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NOVEL ACETYLCHOLINE CHLORIDE BASED WATER IMMISCIBLE DEEP EUTECTIC SOLVENT: FORMULATION, CHARACTERIZATION AND APPLICATION IN DRUG SOLUBILIZATION

BY

SHIKSHA SUBEDI

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Chemistry

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2021

DISSERTATION ACCEPTANCE PAGE

Shiksha Subedi

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

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Director, Graduate School Date

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ABBREVIATIONS

Abbreviation	**Full Name**
α	Hydrogen Bond Donating Acidity Parameter
β	Hydrogen Bond Accepting Basicity Parameter
Π*	Polarizability Parameter
°C	Degree Centigrade
AA-DLLME	Air-Assisted Dispersive Liquid-Liquid Microextraction
AA-ELLME	Air Assisted Effervescence Liquid-Liquid Microextraction
AA-LLME	Air-Assisted Liquid-Liquid Microextraction
AcChCl	Acetylcholine Chloride
BA	Butyric Acid
BPA	Bisphenol A
CA	Caprylic Acid
ChCl	Choline Chloride
DES	Deep Eutectic Solvent
DESs	Deep Eutectic Solvents
DLLME	Dispersive Liquid-Liquid Microextraction
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
DTG	Derivative Thermogravimetry
EE-DLLME	Effervescence Assisted Dispersive Liquid-Liquid Microextraction
FAAS	Flame Atomic Adsorption Spectroscopy

FTIR	Fourier Transform Infrared Spectroscopy
GF-AAS	Graphite Furnace Atomic Adsorption Spectroscopy
HA	Hexanoic Acid
HBA	Hydrogen Bond Acceptor
HBAs	Hydrogen Bond Acceptors
HBD	Hydrogen Bond Donor
HBDs	Hydrogen Bond Donors
HDEs	Hydrophobic Deep Eutectic Solvent
HDESs	Hydrophobic Deep Eutectic Solvents
HPLC	High- Performance Liquid Chromatography
HPLC-FLD	High Performance Liquid Chromatography with Fluorescence Detector
^{1}H NMR	Proton Nuclear Magnetic Spectroscopy
IL	Ionic Liquid
ILs	Ionic Liquids
Kcal mole^{-1}	Kilocalorie per Mole
KJ mole^{-1}	Kilojoules per mole
KT- parameter	Kamlet-Taft Parameter
LLE	Liquid-Liquid Extraction
LLME	Liquid-Liquid Microextraction
LOD	Limit Of Detection
LPME	Liquid Phase Microextraction
Mg	Milligram

mL	Milliliter
PAH	Polycyclic Aromatic Hydrocarbon
RI	Refractive Index
RPM	Revolution Per Minute
SFD-DLLME	Solidification Of Floating Drop Dispersive Liquid-Liquid Microextraction
SFO-DLLME	Solidified Floating Organic Drop Dispersive Liquid-Liquid Microextraction
Td	Decomposition Temperature
TGA	Thermogravimetric Analysis
UA-DLLME	Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction
UV-Vis	Ultraviolet-Visible
VA	Valeric Acid
Weight %	Weight by percentage

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ABSTRACT

NOVEL ACETYLCHOLINE CHLORIDE BASED WATER IMMISCIBLE DEEP EUTECTIC SOLVENT: FORMULATION, CHARACTERIZATION AND APPLICATION IN DRUG SOLUBILIZATION

SHIKSHA SUBEDI

2021

Deep eutectic solvents (DESs) are the emerging class of new and inexpensive solvents composed of two or three safe components capable of self-association through hydrogen bond interaction. The depression in the melting point characterizes DESs compared to those of the individual components. These solvents have properties like the traditionally used ionic liquids (ILs), but they can offset the significant drawbacks of ILs, such as biodegradability, toxicity, and complex synthesis. Due to these remarkable advantages, the research in DESs is increasing exponentially. However, current deep eutectic solvents still have limitations to apply to the real chemical industries due to hydrophilicity and the solid-state nature at room temperature of most solvents. Thus, this study focuses on the formulation of novel water-immiscible DES. The formulation of new DESs using novel hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) and their unique combinations will broaden the scope of green solvent selection for diverse applications.

The first objective of this dissertation is to formulate new water immiscible DESs, pairing acetylcholine chloride (AcChCl) as HBA and fatty acids as HBDs at different molar ratios. Fourier-transform infrared spectroscopy (FTIR) and proton nuclear resonance

spectroscopy (NMR) confirmed the interaction between HBA and HBDs. The freezing point was determined using differential scanning calorimetry (DSC). All synthesized DESs exhibited a lower freezing point than the individual components, thereby confirming the formation of the eutectic mixture.

The in-depth characterization of the prepared DESs was performed. First, the physical properties of DESs, including viscosity, density, conductivity, refractive index, surface tension, and pH, were investigated. These properties were highly dependent on the composition, and the molar ratio of the HBA and HBDs used for DESs preparation. Second, the thermal stabilities of the DESs were determined using thermogravimetric analysis (TGA). DESs showed higher thermal stability than HBDs due to strong intermolecular forces occurring within the solvents.

The electronic and molecular properties of the DESs were studied using Solvatochromic parameters, including polarity and Kamlet-Taft parameters. Changes in the composition of HBDs and the molar ratio of HBA to HBDs change the polarity of the solvents. Increasing the carbon chain length of the organic acid increases the hamlet-Taft parameter α, β. However, with an increase in the chain length of organic acid, the decrease in Π* parameter was observed. The Kamlet-Taft parameters α, β, Π* of formulated DESs were compared with organic solvents and ionic liquids using solvent selectivity triangles. The polarity of some DESs was very close to the polarity of organic solvents, whereas others had polarity comparable to those of ionic liquids.

Lastly, the application of the DESs for solubilizing the poorly water-soluble drugs, such as aspirin, paracetamol, acetanilide, and phenacetin, was evaluated. All selected drugs show higher solubility in formulated DESs than in an aqueous medium. For example, the

solubility of aspirin is 2- to 11- times higher, paracetamol is 1.5- to 5.4 times higher, acetanilide is 7- to 15- times higher, and phenacetin is 15- to 64- times higher in prepared DESs than in water. The overall result obtained showed that the synthesized solvents could replace traditional solvents and would be suitable for diverse engineering and industrial applications.

CHAPTER 1

INTRODUCTION

1.1 Background

Solvents, ordinarily liquids, dissolve or dilute other substances or materials (solutes) without any chemical change. The term `solvent` is derived from Latin, which means `loose.` Solvents are extensively used in various everyday product applications, such as personal care products, paints, pesticides, cleaners, and ink. In most of these applications, organic solvents are used as reaction media. In addition, solvents are also used in many chemical and pharmaceutical industries for extraction, purification, and cleaning processes. Almost 20 million metric tons of organic solvent are produced annually [1]. Such excessive consumption of organic solvent is of environmental concern and is an example of unsustainable practices. More than 80% of the organic waste produced by synthetic chemistry worldwide is attributed to the organic solvent [2]. Furthermore, solvent use consumes about 60% of the overall energy; hence solvent selection plays a major role in chemical synthesis [3]. In recent decades, the concept of sustainability and renewability of resources has been introduced in chemistry. Researchers had started thinking and doing chemistry differently, focusing on preventing the toxicity before it took place. The decade 1990s is marked as the establishment of green chemistry as the legitimate scientific field. The initial idea of green chemistry was originated as a response to the Pollution Prevention act of 1990, even though the idea of safer chemistry was gaining power since the 60s and 70s. In 1998 Paul Anastas and John Warner introduced the guidelines of green chemistry,

publishing a groundbreaking book, “Green Chemistry Theory and Practice. “This book outlines the twelve green chemistry principles that guide the green chemistry movement (Table 1). In simple terms, *green chemistry* can be defined as maintaining environmentally sound practices through designing, manufacturing, using, and disposal of a chemical product and processes. Green chemistry does not involve the clean-up of pollution. Instead, it creates products that never contribute to pollution. The technology is considered green chemistry only if it significantly reduces or eliminates the hazardous chemicals used to clean up environmental contaminants. However, the concept of green chemistry is not limited to the definition proposed by Anastas and Warner and is continuously evolving.

Table 1. Principles of Green chemistry

Number	**Principle**
1.	Waste prevention
2.	Maximize atom economy
3.	Design less hazardous chemical synthesis
4.	Design safer chemicals and products
5.	Use safer solvent and reaction condition
6.	Higher energy efficiency
7.	Use renewable feed stocks
8.	Minimize chemical derivatives
9.	Use catalyst instead of stoichiometric reagents
10.	Design chemical and products to degrade after use
11.	Analyze in real time to prevent pollution

12.	Decrease the potential for accidents

Some big companies are moving a step forward and trying more for the use of the green concept. For example, Pfizer has categorized solvents as preferred, usable, and undesired, depending on their possible toxicity. Solvents like carbon tetrachloride, chloroform, dichloroethane, and pyridine are undesirable due to their carcinogenic ability [4, 5]. Pfizer has also provided a list of solvents alternative to the undesirable solvents. In addition, Marek et al. have introduced chemometrics and multicriteria decision analysis for proper solvent selection for chemical processes [4]. Another approach reported by GSK assesses the solvents based on health, safety, and lifecycle assessment and then reports a score for various solvent categories using a color-coded table [3].

Pharmaceutical industries are regarded as one of the major sources of chemical waste. Production of complex molecules requires many solvents both during synthesis and purification. One study shows that 85% solvent by mass is required to prepare active pharmaceutical reagents (APIs) [6]. More solvent is required as drug manufacturing passes from early-stage development to commercial processes [7]. The oil refining industry is another sector that produces tons of organic waste [8].

There are ways to avoid using hazardous conventional solvents. The first way is to go solventless. The petrochemical industry well adapts this technique. But not all types of reaction can be done by solventless techniques. Another way is to use water as a solvent. However, not all compounds are water-soluble, and the disposal of contaminated aqueous waste poses another problem. Another approach utilizes supercritical fluids that have both gas and liquid-like properties. However, this approach requires expensive equipment, and

the reaction should be set at very high pressure to maintain the solvent in a supercritical state [9]. Thus, finding the proper replacement solvents of any kind is the biggest challenge. The continued search for greener alternative solvents led researchers to introduce new green solvents, like ionic liquids (ILs) and deep eutectic solvents (DESs).

1.2 Ionic liquids (ILs)

ILs are molten organic salts formed by mixing organic cation and organic/inorganic anion with a melting point below 100 °C [10]. The majority of ILs have a melting point in the range of Tg-1.5Tg [11]. These solvents are colorless, odorless, fluid, and easy to handle. The cationic component of IL has a very low degree of symmetry. The possibility of many combinations of the cation moiety with anion leads to the wide range of tunability of ILs exhibiting different properties like viscosity, melting point, conductivity, density, and solubility, among others. This enables the solvent to be designed and tuned according to the specific need to increase efficiency, selectivity, and sensitivity. ILs have negligible vapor pressure and hence, can be used in high vacuum systems. These liquids have emerged as an environmentally benign alternative to volatile organic compounds, such as dimethyl methanamide, trichloromethane, etc. The polarity of IL can be tuned using a suitable cation and anion. Due to their immiscibility with a large number of organic solvents, ionic liquids [12]. One study shows that at least a million binary ILs and 10^{18} ternary ILs are possible [13].

Synthesis of ILs is a two-step process. The first step involves forming the desired cation, which is done by protonating the amine using an acid or quaternization reaction of an amine with a haloalkane, followed by heating. In the second step, anion exchange is carried out by treating halide salt with Lewis's acid-forming Lewis acid-based ILs, shown in figure

1.1. $AlCl_3$ based salts are one of the most widely studied Lewis acid-based ionic liquids. Synthesis of such salt is done simply by mixing Lewis acid and halide salts, resulting in more than one anionic species, as shown in figure 1.2 [14].

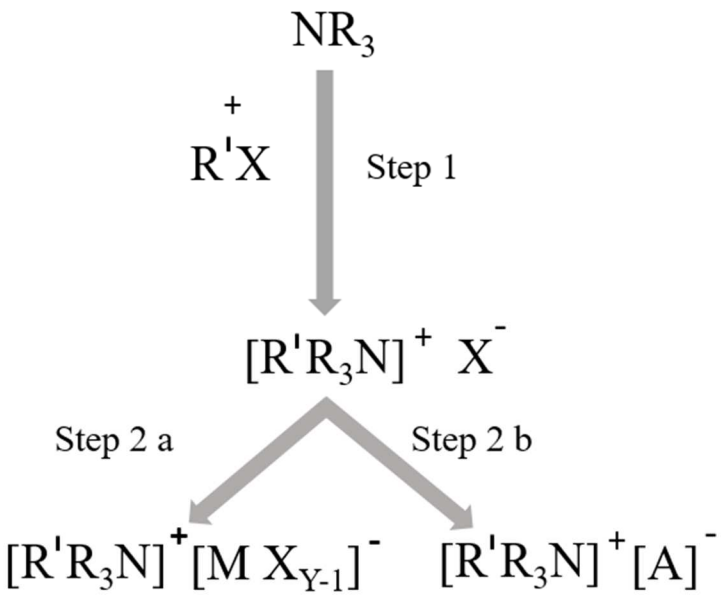

Figure 1.1. Schematic representation of preparation of ionic liquids

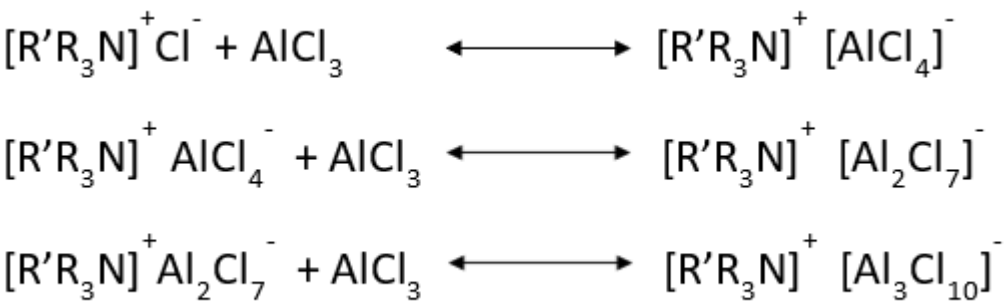

Figure 1.2. A series of reaction between quaternary halide salt and Lewis acid

The earliest reported IL was alkylammonium nitrates, [$EtNH_3$] [NO_3], with a melting point of 12 °C. Paul von Walden investigated these salts due to their low melting point, a prerequisite for conductivity measurement. The first genuine room-temperature ionic liquid based on 1-butyl-pyridinium chloride and ammonium (III) chloride was reported by Osteryoung and Husley. These have exceptionally low lattice energy. The idea of alteration of the cation and the anion to fine-tune the properties of ionic liquids has been noted by Seddon and co-workers and Wilkes and Zaworotko [15]. Changing the anion of the imidazolium cation [C4mim] changes the melting point of the liquid. [C4mim] [Cl] has melting point 41°C, [C4mim] [I] has melting point -72 °C, and [C4mim] [PF6] has melting point of 10 °C. Similarly, changing the alkyl group of the imidazolium cation changes the melting point of the resulting liquid. Melting point of [C_1mim] [AlCl4] is 125 °C [C_2mim] [$AlCl_4$] is 84 °C [C_3mim] [$AlCl_4$] is 60 °C, and [C_4mim] [$AlCl_4$] is 65 °C [16].

1.3 Deep Eutectic Solvent: Formation and Classification

A DESs is formed by mixing two or three components at a specific molar ratio which can associate with each other through hydrogen bond interaction. These solvents have a lower melting point than the individual components. The composition at the lowest temperature of the mixture is represented by the eutectic point (figure 1.3) .DESs is a eutectic mixture of Lewis or Bronsted acids and bases containing various cationic and anionic species[17]. Basically, DESs are eutectic mixture of Lewis or Bronsted acids and bases that contain variety of cationic and anionic species [18]. Unlike ionic solvents, which contain discrete cations and anions, these contain cations and anions along with hydrogen bond donating species. Even though the result is similar, the route of lattice energy reduction is different. The hydrogen bond donor complexes with the anion and withdraws the electron density of

the anion from the cation resulting in a weaker cation/anion interaction. The charge delocalization that occurs between HBDs and HBAs is responsible for the decrease in the melting point of the mixture relative to the melting point of the starting components [19]. The decrease in melting point also depends on the lattice energy of the quaternary ammonium salts and HBDs and the degree of interaction between the anion and the HBD. The higher the interaction between the HBA and HBD, the higher the system's entropy, and the lower is the freezing point [20].

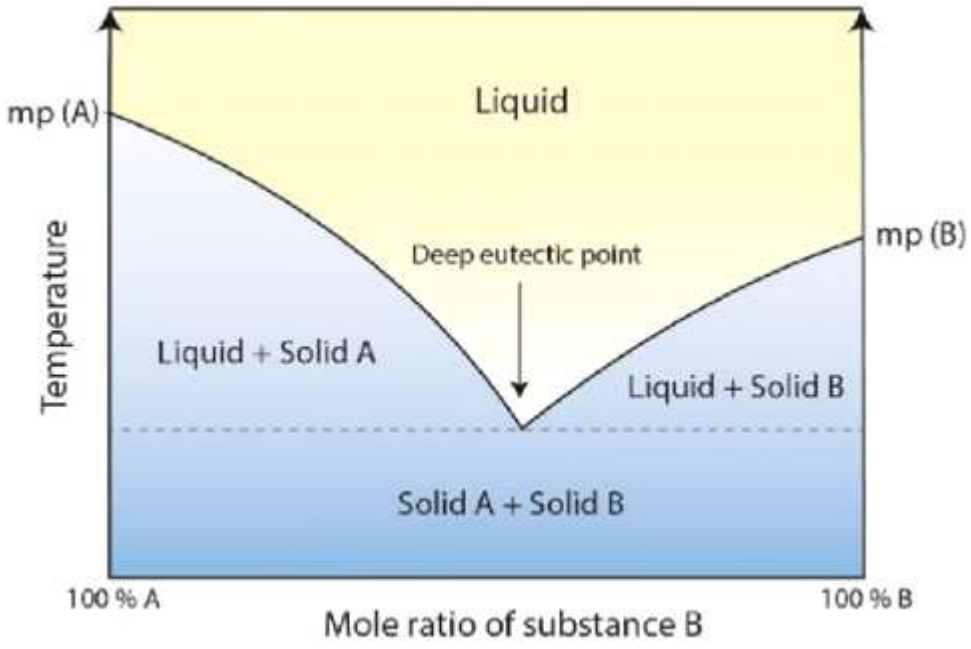

Figure1.3. Diagrammatic representation of Binary Phase diagram of DESs

DES can be described by the general formula

$$Cat^{+}X^{-}zY$$

Where Cat^{+} is any ammonium, phosphonium, or sulfonium cation, X is a Lewis base, Y is Bronsted acid, and z is the number of Y molecules that interact with X. DESs are classified

into four different categories based on the nature of the complexing agent used [18]: Type I (Quaternary salt and metal halides), Type II (Quaternary salts and hydrated metal halide), Type III (Quaternary salts and HBDs) and Type IV (Metal halide and HBDs) [18, 21].

Type I DESs are prepared from metal halide and quaternary ammonium salts. These are analogous to the well-studied metal halide/imidazolium salt systems. Metal includes Al, Sn, Zn, Fe, Ga, and In. Examples include chloroaluminates imidazolium salt melts and ionic liquids formed with imidazolium salts with metal halides, such as CuCl, LiCl, $FeCl_2$, $CdCl_2$, AgCl, $SnCl_2$, $ZnCl_2$, $LaCl_3$, YCl_3, and $SnCl_4$ [18]. Due to the high melting points of metal halides, these DESs have limited application in biomass processing. However, due to the high melting points of metal halides, these DESs have limited application in biomass processing. Type II DESs are prepared using quaternary ammonium salt and metal halide hydrate and are represented by a formula, Cat^+X^- + $zMClx.yH_2O$. Metal includes Cr, Ni, Cu, Co, and Fe. Type II DESs use hydrated metal halides. Metal halides are cheap, readily available, and are insensitive to air and moisture, making them more assessable for industrial application [18].

Table 1.2. Types of DESs, general formula, terms, and example

Type	**General Formula**	**Terms**	**Examples**
Type I	Cat^+X^- + zMClx	M= Sn, Zn, Fe, In, Al, Ga	ChCl+$SnCl_2$
Type II	Cat^+X^-+ $zMClx.yH_2O$	M= Cu, Co, Cr, Ni, Fe	ChCl+$CoCl_2.H_2O$

Type III	Cat+X- + zRZ	Z= OH, COOH, $CONH_2$	ChCl+ glycerol
Type IV	MClx + zRZ	Z= OH, $CONH_2$ M= Al, Zn	$ZnCl_2$+ ethylene glycol

Type III DESs are of great interest to researchers due to their ability to solvate a wide range of transitional metal species [18]. Type III DESs are prepared by mixing quaternary ammonium, phosphonium, or sulfonium salts with HBDs such as amides, amines, carboxylic acids, and alcohols [22]. These are the most versatile type of DESs in terms of variety due to the availability of a large number HBDs available to prepare them. So, there is no limit to the number of DESs that can be synthesized [23]. The very first formulated DESs by Abbot in 2003 were type III DESs, prepared using choline chloride as HBA and urea as an HBD. This resulted in eutectic formation with a melting point of 12 °C, far below the melting point of 302 °C and 133 °C choline chloride and urea, respectively [24].

Type IV DESs are represented by MClx + zRZ, where M is metal like Al and Zn. These kinds of DESs are formed from metal halides and hydrogen bond donors. Due to the high charge density, inorganic cation does not form low melting point eutectics. However, some studies show the formation of eutectics using metal halide and urea Later, Abbot et al. also confirmed the formation of a eutectic system between transitional metal halides and HBDs like urea, acetamide, ethylene glycol, and 1,6- hexanediol [25]. DESs have a very similar physical property to ILs, but they have different chemical properties. They have several advantages over ILs. First, DESs can be prepared from a variety of inexpensive

components that are toxicologically well-characterized. Preparation of DESs involves the simple mixing of two more components with a moderate level of heating. Second, multistep synthesis under harsh chemical conditions is required for ILs synthesis. No purification of the final product is required. Third, DESs by-passes all the problems of waste disposal generally encountered with ILs. These solvents show chemical inertness with water, allowing easy storage for future use. Finally, most DESs are biocompatible, bio-renewable, and biodegradable [18, 26-28]. Some studies have shown ionic liquids to be cytotoxic, phototoxic, and less biodegradable than DESs. On the contrary, DESs have a 100% atom economy [27, 29-31].

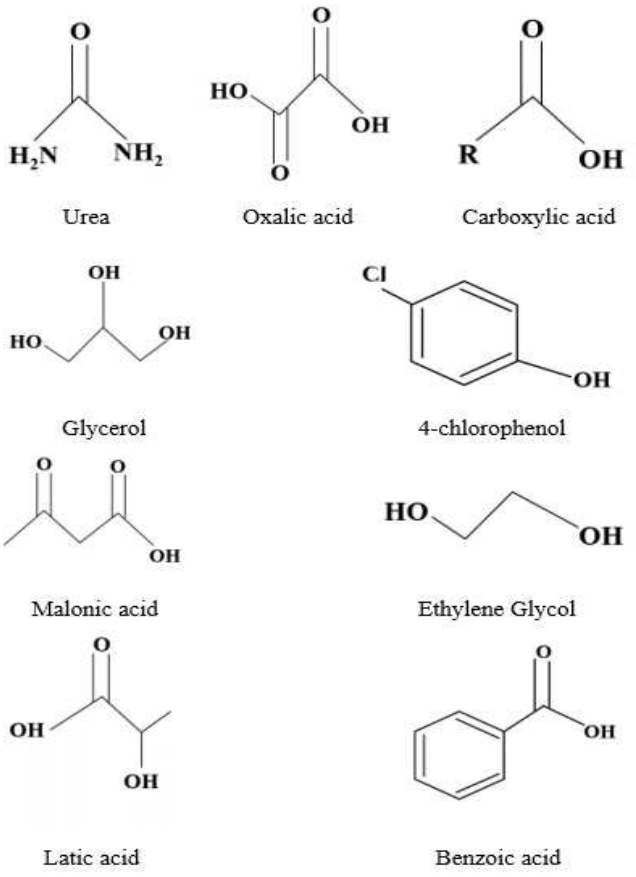

Figure 1.4. Some commonly used hydrogen bond donors (HBDs) of DESs

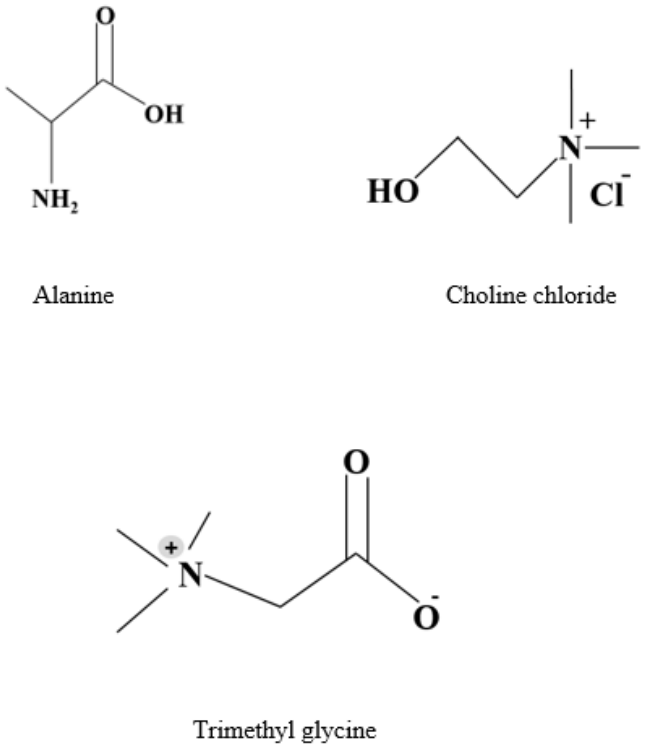

Figure 1.5. Some commonly used hydrogen bond acceptors in DESs

1.4 Scope of research

1.4.1 Problem Statement

Organic solvents are of great environmental concern, specifically if they are hazardous and increase the carbon footprint. In addition, organic solvents have posed a serious concern in the field of occupational health. Exposure to organic solvents may cause headaches, irritation of the eye, nose, and throat, dizziness, unconsciousness, and even death [32]. Some commonly used solvents like n-hexane, benzene, xylenes, and toluene are a neurotoxicant. Excessive exposure to these solvents results in neuropathy, psychosis, brain dysfunction, facial paralysis, and numbness [33]. Alcohols, like methanol, can cause various effects, including retinal damage and necrosis of the cells. Long-term exposure to

solvents like benzene and chlorinated hydrocarbons can cause leukemia, scleroderma, and renal cancer [34-36]. In several studies, diseases like Alzheimer`s disease and Parkinson`s disease have been associated with solvent exposure [37, 38]. Most of these solvents are deleterious and have a very long half-life leading to prolonged adverse effects on the environment. Organic solvents can react in the atmosphere in the sunlight, producing ground-level ozone, adversely affecting plant and animal health [39]. Regardless of the numerous environmental and health concerns, the demand for volatile organic compounds is increasing at a notable pace.

The European Union introduces new rules on climate and energy framework that targets cutting at least 40% of the greenhouse gas emission (from a level of 1990) by 2030 and 80% by 2050 [40]. The increasing environmental and economic impact of conventional solvents and the growing perception of sustainability and green chemistry principles have led to increased interest in developing green and sustainable solvents. During the past decades, a new designer solvent, ILs, and DESs have attracted immense attention from researchers in various chemical fields to replace solvents that present inherent toxicity.

Non-volatility and negligible vapor pressure of ILs qualify them as green solvents [40]. However, the green aspects of ILs are often challenged due to their poor degradability, hazardous toxicity, high cost, and complex synthetic and purification process [41]. However, the green aspects of ILs are often challenged due to their poor degradability, hazardous toxicity, high cost and complex synthetic and purification process [42, 43]. To overcome these drawbacks, a new class of designer solvents, DESs, has emerged. DESs have very similar properties to ILs but are synthetically more accessible, non-toxic, inexpensive, highly pure, and biodegradable [44]. Despite the advantages, the research in

DESs is still in the nascent stage, and most of the currently available DESs are hydrophilic, limiting their application to only polar compounds [18, 44]. So, the truth is that most scientists still use toxic organic chemicals due to the scarcity of green hydrophobic solvents. Figure 1.6 shows the number of publications reporting DESs and HDESs. Most of these articles focus on the hydrophilic DESs, thereby limiting the number of articles published in hydrophobic deep eutectic solvents. Only 9 references noted the concept of HDESs until 2017. However, the number of articles started to increase in 2018, and more than 90 references have already been published in HDESs. As can be observed, the publication in DESs is booming since their first inception in 2003. In 2020, more than 1000 articles were already published, making it the year with the largest number of articles published in this field.

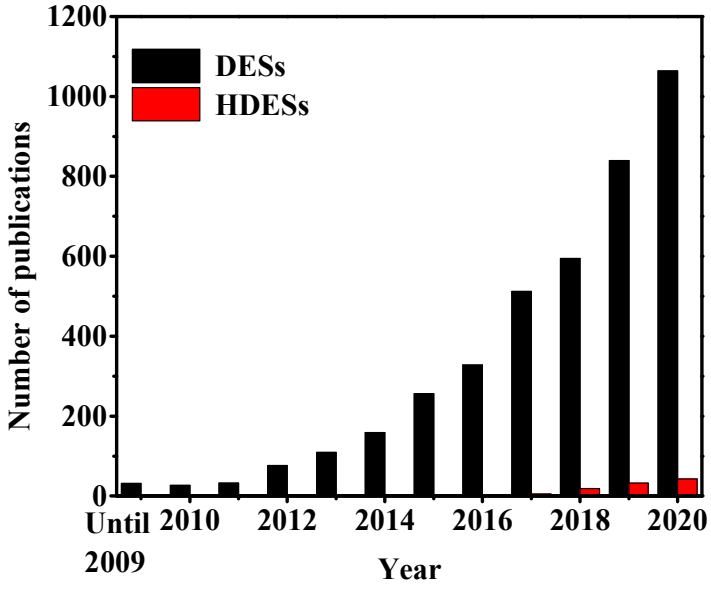

Figure1.6. Comparison of the number of publications on deep eutectic solvent and hydrophobic deep eutectic solvent based on SciFinder^n database. Date accessed: 25 November 2020.

1.4.2 Justification of the study

For the first time in 2015, hydrophobic DESs were first described in the literature. These solvents consist of decanoic acid as hydrogen bond donor (HBD) and quaternary ammonium salts as hydrogen bond acceptor (HBAs). These newly synthesized solvents were tested to extract volatile fatty acids from dilute aqueous samples [45]. Since then, several research articles have been published in the field of hydrophobic DESs. However, most of the HDESs are synthesized using a large alkyl group attached to cations, making them expensive and uncompetitive to existing solvents. Thus, this study primarily focuses on synthesizing HDESs from inexpensive, non-toxic, and biodegradable starting materials. In addition to this, an investigation of physicochemical and thermal properties of the formulated DESs was also performed.

1.4.3 Objectives of Study

The main objective of this study is to design cost-effective water-immiscible solvents. Therefore, the second chapter is focused on the formulation of DESs, using acetylcholine chloride and fatty acids, such as butyric acid, valeric acid, hexanoic acid, and caprylic acid at various ratios. In the third chapter, we investigated the physicochemical and thermal properties of the prepared DESs. We also evaluated how changing the hydrogen bond donors, and their molar ratios in DESs changes the properties of DESs. The fourth chapter determined the Solvatochromic parameters, including polarity and Kamlet- Taft parameters of synthesized DESs. Finally, chapter five explored the applicability of the formulated DESs for solubilizing sparingly water-soluble drugs.

1.4.4 Acetyl choline chloride

Acetylcholine chloride, also known as (2-(acetyloxy)-N, N, N-trimethylethanamonium) chloride, is the acetylated form of choline chloride. The presence of the acetyl group makes it more biodegradable and hence environmentally friendly than choline chloride [46].This is also a neurotransmitter that is present naturally in many organisms, including human beings [47].

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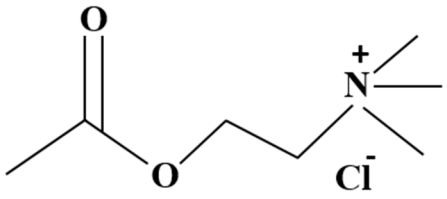


Figure 1.7. Structure of Acetylcholine chloride

1.4.5 Fatty acids

Fatty acids are essential structural components of animals having a carboxylic acid functional group. Fatty acids are classified as a) short-chained, b) medium chained, and c) long-chained fatty acids. Short-chained fatty acids have less than five carbon, medium chained have 6 to 12 carbons, and long-chained have 13 or more carbons

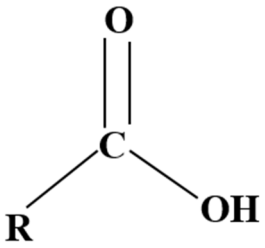


Figure 1.8. Structure of fatty acid

1.5 Hydrophobic Deep Eutectic Solvents: Synthesis and classification

HDESs are synthesized using a similar method as hydrophilic DESs, except that synthesis is done using poorly water-soluble components. The constituents are mixed at a desired ratio at ambient temperature or heated at 60 °C with or without stirring until the homogeneous mixture is formed [48-50]. The hydrophobicity of the HDESs can be tested visually by (i) observing the formation of two distinct phases while in contact with water [51] or (ii) detecting the water content of DESs or DESs constituents in the water phase before and after mixing DESs with water using spectroscopic techniques [45, 52-55]. Based on the type of HBA used, HDESs is divided into two different groups:

1.5.1 Ionic Hydrophobic Deep Eutectic Solvents

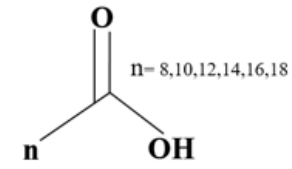
Ionic HDESs are synthesized using ionic compounds like tetra alkyl ammonium and/or phosphorus salts. The very first reported HDESs, based on quaternary ammonium salts and decanoic acid, were ionic HDESs.

The long alkyl chain quaternary ammonium salts like tetrabutylammonium, trioctyl methylammonium, tetraheptylammonium, and tetraoctylammonium salts were used as HBAs as these salts show greater hydrophobicity compared to short-chain quaternary ammonium salt. The hydrophilicity of these solvents was accessed by studying the leaching of DESs components to the water phase and their water uptake [45].Since then, several HDESs were proposed using quaternary ammonium salts as HBAs and long-chain acids and alcohols as HBDs. Sometimes, it is impossible to obtain HDESs in the combination of

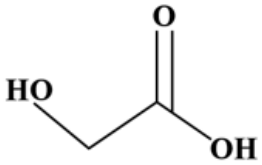
ammonium salts with short-chain acids. In such a case, three compounds can be used for the synthesis [56].

1.5.2 Nonionic hydrophobic deep eutectic solvents

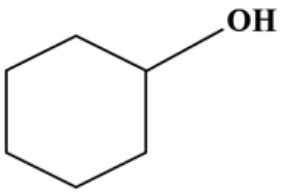
Nonionic HDESs are formulated using nonionic compounds like monoterpenes. Ribeiro et al. synthesized the first natural resource-based HDESs by combining DL-menthol and several natural carboxylic acids like pyruvic, lactic, acetic, and dodecanoic acid [52]. Following this idea, several works were then proposed using menthol as HBA and changing the structure of HBDs such as fatty acids having a carbon chain length from C8 to C18 [57-59], phenolic compounds [60] and therapeutic drugs [61]. In addition to menthol, other terpene-based compounds such as thymol and L-menthol were also combined with several carboxylic acids to synthesize HDESs [58]. Compounds from the same family, long-chain fatty acids, were also used as HBA and HBDs to prepare HDESs [54]. Other HDESs were synthesized using painkillers with organic acids and terpenes [62, 63].



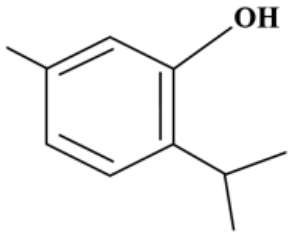
Carboxylic acid



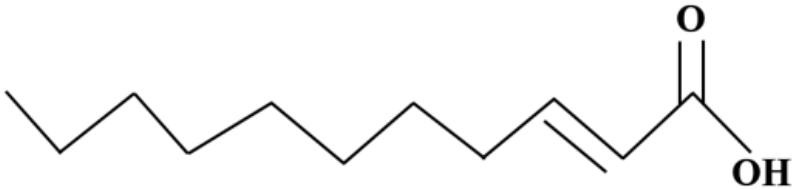
Glycolic acid



Cyclohexanol



Thymol



Undecylenic acid

Figure 1.9. Some commonly used hydron bond donors for HDESs

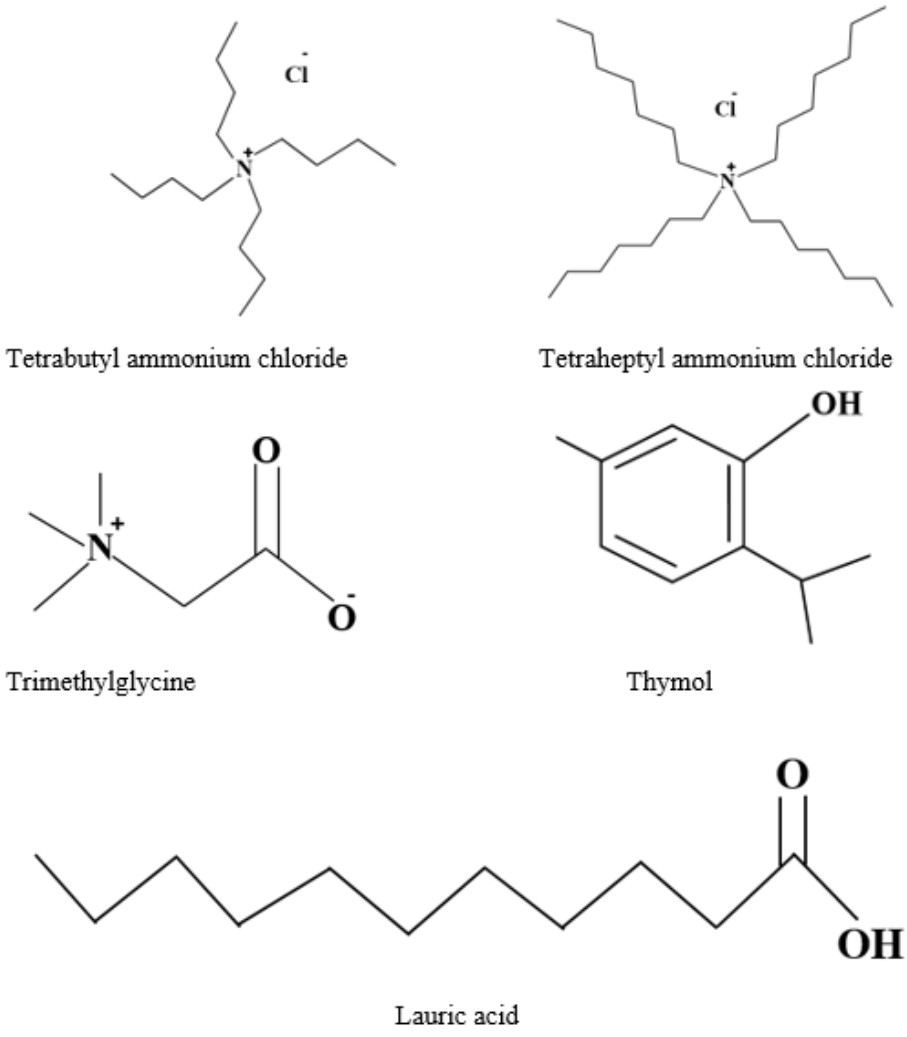


Figure 1.10. Some commonly used hydrogen bond acceptors for HDESs

1.6 Application of HDESs

Since the first inception of HDESs, active research is ongoing to investigate their possible applications in diverse fields. Being immiscible with water, HDESs have been broadly used as potential extraction media to replace conventional solvents. More than 87% of the published articles on HDESs are focused on the application of these solvents in the

extraction process. Figure 1.11 shows the number of articles related to the application of HDESs in the extraction process compared to articles on HDESs. The liquid-phase extraction is done either in the traditional liquid-liquid extraction (LLE) version or the miniaturized LLE version, called the liquid-liquid microextraction technique (LLME). The application of HDESs in LLE is relatively limited due to disadvantages like time-consuming, poor sensitivity, labor-intensive, and requirement of the large volume of HDESs for the extraction process [64]. Other main areas of applications are organic synthesis and materials, water purification, and CO_2 capture. All the applications of HDESs are discussed below.

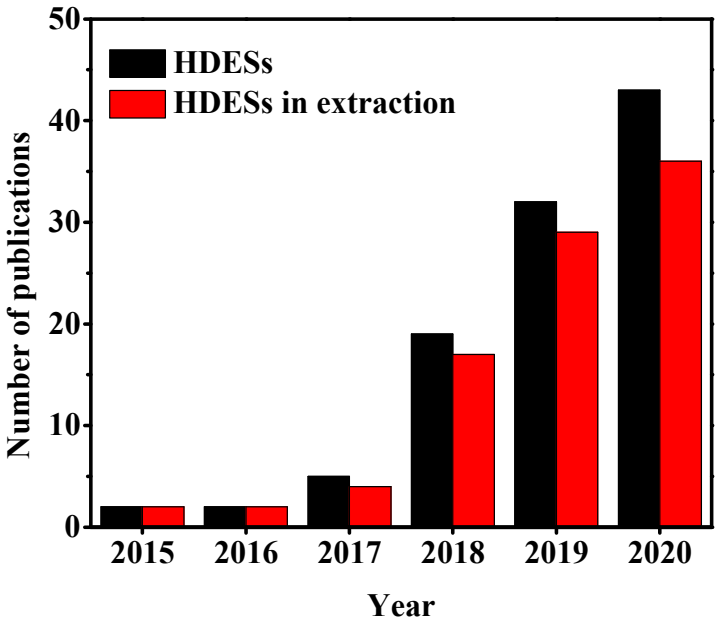


Figure 1.11. Number of references published on hydrophobic deep eutectic solvents and applicability of hydrophobic deep eutectic solvents in extraction process based on SciFinder^n database

1.6.1 Liquid-phase extraction

The first application tested for HDESs was the extraction of volatile fatty acids (VFAs) from diluted aqueous solution via liquid-liquid extraction. The extraction efficiencies of VFAs were higher in the newly synthesized HDESs than in the conventional extraction medium, trimethylamine [45]. Simultaneously, Ribeiro et al. synthesized menthol and naturally occurring acids based HDESs and utilized them to extract biomolecules like tryptophan, isophthalic, caffeine, and vanillin from aqueous solution. The highest partition coefficient was obtained for the separation of caffeine and tryptophan using menthol and the lactic acid at pH 1.18 [52]. Finally, Cao et al. utilize two-phase systems developed with hydrophilic DESs and HDESs to separate several bioactive components present in *Ginkgo biloba* leaves. The two-phase system effectively extracted bioactive components with different polarities simultaneously [56].

Separation of micropollutant Bisphenol A (BPA) from the water phase was reported using both ionic and neutral hydrophobic DESs. HDESs synthesized from tetraoctylammonium bromide, and decanoic acid extracted almost 100% of the BPA from water under the optimal conditions. The same solvent was reused five times for extraction without the loss of its BPA extraction capability [65]. The same group also prepared fatty acid based HDESs and used them to extract the same micropollutant from water. The extraction efficiencies of 76.04%, 88.32%, and 81.81% were obtained for octanoic: decanoic acid (3:1), nonanoic acid: dodecanoic acid (3:1), and decanoic: dodecanoic acid (2:1), respectively. Various Ternary HDESs mixtures were also prepared to extract BPA from an aqueous phase. The highest efficiency of 91.52% was observed with nonanoic acid: decanoic acid: dodecanoic acid (3:1:1) [54]. An et al. synthesized menthol-based HDESs

to extract BPA from the water via liquid-liquid extraction. Under optimized conditions, an excellent recovery rate of 98.2% and 99.0% was obtained for menthol: propionic acid and menthol: formic acid, respectively [66]. HDESs based on natural neutral ingredients and others based on quaternary ammonium salts and organic acids were prepared as a cheap extractant to extract pesticides like imidacloprid, acetamiprid nitenpyram, and thiamethoxam. The extraction efficiencies of up to 80% was obtained using HDESs based on menthol and organic acids [55].

HDESs were also applied to LLE for the extraction of metals and ions like 111ln (III), Cr (IV), Cu (II), 99mTcO-4, and $UO2^{2+}$ [50, 58, 67-69]. Extraction of indium was carried out from hydrochloric acid and oxalic acid solution using hydrophobic solvents composed of quaternary ammonium chloride and fatty acids, decanoic acid, and oleic acid, and ibuprofen. The analyte was measured using a radioactivity gamma counter [67]. HDESs prepared from tetraalkylammonium and tetraalkylphosphonium halide as HBA and monocarboxylic acid HBDs demonstrated to be excellent media for extraction of 99mTcO-4 in the presence of a variety of competing anions without the aid of other formal extracting agents. Schaeffer et al. synthesized terpene-based eutectic solvents and utilized them to extract and separate Cu (II) from other transitional metals in a mildly acidic solution. Other authors also reported the extraction of alkali and transitional metals by HDESs prepared from lidocaine and decanoic acid [62].

1.6.2 Liquid phase microextraction

This miniaturized version of LLE offers numerous advantages like higher sensitivity, short extraction time, simple operation, and low consumption of both real samples and HDESs over the conventional method of extraction. HDESs have been applied as an extraction

solvent to various modes of LPME techniques. For example, Zhu et al. applied LLME to remove synthetic pigments in beverages using HDESs [70]. The proposed method provides high precision, low detection limit, good linearity, and satisfactory recovery of the analytes. Microextraction of diclofenac and ketoprofen from human urine samples was reported via in-situ formation of HDESs. This approach is based on the formation of HDESs between DL-menthol and drugs directly in an aqueous sample, thereby increasing the selectivity and simplicity. For both analytes, recovery values were from 93% to 97% [61]. Another study utilizes HDESs, formed in situ by decanoic acid and DL-menthol for the microextraction of parabens in an aqueous sample. The proposed method has the advantages of short extraction time with acceptable recoveries [71]. Finally, Shishov et al. suggested the simple and novel approach for effective LLME based on the decomposition of DESs. The HDESs prepared from tetrabutylammonium bromide and long-chain alcohols decomposed in an aqueous phase result in situ organic phases. These in situ organic phases provide efficient extraction of 17-β-estradiol with excellent reproducibility and recovery [72].

Farajzadeh et al. first reported HDESs based DLLME for extraction and preconcentration of pesticides from fruit and vegetable juices. In this method, choline chloride and 4-chlorophenol in the molar ratio of 1:2 was used to prepare HDESs, and acetonitrile was used as a dispersing solvent. The proposed method has wide linear calibration ranges and a low limit of detection and quantification[73]. The same method has also been reported for the removal of chlorophenols from wastewater [74], extraction of thorium from water and rock samples [75], tetracyclines from milk [76] and separation of pyrethroids from tea beverages and fruit juices [77].

The conventional DLLME, in which dispersion of HDESs is done by dispersing solvents, was made greener by using air agitation, vortex mixing, ultrasound irradiation, and shaker mixing to dispersion HDESs. Zounr. et al. employed DESs based AA-LLME for separation, preconcentration, and determination of lead in various food and water sample. When tested with the certified reference materials, the developed technique gave satisfactory results and had a low detection limit, low relative standard deviation, and high preconcentration factor than reported literature values [78]. A new DL-menthol-based HDESs were synthesized and used for air-assisted dispersive liquid-liquid microextraction (AA-DLLME) for preconcentration and extraction of benzophenone type UV filters from aqueous samples with relative recoveries of 88.8%- 105.9%. The air-assisted process enhanced the formation of fine droplets of the extraction solvents in the aqueous solution without dispersing solvents [79]. The HDESs based AA-DLLME was also effective for extraction and determination of some pesticide residues in fruits and vegetables [80].

Vortex-assisted dispersive liquid-liquid microextraction (VV-DLLME) was employed by Zhang et al. for the determination of nitrite in water and biological samples. Four ionic, hydrophobic deep eutectic solvents composed of trioctyl methylammonium chloride and oleic acid were used as an extraction solvent. Under the optimized condition, the method has a very good linear range and a low limit of detection and quantification [81]. Using a similar method combined with HPLC-UV, folic acid was extracted from the flour sample with relative recoveries of ≥90% [82] . Another interesting technique, ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME), was used to extract UV filters like 2,4- dihydroxybenzophenone, benzophenone, and 2-hydroxy-4-methoxybenzophenone in swimming pool and river water samples before HPLV-UV detection. This technique

facilitates the extraction of the target analyte by efficiently dispersing the extractant into fine droplets using ultrasound. The proposed method had satisfactory extraction efficiency, good repeatability, acceptable recoveries under the optimized condition [83]. PAHs analytes were isolated from industrial effluents using UA-DLLME using HDESs prepared from natural compounds like thymol, ±camphor, decanoic and 10-decylenic acid [84]. This technique was also used to preconcentration pyrethroid insecticides before determination by HPLC/UV [85]. Under optimized conditions, the proposed method requires only a small amount of HDESs to complete extraction with acceptable recoveries of 80.93%- 109.88 %. Another green extraction method was developed by Ahmadi et al. to extract methylene blue in aqueous samples using shaker assisted dispersive liquid-liquid microextraction (SA-DLLME) with back extraction method combined with UV-Vis detection. The back-extraction method increases the selectivity and decreases the LOD of the method. The method showed great analytical features under the optimized condition [86].

Ravandi et al. reported effervescence-assisted dispersive liquid-liquid microextraction (EE-DLLME) based on HDESs to extract synthetic dyes from food samples. As a result of the effervescence reaction between an effervescent agent and a proton donor agent, carbon dioxide is produced, which facilitates the dispersion of extraction solvent, thereby excluding the use of dispersive solvents [87]. EE-DLLME was also reported by Shishov et al. for separation and preconcentration of ketoprofen and diclofenac in liver samples combined with HPLC-MS/MS [88]. Effervescence can be attained by air-assisted, vortex-assisted, and ultrasound irradiation denoted by AA-, VA-, and UA- ELLME. Zounr et al. reported AA-ELLME for the separation, preconcentration, and determination of lead using graphite furnace atomic adsorption spectroscopy (GF-AAS). HDESs consisting of choline

chloride and phenol were synthesized, and tetrahydrofuran was used for the phase separation. The proposed method was simple, rapid, efficient, less toxic, cheap, and highly accurate than other LPME methods [78]. A novel approach, UA-ELLME, was studied for the determination and speciation of chromium (III) and chromium (VI) in water samples in combination with a micro-sample injection flame atomic adsorption spectrometer (FAAS). Five different HDESs based on choline chloride and phenol at different molar ratios, tetrabutylammonium chloride, and decanoic acid, and Methyltrioctylammonium chloride and decanoic acid, were used as a green microextraction phase. The presented procedure has the advantage of simplicity, low toxicity, low LOD (5.5 μg L-1), and good recovery [89]. Other applications of combination of HDESs with UA-ELLME are extraction of arsenic from water and environmental samples [90], determination of selenium species from water [91] and food samples and extraction of benzene, toluene, ethylbenzene and PAHs from water samples [92].

Yousefi et al. reported the applicability of HDESs in the microextraction method based on solidification of floating drop (SFD-DLLME). HDESs consisting of tetra-n-butyl ammonium bromide (TBAB) and carboxylic acid were synthesized to be used as an extraction solvent to analyze PAHs in environmental water samples before HPLC-FLD. HDESs were dispersed using ultrasonic irradiation without using a disperser. The proposed method had high sensitivity, good repeatability, and good linearity [93]. In another study, SFD-DLLME was used to extract benzoylureas in water samples before analysis by HPLC. During the extraction, ferric chloride ethanol solution was used as a dispersive-demulsified solvent [94]. SFD-DLLME has also been used for extraction and determination of selenium in aqueous sample combined with UV-Vis spectrometry [95]. Naeemullah et al. used

HDESs synthesized from decanoic acid and choline chloride to determine lead in food and water sample by SFO-DLLME. A reasonable recovery rate of more than 95% was observed [96].

1.6.3 Other applications

Another application of HDESs with great potential is CO_2 capture. Dietz et al. proposed PC-SAFT' pseudo-saft' approach for modeling CO_2 solubility in various HDESs. CO_2 solubility was predicted in five different HDESs formed from Decanoic acid and perfluorordecanoic acid, HBD, tetraoctylammonium chloride, tetraoctylammonium bromide, tetrabutylammonium chloride as HBA at different molar ratios. The CO_2 solubilities were predicted reasonably for all the systems under study [97]. Zubeir et al., for the first time, measured the solubility of CO_2 in HDESs consisting of decanoic acid and quaternary ammonium salts with different halide anions and alkyl chain length. They found that measured solubility in HDESs is like those of the fluorinated ILs with the heat of CO_2 absorption in the range of non-polar solvents. The CO_2 solubility increases by increasing the alkyl chain length and lowering the HBD to HBA [98].

Boldrini et al. has used HDESs based on DL-menthol and acetic acid as ecofriendly electrolyte medium in dye-sensitized solar cells. The device displayed excellent power conversion efficiency with higher voltage and lower recombination resistance by improving the eco-compatible and sustainable character of the dye-sensitized solar cell, facilitating the industrial development plan [99]. Furthermore, Murakami et al. reported a new class of triplet-triplet annihilation photon up conversion (TTA-UC) samples based on HDESs with high thermal stability. Depending on the HDESs composition, their UC quantum yields range from 0.11 to 0.21 [100].

Another area of HDESs application in catalysis. Milker et al. synthesized four different DESs, i.e., choline chloride: glycerol (1:1.5), tetraoctyl ammonium bromide: ethylene glycol (1:3), tetrabutylammonium chloride: 4-nitrobenzaldehyde (2.2:1.5) and tetraoctyl ammonium bromide: 1, 5-pentadiol (1:3) and tested the catalytic aldose activity of porcine pancreas lipase for 4-nitrobenzaldehyde and acetone. The result shows the formation of aldol products, especially in HDESs [101]. In another study, HDESs were used as reaction media for the lipase catalyzed esterification of the HDESs compounds itself to synthesize (-) - menthol fatty acid esters. The study shows that enzymatic reaction can be performed in HDESs formed from the substrate without use of the co-solvents [102]. Jiang et al. used DESs as the pore-forming agent to tailor the morphology and performance of polyethersulfone membrane. In addition to the HDESs consisting of tetrabutylammonium chloride and decanoic acid at a molar ratio of 1:2 into the casting solution, the membrane's water flux and rejection ratio were increased, increasing the membrane performance during the phase inversion process. The synthesized DESs were a superior pore former compared to other additives [103]. Dietz et al. proposed HDESs supported liquid membranes for the recovery of furfural and hydroxymethylfurfural from aqueous solution. Addition of HDESs enhanced the transportation of furfural and hydroxymethylfurfural through the polymeric membrane support [53].

CHAPTER 2

NOVEL ACETYLCHOLINE CHLORIDE BASED WATER IMMISCIBLE DEEP EUTECTIC SOLVENTS : FORMULATION AND SPECCTROSCOPIC ANALYSIS

2.1 Introduction

Over the last two decades, the research into room temperature ionic liquids (ILs) is booming. Low vapor pressure and high boiling point facilitate recycling, two essential properties that qualify ILs as a green solvent. However, the green aspect of this solvent is often challenged due to its poor biodegradability, toxicity, high cost, and complex synthetic and purification process [42, 104]. To overcome these drawbacks, a new class of designer solvents, deep eutectic solvents (DESs), has emerged. The most pronounced advantage of DESs over ILs is the ease of preparation and purity of the final product. Various properties of DESs are like those of the ionic solvents, so they can replace many traditional ionic solvents and even some nonionic solvents. DESs are prepared by gentle warming of the individual components until a homogeneous mixture is formed [24] or by freeze-drying the aqueous solutions of the individual counterparts of DESs [105]. The purity of the DESs is determined by the purity of the toxicologically well-characterized starting materials, and no further purification is required. This maintains the relatively low production cost compared to ILs and permits large-scale applications [18, 106].

Because of these properties and their advantage over other ILs, DESs have been used in many areas of chemistry such as electrochemistry [107, 108], enzyme [106]and organic reaction [28, 109], extraction and separation[110]. However, despite the advantages and broad application of DESs, most of the available DESs are water-miscible, limiting their

applicability to polar compounds only. So, most scientists still use toxic organic solvents due to the lack of green hydrophobic solvents. To overcome this problem, Van Osch et al. in 2015 introduced the concept of hydrophobic DESs (HDESs). These solvents consist of decanoic acid as HBD and quaternary ammonium salts as HBAs [45]. HDESs are formulated using a similar method as hydrophilic DESs, except using poorly water-soluble components. The constituents are simply mixed at a desired ratio at ambient temperature or heated at 80 °C with or without stirring until the homogeneous mixture is formed [48, 50, 51].

A lot of research has been preceding to modify the reported HDESs and introduce new ones. Based on the trend, we formulated new water-immiscible DESs based on AcChCl as HBAs and various fatty acid-based HBDs. HBDs used are butyric acid (BA), valeric acid (VA), hexanoic acid and (HA), and caprylic acid (CA). The difference in the carbon chain length of HBDs is expected to influence the physiological properties of DESs with potential for different applications and broaden our understanding of the role of HBDs present in DESs. AcChCl is the acylated form of choline chloride (ChCl), which is more benign and biodegradable than ChCl due to the presence of the acyl group. It is a neurotransmitter that is present in many organisms, including humans [47, 111].

Several methods like sonication, freeze-drying, microwave irradiation, heating and stirring, grinding, and vortex mixing have been reported in the literature to synthesize DESs. The different synthesis mechanisms of DESs are helpful to gain a clear insight on the molecular basis of DESs formation and explore their application in various fields. For example, exploring the freeze-drying method for the synthesis of DESs offers the possibility of incorporating organic self-assemblies in DESs in their pure state with their potential utility

in catalytic application [105]. In addition, other synthesis methods like thermal treatment, vortex mixing, sonication, and microwave irradiation might be helpful to synthesizing single-step DESs. The synthesis method of DESs can be categorized into two broad groups.

2.1.1 Thermal Treatment method

This method utilizes heat for the preparation of DESs. First, individual components of DESs are heated and stirred together until the homogeneous solution is obtained. This can be done in two different ways. Firstly, a component having a lower melting point is melted first, and then a component with a higher melting point is added to the liquid, and the mixture is melted together. In another method, components are added and melted together [112]. To avoid the breakage of hydrogen bonds so formed, the heating temperature is kept as low as possible, usually ranging from 60 to 100 °C with continuous stirring, which can take 30-90 minutes [113].

The first synthesized DESs were prepared by this method. Abbott et al., in 2002, formulated eutectic solvent by heating and stirring choline chloride and urea at a molar ratio of 1:2. The freezing point of this eutectic mixture is 12 °C, which is considerably lower than that of choline chloride (MP= 302 °C) and urea (MP=133 °C) and allows the mixture to be used as an ambient temperature solvent [24]. Another heat treatment technique involves using a rotary device where the components are dissolved in water and evaporated at 50 °C. The liquid thus obtained is kept in a silica gel desiccator until a constant weight is achieved [23].

2.1.2 Non-thermal treatment method

Non- thermal treatment method includes grinding, vortex mixing, and freeze-drying. Vortex mixing involves agitation of the DESs components at a very high speed. Grinding is based on mixing the individual DESs components at room temperature and grinding them using mortar and pestle until a clear liquid is formed [114]. Freeze drying is done by lyophilization of an aqueous solution consisting of individual DESs components [105, 115].The formation of DESs is confirmed by depression in the melting/freezing point of the mixture compared to the melting point of starting components [44]. Generally melting point of the DESs is dependent on various factors. However, the two most important factors are the individual lattice energy of hydrogen bond donor and quaternary ammonium salt, and secondly, the degree to which the anion-hydrogen bond donor interacts with each other. The higher the anion-hydrogen bond interaction, the higher the entropy of the system, resulting in more disorder in a system offering a lower freezing point. Thus, to a certain degree, the depression in the freezing point is the measure of entropy change [11]. Proton NMR is used to check the structure, purity and hydrogen bonding interaction in DESs [116]. FTIR is used for analyzing the functional group of DESs to explain the interaction of the molecules and the stability of these mixtures [117]. A well-established FTIR technique studies the interaction between different groups in a molecule, analyzes and identifies the structures. This technique involves the interaction of infrared radiation (IR) with the constituent by transmitted or reflected IR beam. Hydrogen bond formation changes some properties of the infrared spectrum, like frequency shift and IR intensity of functional groups directly involved in hydrogen bonding [118, 119].

2.2 Experimental

2.2.1 Materials

AcChCl (99%), BA (99%), HA (99%), CA (99%), and deuterated chloroform (99.8%) were purchased from Acros Organics (Fair lawn, NJ). VA (99%) was purchased from Alfa Aesar (Haverhill MA). Ultra-pure water was obtained in the lab with instrument Solution 2000 Water Purification. All the chemicals were used as supplied.

2.2.2 Preparation of Acetylcholine chloride based-DESs.

DESs were prepared using acetylcholine chloride as HBA and butyric acid, valeric acid, hexanoic acid, and octanoic acid as HBDs at a molar ratio of 1:2, 1:3, 1:4, 1:5, and 1:6. AcChCl and different low-carbon fatty acids (Table 2.1) were heated at 60 ˚ C for about 15-45 minutes. The solution was stirred constantly at 600 rpm under atmospheric pressure until the clear homogenous solution was obtained. The temperature was controlled using an oil bath. The prepared DESs 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 HDESs were used without further purification.

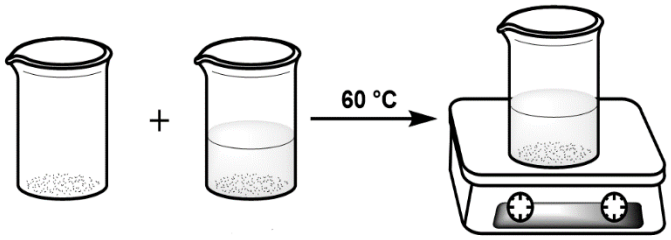


Figure 2.1. Schematic diagram of synthesis of DESs using acetylcholine chloride and fatty acids in molar ratio of 1:2-1:6

Table 2.1. Components, molar ratios, and abbreviation of synthesized DESs.

Components	Mole Ratios	Abbreviation
Acetyl Choline Chloride: Butyric acid	1:2	AcChCl: BA (1:2)
Acetyl Choline Chloride: Butyric acid	1:3	AcChCl: BA (1:3)
Acetyl Choline Chloride: Butyric acid	1:4	AcChCl: BA (1:4)
Acetyl Choline Chloride: Butyric acid	1:5	AcChCl: BA (1:5)
Acetyl Choline Chloride: Butyric acid	1:6	AcChCl: BA (1:6)
Acetyl Choline Chloride: Valeric acid	1:2	AcChCl: VA (1:2)
Acetyl Choline Chloride: Valeric acid	1:3	AcChCl: VA (1:3)
Acetyl Choline Chloride: Valeric acid	1:4	AcChCl: VA (1:4)
Acetyl Choline Chloride: Valeric acid	1:5	AcChCl: VA (1:5)
Acetyl Choline Chloride: Valeric acid	1:6	AcChCl: VA (1:6)

Acetyl Choline Chloride: Caproic acid	1:2	AcChCl: HA (1:2)
Acetyl Choline Chloride: Caproic acid	1:3	AcChCl: HA (1:3)
Acetyl Choline Chloride: Caproic acid	1:4	AcChCl: HA (1:4)
Acetyl Choline Chloride: Caproic acid	1:5	AcChCl: HA (1:5)
Acetyl Choline Chloride: Caproic acid	1:6	AcChCl: HA (1:6)
Acetyl Choline Chloride: Caprylic acid	1:2	AcChCl: CA (1:2)
Acetyl Choline Chloride: Caprylic acid	1:3	AcChCl: CA (1:3)
Acetyl Choline Chloride: Caprylic acid	1:4	AcChCl: CA (1:4)
Acetyl Choline Chloride: Caprylic acid	1:5	AcChCl: CA (1:5)
Acetyl Choline Chloride: Caprylic acid	1:6	AcChCl: CA (1:6)

2.2.3 Characterization of DESs: DESs is the single mole ratio that has the most significant freezing point depression. Thus, the formation of DESs was confirmed by the

mixture's melting/freezing point determination and the starting components. The freezing point of components was determined using TA Instruments Q200 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA). Samples were weighed and placed in sealed Tzero aluminum hermetic pans heating at the rate of 1°C/min. Measurements were performed from -50 °C to 100 °C. A continuous nitrogen purge at a rate of 20ml/min was set to avoid moisture contamination. To ensure the accuracy of the measurements instrument was calibrated using an indium standard. FTIR was used for analyzing the functional group of HDESs to explain the interaction of the molecules and the stability of these mixtures. The FTIR spectra of HDESs and their components were recorded using Nicolet 380 Thermo Fischer-Scientific FTIR spectrometer (Waltham, MA). The spectrum measurement ranges from 4000-700 cm^{-1} at a resolution of 8 cm-1. Spectra were processed with EZ OMNIC software by Thermo-Fisher Scientific. To further confirm the interaction between HBA and HBDs, 1H NMR spectra of HDES and their components were recorded with a Bruker 600 MHz spectrometer (Billerica, MA) at room temperature. Deuterated chloroform was used as a solvent. HDESs and their components were dissolved in deuterated chloroform, and the ^{1}H NMR parameters were set as 90° pulse angle, 25 pulse rate, and 128 scans.

2.3 Result and Discussion

2.3.1 Evaluation of DESs Synthesis

Table 2.2 presents the results of the mixing of AcChCl with different acids. Most of the solvents obtained were colorless, while others were yellowish. DESs at molar ratio 1:2 appeared comparatively more viscous than the DESs at other molar ratios. DESs appeared less viscous and more transparent with the increase in the molar ratio of the fatty acids. The

higher the molar ratio of fatty acid, the less viscous the solvent is. The high viscosity of DESs might be due to high charge transfer and strong hydrogen bonding between the cation and the chloride anion.

Table 2.2. Physical Characterization of formulated DESs.

DESs	**Physical characterization of DESs at room temperature**				
	Molar ratio 1:2	Molar ratio 1:3	Molar ratio 1:4	Molar ratio 1:5	Molar ratio 1:6
AcChCl: BA	Viscous, transparent liquid	Transparent liquid, viscous	Transparent liquid	Transparent liquid	Transparent liquid
AcChCl: VA	Viscous, yellow liquid	Yellowish-transparent liquid, viscous	Transparent liquid	Transparent liquid	Transparent liquid
AcChCl: HA	Viscous, Transparent liquid	white liquid, viscous	Transparent liquid	Transparent liquid	Transparent liquid
AcChCl: CA	Viscous, Transparent liquid	White viscous liquid	Yellowish-transparent liquid	Transparent liquid	Yellowish-transparent liquid

To test the miscibility of formulated HDESs in water, an equal volume of DES and water was mixed, vortexed for 5 minutes, and left to equilibrate. After exactly 12 hours of mixing,

the solution observation was done, showing two distinct layers of HDESs and water conforming to the hydrophobic nature of DESs.

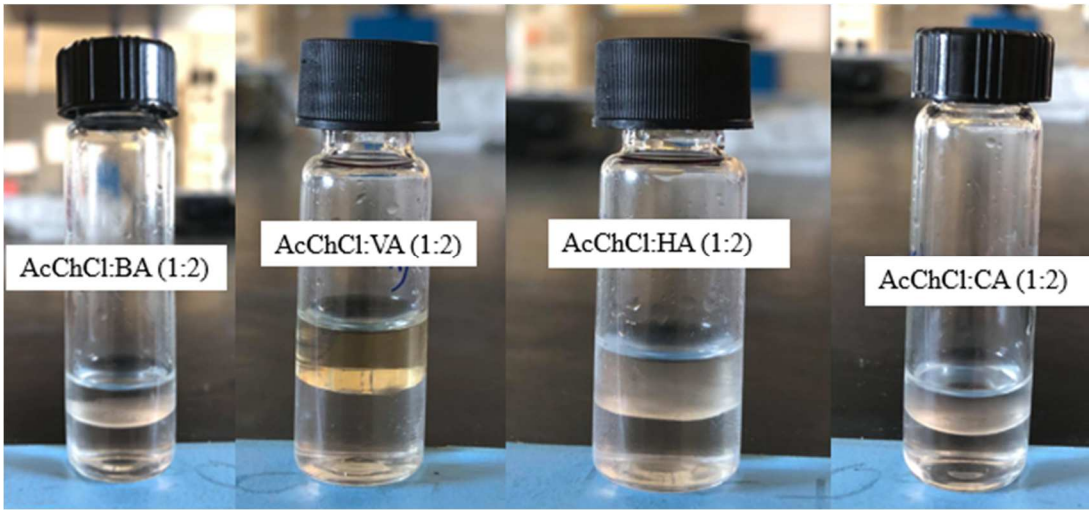


Figure 2.2. The formation of two distinct layer of 1:2 AcChCl: acid in water, with HDESs being the top layer, demonstrating the hydrophobic nature of solvent.

2.3.2. Freezing Point Depression

The melting point of AcChCl, BA, VA, HA, and CA are 150 ˚C, -3.91 ˚C, -30.62 ˚C, -1.23 ˚C, and 18.55 ˚C, respectively. As convention [24], all the formulated DESs have lower melting points than their components, confirming the formation of eutectic mixtures. The depression in the melting point is originated from the interaction between the halide anion of the acetyl choline chloride with the proton of the donor molecule. It is affected by the type of interaction involved during DESs formation, the change in entropy, and the lattice energy of DESs [44] . The higher the magnitude of the interaction, the more significant the depression in the melting point of the mixture. This interaction generally lowers the lattice energy of both donor and acceptor molecules [18]. Figure 2.3 gives the summary of the freezing point of the formulated HDESs. Freezing point depression depends on the type

and mole fraction of the fatty acid used in the mixture. For the HDESs formulated in this study, the depression in melting point is most significant as fatty acid content increases, except for AcChCl: HA (1:4) and AcChCl: HA (1:5), which has the melting point of -4.1, ˚C and -4.4 ˚C respectively. All other DESs followed the trend. For instance, AcChCl: BA (1:2) has a melting point of -17.1 ˚ C higher than that of the melting point of DESs at other molar ratios. The highest depression in melting point for AcChCl: VA was observed at molar ratio 1:2 followed by at molar ratio 1:3, which is -38.3 ˚C and 37.7 ˚C, respectively. A similar trend is observed for DESs prepared using other fatty acids. The melting point of AcChCl: HA (1:2), AcChCl: CA (1:2) is -16.1 ˚C, and -1.3 ˚C, which is higher than that of DESs formed from similar fatty acids at other molar ratios. The lowest melting point depression is observed at a mole ratio of 1:6 (HBA: HBD) for all synthesized DESs.

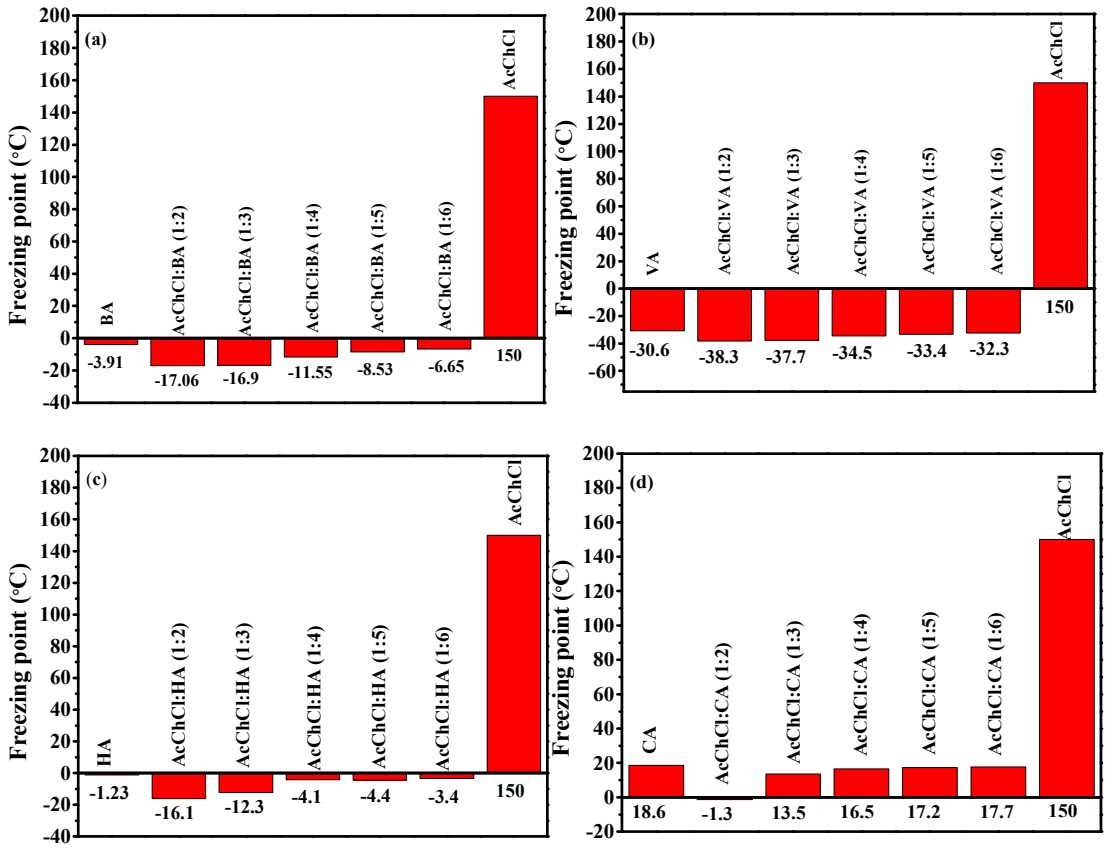


Figure 2.3. Freezing point of (a) AcChCl and different ratios of AcChCl with BA (b) AcChCl and different ratios of AcChCl with VA. (c) AcChCl and different ratios AcChCl with HA. (d) AcChCl and different ratios of AcChCl with CA.

2.3.3 FTIR Spectroscopy

The FTIR spectrum of the pure AcChCl, fatty acids, and DESs are shown in figure 2.4. When compared with the pure components, the FTIR spectra of the DESs display changes in the frequency shifts, absorbance values, and bandwidth. However, some spectral characteristics of DESs overlap with those of AcChCl and fatty acids. The characteristic carbonyl peak of fatty acids is seen at 1711 cm^{-1} for BA, 1708 cm^{-1} for VA and HA, and 1713 cm^{-1} for CA, and ammonium identity peak is at 958 cm^{-1} from the acceptor. The FTIR spectrum of all donors shows the O-H stretching broadband around 3400-2800 cm^{-1} with

the overlapping peak of C-H stretching vibrations (2969, 2879 cm^{-1} for BA, 2962,2875 cm^{-1} for VA, 2959, 2874 cm^{-1} for HA, and 2922, 2859 cm^{-1} for CA). In HDESs, the stretching vibration absorption peak of the hydroxyl group was broader (3700-2225 cm^{-1}) compared to the HBDs due to the formation of a hydrogen bond between coordinated acid and AcChCl. The shift of the bands corresponding to the stretching vibrations of the C=O groups towards higher wavenumber for all HDESs further proves the interaction between the HBA and HBDs. The C-O stretching vibration of BA at 1285 cm^{-1} displayed between 1235-1239 cm^{-1} for BA based HDESs, VA at 1275 cm^{-1} shifts between 1229-1235 cm^{-1} for VA based HDESs, HA at 1290 cm^{-1} appears between 1230-1241 cm^{-1} for HA-based HDESs and CA at 1281 cm^{-1} shifts between 1229-1235 cm^{-1}. All these spectrum changes suggest hydrogen bond formation. The frequency of the CH_3 group in AcChCl shifts from 1483 to 1467-1480 cm^{-1} in VA-based DESs.

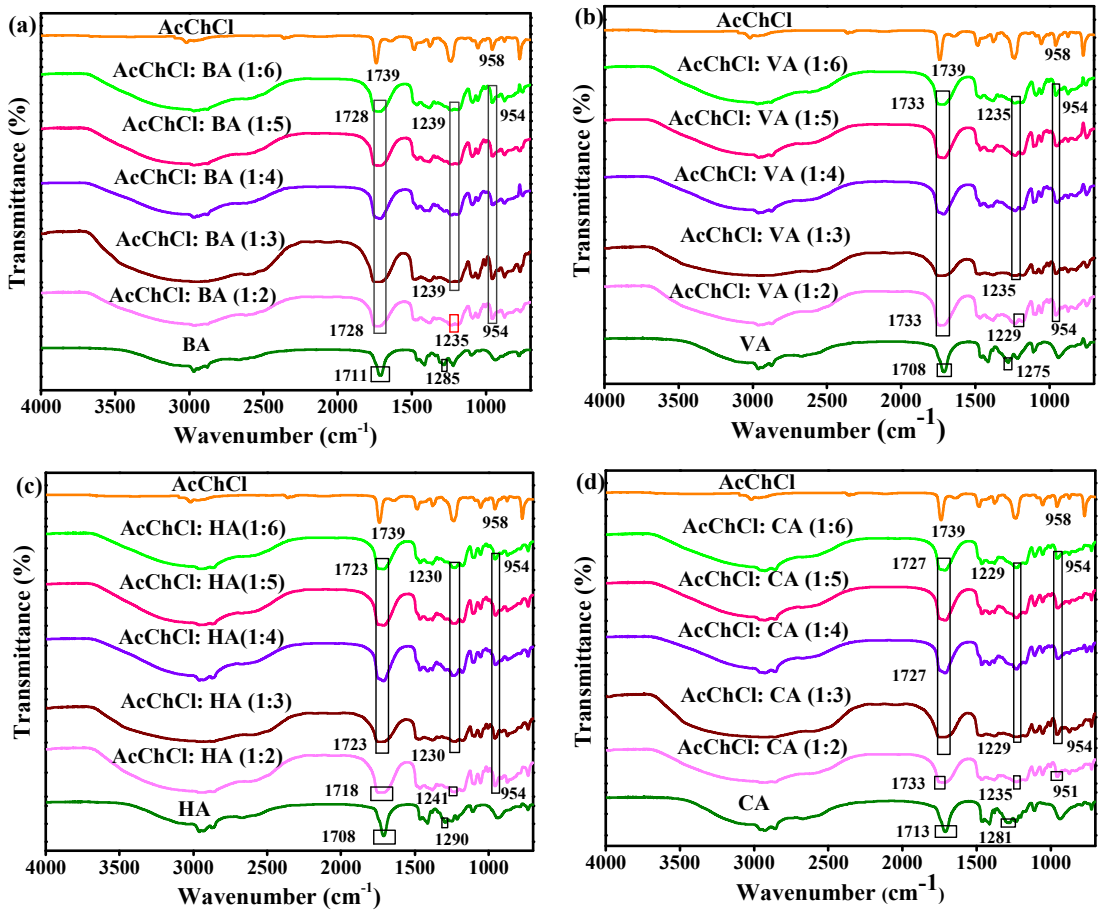


Figure 2.4. FTIR spectrum of (a) AcChCl and different ratios of AcChCl with BA (b) AcChCl and different ratios of AcChCl with VA. (c) AcChCl and different ratios AcChCl with HA. (d) AcChCl and different ratios of AcChCl with CA. The extended box shows similar wavenumber for the HDESs.

2.3.4 ^{1}H NMR Spectroscopy

Very few NMR spectral studies have been reported in the literature to confirm hydrogen bonds in eutectic systems. However, the shifts in the ^{1}H NMR signal of DESs compared to

the individual components evidence hydrogen bonds formation. Table 2.3 shows the chemical shift values of the formulated HDESs. A slight change in the chemical shift value of the HDESs compared to that of its constituents was observed. The shifts in the proton frequency around the quaternary nitrogen of AcChCl were seen in the presence of HBDs, thereby confirming the interaction between the HBAs and HBDs. The frequency of proton around the quaternary nitrogen of AcChCl is changed from 3.540 to 3.495, 3.460, 3.450, 3.450, and 3.465 ppm for AcChCl: BA (1:2), AcChCl: BA (1:3), AcChCl: BA (1:4), AcChCl: BA (1:5), and AcChCl: BA (1:6), respectively. Similarly, the chemical shift value of proton around nitrogen decreases to lower the lower field in VA, HA, and CA-based HDESs as observed in BA-based HDESs.

The loss of the hydroxyl proton (–OH) signal in the accepter molecule also confirms the interaction between donor and acceptor molecules. For all HDESs, the peak due to hydroxyl proton of fatty acids at 12 ppm is conspicuously absent in HDESs at all molar ratios.

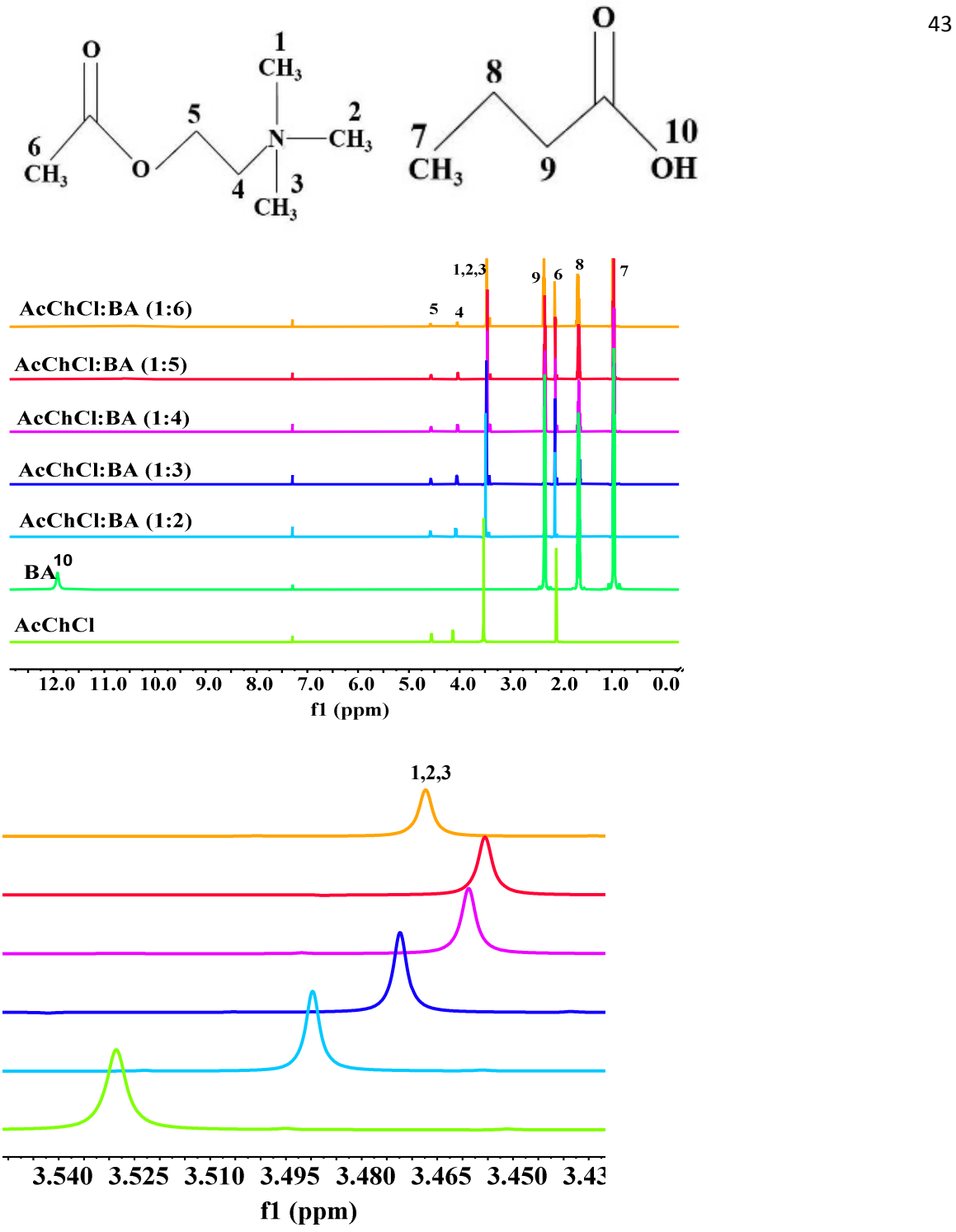


Figure 2.5. Full range ^{1}H NMR spectra of a) AcChCl and different ratios of AcChCl with BA. Expansion of NMR spectra showing 1, 2,3 Upfield (bottom).

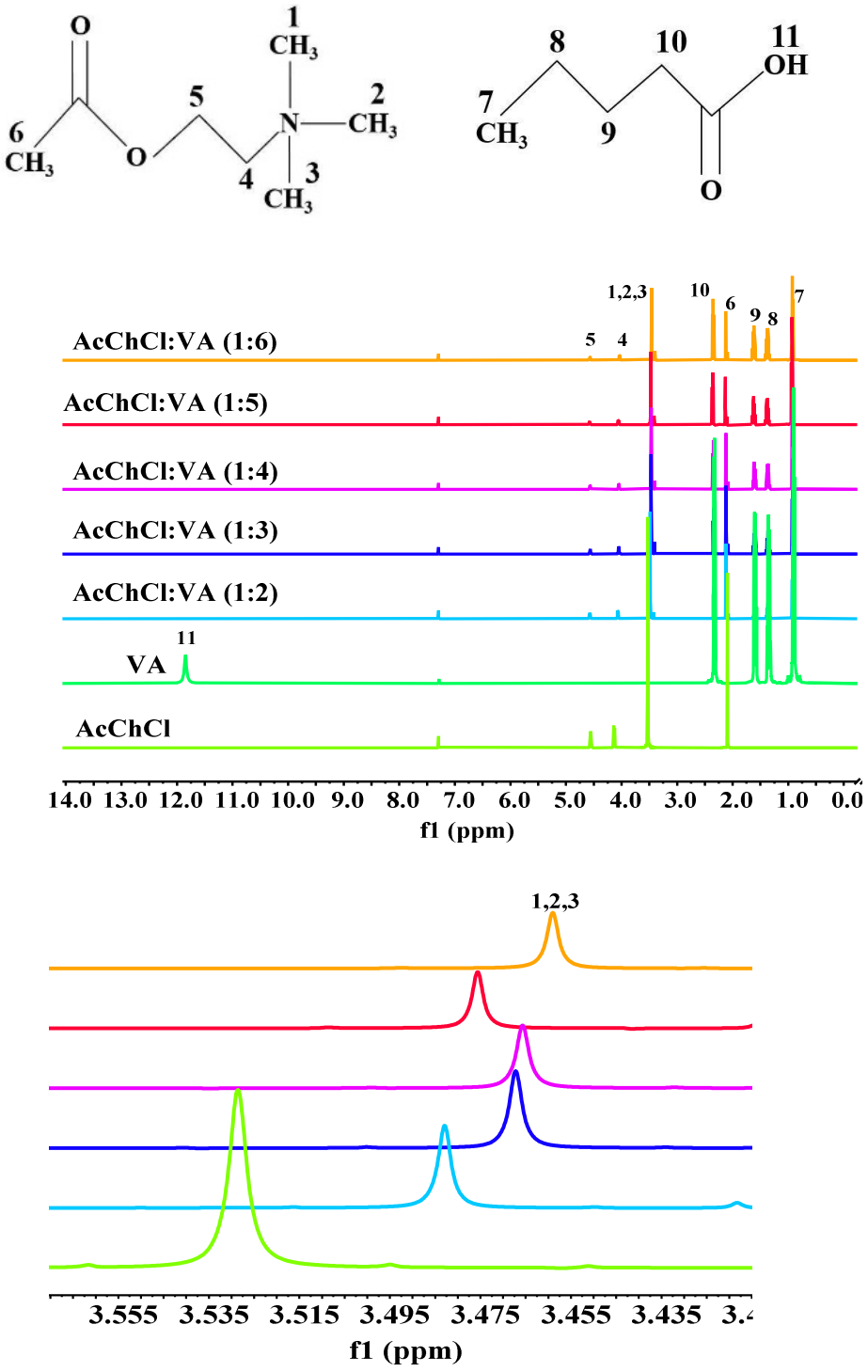


Figure 2.5. Full range ^{1}H NMR spectra of b) AcChCl and different ratios of AcChCl with VA. Expansion of NMR spectra showing 1, 2,3 Upfield (bottom).

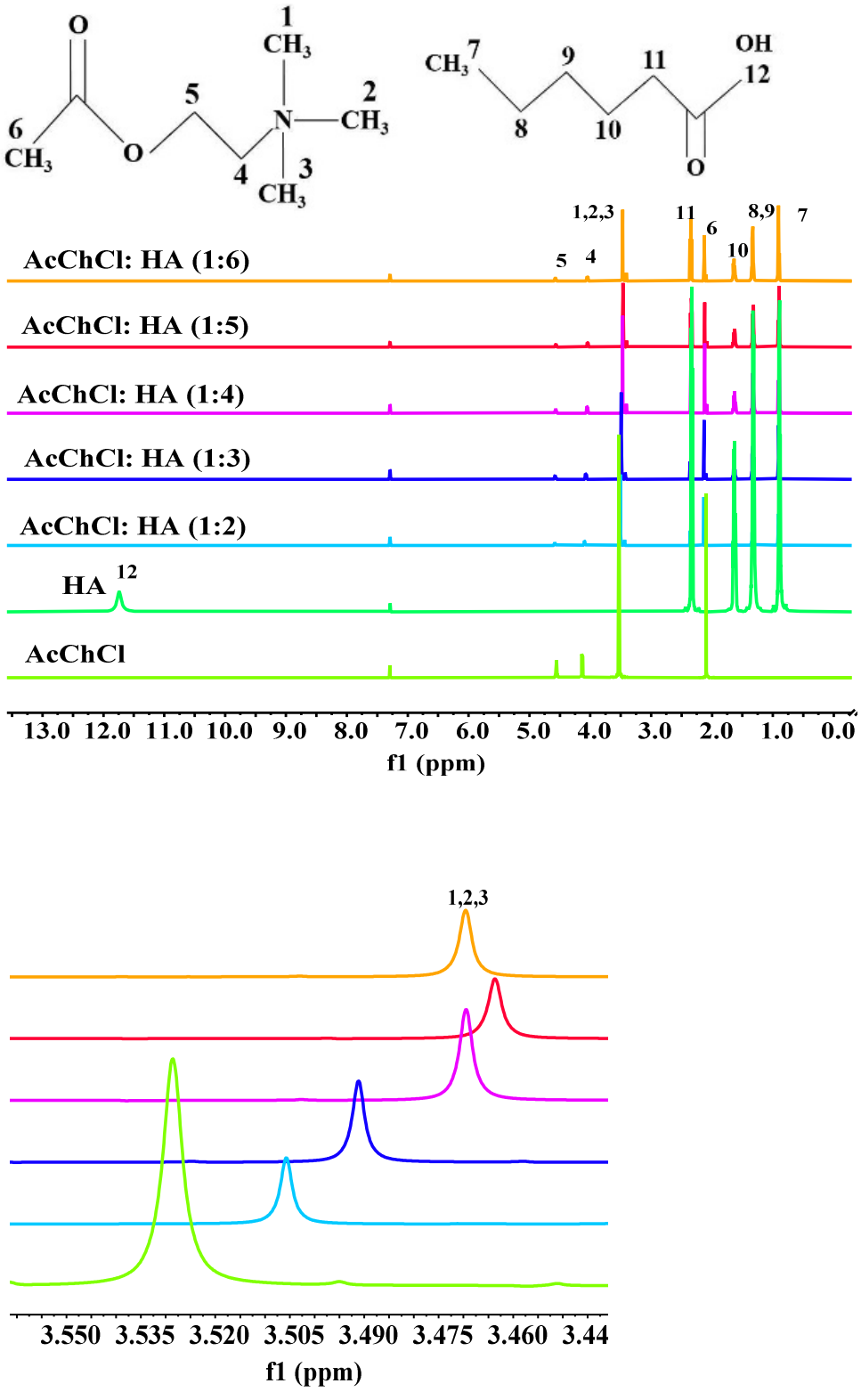


Figure 2.5. Full range ^{1}H NMR spectra of c) AcChCl and different ratios of AcChCl with HA. Expansion of NMR spectra showing 1, 2,3 Upfield (bottom).

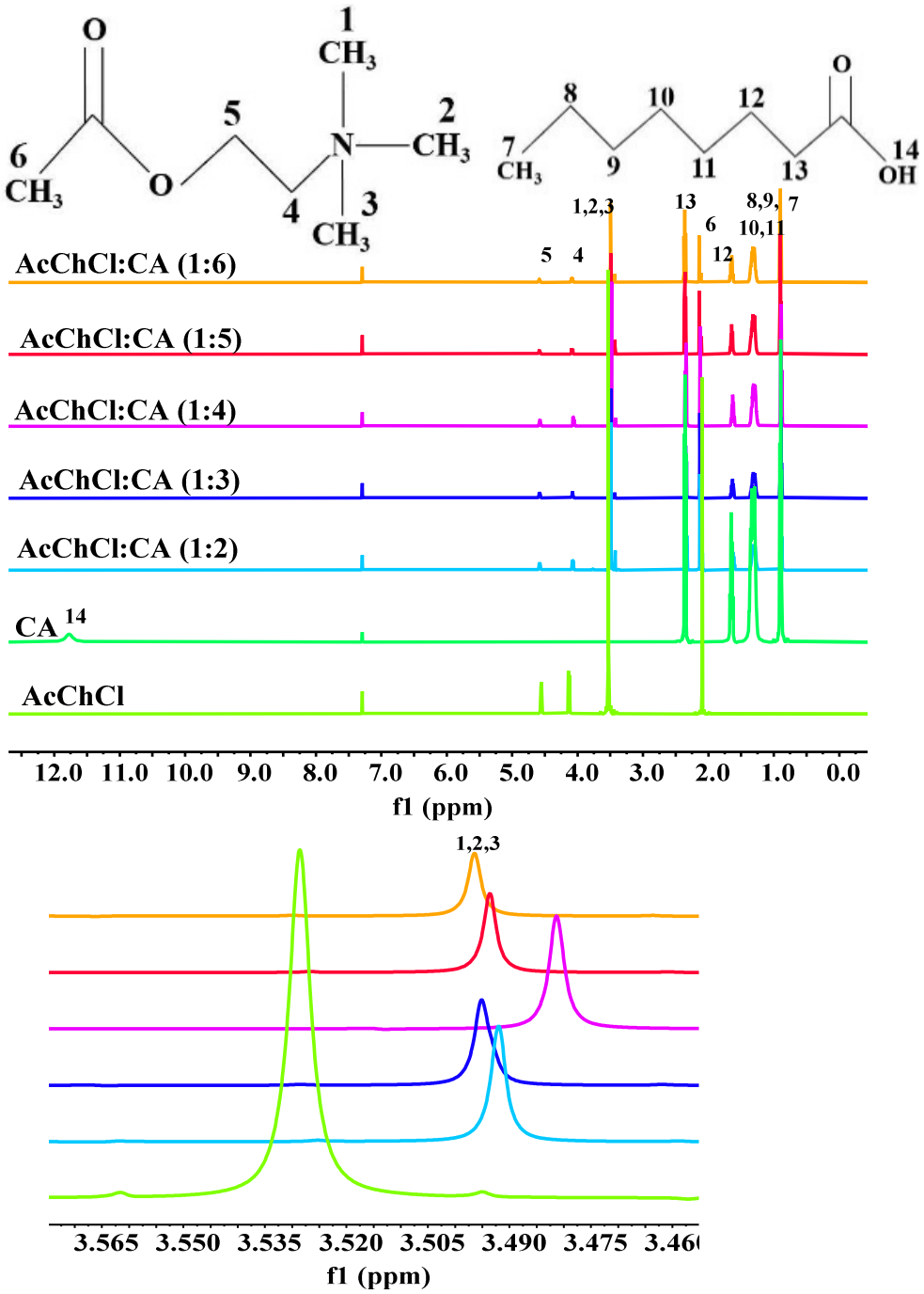


Figure 2.5. Full range ^{1}H NMR spectra of d) AcChCl and different ratios of AcChCl with CA. Expansion of NMR spectra showing 1, 2,3 Upfield (bottom).

Table 2.3. Frequency shifts of NMR spectra of DESs and its constituents

Proton	**AcChCl and BA**	**AcChCl: BA (1:2)**	**AcChCl: BA (1:3)**	**AcChCl: BA (1:4)**	**AcChCl: BA (1:5)**	**AcChCl: BA (1:6)**
1,2,3	3.528	3.489	3.472	3.458	3.455	3.466
4	4.129	4.072	4.005	4.035	4.033	4.031
5	4.555	4.559	4.563	4.538	4.564	4.567
6	2.094	2.123	2.119	2.113	2.115	2.122
7	0.961	0.966	0.963	0.961	0.964	0.977
8	1.662	1.666	1.663	1.665	1.664	1.669
9	2.321	2.321	2.321	2.321	2.323	2.338
10	11.871	-	-	-	-	-
Proton	**AcChCl and VA**	**AcChCl: VA (1:2)**	**AcChCl: VA (1:3)**	**AcChCl: VA (1:4)**	**AcChCl: VA (1:5)**	**AcChCl: VA (1:6)**
1,2,3	3.528	3.483	3.467	3.465	3.476	3.459
4	4.129	4.053	4.056	4.052	4.057	4.052
5	4.555	4.565	4.561	4.569	4.567	4.563
6	2.094	2.121	2.117	2.119	2.13	2.123
7	0.901	0.916	0.916	0.919	0.931	0.925
8	1.354	1.367	1.367	1.371	1.384	1.379
9	1.593	1.601	1.601	1.608	1.621	1.615
10	2.326	2.337	2.339	2.344	2.357	2.351
11	11.871	-	-	-	-	-

Proton	AcChCl and HA	AcChCl: HA (1:2)	AcChCl: HA (1:3)	AcChCl: HA (1:4)	AcChCl: HA (1:5)	AcChCl: HA (1:6)
1,2,3	3.528	3.505	3.491	3.469	3.463	3.469
4	4.129	4.091	4.074	4.047	4.047	4.047
5	4.555	4.559	4.556	4.565	4.565	4.561
6	2.094	2.133	2.131	2.12	2.12	2.127
7	0.891	0.908	0.908	0.899	0.899	0.906
8,9	1.316	1.33	1.33	1.321	1.321	1.329
10	1.628	1.635	1.635	1.628	1.628	1.638
11	2.332	2.346	2.346	2.339	2.339	2.348
12	11.871	-	-	-	-	-
Proton	**AcChCl and CA**	**AcChCl: CA (1:2)**	**AcChCl: CA (1:3)**	**AcChCl: CA (1:4)**	**AcChCl: CA (1:5)**	**AcChCl: CA (1:6)**
1,2,3	3.528	3.492	3.495	3.481	3.494	3.497
4	4.129	4.079	4.079	4.061	4.079	4.079
5	4.555	4.577	4.577	4.577	4.59	4.588
6	2.094	2.129	2.132	2.128	2.138	2.141
7	0.894	0.887	0.891	0.886	0.896	0.899
8,9,10,11	1.323	1.305	1.305	1.305	1.307	1.309
12	1.647	1.626	1.629	1.629	1.643	1.643
13	2.357	2.357	2.347	2.345	2.358	2.361
14		-	-	-	-	-

2.4 Conclusion

In summary, twenty different DESs were formulated using the thermal treatment method using Acetylcholine chloride as HBA and BA, VA, HA, and CA as HBDs. The freezing point of all mixtures formed was lower than the freezing point of the individual components. Depression in melting point depends upon the composition and molar ratio of the HBDs used. Different DESs took different times to form the homogenous solution, which was between 15-45 minutes. Band bordering and frequency shifts observed in FTIR, and NMR spectra confirm the formation of DESs.

CHAPTER 3

CHARACTERIZATION OF DEEP EUTECTIC SOLVENTS

3.1 Introduction

Because of the green properties and varieties, DESs have been used in many areas of chemistry such as, electrochemistry [107, 108], enzyme [106], organic reaction [28, 109], extraction, and separation [110]. Various properties of DESs are like those of the ionic solvents to replace many traditional ionic solvents and even some nonionic solvents. However, the application of DESs is still in the primeval stage, and the replacement of molecular solvents and ionic solvents by DESs requires knowledge about their physical, chemical, and thermal properties. So, there is a significant need to measure DESs properties and develop a reliable technique to measure them to manage their future application properly. Thus, in this chapter, some properties like pH, viscosity, conductivity, density, surface tension, refractive index, octanol-water partition coefficient, and decomposition temperatures are determined. Density is a fundamental physicochemical property of a solvent. Knowledge about density is essential to design chemical processes that involve fluid mechanics and mass transfer calculations. Density also dramatically affects the dissolution, reaction, and separation processes, determining their feasibility. For example, most hydrophilic DESs have a density higher than the density of water [22, 44].

Viscosity helps to understand the mass transport phenomena and the conductivity for ionic fluids, thereby affecting their suitability for the application. It also influences the dissolution and extraction investigating the variation of viscosity with temperature is crucial to decrease energy requirements for processing DESs. Viscosity-temperature profile of DESs typically follows Arrhenius behavior. As temperature increases, viscosity

decreases [18, 44, 120]. The viscosity of most DESs are high (≥ 100 cP) at room temperature [121].

pH is the measure of acidity and basicity of the substance. This property is essential to finding a substance's chemical characteristics and is critical in managing chemical reactions. pH also affects the extraction and separation.

Conductivity measures the ability of the material to conduct electricity. It also indicates how much material is resistive for a motion of electrons within its molecule. Information on the conductivity is needed to understand the electrical properties of electrolytes, such as carrier mobility, carrier concentration, and longevity of excess carrier. Determination of electrical conductivity is also crucial for designing, controlling, and optimizing the electrolysis processes and producing the electrochemical power sources. It is also used to evaluate the characteristic quantities like dissociation constant [122-124]. In addition to this, it is also a helpful measurement in various industries [125].

Refractive index (RI) is a dimensionless property that measures the bending of a ray of light when it passes from one medium to another medium. It measures the relative speed of the light in a medium to the speed of light in a vacuum. Knowing RI of HDESs is vital in checking the purity of materials, in measuring the concentration of solutes in solution, and in building materials with photo application [125, 126]

Surface tension is the fundamental physical property of DESs for its application in the interface, collide, heat, momentum, and mass transfer processes like distillation, absorption, extraction, and separation [77, 127]. Determination of surface tension is needed to solve many industrial-related problems and develop new separation technology. It

provides crucial information on molecular influence on the intensity of interactions in the mixture [128]. Surface tension arises due to the discontinuity of the electrostatic force on the surface. In the bulk of the liquid material, molecules move to respond to both attractive and repulsive forces from each other. Such forces are unbalanced on the surface and result in a robust and attractive force among the molecules on the surface [119]. To confirm the potential use of DESs as alternative solvents, one of the most relevant properties that need to be determined is thermal stability. It determines the maximum temperature at which DESs can maintain their liquid state without decomposition and thus their range of use as solvent [116]. Therefore, knowledge of thermal stability is essential to determine the application of DESs at high temperatures. In addition, it is also necessary to set the feasible operating range of the new solvents [129].

3.2 Experimental

3.2.1 Materials

AcChCl (99%), BA (99%), HA (99%), CA (99%), and deuterated chloroform (99.8%) were purchased from Acros Organics (Fair lawn, NJ). VA (99%) was purchased from Alfa Aesar (Haverhill MA). Ultra-pure water was obtained in a lab using instrument Solution 2000 Water Purification.

3.2.2 Methods

3.2.2.1 Synthesis

All DESs are prepared by thermal treatment method by heating AcChCl and fatty acids at ~60 °C for about 15-45 minutes until a homogeneous solution is formed. The solution was

cooled at room temperature, kept in close sealed vials, and then stored in a desiccator to prevent moisture absorption.

3.2.2.2 Density

The gravimetric method was used to determine the density of the synthesized DESs. Measurements were taken at room temperature and pressure. For calibration, a 1.00 mL pipet was used to dispense 1.00 g mL^{-1} of water. Then 1 mL of the DESs was slowly pipetted using the same pipette into a pre-weighed vial, and the mass was measured using Mettler Toledo analytical balance (Columbus, OH). Densities were determined in triplicate.

3.2.2.3 Refractive Index

Refractive Indices of all synthesized DESs were measured in triplicate using Bausch and Lomb Abbe-3L refractometer (Rochester, NY) at room temperature. Deionized water was used for the calibration before each experiment.

3.2.2.4 Surface Tension

The surface tension of the synthesized DESs was recorded with a Fischer Scientific tensiomat 21 (Dubuque, Iowa) using the Du Nouy ring method at room temperature. A flame-dried platinum-iridinium ring was used for the measurement. All glassware was washed and dried using deionized water and acetone before each measurement. Tensiometer was calibrated using pure deionized water.

3.2.2.5 pH

The pH of the DESs was determined using a Mettler Toledo FEP20 pH meter (Columbus, OH) as a function of temperature from 25 to 65 °C for every 10 °C intervals. The temperature was controlled using an oil bath. For calibration, standard buffer solutions of pH 4, 7, and 10 were used.

3.2.2.6 Conductivity

The conductivity of synthesized DESs was measured using Oakton Cond 6+ conductivity probe as a function of temperature from 25 °C to 65 °C for every 10 °C intervals. The probe was calibrated using 1000 ppm sodium chloride solution. All the measurements were taken in triplicate.

3.2.2.7 Viscosity

A Brookfield DV-III Ultra rheometer (Toronto, Canada) was used to determine the viscosities as a function of temperature ranging from 25 to 65 °C at 10 °C increments. Temperature variation was obtained using an external water bath and Brookfield TC- 502 circulator (Toronto, Canada). All the measurements were taken in triplicate with a CP40 spindle at a speed of 1 rpm.

3.2.2.8 Decomposition Temperature

Decomposition temperature was analyzed using thermogravimetric analysis (TGA). TGA was done with Seiko 220 TG/DTA (Tokyo, Japan) instrument with an aluminum reference pan. 6-12 mg of sample was weighed into the aluminum pan for measurement. Sampling was done at room temperature to 350 °C at a heating rate of 10 °C/min under a nitrogen

atmosphere. The high purity nitrogen purging system (flow= 20mL/min) provided an inert environment to prevent unwanted oxidation.

3.3 Results and Discussion

3.3.1 Synthesis

DESs were synthesized by mixing acetylcholine chloride and HBDs at different molar ratios (Table 2.1) by heating at 60 °C for about 15-45 minutes until a homogeneous mixture was formed. Then, the prepared solution was cooled at room temperature and stored in a desiccator to prevent any moisture absorption. The components and molar ratios of the synthesized DESs are shown in Table 2.1 of chapter 1.

3.3.2 Density

Density of AcChCl, BA, VA, HA and CA are 1.20 g/mL, 0.964 g/mL, 0.940 g/ml, 0.927 g/mL, and 0.910 g/ mL, respectively. Opposite to hydrophilic DESs, the density of hydrophobic DESs is similar or lower than water depending on the components used [65]. The low density of HDESs can be explained by the low density of the components used to prepare the HDESs [45].

Figure 3.1 shows the densities of all formulated HDESs. This graph also shows that the molar ratios of the HBDs used have significant effects in modulating the densities of the synthesized DESs. Density decreases with increasing the molar ratio of HBDs. Figure 3.2 shows the effect of the amount of AcChCl on the densities of the product. The more AcChCl in the system, the higher the density it will have. This is due to an increase in the molar volumes of the HDESs as more HBD was added relative to the mass. On the other

hand, the addition of HBDs might have also increased, resulting in a slight decrease in the density.

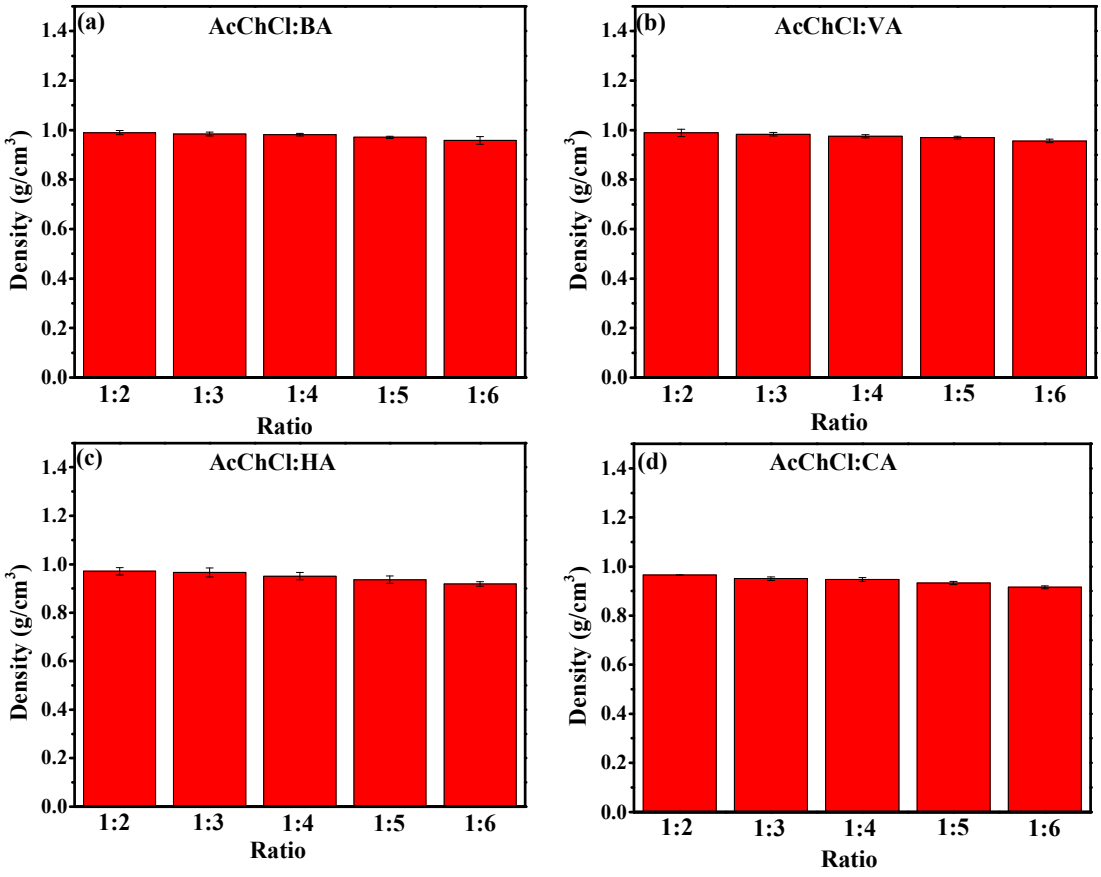


Figure 3.1 Density of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA at molar ratio 1:2-1:6

Also, figure 3.2 demonstrates that increasing the alkyl chain length decreases the density of HDES. This might be because increasing the alkyl chain length increases the molar mass and the molar volume. Due to the higher molar volume, the density decreases.

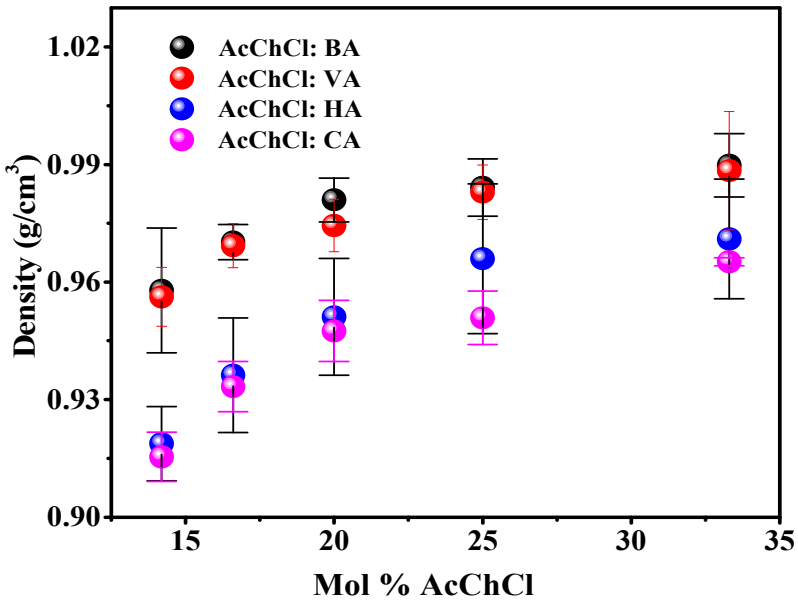


Figure 3.2. Density of DESs as a function of molar % of AcChCl

3.3.3 Refractive index

Figure 3.3 shows the refractive indices of the formulated HDESs. Figures 3.3 and 3.4 reveals that the refractive indices values of HDESs are significantly affected by the HBDs used. As expected, RI decreases with increasing the content of HBDs. The refractive indices values of AcChCl and BA decreases from 1.453 (AcChCl: 2BA) to 1.424 (AcChCl: 6BA), AcChCl and VA ranges from 1.451 (AcChCl: 2VA) to 1.423 (AcChCl: 6VA), AcChCl and HA ranges from 1.450 (AcChCl: 2HA) to 1.419 (AcChCl: 6CA) and AcChCl and CA ranges from 1.449 (AcChCl: 2CA) to 1.417 (AcChCl: 6CA). This decrease in refractive indices is attributed to the density of the solvent. The denser the medium, the greater the difficulty in light penetration. Thus, the higher the density, the higher the solvent's refractive index and vice versa. . Figure 3.4 also shows that the refractive indices decrease with increase in the alkyl chain length of the HBDs. Increasing the alkyl chain

length decreases the density of the solvent thereby decreasing the refractive indices of the HDESs. Figure 3.5 demonstrates the strong linear relationship between densities and refractive, with correlation coefficient values ranging from 0.94851 for BA-based HDESs to 0.98134 for HA-based HDESs

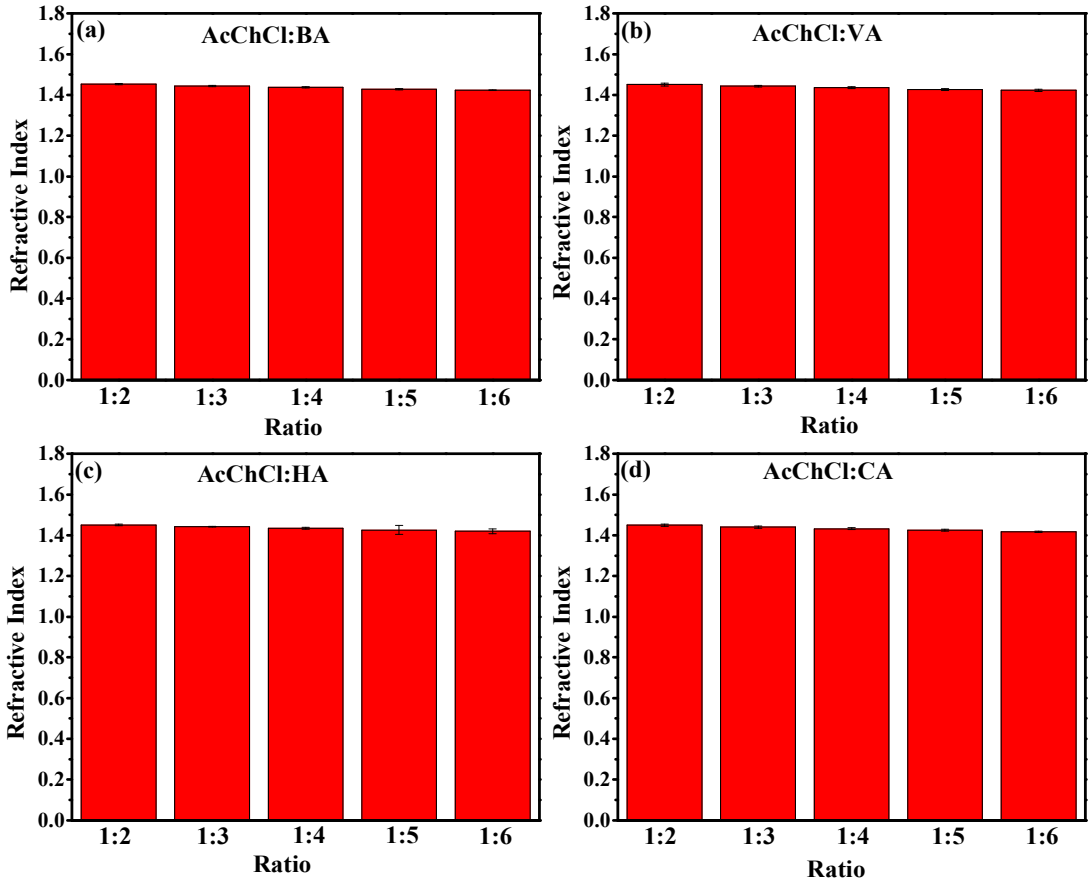


Figure 3.3. Refractive index of (a) AcChCl with BA (b) AcChCl with VA. (c) AcChCl with HA (d) AcChCl with CA at molar ratio 1:2-1:6

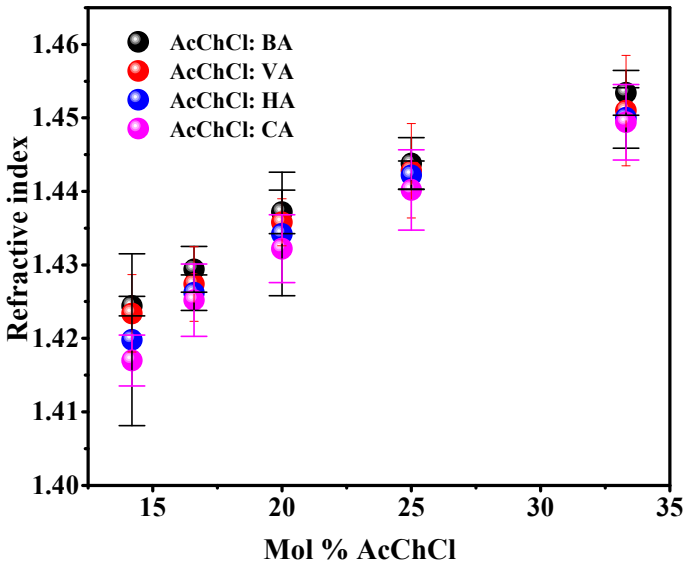


Figure 3.4. Refractive Index of DESs as a function of molar % of AcChCl

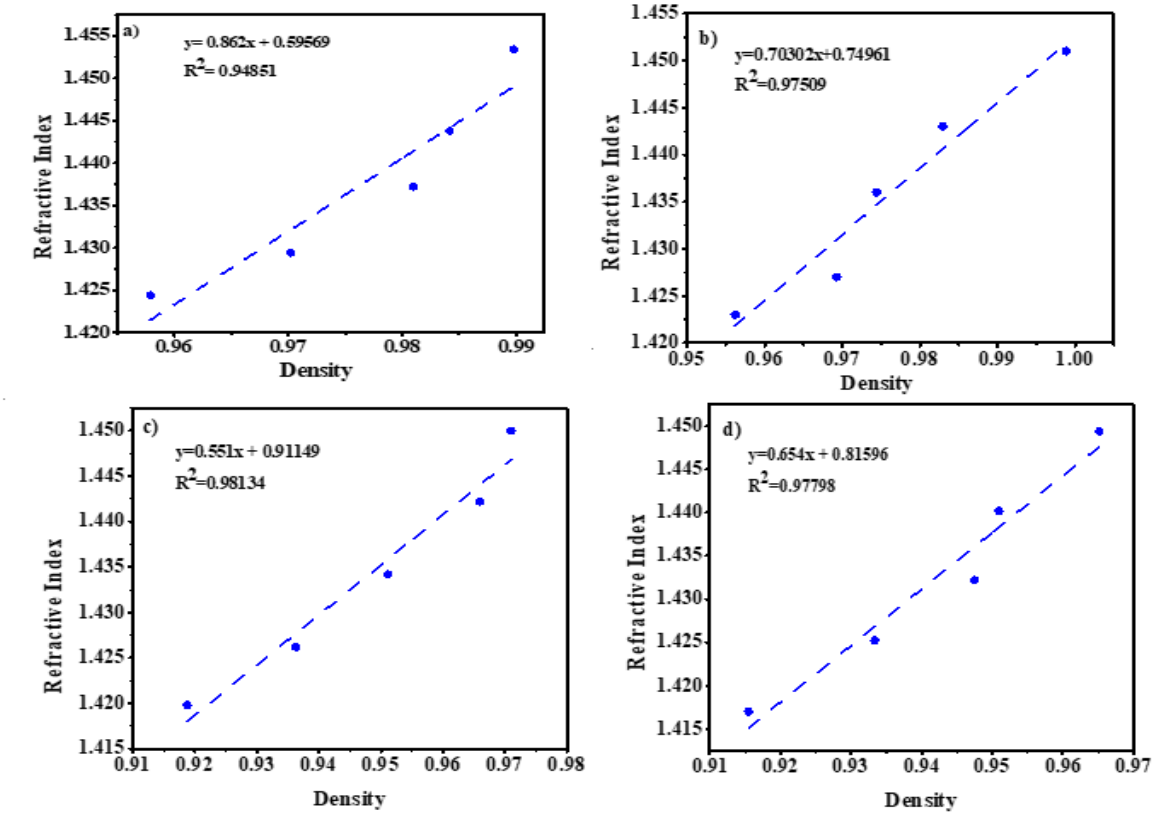


Figure 3.5. Plot of refractive index Vs density. A strong relationship exists between the two physical properties.

3.3.4 Viscosity

The viscosity of the HDESs is mainly governed by the chemical nature of the HDESs components and temperature. The higher viscosity of HDESs can be explained by an extensive hydrogen-bonding network between the HDESs components. This results in the lower mobility of species within the DESs. In addition, the large ion size, tiny void volume, and other forces such as van der Waals or electrostatic interaction may also contribute to the higher viscosity of HDESs [44].

The viscosities of AcChCl based DESs closely depend on the type of HBDs used. The viscosities decrease with increasing the molar ratios of fatty acids, as shown in figure 3.6 and figure 3.7. This is expected as the strength of the hydrogen bonding between the components of HDESs reduces with increasing fatty acids. This is also in agreement with the density measurement. The decrease in density is due to an increase in volume, which reduces viscosity.

Figure 3.7 shows that the viscosities of the synthesized DESs increase with the increase in the alkyl chain length. This behavior is attributed to entropy loss due to acid's more restricted conformational freedom as alkyl chain length increases [26, 116].

Figure 3.6 shows the Viscosity of HDESs as a function of temperature from 25 °C to 65 °C. The immense variation in viscosity with temperature indicates that HDESs are extremely sensitive towards temperature. The viscosity-temperature profile follows an Arrhenius- like behavior. As temperature increases, viscosity decreases. This is due to a decrease in hydrogen bonding interaction which reduces the internal resistance of

molecules, causing the easy molecules to flow and thus be less viscous. The viscosity-temperature relationship is modeled well by the Arrhenius equation,

$$\mu=\mu_0 \, ^{E\mu/RT} \quad \ldots\ldots (3.1)$$

Where μ is dynamic velocity, μ_0 is pre-exponential constant, Eμ is the activation energy, R is the gas constant, and T is the temperature in Kelvin. Thus, μ_0 and Eμ are the model parameter that depends on the nature of HBDs. These parameters, along with the regression coefficient, are shown in Table 3.1.

Table 3.1. Model parameters for viscosity

HDESs	**E^{μ}/RT**	**μ_0**	**R^2**
AcChCl: BA (1:2)	3434.9	-6.916	0.961
AcChCl: BA (1:3)	2917.3	-5.934	0.879
AcChCl: BA (1:4)	2438.3	-4.895	0.907
AcChCl: BA (1:5)	2305.5	-4.779	0.965
AcChCl: BA (1:6)	1701	-3.092	0.977
AcChCl: VA (1:2)	3135.7	-5.797	0.924
AcChCl: VA (1:3)	3007.1	-6.110	0.888
AcChCl: VA (1:4)	1971.7	-3.315	0.945
AcChCl: VA (1:5)	2031.1	-3.824	0.994
AcChCl: VA (1:6)	2373	-5.205	0.960
AcChCl: HA (1:2)	5060	-11.819	0.957
AcChCl: HA (1:3)	3242.6	-6.709	0.976

AcChCl: HA (1:4)	2162.8	-3.710	0.794
AcChCl: HA (1:5)	2116.4	0.774	0.774
AcChCl: HA (1:6)	2353.5	-4.919	0.771
AcChCl: CA (1:2)	4329.3	-9.382	0.979
AcChCl: CA (1:3)	3923	-0.867	0.997
AcChCl: CA (1:4)	3223.1	-6.854	0.973
AcChCl: CA (1:5)	3237.3	-7.193	0.975
AcChCl: CA (1:6)	3145.6	-7.183	0.960

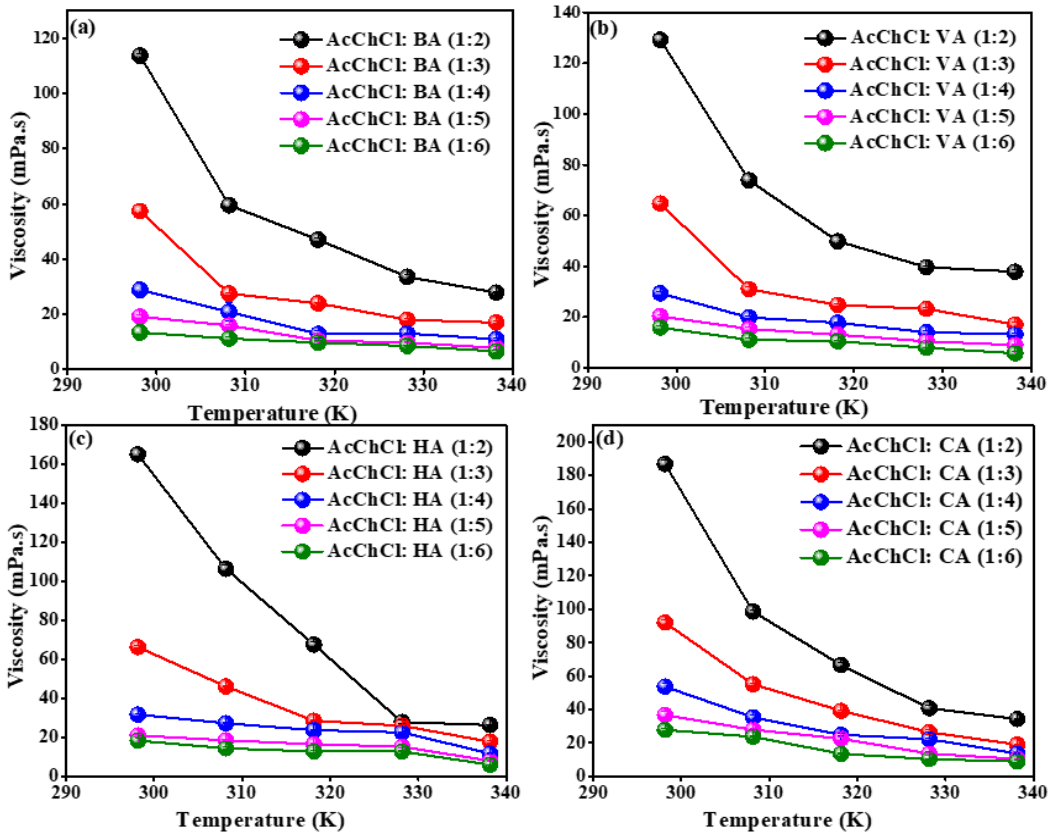


Figure 3.6. Viscosity of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA from temperature 298 K to 338 K.

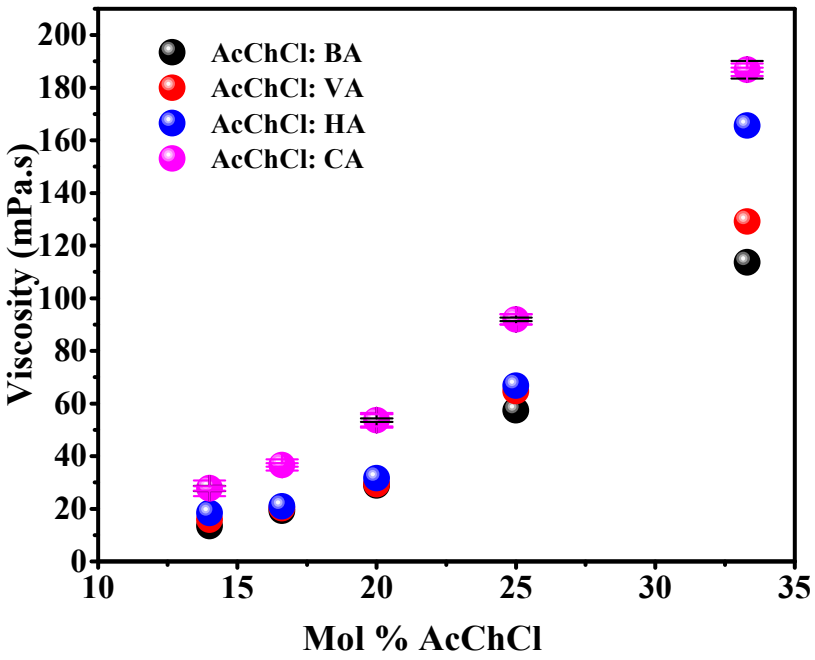


Figure 3.7. Plot of viscosity Vs molar % of AcChCl. Viscosity increases with increase in alkyl chain length of organic acids.

3.3.5 pH

Figure 3.8 shows the pH of the formulated HDESs. Results obtained indicate the acidic nature of HDESs at room temperature. The results show that pH depends on the chemical nature of the HBDs and the molar ratios of the HBA/HBD used. The higher the pH value, the lower the availability of hydrogen ions that are not involved in hydrogen bonding. The pH of DESs increases with increasing the AcChCl content. A pH of HDESs was studied as a function of temperature from 25 to 65 °C. The pH-temperature plot demonstrated a linear relationship and fit to following simple linear model,

$$\mathbf{pH = a + b\,T} \quad \text{(3.2)}$$

Where T is the temperature in kelvin, a and b are the constants that depend on the molar ratio and type of HBDs.

The values of constants along with the regression coefficient are shown in table 3.2. The increase in temperature increases the kinetic energy of DESs molecules, thereby weakening the intermolecular hydrogen bonding strength. The collision between molecules also causes an equilibrium shift of the components of DESs molecules resulting from the dissociation of more H+ ions, leading to a decrement of pH value. The average uncertainty associated with the pH measurement is ±0.03.

Table 3.2. Model parameter for pH

DESs	**a**	**b**	**R^2**
AcChCl: BA (1:2)	3.866	-0.0091	0.920
AcChCl: BA (1:3)	4.251	-0.0107	0.927
AcChCl: BA (1:4)	3.302	-0.0087	0.942
AcChCl: BA (1:5)	3.565	-0.0098	0.976
AcChCl: BA (1:6)	3.736	-0.0105	0.960
AcChCl: VA (1:2)	8.879	-0.0218	0.973
AcChCl: VA (1:3)	9.877	0.0262	0.952
AcChCl: VA (1:4)	10.125	-0.0275	0.912
AcChCl: VA (1:5)	10.303	-0.0287	0.879
AcChCl: VA (1:6)	10.894	-0.0311	0.847
AcChCl: HA (1:2)	10.017	-0.0233	0.999
AcChCl: HA (1:3)	9.193	-0.0221	0.994

AcChCl: HA (1:4)	12.486	-0.0345	0.849
AcChCl: HA (1:5)	12.709	-0.0363	0.844
AcChCl: HA (1:6)	8.556	-0.0236	0.965
AcChCl: CA (1:2)	7.168	-0.0139	0.998
AcChCl: CA (1:3)	9.044	-0.0218	0.994
AcChCl: CA (1:4)	6.352	-0.0141	0.978
AcChCl: CA (1:5)	5.131	-0.0117	0.882
AcChCl: CA (1:6)	3.474	-0.0069	0.934

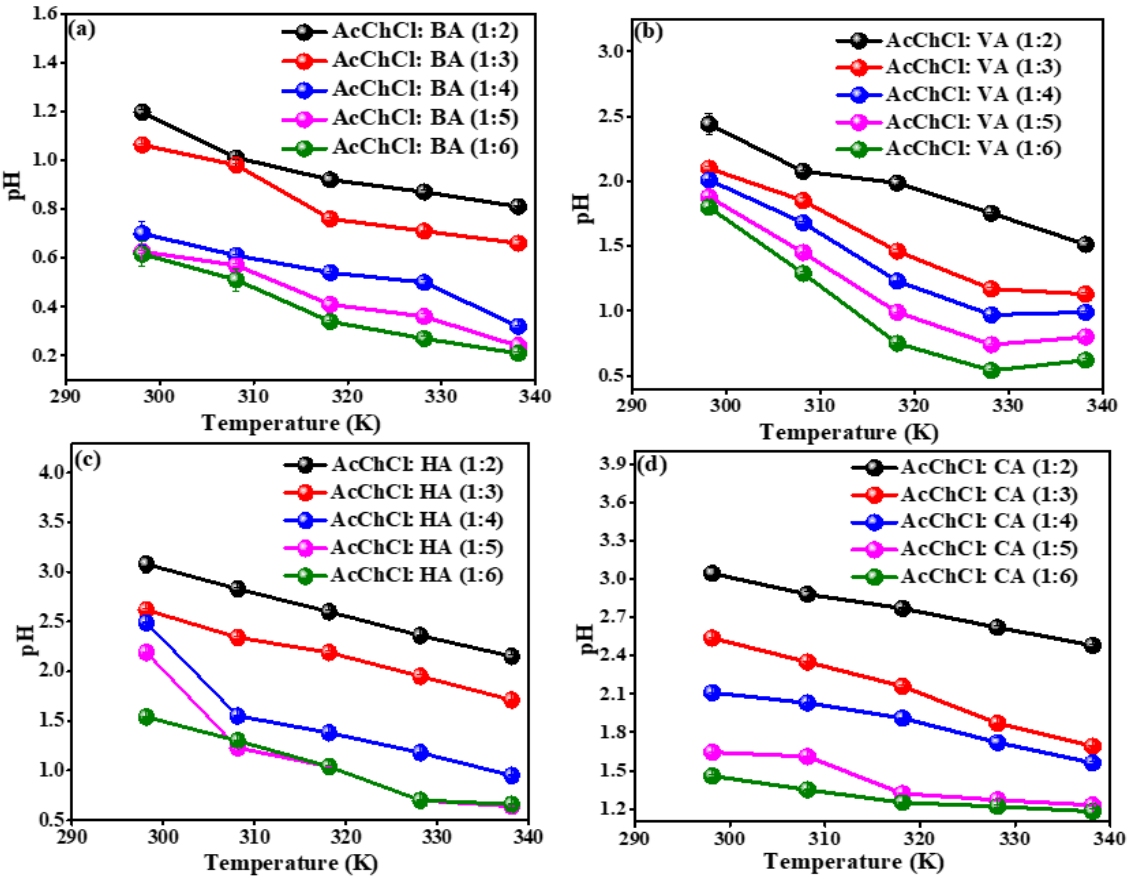


Figure 3.8. pH of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA at temperature 298 K to 338 K.

3.3.6 Conductivity

As expected, most formulated HDESs exhibit lower conductivity owing to their higher viscosity at room temperature. Considering that HBA/HBD molar ratio impacts viscosity, this parameter also affects conductivities. The conductivity of DESs increases with increasing the HBDs content. This can be attributed to the increase in charge carriers in the solution [124]. Figure 3.9 shows the conductivities of HDESs at a temperature ranging from 25 °C to 65 °C. The conductivity of the synthesized DESs shows strong temperature dependence behavior. The conductivity of all synthesized DESs increases with an increase in temperature. Conductivity is related to ion mobility and the number of charge carriers. Ions move faster at higher temperatures due to relatively low viscosity [130] .

The effect of temperature on the conductivity is modeled using an Arrhenius equation,

$$\text{K} = \text{k}_0 \exp\left[-E_\text{k}/RT\right] \quad (3.3)$$

Where K is conductivity in µS/cm, k0 is constant, Ek is the activation energy of conductivity, R is the gas constant, and T is the temperature in Kelvin. The model parameters are shown in Table 3.3. As shown, there is a strong relationship between conductivity and temperature.

Table 3.3. Model parameter for Conductivity

HDESs	E_k/R	k_0	R^2
AcChCl: BA (1:2)	-635.59	9.095	0.955
AcChCl: BA (1:3)	-700.92	9.3227	0.943
AcChCl: BA (1:4)	-755.39	9.511	0.979

AcChCl: BA (1:5)	-782.28	9.616	0.987
AcChCl: BA (1:6)	-584.45	9.032	0.915
AcChCl: VA (1:2)	-539.74	8.1651	0.829
AcChCl: VA (1:3)	-412.09	7.817	0.955
AcChCl: VA (1:4)	-409.72	7.845	0.963
AcChCl: VA (1:5)	-359.13	7.717	0.951
AcChCl: VA (1:6)	-355.33	7.728	0.987
AcChCl: HA (1:2)	-1313.6	9.999	0.931
AcChCl: HA (1:3)	-844.7	8.620	0.942
AcChCl: HA (1:4)	-888.46	8.806	0.979
AcChCl: HA (1:5)	886.36	8.848	0.984
AcChCl: HA (1:6)	-774.58	8.549	0.999
AcChCl: CA (1:2)	-2471	12.707	0.979
AcChCl: CA (1:3)	-1812	10.833	0.995
AcChCl: CA (1:4)	-1632.7	10.38	0.984
AcChCl: CA (1:5)	-1455.8	9.931	0.977
AcChCl: CA (1:6)	-1335.9	9.683	0.991

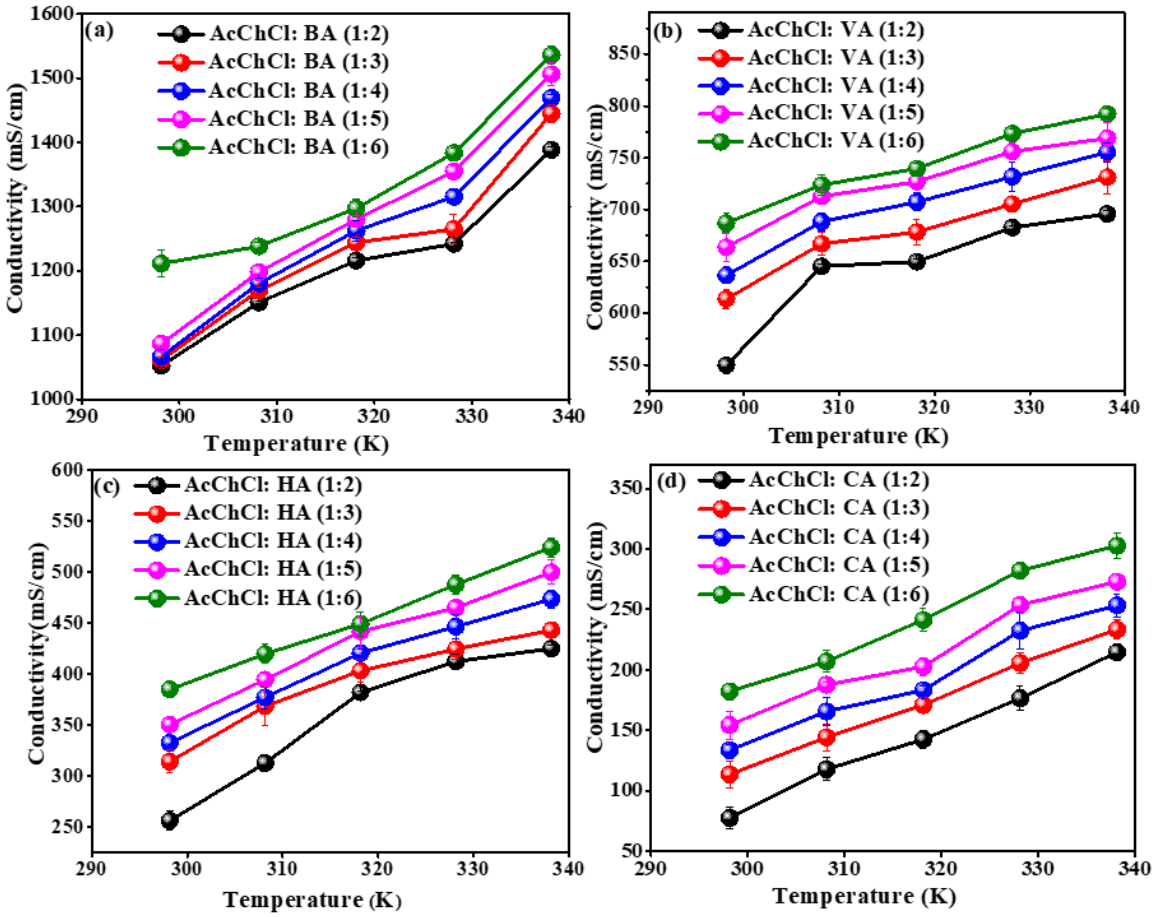


Figure 3.9. Conductivity of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA at temperature 298 K to 338 K

3.3.7 Decomposition Temperature

The thermal stabilities of HDESs and their components were determined using thermogravimetric analysis (TGA). The hydrogen bond plays a crucial role in the thermal decomposition of DESs. A strong hydrogen bond hinders the escape of molecules and requires greater energy to break the bond between HBA and HBDs. Also, the more stable the HBDs are, the greater would be the decomposition temperature of DESs [131]. The thermal properties of HDESs also depend on the type and molar ratios of components used

for the HDESs preparation. Almost all TGA and DTA thermograms show mass loss in three different steps: (1) loss of water content together with weakly interacted HBDs, (2) HDES, and (3) AcChCl.

The thermal decomposition profile of DESs and their constituents are studied using T1/2 temperature. T1/2 is the temperature at which 50% of the initial mass is lost during the TGA scanning experiment.

The T1/2 of all the HDESs is greater than their corresponding HBDs and lower than that of HBA, implying that the thermal properties of HDESs are entirely different from those of individual components. The only exception is AcChCl: CA (1:6) AND AcChCl: CA (1:5), which has the same $T_{1/2}$ as caprylic acid. The increased $T_{1/2}$ is the qualitative indicator of strong intermolecular interaction occurring with the increase in thermal stability. Hydrogen bonding is a major force that contributed to HDESs thermal stability. Also, the $T_{1/2}$ value decreases with the increase in the molar ratio of the HBDs. The $T_{1/2}$ value for AcChCl: 2BA is 205.5 °C, AcChCl: 2BA is 152.5 °C, AcChCl: 2BA is 144 °C, AcChCl: 2BA is 128 °C, AcChCl: 2BA is 113.7 °C. The higher stability of HDESs at molar ratio 1:2 is due to the stronger intermolecular force of attraction between HBA and HBDs. This result is in agreement with the result obtained with the freezing point of the HDESs. The high decomposition rate at low temperature may be due to the presence of excess donor molecules which do not interact with limiting acceptor molecules.

The DTG plot of HBDs shows that the decomposition temperature of the HBDs increases with an increase in the alkyl chain length (figure 3.11). The decomposition temperature of AcChCl is the greatest of all the HBDs used for the formulation of HDESs (figure 3.10). The DTG plot shows the broad decomposition profile attributed to the simultaneous loss

of water and acid. This plot shows that the one set temperature of all HDESs is greater than HBDs (figure 3.12). The DTG plot of AcChCl 1:2 BA shows the decomposition of HDESs at an onset temperature of 201°C to an end set temperature 281 °C, AcChCl: 2VA shows decomposition from 183 °C to 279 °C, AcChCl: 2HA shows decomposition from 220 °C to 283 °C and AcChCl l: 2 CA shows decomposition from 216 °C to 294 °C. At first decomposition cycle, AcChCl: 2BA loses about 43% of the solvents at 171 ° C, AcChCl: 3BA loses about 47% at 175 °C, AcChCl: 4BA loses about 55% at 168 °C, AcChCl: 5BA loses about 69% at 196 °C, and AcChCl: 6BA 73% at 164 °C. This high weight loss is due to the loss of water and acids.

The magnitude of difference between T_f and T_d is used to determine the operating range of the solvents. Td is the temperature at which 10% of the original mass has been lost during TGA scanning. The higher the difference between T_f and Td, the wider is the operating range of the solvent. The operating range of the synthesized DESs ranges from 83.56 °C to 141.14 °C. The operating range of some synthesized DESs such as, AcChCl: VA (1:2) (138.7 °C), AcChCl: VA (1:3) (128.53 °C), AcChCl: VA (1:4) (133.4 °C), AcChCl: VA (1:6) (127.34 °C) , AcChCl: HA (1:2) (141.14 °C), AcChCl: HA (1:3) (132.32 °C), AcChCl: CA (1:2) (143.26 °C), and AcChCl: CA (1:3) (130.48 °C) is higher than the common solvents like acetonitrile (125.45 °C), Cyclohexane (87.3 °C), carbon tetrachloride (99.4 °C) and t-butyl alcohol (108.1°C) [132].

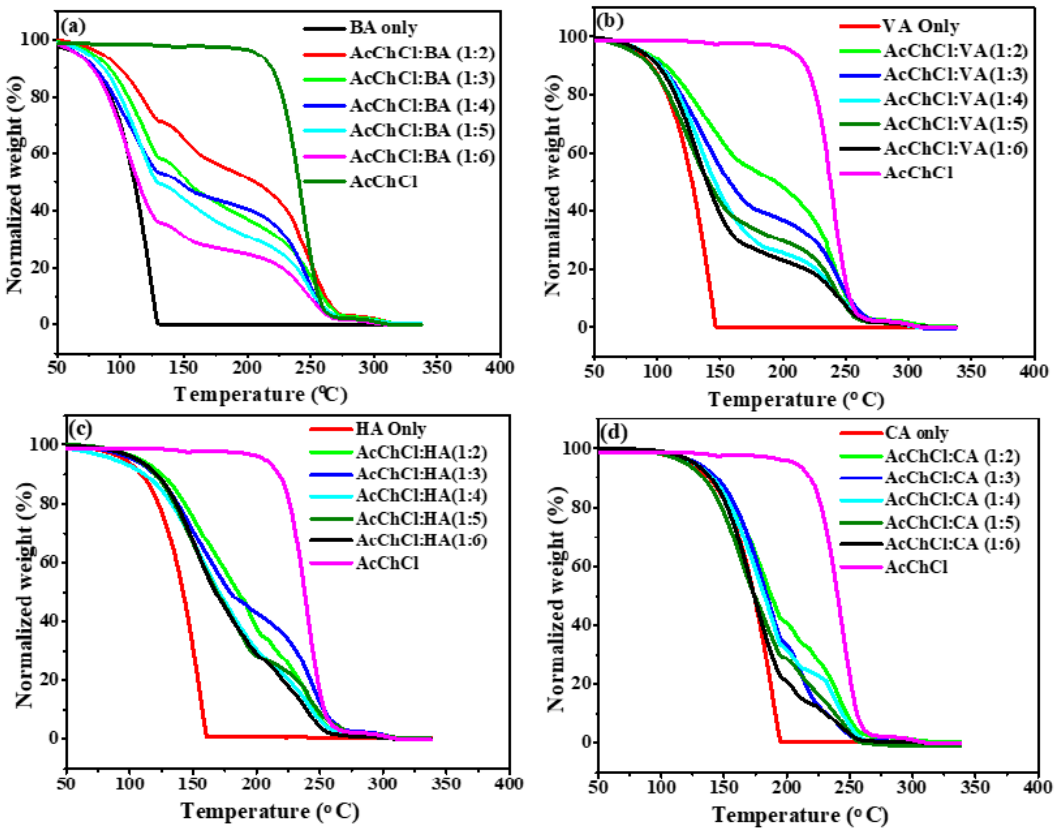


Figure 3.10. TGA plot of (a) AcChCl with BA (b) AcChCl with VA(c) AcChCl with HA (d) AcChCl with CA.

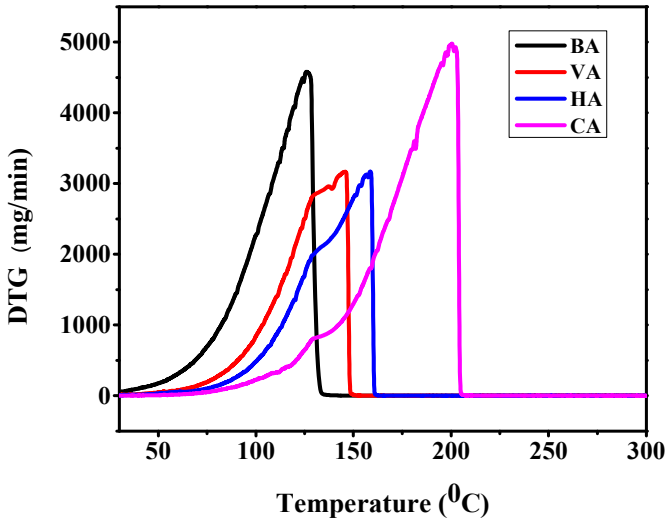


Figure 3.11. DTG plot of HBDs used for DESs formulation

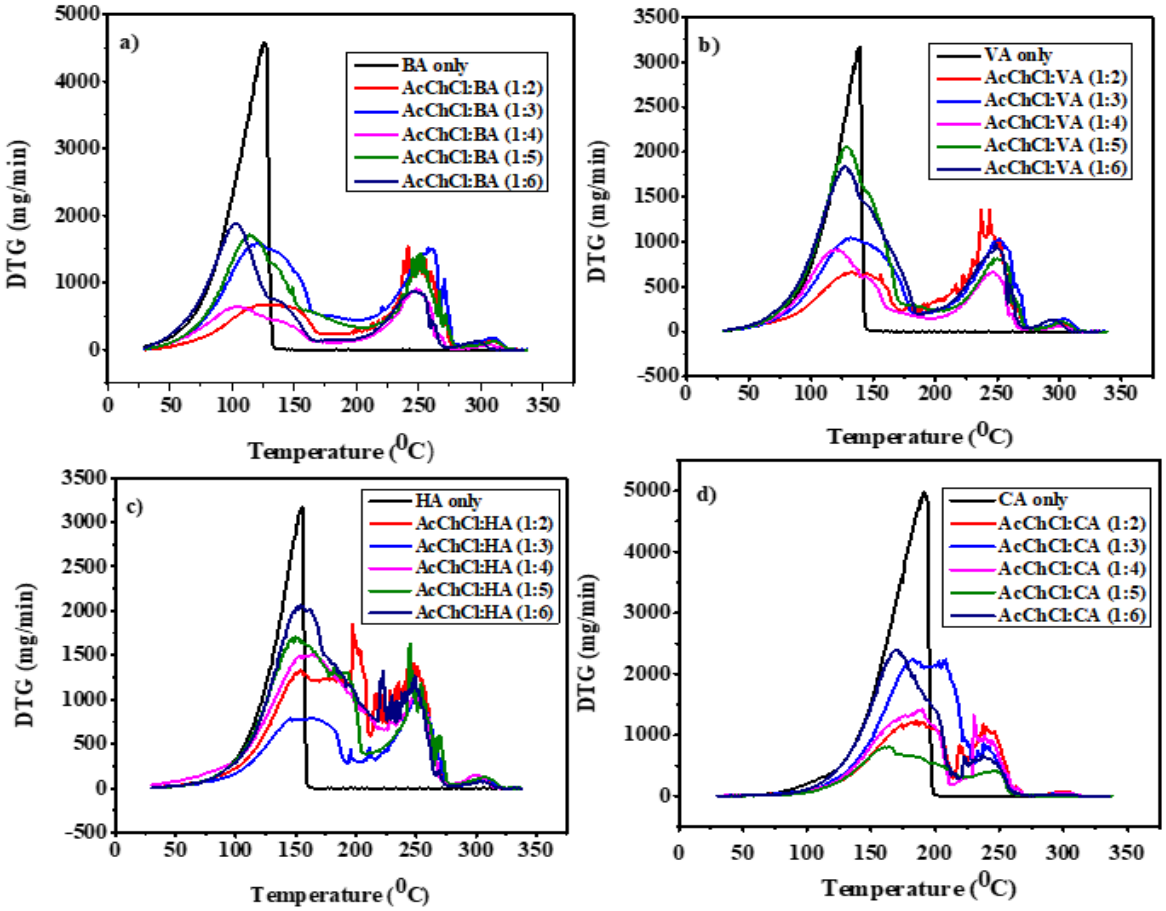


Figure 3.12. DTG plot of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA.

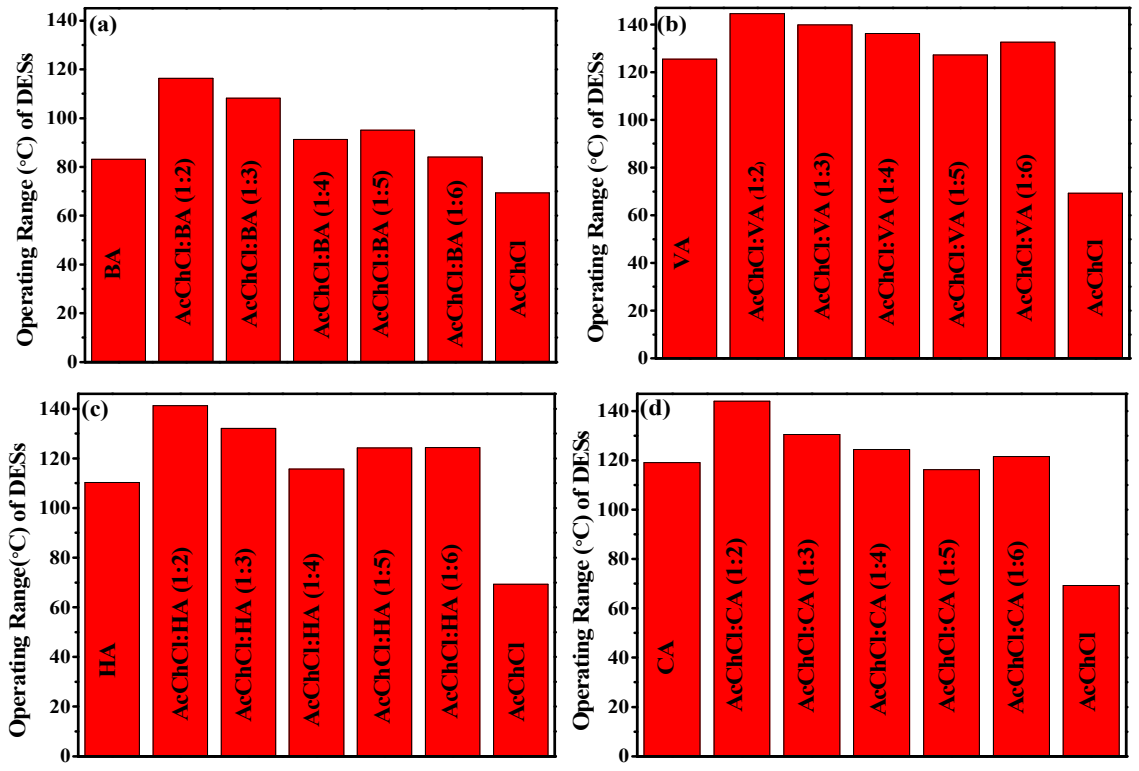


Figure 3.13. Operating temperature range of (a) AcChCl with BA (b) AcChCl with VA(c) AcChCl with HA (d) AcChCl with CA.

3.3.8 Surface tension

Figure 3.14 shows the surface tension of HDEs at all molar ratios. Surface tension of synthesized DESs is expected to follow the similar trend to viscosity. The higher the strength of intermolecular interaction between the HBA and HBDs, the higher is the surface tension. The nature of HBD, alkyl chain length of HBDs and molar ratio of HBA/HBDs also has impact on surface tension of DESs. Increasing the alkyl chain length of HBDs increases the surface tension of the HDESs. Surface tension values of the synthesized DESs is higher than the organic solvent and is comparable to those of imidazole based ILs [133, 134]. The higher value of surface tension is the indication of

strong hydrogen bond formation between the HBA and HBDs. Surface tension value decreases with decrease in the AcChCl content.

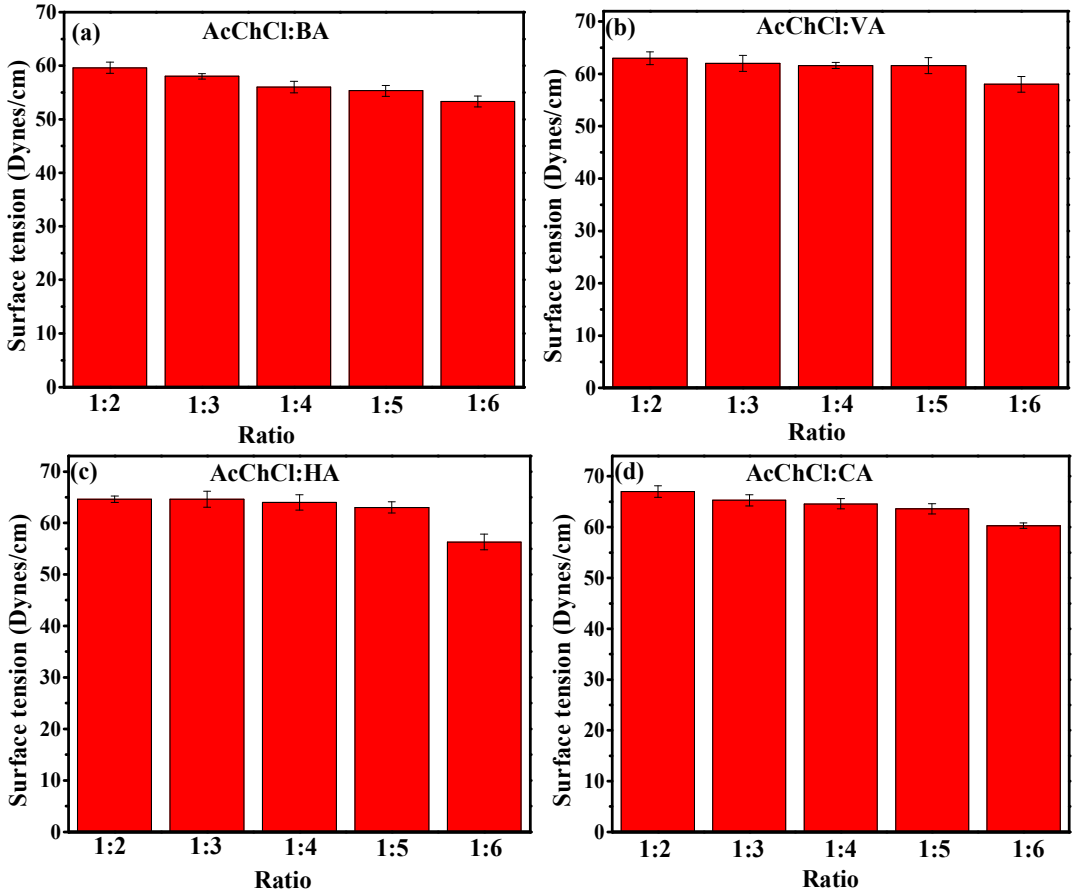


Figure 3.14. Surface tension of (a) AcChCl with BA (b AcChCl with VA (c) AcChCl with HA (d) AcChCl with CA at different molar ratios.

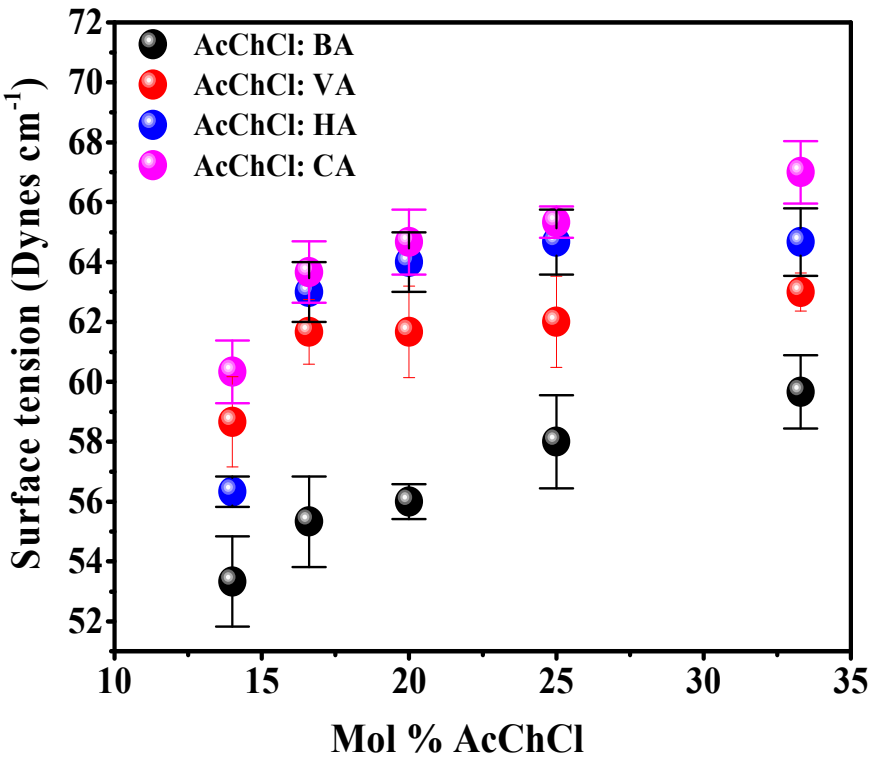


Figure 3.15. Plot of surface tension Vs molar percentage of AcChCl

3.4. Conclusion

In Summary, a detailed investigation of the physicochemical and thermal properties of HDESs and their individual components was performed. The effect of the type and molar ratio of HBDs on the physical and thermal properties of HDESs were also determined. The density of solvents showed a strong linear relationship with the refractive index of solvents. The density of the formulated HDESs was less than the density of water. The temperature dependence of the physical properties was fitted using a simple linear and Arrhenius equation. A very wide range of viscosity (13-186.8 mPa/s), conductivity (48-1388 µS/cm), PH (0.61-3.08) was observed at room temperature. The thermal stability profiles of HDESs were also investigated through TGA, which showed that HDESs was thermally stable. This indicates that these novel HDESs have strong potential in various industrial applications.

CHAPTER 4

SOLVATOCHROMIC PARAMETERS OF FATTY ACIDS BASED DEEP EUTECTIC SOLVENTS

4.1 Introduction

Deep eutectic solvents are new generation alternative solvents formed by mixing two or more appropriate compounds, such as quaternary ammonium salts and hydrogen bond donors (HBD), with depressed melting points compared to the individual components [20, 24]. The depression in melting point is due to the strong hydrogen bond interaction of HBDs with hydrogen bond acceptor (HBA), which leads to a decrease in the cation-anion exchange of HBA species, resulting in a significant depression in the melting point of the mixture [135].

With growing environmental awareness across the globe, the interest in the formulation and characterization of DESs is increasing. These solvents have shown significant advantages over conventional ionic and molecular liquids, especially ease of preparation, toxicity, and cost [136]. Other advantages of DESs are negligible vapor pressure, high biodegradability, and diversity of combinations of the starting material, which facilitates the alternation of physical and chemical properties of the solvent, thereby presenting an extensive industrial application. In addition, natural and renewable sources, such as amino acids, organic acids, polyols, carbohydrates, can be used for DESs preparation [126, 137, 138]. Due to these promising advantages, DESs have been studied in a wide range of applications, such as electrochemistry, organic synthesis, separation processes, nanotechnology, and catalysis [44, 108, 126, 139, 140]. Even though the enormous potential of the application of DESs, detailed knowledge on polarity is still lacking.

Understanding the polarity of DESs greatly eases the challenging solvent selection step of the chemical process. Therefore, knowing the polarity of DESs is essential to use them as green alternatives to molecular and ionic solvents. It also plays an indispensable role in developing chemical processes that involve synthesis, extraction, and separation processes. Spectrometric and chromatographic methods have been used to determine the polarity of solvents [141]. However, the spectrometric method is the most commonly utilized method for polarity determination [142].

4.2 Solvatochromism

Solvatochromism is a term used to describe the change in position and intensity of a UV/ Vis absorption band due to a change in the polarity of the medium. The position, intensity, and shape of the absorption bands of organic compounds are usually modified when measured in the solvent of different polarities. These changes are attributed to the physical intermolecular solute solute-solvent interaction forces, such as dipole-dipole, ion-dipole, dipole-induced dipole, and hydrogen bonds, which alter the energy difference between a ground state and excited state of the absorbing species containing chromophore [143]. In addition, the solvent effect on spectra arising from electronic transitions is dependent on the chromophore and the nature of transition [144]. Solvatochromic analysis studies both bulk and localized polarity in chemical and biological solvents [145].

Electromagnetic waves within the UV/Vis and near-IR are usually used to explore the effect of solvent polarity on the electronic excitation of the dyes. The chemical interaction between the dyes and solvent molecules causes a change in the polarity of the probes. The change in the absorption band observes this change. The change in absorption band to longer wavelength is called red-shift, bathochromic shift, or positive solvatochromism.

Change to a shorter wavelength is denoted as blue shift, hypsochromic shift, or negative solvatochromism. The shift in the wavelength of the absorption band is used for the calculation of the polarity.

Polarity is the sum of all specific and non-specific intermolecular interactions between solvent and solute, except the interactions that lead to the solute's chemical transformations [146] . This property is a combination of both physical and chemical phenomenon. It comprises several interacting components, such as columbic interactions, dipole interactions, permanent and induced hydrogen bonding, Л-interaction, van der Waals forces, and electron pair donor-acceptor interactions. Polarity is described in terms of the potential behaviors of the solvent in relationship with the solute. Thus, this is not an absolute property of the pure liquid. Polarity scales are estimates, and different scales give different polarities of the same solvents. Depending on the various measurement techniques used, different relative polarities can arise [146-148].

Polarity determination by spectrometry uses organic compounds called probes. Detailed information on the chemical nature of the solvation system of a solute can be obtained using probes since the transition energy of the indicator depends on the solvation sphere composition and the solvent properties. It also provides information about polarity and hydrogen bonding capabilities [146]. The change in polarity of the probes in solvents can be used to determine the polarity scale of the solvent. Some commonly used probes are 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridnio) phenoxide, (N- methylpyrdino) vinyl, 4,6 dichloro [(N-methylpyrdinio) vinyl] phenolate, 1-ethyl-4-cyano iodide, merocyanines, and ketocyanines [149, 150]. The possibility of probable use of probe was first discovered by Brooker et al. in 1951 [151]. However, the first comprehensive solvent scale was set up by

Kosower in 1958. The scale was called the Z scale and was based on the shift of the wavelength of maximum absorbance [146].

Various empirical solvent polarity scales have been developed due to the lack of theoretical expressions for calculating solvent effects and the inadequacy of defining solvent polarity. The most commonly used scale to characterize solvents is the ET (30) polarity scale of Dimroth and Reichardt [135, 148] and the scale of Kamlet and Taft (KT) [151, 152]. KT is a multiscale parameter and is used to describe the solvent di-polarity/ polarizability, acidity, and basicity by using the following equation:

$$\mathbf{Umax = \upsilon 0 + s\Pi^* + a\alpha + b\beta} \quad \text{..... (4.1)}$$

Where, υ_{max} is the frequency of maximum absorption of dyes in the solvent, υ_0 is the intercept, Π* represent the solvent dipolarity/ polarizability, α hydrogen- bond donating ability of HBD, β hydrogen- bond accepting capability of HBA, s, a, and b are solvent independent coefficient determined by nature of indicator dye [149]. The α- value is the ability of the HBD to donate proton to the solute [153, 154]. β- value measures the potential to receive proton from HBD [155, 156]. The Π* value is the indicator of the ability of solvent to hold a charge due to its dielectric effect [155].

To get a clear insight into the polarity of the solvent, the polarity of formulated DESs was collectively studied using the scale of Dimorth and Reichardt and the scale of Kamlet and Taft. In addition, ET(NR), ETN, hydrogen bond acidity, hydrogen bond basicity, and polarizability were determined by measuring the Solvatochromic responses of ultraviolet-visible (UV-Vis) absorbance probe, such as the Nile red (NR), 4-nitroaniline (4NA), and N, N- diethyl-4-nitroaniline (DENA).

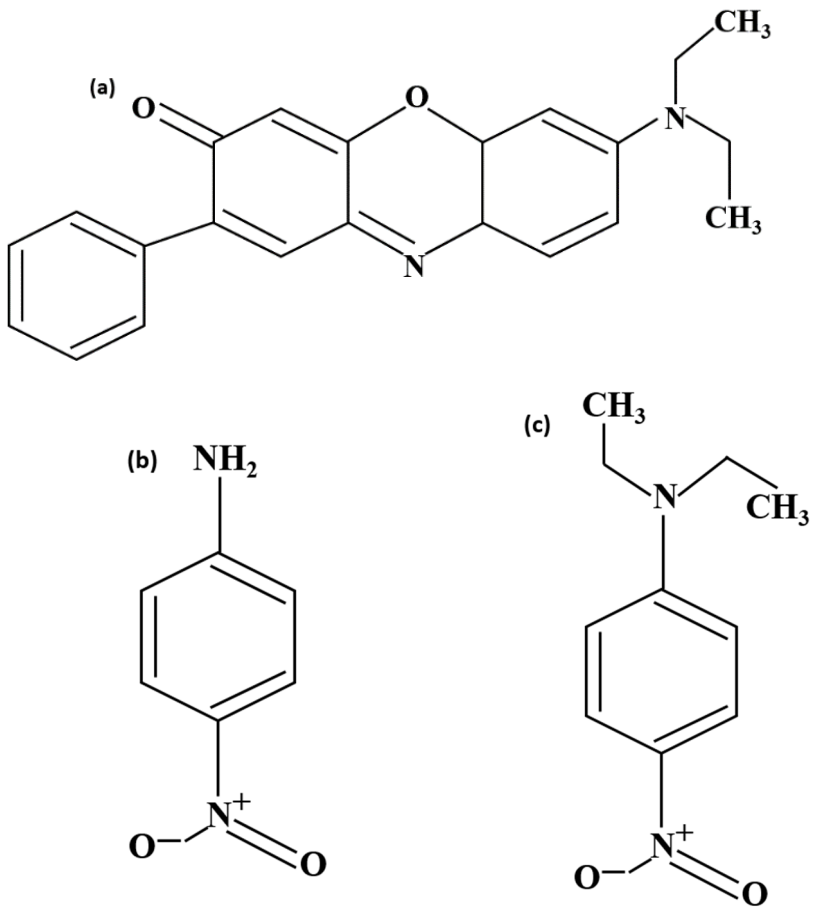


Figure 4.1. Dyes used for Solvatochromic studies of solvents, a) Nile red, b) 4-nitroaniline, c) N, N- diethyl-4-nitroaniline

4.3. Experimental

4.3.1 Materials

AcChCl (99%), BA (99%), HA (99%), CA (99%), and deuterated chloroform (99.8%) were purchased from Acros Organics (Fair lawn, NJ). VA (99%) was purchased from Alfa Aesar (Haverhill MA). HPLC grade methanol, Nile Red (99%), 4-nitroaniline (99%), N, N-diethyl-4-4-nitroaniline (99%) were purchased from Thermo- Fischer Scientific

(Dubuque, IA). Ultra-pure water was obtained in a lab with instrument Solution 2000 Water Purification. All the chemicals were used as supplied. Maximum absorption wavelength (λ_{max}) was determined using Synergy/H1 microplate reader, Biotek.

4.3.2 Methods

4.3.2.1 Synthesis

DESs were prepared by heating acetylcholine chloride as HBA and butyric acid, valeric acid, hexanoic acid, and octanoic acid as HBDs at 60 ˚ C for about 15-45 minutes molar ratio of 1:2, 1:3, 1:4, 1:5, and 1:6. AcChCl and. The prepared DESs were cooled at room temperature and then kept in sealed laboratory vials and stored in a desiccator to prevent moisture absorption.

4.3.3 Methodology

Individual solution of 10^{-4}M NR, 4NA, DENA was prepared in methanol. The required number of probes was weighed using Mettler Toledo balance. The concentration of each dye was kept low to neglect the solute-solute interaction in the observed solvent effect [144]. 100 µL of each probe were pipetted in a microplate in triplicate.

The residual methanol was evaporated at room temperature. DESs were then added to the microplate, and a UV-Vis spectrophotometer (Synergy|H1 microplate reader, Biotek) was used to determine the maximum absorption wavelength (λmax) from the absorption spectra. Absorbance readings were collected by spectral scanning of DES in Nile red (400-650 nm), N, N-diethyl-4-nitroaniline (300-500 nm), and 4-nitroaniline (300-500nm) at 1nm intervals in triplicate. To determine the effect of temperature in the electronic transition of the probes, the experiment was carried out at a temperature, 25˚C to 45˚C for

every increment of 5°C. All measurements were carried out in triplicate, and average values were taken for further calculation.

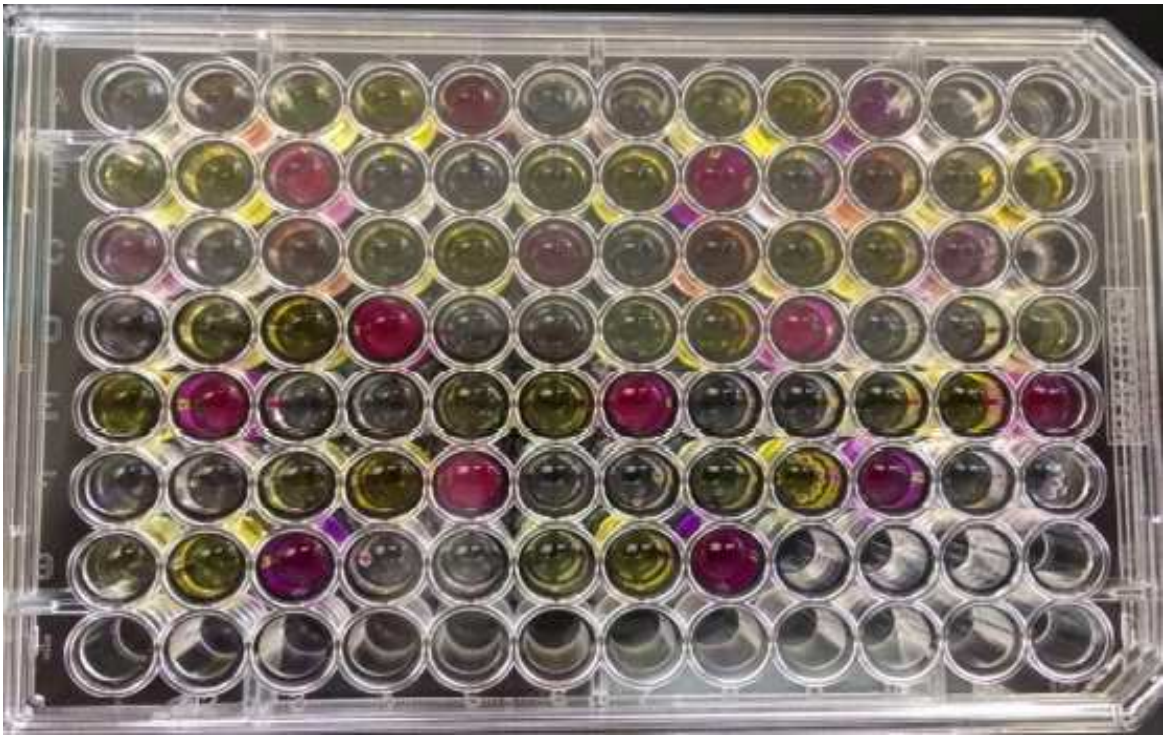


Figure 4.2. Microplate containing DESs samples dissolved in Nile red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes

4.4 Result and Discussion

4.4.1 Solvatochromic Parameters

4.4.1.1 Nile Red E_T(NR) and normalized ET^N

The molar electronic transition energy of dissolved Nile Red (E_T(NR)) were calculated using wavelength of maximum absorbance (λ_{max}) using equation 4.2.

$$hc \times V_{max} \times N_A \quad \text{(4.2)}$$

$(2.8591 \times 10^{-3}) V_{max}(cm^{-1})$……………………………………………………………............(4.3)

$$= \frac{28591}{\lambda\, max(nm)}$$

Where, h= Plank`s constant, c= speed pf light, Vmax= Wavenumber, A= Avogadro`s constant, λ_{max} = Wavelength of maximum absorbance.

The value of E_T(NR) ranges from 30.7 Kcal mole-1 for tetramethyl silane (TMS) to 63.1 kcal mole^{-1} for water. Consequently, TMS is the least polar solvent and water is the most polar solvent. Later, in 1983, Reichardt et al. introduced a new normalized E scale (E_T^N) to avoid the non-SI unit kcal/mol and conversion of the E_T(NR) values into KJ/mole with the similar assumptions of TMS as least polar solvent and water as most polar [157]. E_T^N scale is easier to conceive as it ranges from 0.00 for TMS, extremely non-polar solvent, to 1.00 for water, extremely polar solvent [158]. E_T^N is calculated using equation 3

$$E_T^N = \frac{E_T(slovent) - E_T(TMS)}{E_T(water) - E_T(TMS)} \quad \text{……… (4.4)}$$

$$= \frac{E_T(slovent) - 30.7}{32.4} \quad \text{……… (4.5)}$$

E_T^N is used to study the overall polarity, dipolarity/polarizability and HBD ability, arising from the interaction between the solvent and the dye [159].

4.4.1.2 Kamlet-Taft parameters

Kamlet-Taft parameters α, β, and π*values of DES were calculated using the equations 4.6 to 4.9 with υ referring to the wavenumber,

$$\nu\,(\text{dye}) = \frac{1}{\lambda_{max}\,(\text{dye})} \times 10^{-4} \quad \text{.....(4.6)}$$

$$\alpha = 0.00649E_T\,(NR) - 2.03 - 0.72\pi^* \quad \text{.....(4.7)}$$

$$\beta = \frac{(1.035\,\nu_{N,N-diethyl1-4-nitroaniline} + 2.64 - \nu_{4-nitroaniline})}{2.80} \quad \text{..... (4.8)}$$

$$\pi^* = 0.314(27.52 - \nu_{N,N-diethyl1-4-nitroaniline}) \quad \text{.....(4.9)}$$

Where, α= hydrogen-bond donor acidity, β= hydrogen bond accepter basicity π*= dipolarity/polarizability parameter υ_{DENA}=Wavenumber of 4-nitroaniline, υ_{4NA}= Wavenumber of N, N-diethyl-4-nitroaniline.

The α parameter is least for non-HBD solvents such as aliphatic and aromatic solvent and largest for hexafluoro-isopropyl alcohol, ranging from 0.00 to 1.96. The value of parameter β ranges from 0.00 for cyclohexane to 1.00 for hexamethylphosphoric acid triamide. The π* scale ranges from 0.00 for cyclohexane to 1.00 for dimethyl sulfoxide [158]. Figure 4.3 shows the Nile red, 4-nitroaniline and N, N- diethyl-4-nitroaniline dissolved in AcChCl: BA (1:2). DESs were completely dissolved in the probe without any aggregation left in the solution. This proves that the polarity of formulated DESs can be measured using the dyes selected.

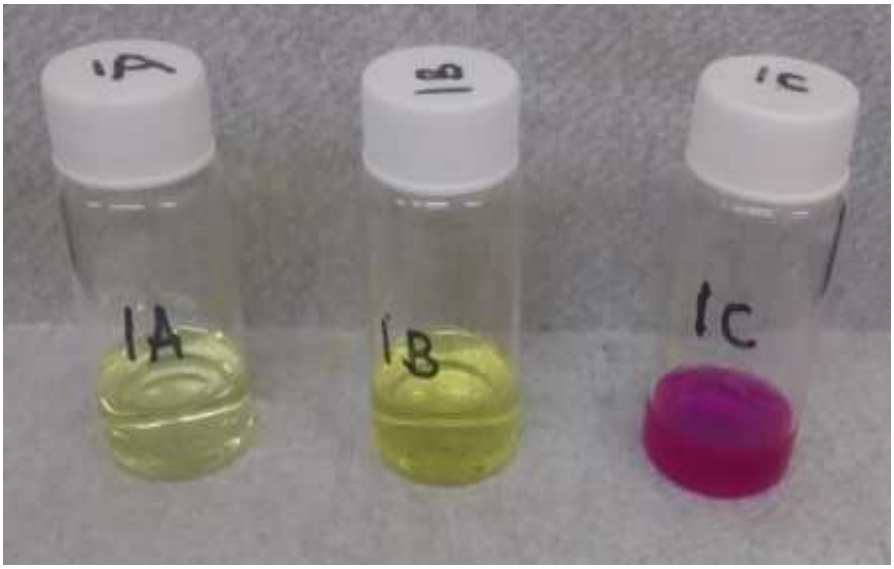


Figure 4.3. Light yellow, yellow and purple color of 4-nitroaniline, N,N-diethyl-4-nitroaniline and Nile red dyes dissolved in DESs.

The empirical polarity parameters ET (NR) and ETN are calculated using the wavelength of the maximum absorbance wavelength using Nile red as the Solvatochromic probe. Nile red was used due to the acidic nature of the HDESs instead of Penta-tert-butyl substituted betaine dye by Dimroth and Reichardt. In an acidic medium, the latter becomes protonated and losses its absorption band. In addition, other properties like low basicity, higher solubility in diverse polarity solvents, and higher photochemical stability make the Nile red a perfect Solvatochromic probe for studying the solvent polarity of the HDESs under study [160].

The E_T (NR) and E_T^N values of the formulated DESs are shown in table 4.1. To validate the experiment performed, the E_T^N value of the methanol was determined and compared with the published literature value. The ET(NR) value of the methanol was found to be 51.64 kcal.mole-1, which is similar to a literature value of 52.02 kcal.mole-1 and is

consistent with ET(30) and ET(26) values [160] . As can be seen the E_T (NR) values of the synthesized HDESs ranges from 50.85 kcal.mole^{-1} for AcChCl: BA (1:6) to 52.93 kcal.mole^{-1} for AcChCl: CA (1:2) at room temperature.

Table 4.1. Solvatochromic polarity (E_T(NR) and E_T^N) and Kamlet-Taft parameters of DESs at 25 °C.

DESs	**E_T (NR) kcalmol^{-1}**	**E_T^N**	**α**	**β**	**Π***
AcChCl: BA (1:2)	51.14669	0.631071	0.613405	0.520071	0.938909
AcChCl: BA (1:3)	51.11621	0.63013	0.615967	0.560997	0.932605
AcChCl: BA (1:4)	51.02499	0.627314	0.623705	0.361225	0.913634
AcChCl: BA (1:5)	50.87367	0.622644	0.618452	0.351175	0.90729
AcChCl: BA (1:6)	50.84351	0.621713	0.618022	0.353671	0.905169
AcChCl: VA (1:2)	51.59278	0.671622	0.689986	0.55449	0.888194
AcChCl: VA (1:3)	51.57727	0.670138	0.709003	0.575273	0.856156
AcChCl: VA (1:4)	51.57727	0.669644	0.709599	0.54881	0.849717

AcChCl: VA (1:5)	51.51532	0.66915	0.717887	0.564008	0.836806
AcChCl: VA (1:6)	51.4844	0.668656	0.726205	0.544969	0.823853
AcChCl: HA (1:2)	52.46055	0.650125	0.862059	0.761921	0.711987
AcChCl: HA (1:3)	52.41247	0.648198	0.846155	0.715601	0.729742
AcChCl: HA (1:4)	52.39645	0.646277	0.848304	0.660969	0.725314
AcChCl: HA (1:5)	52.38046	0.645797	0.866483	0.692391	0.698623
AcChCl: HA (1:6)	52.36447	0.645318	0.870277	0.657083	0.691913
AcChCl: CA (1:2)	52.9463	0.686614	0.937165	0.790735	0.651458
AcChCl: CA (1:3)	52.91364	0.685606	0.939928	0.764492	0.644676
AcChCl: CA (1:4)	52.88101	0.684599	0.942703	0.74666	0.637881
AcChCl: CA (1:5)	52.86472	0.684096	0.946546	0.737373	0.631076
AcChCl: CA (1:6)	52.84843	0.683593	0.955314	0.701168	0.617429

Figure 4.4(a-b) show the change in the E_T (NR) and E_T^N values with a change in the hydrocarbon chain length of the HBDs. Generally, at room temperature, for the same cation, E_T (NR) values decreased in the order of HBDs: Caprylic acid> Hexanoic acid > Valeric acid> Butyric acid. The E_T (NR) value for AcChCl: CA (1:2) is 52.95 kcal. $Mole^{-1}$ which is greater than AcChCl: HA (1:2) (52.46 $Kcal.mole^{-1}$) followed by AcChCl: VA (1:2) (51.76 kcal. $mole^{-1}$) and AcChCl: BA (1:2) (51.15 kcal. $mole^{-1}$). The higher ET (NR) value of the CA-based HDESs might be due to the higher interaction of the HBDs with the Solvatochromic probe. Similar results were observed for the E_T^N parameter.

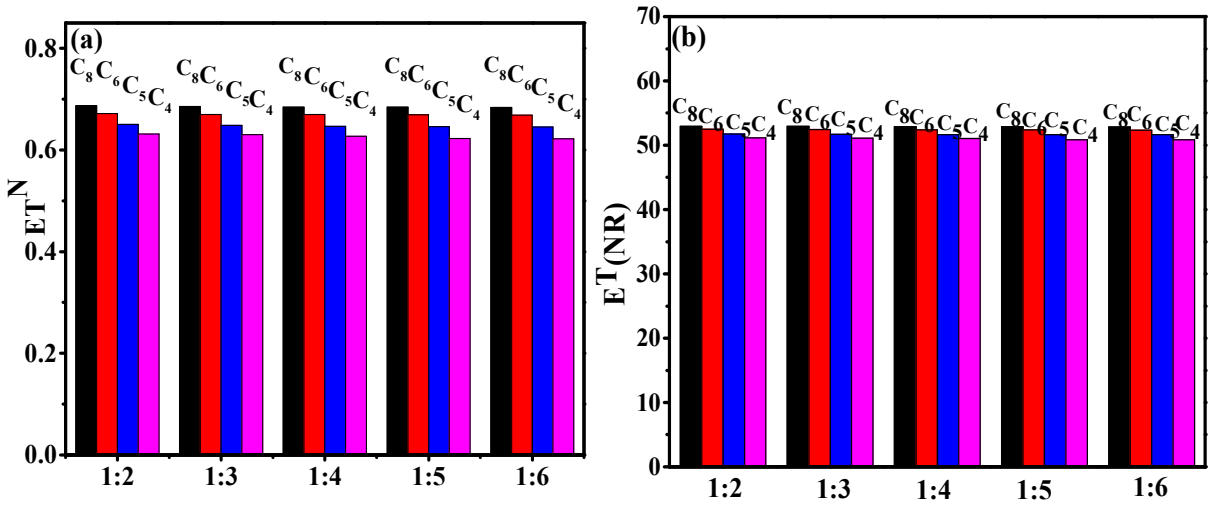


Figure 4.4 (a). E_T^N parameter of DESs composed of AcChCl and different carboxylic acids, namely butyric (C_4), valeric (C_5), hexanoic (C_6), and octanoic (C_8) acids in a mole ratio of 1:2- 1:6, at 298K. (b) E_T (NR) parameter of DESs composed of AcChCl and different carboxylic acids, namely butyric (C_4), valeric (C_5), hexanoic (C_6), and octanoic (C_8) acids in a mole ratio of 1:2- 1:6, at 298K

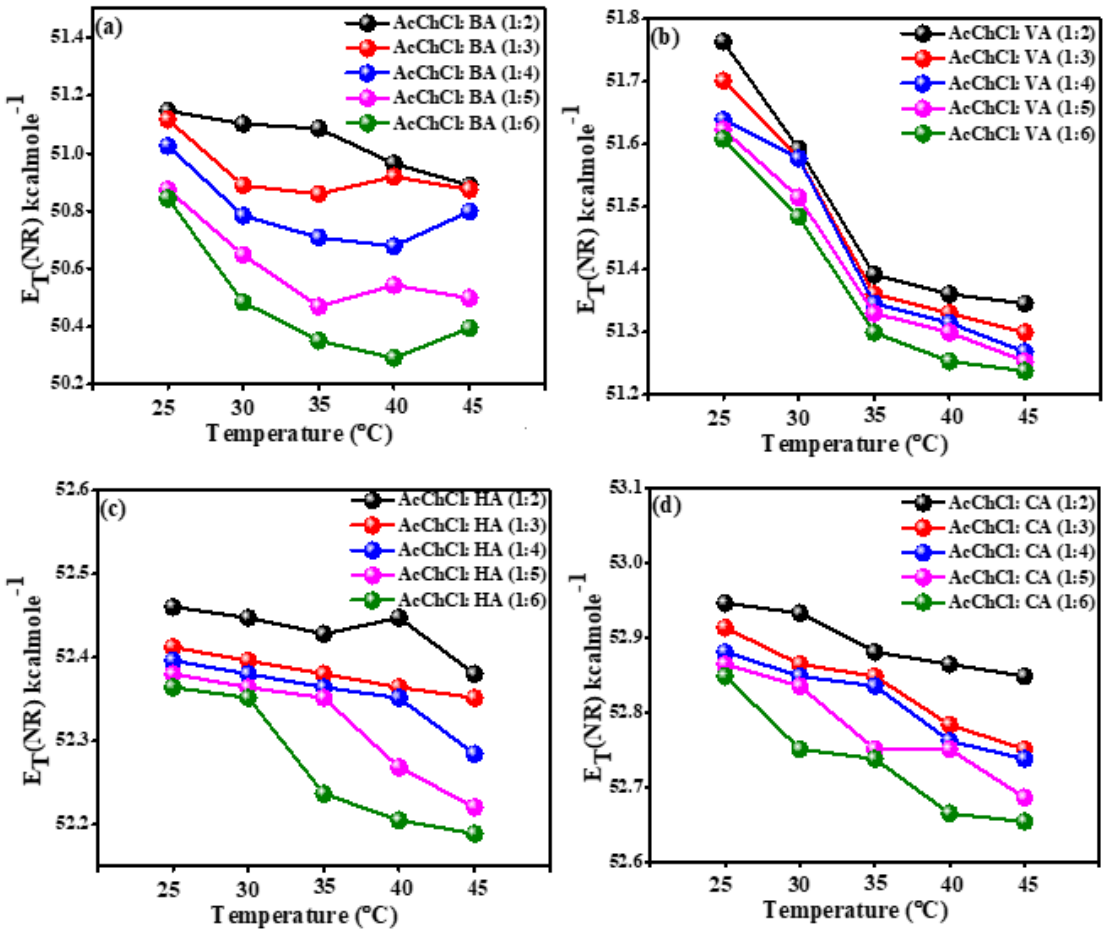


Figure 4.5. E_T(NR) parameter of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA. (d) AcChCl with CA at ratio 1:2-1:6 from temperature 25 °C to 45 °C.

Figure 4.5 shows the change in E_T (NR) values with the change in mole ratios of the HBDs. The E_T (NR) value decreases slightly with increasing the molar ratio of the HBDs. The E_T (NR) value of the BA-based HDESs decreases from 51.15 kcal. Mole^{-1} for AcChCl: BA (1:2) to 50.84 kcal. Mole^{-1} for AcChCl: BA (1:6), 51.76 kcal. Mole^{-1} for AcChCl: VA (1:2) to 51.61 kcal. Mole^{-1} for AcChCl: VA (1:6), 52.46 kcal. Mole^{-1} for AcChCl: HA (1:2) to 52.36 kcal. Mole^{-1} for AcChCl: HA (1:6), 52.95 kcal. Mole^{-1} for AcChCl: CA (1:6) to 52.85 kcal. Mole^{-1} for AcChCl: CA (1:6). Thus, changing the composition of HBDs and the molar ratio of HBA and HBDs can change the polarity of the formed DESs. This property is a

good indicator for selecting appropriate HBD and molar ratio of HBD and HBA for formulating DESs needed for the particular application. A slight change in temperature changes the ET (NR) values of the DESs. No regular trend was obtained with changing the temperature. However, most of the time, the polarity of solvent usually decreases with an increase in the temperature. This might be due to the thermal reorientation of the dipoles, which reduces the dielectric constant [160, 161].

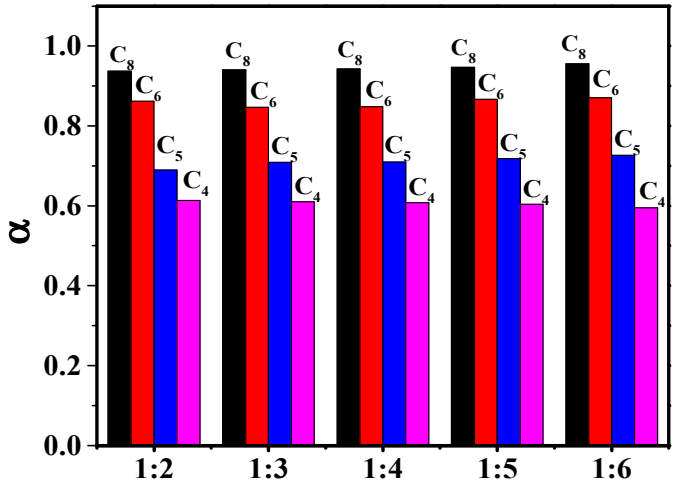


Figure 4.6. Kamlet-Taft parameter α of DESs composed of AcChCl and different carboxylic acids, namely butyric (C_4), valeric (C_5), hexanoic (C_6), and octanoic (C_8) acids in a mole ratio of 1:2- 1:6, at 298K.

The Kamlet-Taft parameters α, β, and Π* were determined using Solvatochromic probes, DENA and 4NA. By combining wavelength of maximum absorbance of these probes within each of the DESs in the temperature range, 25-45 °C with E_T(NR), α, β, and Π* values were calculated. Table 4.1 depict the α, β, and Π* values of the formulated DESs.

The Kamlet- Taft α-parameter is the capacity of solvent to donate proton in solvent to solute hydrogen bonding. Figure 4.6 shows the α values of the formulated DESs, for a better comparison of the obtained data. The α value of the synthesized HDESs ranges from 0.613405 to 0.955314 at 25 °C. The high acidity of solvent is attributed to the carboxylic functional group. The Result shows α parameters are highly dependent on the HBDs. The α value increases with increasing hydrocarbon chain length. For the same HBA used, α value decreases from 0.937165 for AcChCl: CA (1:2) to 0.613405 for AcChCl: BA (1:2). No regular trend was observed on changing the molar ratio of HBDs. However, changing the molar ratio of HBA to HBDs slightly changes the α parameter of DESs, signifying that a slight change in the HBA to HBD molar ratio affects the α parameter. The α is 0.61345 for AcChCl: BA (1:2), 0.615965 for AcChCl: BA (1:3), 0.62375 for AcChCl:BA (1:4), 0.618452 for AcChCl: BA (1:5), and 0.618022 for AcChCl: BA (1:6).

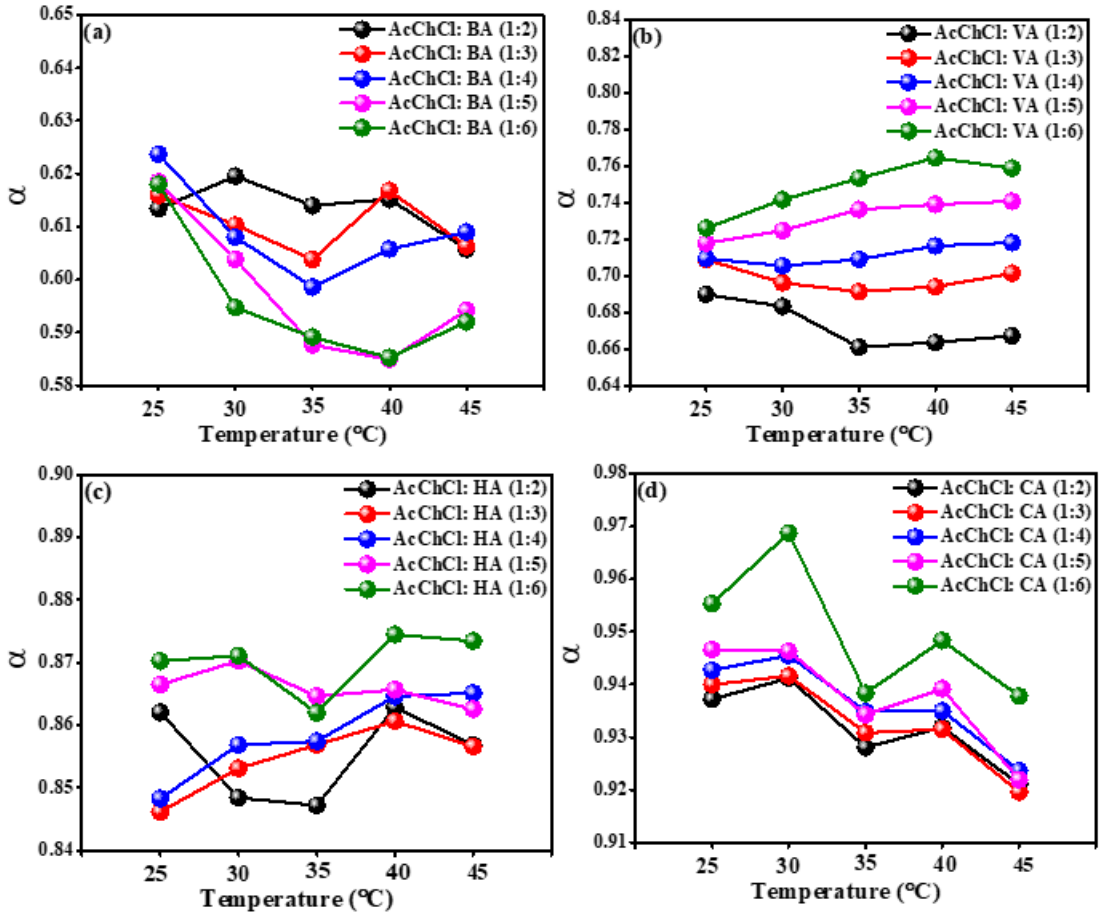


Figure 4.7: Kamlet-Taft α parameter of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA. (d) AcChCl with CA at ratio 1:2-1:6 from temperature 25 °C to 45 °C.

Figure 4.7 shows the effect of temperature on α parameter. The α Vs temperature does not follow any trend to make any conclusions. However, change in temperature changes the α-value significantly.

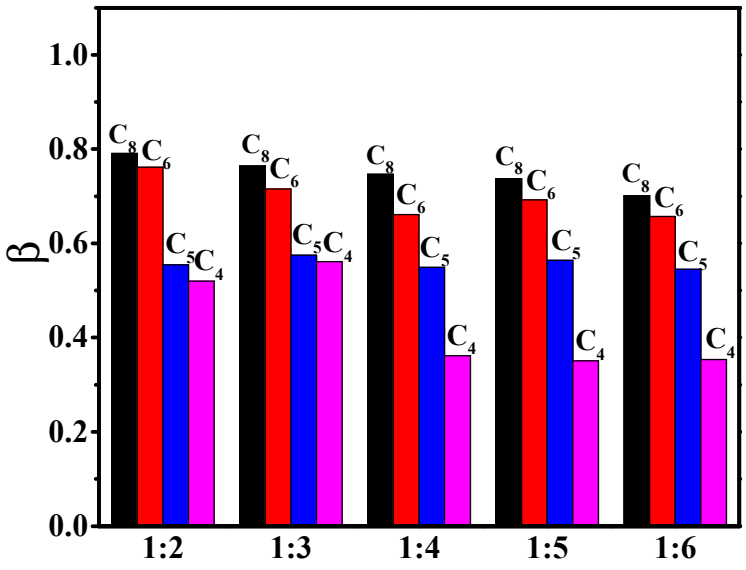


Figure 4.8. Kamlet-Taft parameter β of DESs composed of AcChCl and different carboxylic acids, namely butyric (C_4), valeric (C_5), hexanoic (C_6), and octanoic (C_8) acids in a mole ratio of 1:2- 1:6, at 298K

The Kamlet-Taft β parameter measures the solvent capacity to accept protons or its hydrogen-bond basicity. To better compare the obtained results, figure 4.8 shows the β value of DESs with change in the alkyl chain length of the HBDs at different molar ratios. The broad range of β value of 0.353671 for AcChCl: BA (1:6) to 0.790735 for AcChCl:

CA (1:2) was observed for the studied DESs. For DESs, the β parameter increases with an increase of the alkyl chain length. This agrees with the previous observations concerning the effects of the alkyl chain length on the β value of the DESs composed of ammonium-based salts and carboxylic acids [135]. The trend might be related to the weaker cation-anion interaction as the alkyl chain increases, thus leaving the salt anion more prone to accept a proton from HBD species [162].

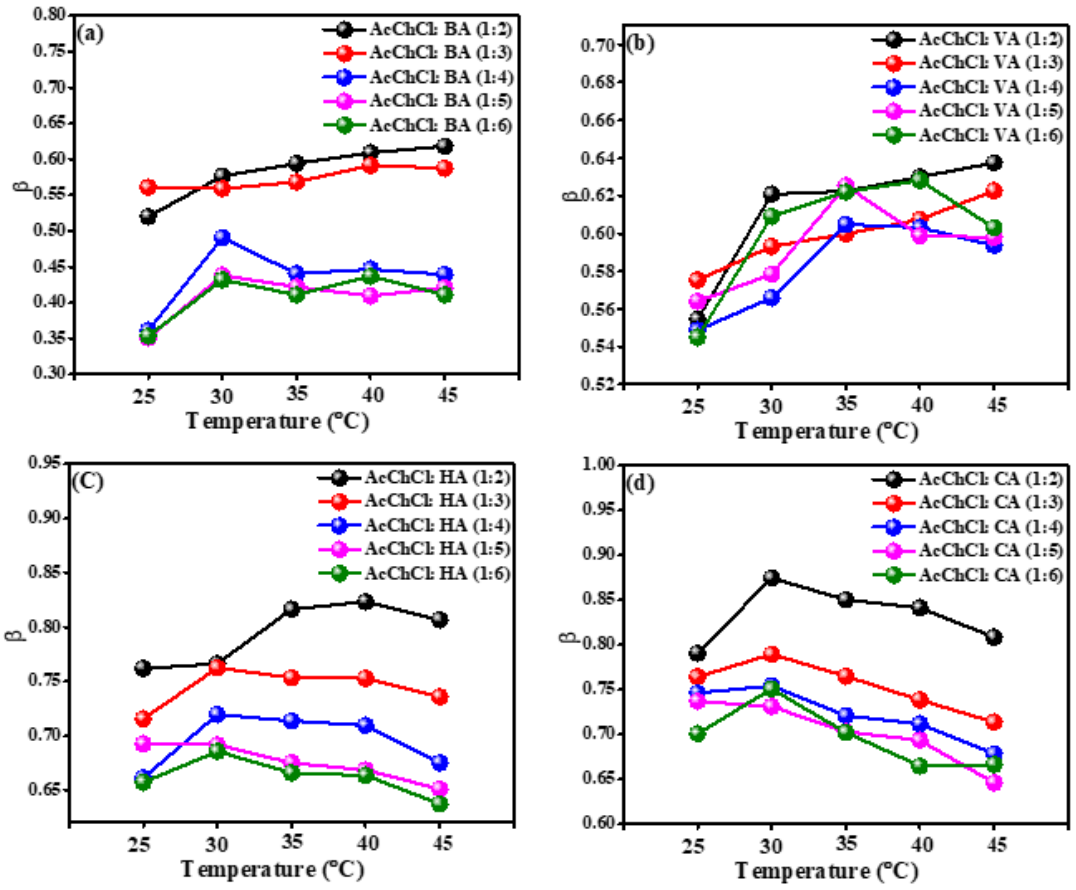


Figure 4.9: Kamlet-Taft β parameter of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA. (d) AcChCl with CA at ratio 1:2-1:6 from temperature 25 °C to 45 °C.

Figure 4.9 shows that changing the molar ratio of HBD changes the β parameter of the formulated DESs. The β parameter for AcChCl: CA (1:2) is 0.790735, AcChCl:CA (1:3) is 0.764492, AcChCl: CA (1:4) is 0.74666, AcChCl: CA (1:5) is 0.737373, AcChCl: CA is 0.701168. Even though, β value decreases with an increase in the molar ratio of HBD for CA-based DESs, no regular trend was observed for BA, VA, and HA based DESs to make any conclusion.

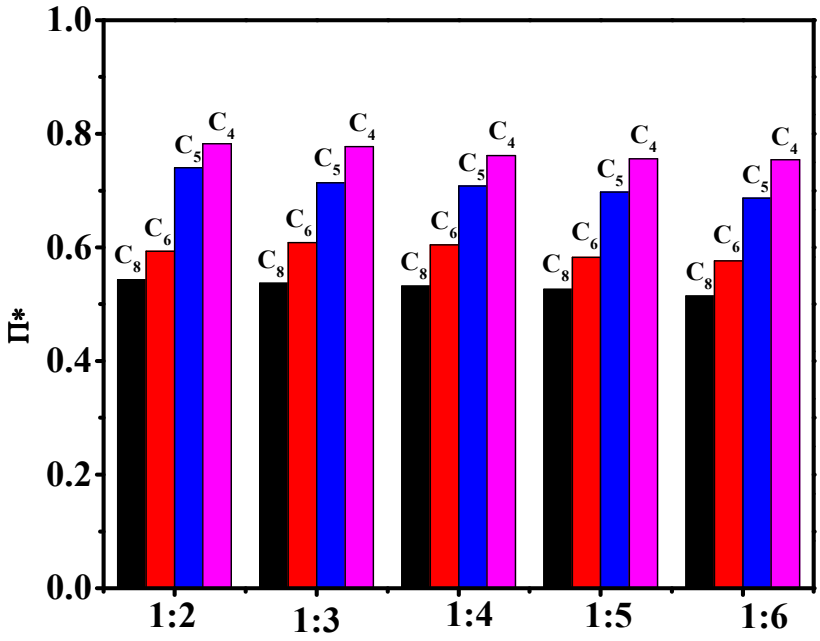


Figure 4.10: Kamlet-Taft parameter Π* of DESs composed of AcChCl and different carboxylic acids, namely butyric (C_4), valeric (C_5), hexanoic (C_6), and octanoic (C_8) acids in a mole ratio of 1:2- 1:6, at 298K

The Kamlet-Taft dipolarity/polarizability is related to nonspecific interactions, such as polarizability, dipole-dipole, and dipole-induced dipole interactions occurring between solute and solvents. The Π* value was determined using molecular probe DENA and 4NA.

Figure 4.10 depicts the Π* values of DESs for a better comparison of the obtained results. According to the data shown in Table 4.1, the Π* values range from 0.938909 for AcChCl: BA (1:2) to 0.617429 for AcChCl: CA (1:6). The Π* value decreases with the increase in the alkyl chain length of the organic acid employed, following the opposite behavior observed in the hydrogen-bond basicity and hydrogen bond acidity. The Π* parameter for AcChCl: BA (1:2) is 0.938909, AcChCl: BA (1:3) is 0.932605, AcChCl: BA (1:4) is 0.913634, AcChCl: BA (1:5) is 0.90729, AcChCl: BA (1:6) is 0.905169. The highest value of BA based DESs might be due to the presence of permanent dipoles and delocalized bonds [163]. Since Π* parameter comprises the ion-dye nonspecific interaction, the result obtained suggest that less nonspecific interaction takes place between the DESs and the dye as the alkyl chain length of HBD increases. The Π* values of methanol, glycerol, and ethanol are 0.58,0.62, and 0.51, which shows that most of the studied DESs have a higher ability to establish nonspecific interaction with solute than organic molecular solvents[164, 165].

Figure 4.11 shows the variation in Π* value with molar ratio of organic acid. As the figure depicts, the Π* value decreases with increase in the HBD content in the DESs, except for AcChCl: HA (1:2), which has Π* value less than AcChCl: HA (1:3) and AcChCl: HA (1:4). For VA based DESs, the Π* parameter is 0.888194 for AcChCl: VA (1:2), 0.856156 for AcChCl: VA (1:3), 0.849717 for AcChCl: VA (1:4), 0.836806 for AcChCl: VA (1:5) and 0.823853 for AcChCl: VA (1:6). No regular change in Π* value was observed with change in temperature to make any conclusion.

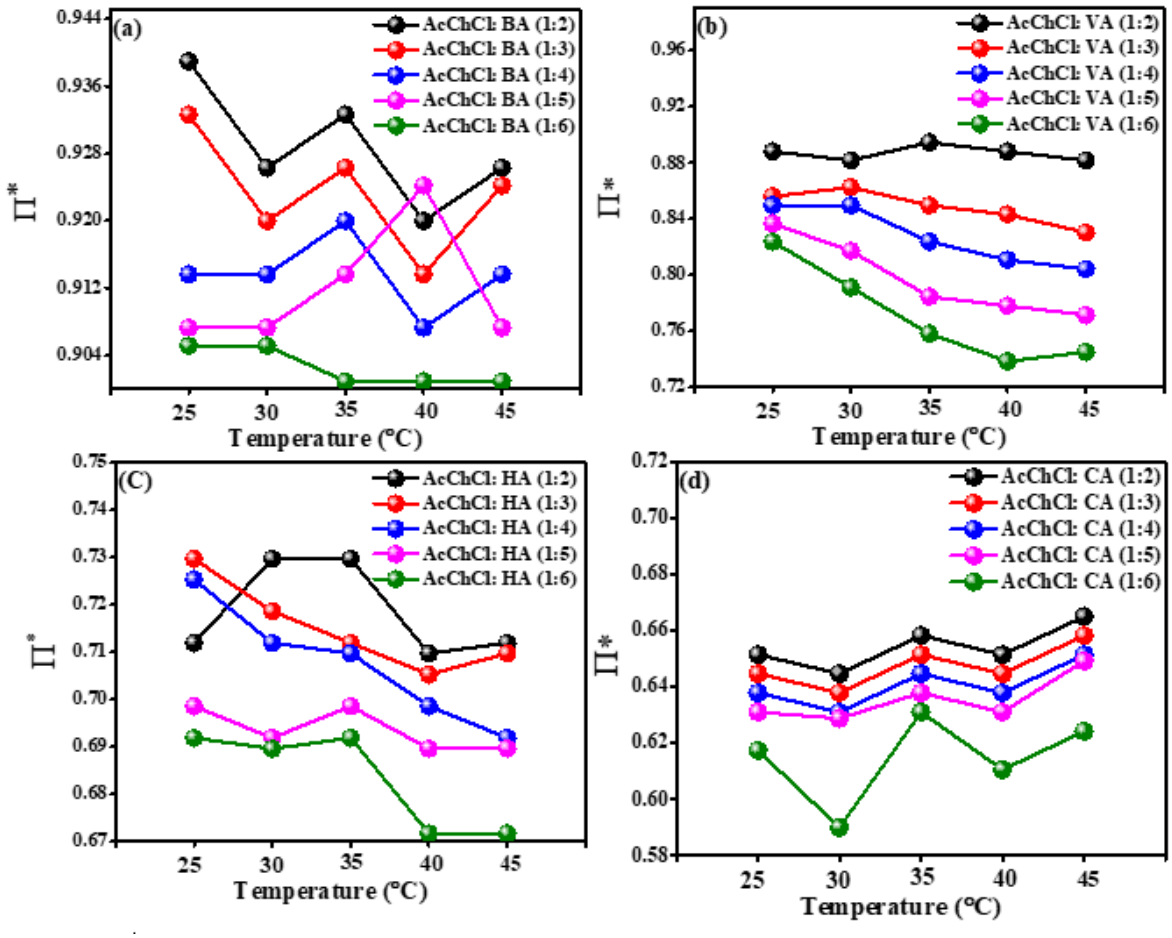


Figure 4.11: Kamlet-Taft Π* parameter of (a) AcChCl with BA (b) AcChCl with VA (c) AcChCl with HA. (d) AcChCl with CA at ratio 1:2-1:6 from temperature 25 °C to 45 °C.

The polarities of the formulated DESs were compared with the polarities of some selected organic solvents and ionic liquids available in the literature [166-169]. The solvent selectivity triangle (SST) introduced by Snyder was used to characterized and group the solvent according to α, β, and Π* value [170, 171]. Figure 4.12 shows that the polarity of the formulated DESs is quite scattered. Some DESs have polarity values closer to ionic liquids, whereas others have polarity comparable to organic solvents. This is further

confirmation that the polarity of DESs can be customed according to HBD and the ratio between HBD and HBA.

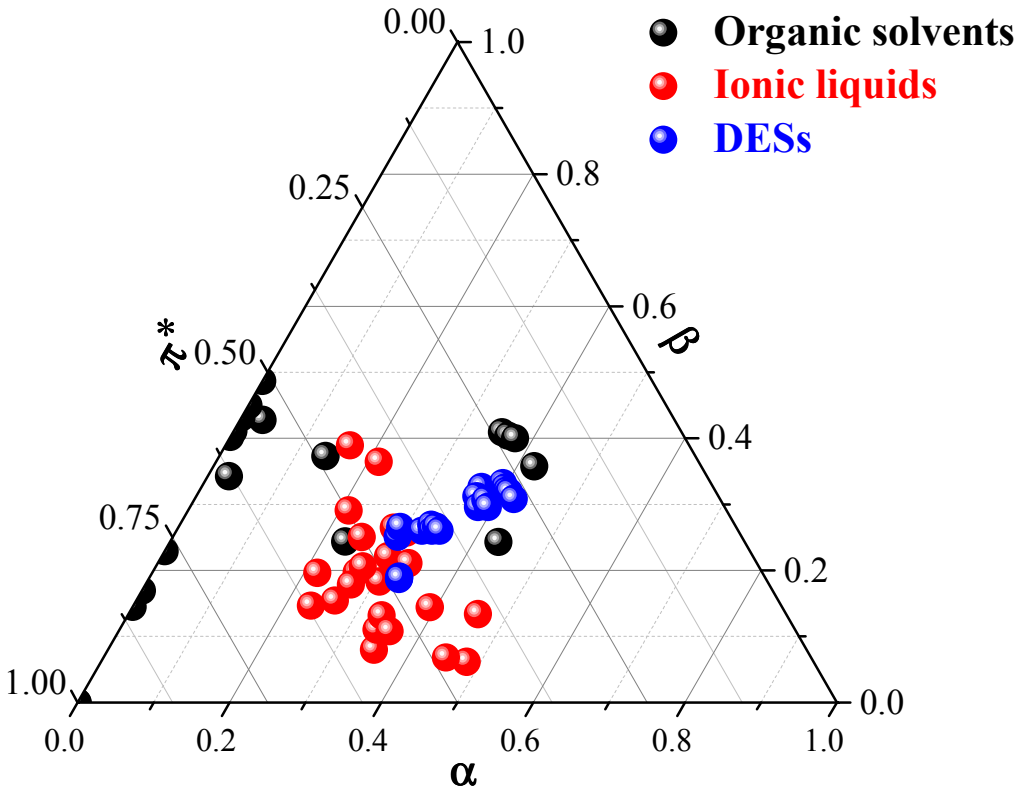


Figure 4.12. Comparison of Kamlet -Taft parameters, α, β, and Π* of formulated DESs (blue) with selected organic solvents (black) and ionic liquids (red).

4.5 Conclusion

To better understand the DESs formulated, polarity and the influence of the chemical composition of DESs, their molar ratio, and temperature on polarity were studied. The result shows that changing the composition of HBD and their molar ratios significantly

changes the polarity of the solvent prepared. This is a good indicator that DESs can be tailored according to the need for solvent-dependent chemical reactions. The kamlet-Taft parameter α, β increases with the increase in carbon chain length of the organic acids. However, the opposite behavior, decrease in Π* parameter with the increase in chain length of organic acid was observed.

CHAPTER 5

APPLICATION OF DEEs IN DRUG SOLUBLILIZATION

5.1 Introduction

Deep eutectic solvents (DESs) are prepared by mixing two or compounds, generating a eutectic mixture with a melting point that is much lower than the individual mixture [44]. DESs is a promising class of solvent that can replace some of the traditional volatile organic solvents due to their ease of preparation, high solubility in a substrate, biodegradable, less toxic, highly tunable, low cost, and negligible vapor pressure [18, 20, 172]. To that end, after the inception of these new designer solvents in early 2000, the research into these solvents is increasing exponentially. Due to the emerging need for green practices in the scientific community, there is a huge demand for the application of DESs. These solvents have been successfully utilized in the various field of the chemical, biotechnology, and electrochemical industries [94, 173-176] and are considered as new alternative green solvent in extraction, separation, and purification process [177]. Eutectic mixtures have also long been utilized in the pharmaceutical fields in applications such as the medium for enzymatic reactions, drug delivery, and drug Solubilization [178, 179].

The major problem of pharmaceutical science, limited bioavailability, and permeability of the poorly soluble drugs that result in poor pharmacokinetics can be addressed by utilizing DESs as a drug delivery system [180-182]. In addition, due to the potential risk of residual solvent in drugs to human health even after the purification enforces drug industries to limit the residual solvents in the final pharmaceutical products. The solvent removal process can be expensive and time-consuming, depending on the amount of residual solvent. Thus,

using the low toxicity solvent can be appropriate alternatives to the organic solvents. Rather than developing a new drug with higher bioavailability, which can be lengthy and expensive, improving the efficiency of the existing drugs is the primary goal of the pharmaceutical industry [183].

Oral drug administration is the most convenient mode of drug delivery. For children, elderly patients, and patients who have difficulty swallowing the drug in solid form, liquid formulations are an essential mode of drug administration. The most accessible and most straightforward type of liquid formulation is an aqueous solution. However, approximately 40% of the approved drugs and nearly 90% of the drug under development are poorly soluble in water [184]. Many of the drugs decompose and are not stable in aqueous solution [185]. Ester-containing pharmaceutical agents, such as aspirin, hydrolyze upon prolonged storage in water [185]. Lu et. al. found the stability of aspirin to be 8.2 times higher in DESs than in water [186]. Another study shows the increase in stability of imipenem and clavulanic acid by 7 and 2.5-fold, respectively, on using DESs as solvent for solubilization [187]. Even though, organic solvents can be utilized to prevent these problems, but they are not recommended due to their toxicity, unpleasant odor, and environmental concerns.

The bioavailability of the drug can be enhanced by the increasing the solubility [188]. Several approaches have been proposed in the literature to improve drug solubility and bioavailability, but, it still remains the most challenging aspects of the drug development, especially for oral drug delivery system [189]. Only a few reports are available on the solubility of drugs in DESs in literature [44, 137, 186, 190]

The prominent and widely accepted non-steroidal anti-inflammatory drugs commonly used to reduce fever, joint pain, headache, and rheumatism are water-insoluble or poorly water-

soluble. Besides the analgesic, antipyretic and anti-inflammatory properties, these drugs are also used in the presentation of cardiovascular diseases [191]. Thus, in this study, we have explored the possibility of using formulated DESs to improve the solubility of anti-inflammatory drugs for potential non-aqueous liquid administration.

Four model drugs, Aspirin, Acetanilide, Acetaminophen, and Phenacetin were chosen for the solubility experiment due to their poor solubility in the aqueous medium. Acetaminophen is an analgesic and antipyretic drug widely used to treat fever, headache, and other mild to severe pain. Due to the low solubility of the drug in water (14 mg/mL at 298.15 K) [192, 193], different methods to improve the solubility to develop a homogeneous liquid form of pharmaceutical dosage have been proposed. This includes but is not limited to the addition of cosolvents, surfactants, cyclodextrins, and pH adjustment [194]. Among these methods, the addition of cosolvents has been widely employed for increasing the solubility of ACP. However, some studies show the higher deviations of co-solvency technique from ideal dissolution behavior that can be explained in terms of possible preferential solvation effect for ACP in the solvent mixture [195]. Thus, there is high demand for universal dissolver for these drugs.

Aspirin, also known as acetylsalicylic acid, is used as a fever reducer, relieve mild to severe pain arising from headache, muscle ache, toothache, and the common cold. Aspirin is also a well-accepted medication for the secondary prevention of cardiovascular diseases [196]. However, aspirin is only sparingly soluble in water (4.6 mg in 1mL) [193]. Therefore, other pain-relieving and fever-reducing drugs, such as phenacetin and acetanilide, were used as model drugs to determine drugs' solubility in formulated DESs. The solubility of acetanilide in water is 6.9 mg in 1 mL, and phenacetin is 0.76 in 1mL [197, 198].

The type and molar ratio of HBA to HBD is important factor in determining whether the DESs formation will be successful as these parameters significantly affects the physical, chemical, and thermal properties of the DESs. Thus, the effect of change of molar ratio and type of HBDs were also studied.

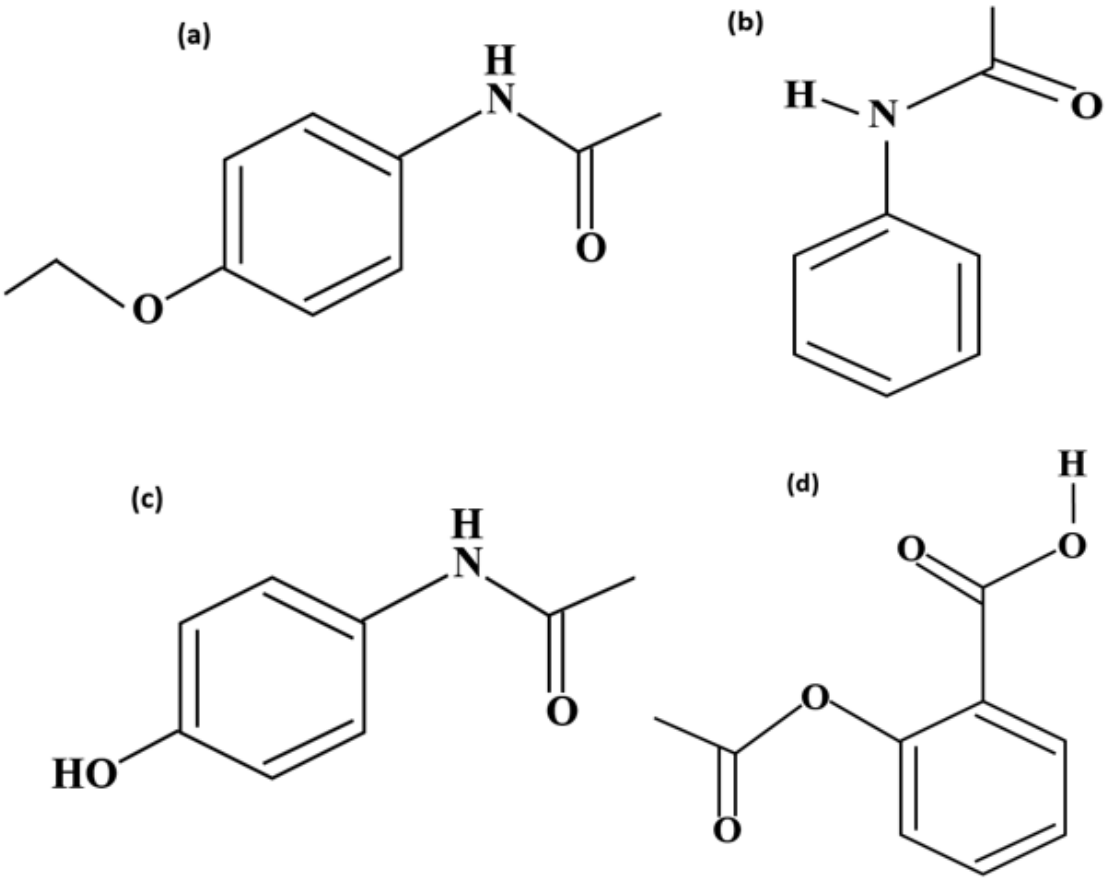


Figure 5.1. Chemical structure of model drugs used for solubility test, a) phenacetin, b) acetanilide, c) paracetamol, d) aspirin

5.2 Experimental

5.2.1 Materials

Aspirin (99%), acetanilide (99%), paracetamol (99%) and phenacetin (99%) were purchased from Sigma Aldrich. Ultra-pure water was obtained in lab using instrument Solution 2000 Water Purification.

AcChCl (99%), BA (99%), HA (99%), CA (99%) and deuterated chloroform (99.8%) were purchased from Acros Organics (Fair lawn, NJ). VA (99%) was purchased from Alfa Aesar (Haverhill MA). Ultra-pure water was obtained in lab using instrument Solution 2000 Water Purification. The chemicals were used as received.

5.2.2. Methods

5.2.2.1 Synthesis

All DESs are prepared by heating AcChCl and fatty acids at ~60 °C for about 15-45 mins until a homogeneous solution is formed. Solution was cooled at room temperature and kept in close sealed vials and then stored in desiccator to prevent any moisture absorption.

5.2.2.2 Drug solubility screening

Aspirin, acetanilide, paracetamol, and phenacetin were chosen as model drugs for the solubility experiment due to their poor solubility in the aqueous medium. To determine the solubility, an excess amount of drug was added to DESs and vortexed until the excess solid remained undissolved. The resulting solution was left standing for at least 24 hours to ensure the equilibrium was attained. The solution was filtered through a nylon syringe filter

with a 0.22 µm membrane. Then the solution was diluted using acetonitrile before HPLC analysis. The drug concentration was then determined using UHPLC (Ultimate 3000 Thermo Fischer UHPLC system) using the C18 column. UV/Vis detector at different wavelengths was used for detection for another model drug. The isocratic elution of acetonitrile and water mixed with 0.1% formic acid was used as the mobile phase. Four different aliquots of the standards at concentration 1000 ppm, 500 ppm, 250 ppm, and 125 ppm were injected, and a calibration curve of signal Vs. Concentration was drawn. Every experiment was carried out three times to ensure the reproducibility of the results. Thermo Scientific Chromeleon software was used for the integration of the chromatogram.

5.3 Result and discussion

The solubility of model drugs in all formulated DESs is evaluated. The calibration curve, drawn by plotting Signal Vs. concentration, for all drugs is shown in figure 5.2. The plot shows a good correlation coefficient (R2) value of 0.9912, 0.9924, 0.9981, and 0.9896 for aspirin, paracetamol, acetanilide, and phenacetin. The closer the value of R^2 to 1.00, the more accurately the curve represents detector response.

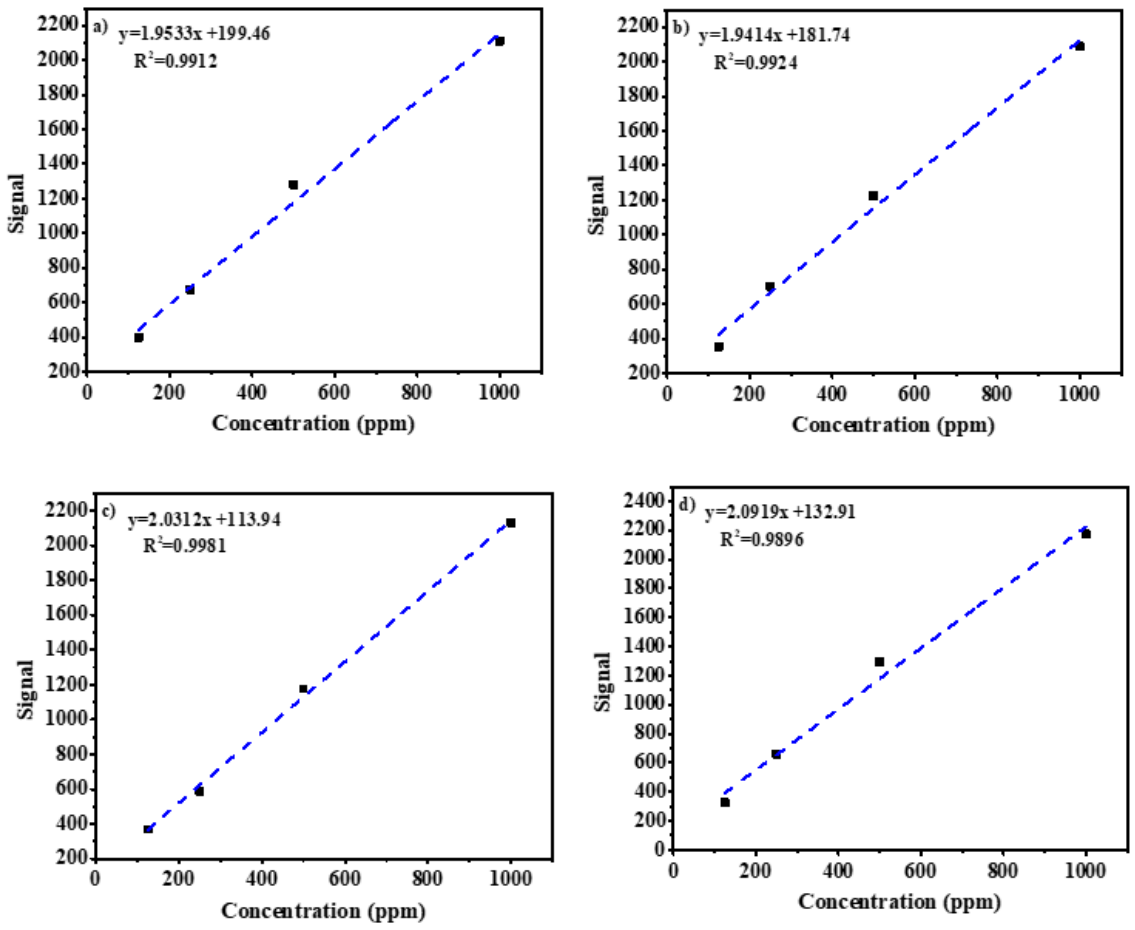


Figure 5.2. Calibration curve for a) aspirin, b) paracetamol, c) acetanilide and d) phenacetin

Figure 5.3 shows the solubility of the aspirin in formulated DESs. From the graph, it is seen that solubility of aspirin changes with changing the molar ratio of the HBDs. For butyric acid based DESs, solubility of AcChCl: BA (1:2) is 18.92 mg/mL, AcChCl: BA (1:3) is 18.75 mg/mL, AcChCl: BA (1:4) is 15.062 mg/mL, AcChCl: BA (1:5) is 16.52 mg/mL, and AcChCl: BA (1:6) is 12.45 mg/mL. No regular trend was observed on increasing the molar ratio of HBDs. However, most of the time solubility value decreases with an increase in the molar ratio of the HBDs. For hexanoic acid based DESs, solubility

value decreases in the order AcChCl: HA (1:2) (30.27 mg/mL) > AcChCl: HA (1:3) (24.98 mg/mL)> AcChCl: HA (1:4) (24.32 mg/mL)> AcChCl: HA (1:5) (21.44 mg/mL) > AcChCl: HA (1:6) (16.06 mg/mL). The solubility is greatest at mole ratio 1:2 followed by 1:3 (HBA: HBD) compared to solubility at other molar ratios for all DESs formulated.

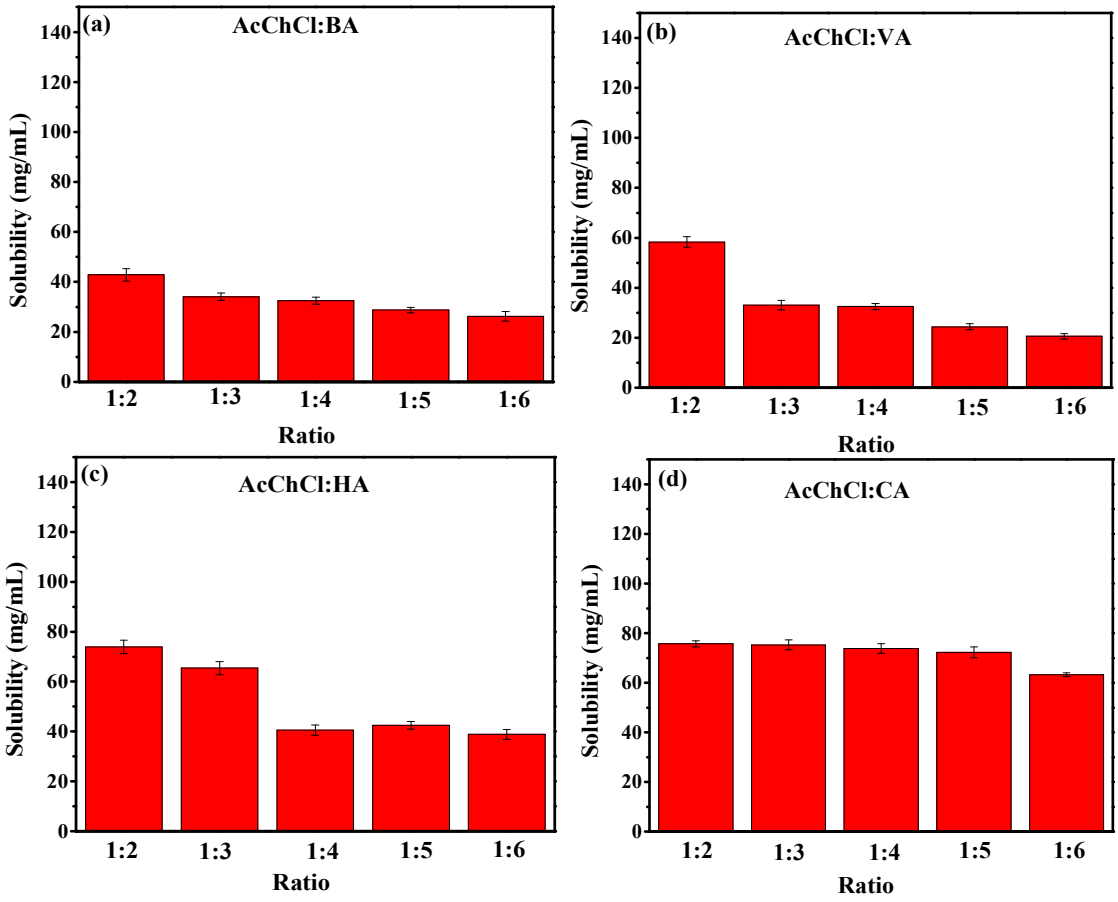


Figure 5.3. Solubility of aspirin in formulated DESs.

Figure 5.4 shows the change in solubility with the chain in alkyl chain length of the HBDs and the change in molar percentage of AcChCl. For all formulated DESs, the solubility of aspirin is greatest in caprylic acid-based DESs followed by hexanoic acid-based DESs at all molar ratios. The solubility value increases in the order of AcChCl: BA (1:2) < AcChCl:

VA (1:2) <AcChCl: HA (1:2) <AcChCl: CA (1:2). Solubility of aspirin in AcChCl: BA (1:5) and AcChCl: BA (1:6) is higher than valeric acid based DESs at a similar ratio.

Solubility is higher in all the formulated DESs compared to the water. The lowest solubility value is observed in AcChCl: VA at mole ratio 1:4, which is 9.45mg/mL, approximately 2 times greater than the solubility of aspirin in water (4.6 mg/ml). The highest solubility of 50.89 mg/mL was noted for AcChCl: CA (1:2), which is approximately 11 times the solubility of aspirin in water. The solubility of aspirin in some of the formulated DESs is even higher than in choline chloride-based DESs [186].

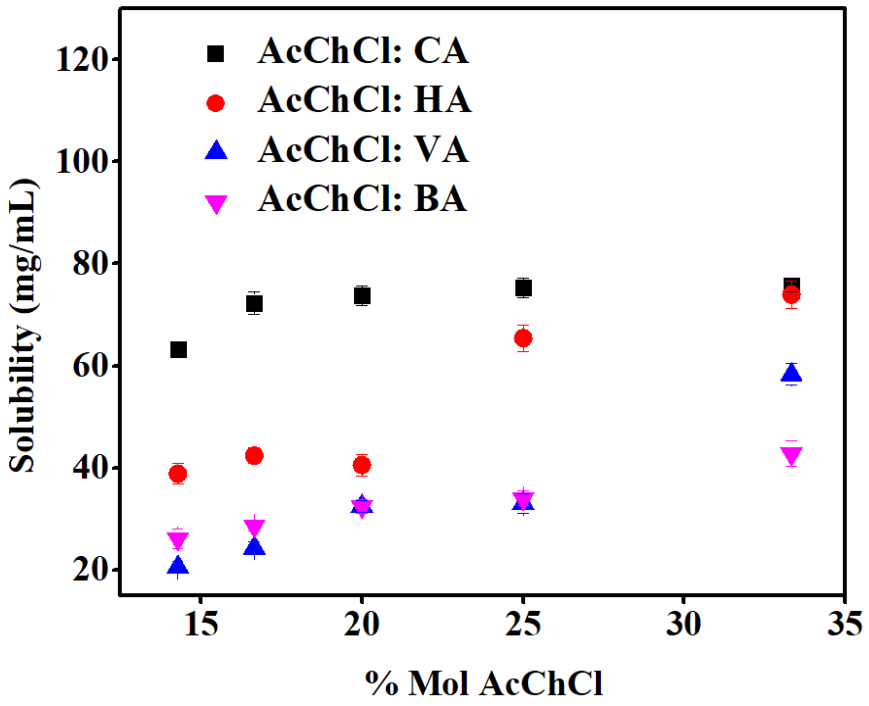


Figure 5.4. Plot of solubility Vs molar percentage of AcChCl for aspirin

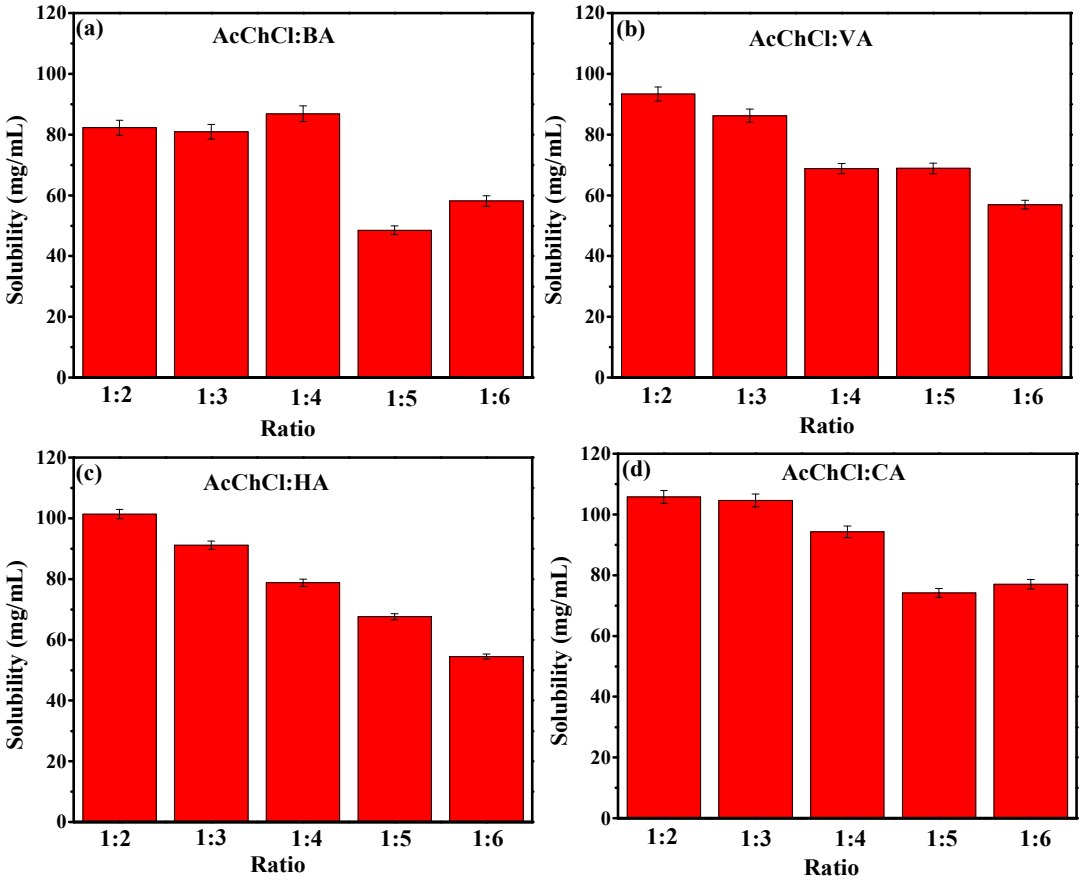


Figure 5.5. Solubility of paracetamol in formulated HDESs.

Figure 5.5 depicts the solubility of paracetamol in formulated DESs at room temperature. The HBA/HBD molar ratio clearly influence the solubility of drug. Solubility decreases with increase in the molar ratio of the organic acid for BA, VA, HA, and CA based DESs, except, AcChCl: HA (1:5), which has solubility higher solubility than AcChCl: HA at mole ratio 1:4. For valeric acid based DESs, solubility of paracetamol decreases in the order, AcChCl: VA (1:2) (58.32 mg/mL)> AcChCl: VA (1:3) (33.08 mg/mL) >AcChCl: VA (1:4) (32.51 mg/mL)> AcChCl: VA (1:5) (24.38 mg/mL)> AcChCl: VA (1:6) (20.62 mg/mL). Minimum change in solubility was observed for CA based DESs at molar ratio of HBA: HBD from 1:2 to 1:5. For AcChCl: CA (1:2) solubility is 75.75 mg/mL, AcChCl: CA (1:3)

solubility is 75.27 mg/mL, AcChCl: CA (1:4) is 73.82 mg/mL, AcChCl: CA (1:5) is 72.29 mg/mL.

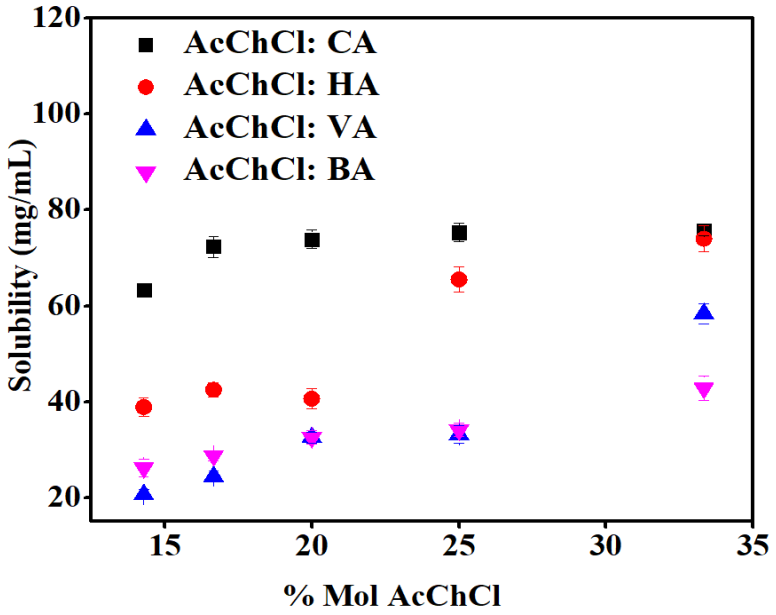


Figure 5.6. Plot of solubility Vs molar percentage of AcChCl

Figure 5.6 shows the change in solubility of paracetamol with change in the molar percentage of AcChCl and alkyl chain length of organic acids in DESs. Figure demonstrates that solubility of the paracetamol is greatest in CA based DESs at all molar ratios compared at other molar ratios. At molar ratio of HBA to HBD at 1:2, the solubility decreases in the order, AcChCl: CA (75.75 mg/mL) < AcChCl: HA (73.93 mg/mL) < AcChCl: VA (58.32 mg/mL) < AcChCl: BA (42.81 mg/mL). Higher solubility value of paracetamol was obtained for all formulated DESs in comparison to that of the water. The least solubility value of 20.62 mg/mL was achieved in AcChCl: VA (1:6) and greatest value of 75.75 mg/mL was obtained for AcChCl: CA (1:2). These values are approximately 1.5 times to 5.4 times greater than the solubility value of paracetamol in water (14mg/mL).

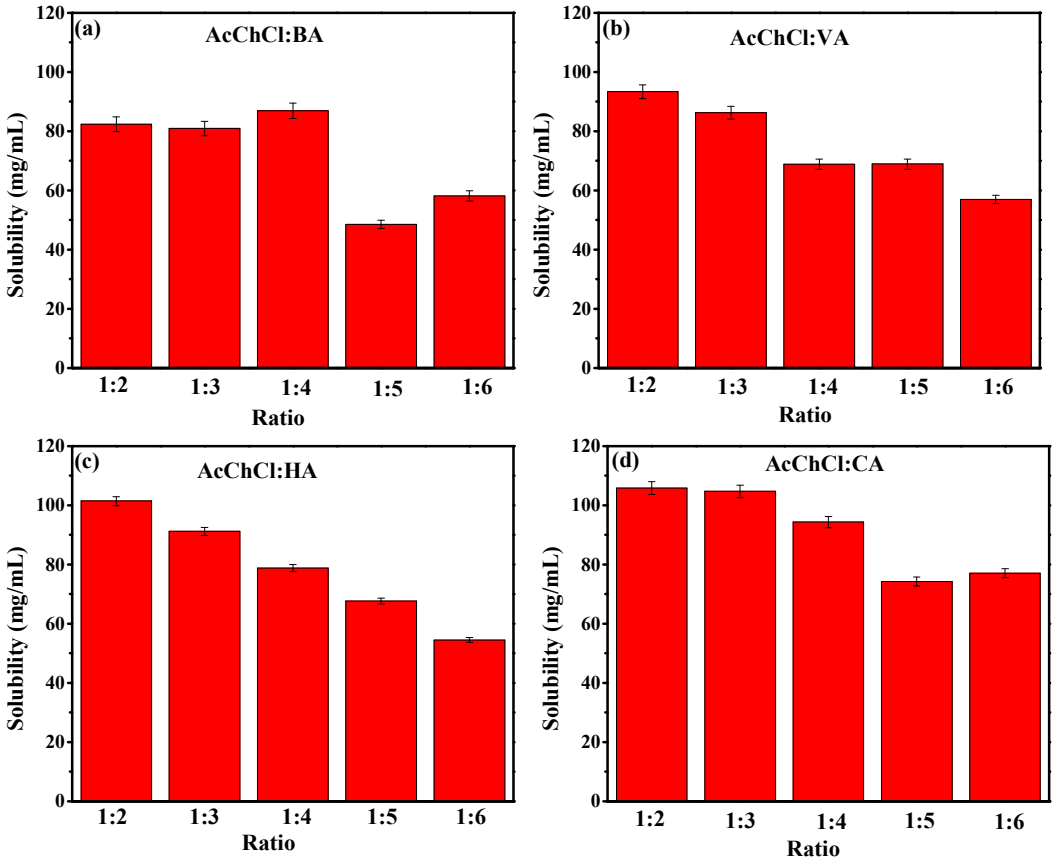


Figure 5.7. Solubility of acetanilide in formulated HDESs

Figure 5.7 shows the solubility profile of acetanilide in DESs. Figure depicts that the solubility of acetanilide changes with change in the molar ratio of HBDs, though no regular decrease or increase in solubility value on changing the HBD molar ratio was observed except for HA based DESs. The solubility decreases in the order, AcChCl: HA (1:2) (101.40 mg/mL) < AcChCl: HA (1:3) (91.16 mg/mL) < AcChCl: HA (1:4) (78.79 mg/mL) < AcChCl: HA (1:5) (67.63 mg/mL) < AcChCl: HA (1:6) (58.48 mg/mL). For BA based DESs, the least solubility of 48.49 mg/mL was observed for AcChCl: BA at molar ratio of 1:5 followed by 58.15 mg/mL for AcChCl: BA at mole ratio 1:6. On contrary to the results obtained earlier, the highest solubility was obtained for AcChCl: BA at molar ratio 1:4.

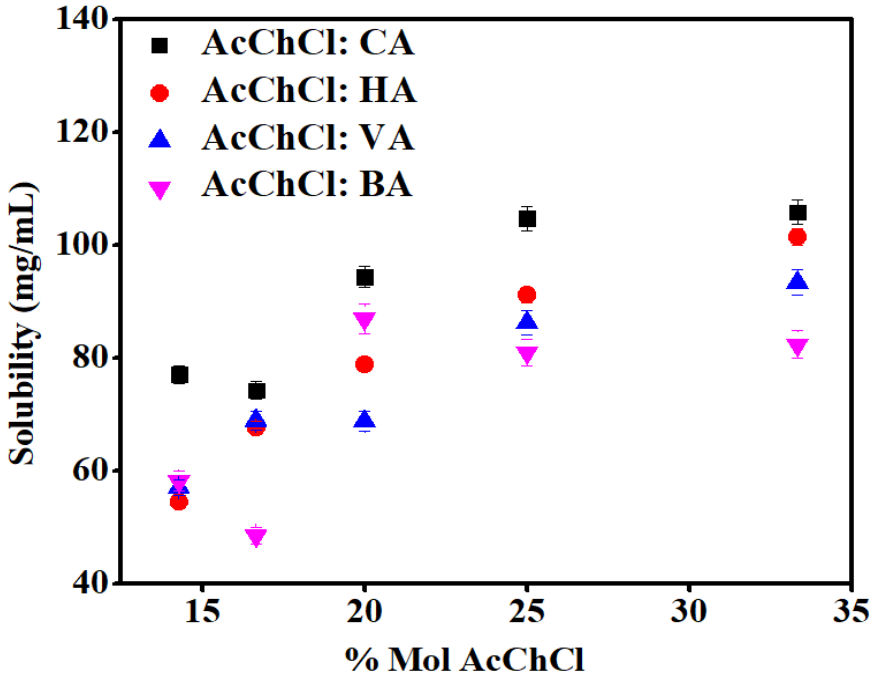


Figure 5.8. Plot of solubility Vs molar percentage of AcChCl for acetanilide

Figure 5.8 shows the change in solubility of acetanilide with change in the alkyl chain length of the HBDs. The solubility in CA based DESs were always greater in comparison to the solubility value of HA, VA and BA based DESs. The solubility value of 105.79 mg/mL was observed for AcChCl: CA (1:2), 101.40 mg/mL AcChCl: HA (1:2), 93.34 mg/mL for AcChCl: VA (1:2) and 82.30 mg/mL for AcChCl: BA (1:2). These solubility values are significantly higher than the solubility of acetamide in water which is 6.9 mg/mL. The lowest solubility of paracetamol was observed in AcChCl: BA (1:5), 48.50 mg/mL, which is about 7 times higher than the solubility in water. The highest solubility

value of 105. 79 mg/mL, obtained for AcChCl: CA (1:2) is approximately 15 times the solubility in aqueous medium.

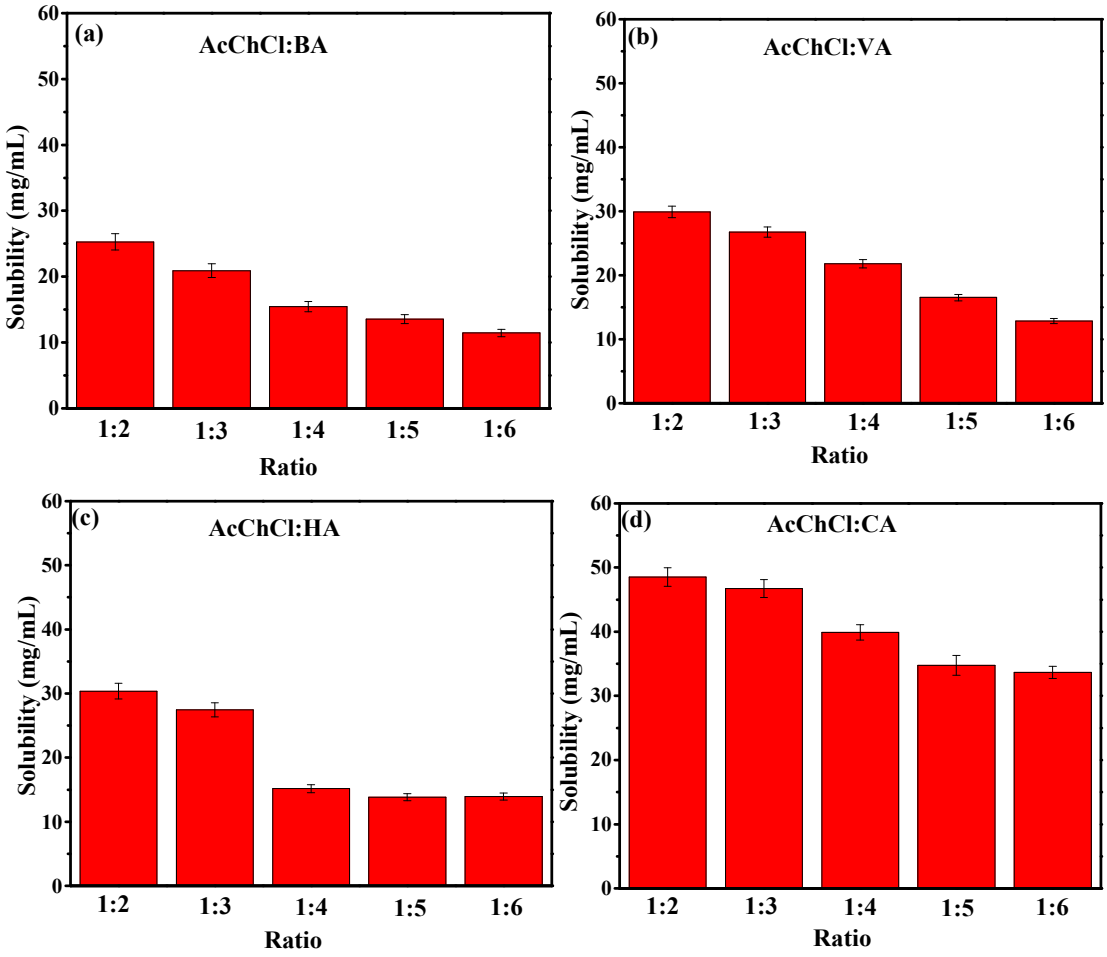


Figure 5.9. Solubility of phenacetin in formulated HDEs.

Figure 5.9 shows the solubility of phenacetin in formulated DESs. Figure depicts the solubility value decreases with increasing the molar ratio of organic acid in the DESs, except for AcChCl: HA at molar ratio 1:5 and 1:6, for which the solubility is 13.83 mg/mL and 13.93 mg/mL, respectively. For BA based DESs, the solubility decreases in the order, AcChCl: BA (1:2) (25.28 mg/mL) > AcChCl: BA (1:3) (20.89 mg/mL)> AcChCl: BA (1:4) (15.45 mg/mL) >AcChCl: BA (1:5) (13.56 mg/mL) >AcChCl: BA (1:6) (11.44mg/mL).

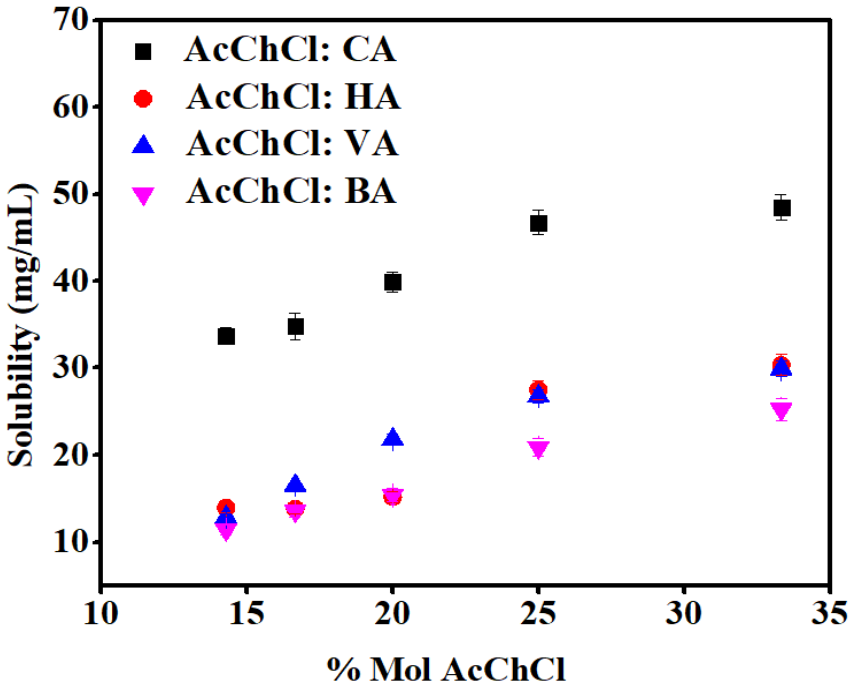


Figure 5.10. Plot of solubility Vs molar percentage of AcChCl for phenacetin.

Figure 5.10 shows the change in solubility of phenacetin in DESs with change in the alkyl chain length of HBDs. The figure illustrates that the solubility of CA based DESs are significantly higher than the solubility of HA, VA and BA based DESs. No regular increase or decrease in solubility with change in alkyl chain length of HBD was observed to make make any conclusion. However, at 1:2 and 1:3 molar ratio of HBA: HBD the solubility decreases in the order, AcChCl: CA (1:2) > AcChCl: HA (1:2) > AcChCl: VA (1:2) > AcChCl: BA (1:2). The solubility is 48.50 mg/mL for AcChCl: CA (1:2), 30.36 mg/mL for AcChCl: HA (1:2), 29.89 mg/ml for AcChCl: VA (1:2), 25.28 mg/mL for AcChCl: BA (1:2). The least solubility is obtained in AcChCl: BA (1:2) which is 11.44 mg/mL and the

greatest solubility is obtained in AcChCl: CA (1:2), 48.51 mg/mL. These solubility values of phenacetin are approximately 15 to 64 times higher than the solubility of phenacetin in water (0.76 mg/mL).

5.4 Conclusion

In summary, the solubility of all model drugs was higher in the formulated DESs than in an aqueous solution. Aspirin showed 2-to-11-fold higher solubility in DESs than in water, and paracetamol has 1.5 times to 5.4 times higher solubility in DESs compared to water. The solubility of acetanilide is approximately 7 to 15 times higher in DESs than in an aqueous solution. Phenacetin showed the solubility increment by 15 to 64-fold in DESs than in an aqueous solution. The solubility values of the drugs are greatly dependent on the type of components used in DESs preparation, and the molar ratio of HBA and HBD used. However, most of the time, no regular increase or decrease in solubility value with changing the molar ratio of HBDs was seen. This shows the difficulty in predicting solute solubility in DESs. To this end, we can conclude that solubility is both DESs and drug-dependent, and the formulated DESs can be a promising alternative for the non-liquid administration of poorly water-soluble drugs.

CHAPTER 6

CONCLUSION AND FUTURE WORK

The main aim of this dissertation was to design and characterize novel water immiscible DESs utilizing non-toxic, cheap, and biodegradable components. In addition, the applicability of the formulated DESs for solubilizing poorly water-soluble was also explored. Twenty different sets of solvents were prepared using quaternary ammonium salt, acetylcholine chloride, as HBA, and different fatty acids as HBDs.

To confirm the formation of the eutectic mixture, the spectroscopic analysis of the solvents was carried out. The freezing point of formulated DESs was lower than the freezing point of the starting material. The freezing point of the DESs changes with the change in the composition and molar ratio of the components used for DESs synthesis. The highest depression in melting point was observed at a mole ratio of 1:2 (HBA: HBD) for all DESs. FTIR spectrum of the DESs showed the change in the frequency shifts, absorbance values, and bandwidth compared with the pure components. Similarly, 1H NMR shows the shift in the signal frequency of DESs compared to the individual components. The frequency shift of the proton around the quaternary nitrogen of AcChCl and the loss of the signal of hydroxyl proton (-OH) in the donor molecule confirms the interaction between the donor and acceptor molecule.

In chapter 3 of this dissertation, the comprehensive characterization of the DESs was carried out. The result showed that the DESs significantly depend on the composition and molar ratio of HBA and HBDs. The density of the DESs was found less than that of water. Density and refractive indices values show the perfect linear relationship to each other.

DESs showed higher viscosity at a molar ratio of 1:2 (HBA: HBD) for all types of formulated DESs. The viscosity decreases with increasing the molar ratio of the fatty acid. The higher viscosity is explained by, the higher hydrogen bonding network between HBA and HBDs. Viscosity showed strong temperature dependence behavior; increasing the temperature decreases the viscosity. PH measurement confirmed the acidic nature of the DESs. As expected, DESs exhibited lower conductivity owing to their higher viscosity. The conductivity of all DESs increased with an increase in temperature. All formulated DESs showed higher thermal stability compared to that of HBDs used for preparation. The thermal properties were also dependent on the type and molar ratio of components used for the preparation. The operating range of the synthesized DESs was also determined, which shows that DESs had a higher operating range than some commonly used solvents.

In another chapter, Solvatochromic parameters, such as polarity and Kamlet-Taft parameters of DESs were studied. This parameter was also highly dependent on the composition and molar ratio used for DESs synthesis. The hamlet-Taft parameter α, β increases with the increase in carbon chain length of the HBDs. However, the opposite behavior decreased in Π^* parameter with an increased chain length of HBDs. The solvent selectivity shows that the polarity of DESs is quite scattered. The polarity of some DESs is like organic solvents, whereas the polarity of other DESs is comparable to those of ionic liquids. This is a good indicator as it shows that the polarity of the DESs can be tailored as per the task requirement.

The last part of this dissertation deals with the application of the formulated DESs. The solubility of the poorly water-soluble drug was tested in the DESs. The result showed the higher solubility of the selected drugs, aspirin, paracetamol, acetanilide, phenacetin, in the

DESs than in aqueous solution. For example, the solubility of aspirin was 2- to 11- fold higher in DESs than in aqueous solution. Similarly, the solubility of paracetamol, acetanilide, and phenacetin was 1.5- to 5.4-, 7- to 15-, and 15- to 64- times higher in formulated DESs than in water, respectively.

Further work is required to confirm the `green` nature of the DESs. Even though DESs is considered green solvent based on the starting material used for the preparation, this assumption may or may not be correct since this assumption did not consider the possible synergetic effect of combining the components. A relatively less amount of work on DESs is focused on studying the toxicity of DESs, which limits its scope of application.

Hydrophobic deep eutectic solvents are still in the nascent stage of development. Thus, the formulation of other novel HDESs is undoubtedly an important area for future research. In this study, only four model drugs were chosen to study the solubility parameter. In-detailed study of other drugs could be carried out using the same solvents or synthesizing the other new solvents in the future. The other area for research could be the application of DESs for the extraction of water-insoluble components.

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