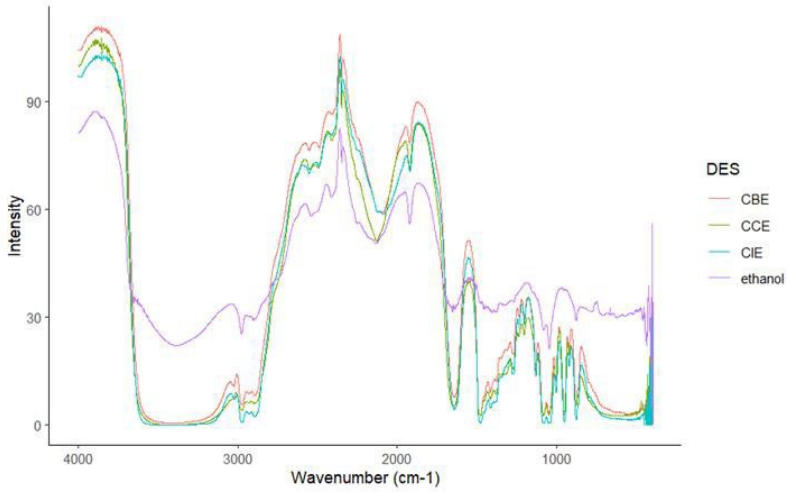


Figure 4.19. IR spectra of solvents with ethanol as HBD

Clustering analysis revealed ChI-ethanol and ChBr-ethanol emerged as a distinct cluster, separate from ethanol (Figure 4.20). This suggests that, despite sharing the same HBD, these DES possess unique structural features stemming from HBA diversity. This observation aligns with the outcomes from the physicochemical clustering, underscoring the correlation between structural nuances evident in IR spectra and the inherent properties of the DES.

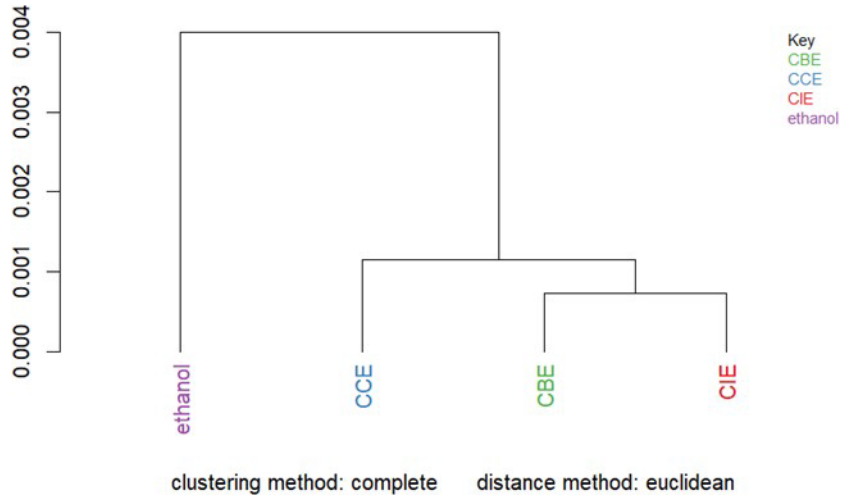


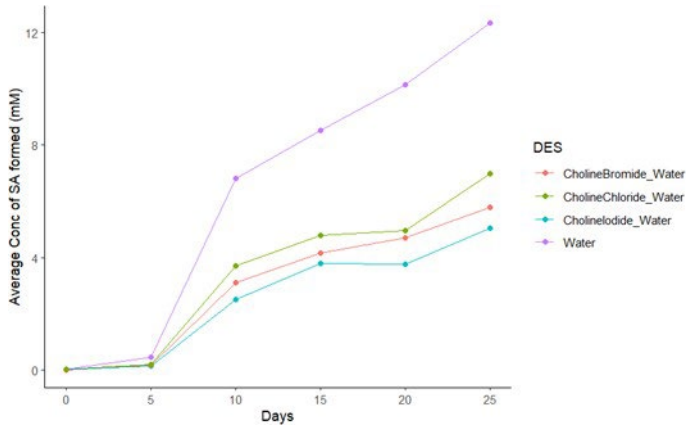
Figure 4.20. Clustering analysis of solvents with ethanol as HBD based on the IR spectra.

4.4.3 Influence of Solvent Structural Changes as A Result Of HBA Changes On Activity

The stability of acetyl salicylic acid revealed intriguing correlations when examined in the context of solvent structural variations. These correlations were particularly noticeable when considering the variations in the HBA while keeping the HBD constant.

When water served as the HBD, the stability of acetyl salicylic acid was found to be highest in the ChI-water, followed by ChBr-water and ChCl-water (Figure 4.21A and B). The least stability was observed in water.

A



B

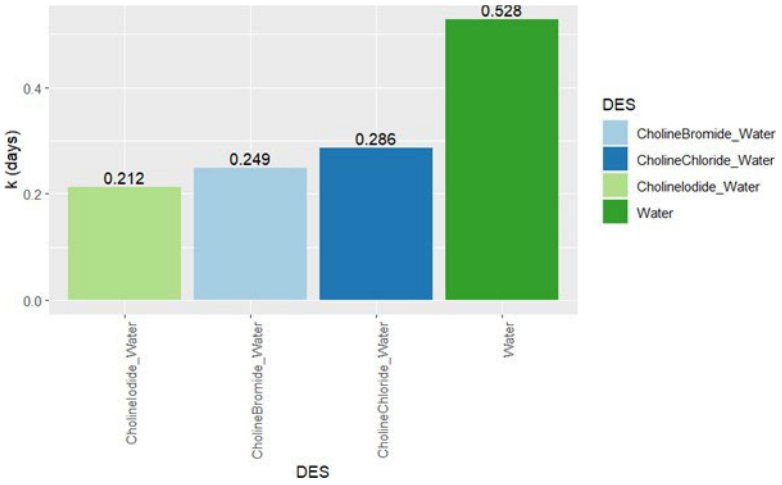


Figure 4.21. Stability of acetyl salicylic acid in solvents with water as HBD. A) Concentration of salicylic acid formed over time. B) Rate of degradation of acetyl salicylic acid in solvent

A similar trend was observed when ethanol was employed as HBD. The stability of acetyl salicylic acid was greatest in the ChI-ethanol, followed by ChBr-ethanol and ChCl-ethanol, with the least stability observed in pure ethanol (Figure 4.22).

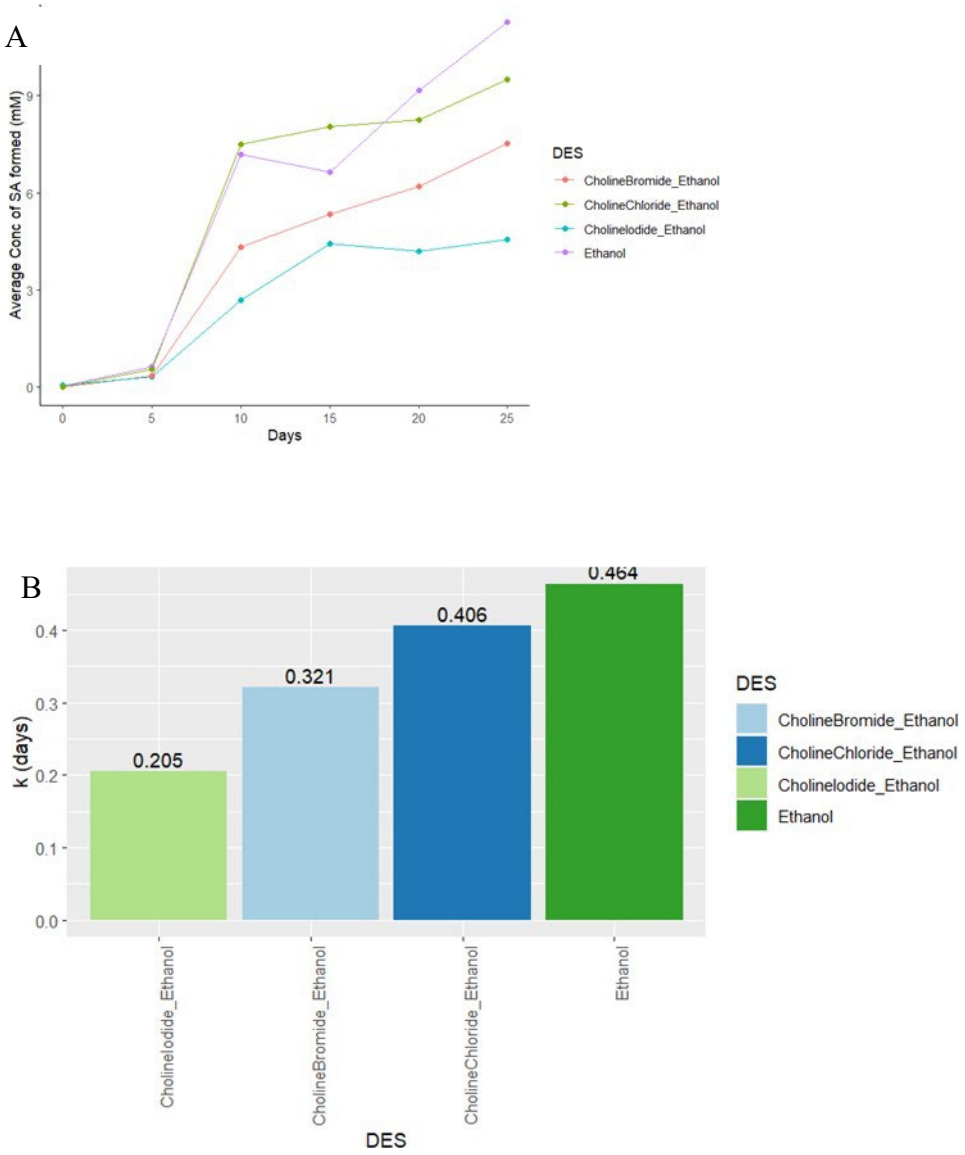


Figure 4.22. Stability of acetyl salicylic acid in solvents with ethanol as HBD. A) Concentration of salicylic acid formed over time. B) Rate of degradation of acetyl salicylic acid in solvent

The observed trend underscores the significance of hydrogen bonding in contributing to the stability of acetyl salicylic acid in the solvent. In a hydrogen-bond-rich environment, ASA molecules are better encapsulated, shielded from potential degradation triggers, enhancing their stability. Particularly, the presence of ChI or ChBr creates a more protective

environment for ASA. This enhanced protection, especially in the presence of ChI or bromide, stems from a richer and more protective hydrogen bonding network than what is available in pure water. Again, the nature of the HBA further elucidates this trend. ChI, with its larger and more polarizable iodide ion, can induce stronger dispersion forces and form more effective hydrogen bonds than ChCl. Consequently, a DES with ChI can potentially furnish a more favorable environment for ASA's stability. ChBr, being intermediate in size and polarizability between iodide and chloride, also provides an environment that reflects its position in the observed stability trend.

In comparison, water, while being a proficient HBD/acceptor, lacks the supplementary stabilizing interactions inherent to DES. As a result, ASA dissolved in pure water misses out on the protective hydrogen bonding network evident in DES, potentially leading to its reduced stability or expedited degradation.

Correlation analysis revealed a significant negative correlation between the activity and density of the solvents (Figure 4.23). This suggests that as the density of the solvent increases, the activity decreases. Density can be linked to the extent of hydrogen bonding within the solvent. A higher density indicates a more densely packed molecular structure, which is often a result of extensive hydrogen bonding. Hydrogen bonds can restrict the mobility of molecules within the solvent, potentially leading to a decrease in activity.

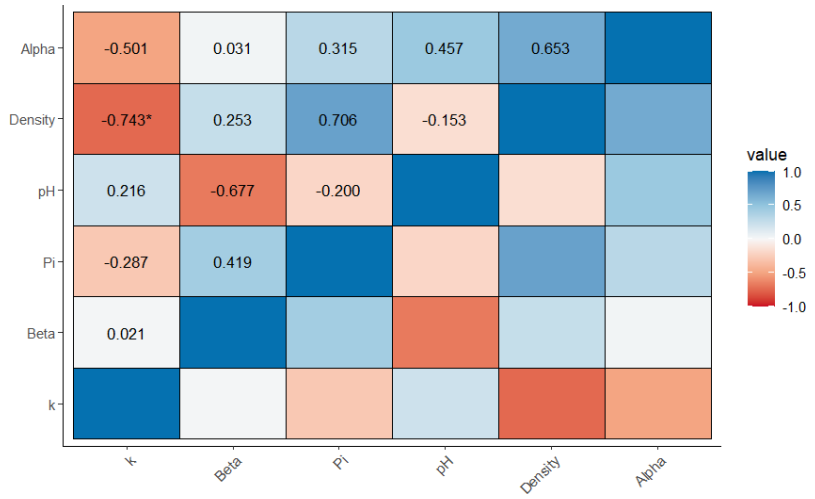


Figure 4.23. Correlation analysis of measured physicochemical parameters with the rate of degradation.

These findings highlight the critical role of the HBA-HBD pairing in the stability of pharmaceutical compounds within DES. The unique intermolecular interactions facilitated by the HBA-HBD pairing in DES could play a crucial role in enhancing the stability of pharmaceutical compounds, providing valuable insights for the design of more effective and efficient pharmaceutical solvents.

4.5 Summary

The study of DES and their structural-activity relationships revealed significant insights into the effects of changing HBD and HBA. When the HBD varied while the HBA remained constant, distinct structural differences were observed in the solvents. The choice of HBD influenced the extent of hydrogen bonding, as reflected in the width of the OH vibration peaks in the IR spectra. Solvents with stronger hydrogen bonding, such as those where water served as the HBD, displayed broader peaks. These structural changes had a direct impact on the stability of acetyl salicylic acid, with the compound showing the

highest stability in DES where water was the HBD. Similarly, changing the HBA while keeping the HBD constant led to notable structural variations in the solvents. Regardless of whether water or ethanol was used as the HBD, the DES exhibited unique structural attributes compared to the pure solvents. These structural variations were found to influence the stability of acetyl salicylic acid within the solvents. The stability was highest in the DES and lowest in the simpler solvents such as methanol, ethanol, and water alone. Furthermore, correlation analysis revealed a significant negative correlation between solvent density and activity, suggesting that solvents with a higher density, indicative of more extensive hydrogen bonding, tend to decrease the stability of acetyl salicylic acid. In conclusion, both the choice of HBD and HBA in DES play a crucial role in determining the solvents' structural characteristics and their activity. These findings underscore the importance of careful selection of HBD and HBA in the design of DES for specific applications.

CHAPTER 5. CONCLUSION AND SUMMARY

The quest for eco-friendly and alternative solvents has gained momentum due to rising concerns about the safety, environmental, and health risks associated with certain traditional solvents. Consequently, stringent regulations have been enacted to limit the use of these potentially hazardous substances. Among the burgeoning alternatives, DES have emerged as a promising candidate. The surge in research focusing on the practical applications of DES further underscores their significance.

An important characteristic of DES is their remarkable tunability. By carefully selecting and combining various components in specific ratios, the physicochemical properties of the DES can be modulated to cater to distinct applications. Central to the formation of DES is the intricate interplay of HBA and HBD, with hydrogen bonding serving as the driving force.

This project pivots around the inherent tunability of DES. It postulates that by altering the components and their ratios in DES, the solvent's structure or the degree of hydrogen bonding can be altered. The solvent structural changes are manifested in physicochemical measurements as well as in IR and NMR spectral data. Moreover, these structural shifts, resulting from the inherent tunability, are hypothesized to significantly impact the solvent's functional behavior in a structure-activity relationship. As such in this project, DES were formulated followed by characterization using physicochemical measurements. Advanced spectroscopic techniques, including Infrared (IR) and Nuclear Magnetic Resonance (NMR), were employed to provide structural changes within the solvent as a result of alteration in the components. The project employed the use of correlation and clustering to reveal the similarities/dissimilarities between the solvents from the measured physicochemical

properties and spectroscopic information from IR and NMR. After, a structural-activity relationship was elucidated using the ability of the solvents to stabilize acetyl salicylic acid with the rate of degradation used as activity measurement. Lastly, correlation plots were utilized to provide information on which physicochemical properties is associated with the observed activity.

The first part of the project begun with the exploration of the influence of different mole ratios on the structure of the solvents and its activity. In this, ChCl-water in the ratios 1:2, 1:5 and 1:10 were selected as the DES. Physicochemical properties highlighted the significant changes in solvent behavior with variation in molar ratios. Specifically, increased ChCl concentrations led to variations in viscosity, basicity, and density. Such variations may play a crucial role in dictating the solubilizing capacity and stabilizing abilities of the solvent towards drugs. IR Spectroscopy provided evidence for the presence and varying strengths of hydrogen bonding within the different DES compositions. This was particularly evident in the OH vibration region, suggesting that ChCl-water in the ratios 1:2 and 1:5 had stronger hydrogen bonding compared to 1:10. NMR studies supported these findings, where downfield shifts in the trimethylammonium protons indicated the presence and extent of hydrogen bonding. Importantly, it was observed that the DES with ChCl-water at 1:2 had the most pronounced hydrogen bonding.

Drug stabilization studies using acetyl salicylic acid as a model revealed that a solvent with more robust hydrogen bonding, specifically ChCl-water 1:2, offered better protection against hydrolytic degradation. The implication of this finding is that the strength of hydrogen bonding in the solvent greatly influenced its physicochemical properties, which, in turn, played a pivotal role in determining the solvent's utility, particularly its drug-

stabilizing abilities. DES, especially those with pronounced hydrogen bonding, can offer enhanced stability to drugs prone to hydrolytic degradation. This is of considerable pharmaceutical significance, potentially paving the way for new drug formulations that can offer enhanced shelf life and efficacy. The overarching theme of this study was the structure-activity relationship. The findings highlighted a direct correlation between solvent structure (as influenced by HBD-HBA ratios) and its activities, chiefly its drug-stabilizing abilities.

For the second part, the purpose was to elucidate the influence of HBA/HBD variations on the structure and activity of the solvent. Structurally, it has been revealed that, the formation of DES is as a result of hydrogen bonding between the hydrogen of the HBD and the "halide" forming a bridge between the bonding of the protons from the HBD to the nitrogen on the choline moiety. From the structural perspective of hydrogen bonding in choline related DES, it is to be expected that variations in the HBA specifically with the halide can affect solvent structure and hydrogen bonding. Again, variations in the HBD can also influence solvent structure. For the HBA component, ChI, ChBr and ChCl were selected. In terms of size, iodide ions are larger than bromide ions, which are larger than chloride ions. Larger ions are generally more polarizable, meaning their electron clouds can be more easily distorted to create temporary dipoles that can interact with other molecules. This can increase their reactivity and ability to participate in non-covalent interactions like hydrogen bonding. For the HBD component, water, methanol and ethanol were selected. Water is a small molecule with two hydrogen atoms and one oxygen atom. Each of its hydrogen atoms can form a hydrogen bond with the electronegative atom (like oxygen, nitrogen, or fluorine) of another molecule, and its oxygen atom can form two hydrogen bonds with the

hydrogen atoms of other molecules. This means that each water molecule can form up to four hydrogen bonds with other water molecules. In contrast, both methanol and ethanol have one -OH group, like water, so they can also form hydrogen bonds. However, they also have a nonpolar part (the CH_3 group in methanol and the C2H5 group in ethanol), which cannot form hydrogen bonds. This reduces their overall ability to form hydrogen bonds compared to water. Each methanol or ethanol molecule can form a maximum of two hydrogen bonds with other molecules.

The key findings from this study were that water was a better HBD as compared to ethanol and methanol. Solvents formed with water as the HBD exhibited the lowest activity and IR spectra revealed a broad peak at the OH regions. Another key finding was that ChI was the better HBA. When ChI was used as the HBA, the activity was the lowest. Again, with HBDs like water and ethanol, a broad peak at the OH regions were revealed. The study found a significant negative correlation between density and activity.

Overall, the study highlights the significance of hydrogen bonding characterized by HBDs and HBAs, as a fundamental factor driving interactions and dictating the solvent's behavior. This study underscores the significance of understanding the intricate structure-activity relationships in DES to harness their full potential in diverse applications. This study also underscores the significance of the HBA-HBD pairing in DES' stability and, by extension, in the stability of pharmaceutical compounds. The ability to tailor solvent properties by carefully selecting HBDs and HBAs offers promising prospects for enhancing the stability of pharmaceuticals, thereby impacting drug formulation and efficacy. It is proposed that, further studies employing advanced computational studies, application- specific

investigations including stability and solubility studies on a wide range of compounds be carried on these potential solvents to confirm their structure and utility.

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