2017

Deep Eutectic Solvents Synthesis, Characterization and Applications in Pretreatment of Lignocellulosic Biomass

Ganesh Degam
South Dakota State University

Recommended Citation

Degam, Ganesh, "Deep Eutectic Solvents Synthesis, Characterization and Applications in Pretreatment of Lignocellulosic Biomass" (2017). *Electronic Theses and Dissertations*. 1156.
https://openprairie.sdstate.edu/etd/1156

DEEP EUTECTIC SOLVENTS SYNTHESIS, CHARACTERIZATION AND APPLICATIONS IN PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

BY

GANESH DEGAM

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Chemistry

South Dakota State University

2017

DEEP EUTECTIC SOLVENTS SYNTHESIS, CHARACTERIZATION AND APPLICATIONS IN PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

This dissertation is approved as a creditable and independent investigation by a candidate for the DOCTOR OF PHILOSOPHY in chemistry degree and is acceptable for the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusion of the major department.

Douglas E Raynie, Ph.D. Date
Dissertation Advisor
Department Head, Department of Chemistry & Biochemistry

Dean, Graduate School Date

ACKNOWLEDGEMENTS

There are literally a number of people involved in the fulfillment of this dissertation work. List they remain unnoticed. I would like to devote some space here to acknowledge their valuable contribution.

I would like to express my gratitude to my advisor, Dr. Douglas E Raynie for sharing his vast knowledge in analytical chemistry and for his never-ending support and encouragement all through. His strong belief in science combined with a systematic approach, tremendous enthusiasm, and convivial criticism has been of great help and a real inspiration to me.

I would like to thank the members of my graduate committee, Drs. Jihong Cole-Dai, Fathi Halaweish, and Francis Ting for their encouragement, useful comments, remarks, and engagement through the entire learning process.

I extend my thanks to the graduate and undergraduate students of the green chemistry lab for their help and wholehearted support throughout my work.

I find no words to express my thanks to my wife Nikitha Gundeti, who has encouraged and supported me through these tough times nothing could have been possible without her affection and encouragement.

Last but not least I extend my sincere gratitude to Almighty for giving me this life and allow me to live in a wonderful world of green chemistry.

This research was sponsored by the Army Research Laboratory and was accomplished under Cooperative Agreement Number W911NF-08-2-0024. The views

and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the Army Research Laboratory or the U.S. Government. The U.S. Government is authorized to reproduce and distribute reprints for Government purposes notwithstanding any copyright notation herein.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

^1H NMR	Proton Nuclear Magnetic Spectroscopy
2-D	Two Dimensional
4-NOPD	4-nitro-o-phenylenediamine
α	Hydrogen Bond Donating Acidity Parameter
β	Hydrogen Bond Accepting Basicity Parameter
π*	Dipolarity/Polarizability Parameter
ΩM cm^{-1}	Megohm per Centimeter
µg	Microgram
µL	Microliter
µU	Micro Units
ΔT_f	Freezing Point Difference
°C	Degree Centigrade
%	Percentage
Acetone-d6	Deuterated Acetone
AcChCl	Acetylcholine Chloride
cm^{-1}	Per Centimeter
ChCl	Choline Chloride
CO_2	Carbon Dioxide
DCM	Dichloromethane
DES	Deep Eutectic Solvent
DESs	Deep Eutectic Solvents
DMSO	Dimethyl Sulfoxide

D_2O	Deuterium Oxide
EBS	EMIM Benzene Sulfonate
EEBS	EMIM Labs
e.g.	Example
EHS	Environmental Health and Safety
EMIM	1-Ethyl-3-methylimidazolium
EMIMAc	1-Ethyl-3-methylimidazolium Acetate
ETS	EMIM Toluene Sulfonate
EtOH	Ethanol
EXS	EMIM Xylene Sulfonate
FBS	Fetal Bovine Serum
FTIR	Fourier Transform Infrared Spectroscopy
G	Glycerol
g	Gram
HBA	Hydrogen Bond Accepting
HBD	Hydrogen Bond Donor
HBDs	Hydrogen Bond Donors
Hr	Hour
IL	Ionic Liquid
ILs	Ionic Liquids
kcal $mole^{-1}$	Kilocalorie per Mole
kg	Kilogram
kj $mole^{-1}$	Kilojoules per Mole

K_{OW}	Octanol-Water Partition Coefficient
LD_{50}	Lethal Dose, 50 %
LDH	Lactate Dehydrogenase
M	Molar
MeOH	Methanol
mg	Milligram
mg g^{-1}	Milligram per Gram
MHz	Megahertz
mL	Milliliter
mm	Millimeter
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
MT	Metric Ton
NaCl	Sodium Chloride
NMR	Nuclear Magnetic Resonance Spectroscopy
OSHA	Occupational Safety and Health Administration
Pa.s	Pascal-second
PCG	Prairie Cordgrass
Pet. Ether	Petroleum Ether
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RCP	Reverbants Colonies per Plate
RI	Refractive Index
RPM	Rounds Per Minute
RPMI	Roswell Park Memorial Institute

RTILs	Room Temperature Ionic Liquids
SDS	Safety Data Sheet
SST	Solvent Selectivity Triangle
SWG	Switchgrass
T_d	Decomposition Temperature
T_f	Freezing Temperature
T_g	Glass Transition Temperature
T_m	Melting Temperature
THF	Tetrahydrofuran
TSB	Tryptic Soy Broth
QAS	Quaternary Ammonium Salt
QASs	Quaternary Ammonium Salts
U	Urea
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
VOC	Volatile Organic Carbon
Wt %	Percentage by Weight

ABSTRACT

DEEP EUTECTIC SOLVENTS SYNTHESIS, CHARACTERIZATION AND APPLICATIONS IN PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

GANESH DEGAM

2017

There has been an increased interest in green solvents and biofuels with the growing environmental awareness across the globe. Conventional methods of biofuel production involve the use of large quantities of molecular solvents and ionic liquids (ILs), but they have the drawbacks of high vapor pressure (organic solvents), toxicity, and recyclability in terms of a sustainability point of view. Deep eutectic solvents (DESs) have recently emerged as green alternatives to molecular solvents and ionic liquids (ILs). They are defined as eutectic mixtures formed between quaternary ammonium, phosphonium or sulfonium salts and hydrogen bond donors (HBDs) with the freezing temperature lower than their individual components.

Sixteen different novel DESs were synthesized by varying the components (ChCl, AcChCl, U, and G) and their composition, employing various methods such as such as mixing (vortex), heating and mixing (thermal treatment, microwave irradiation, sonication, and shaking incubation), and evaporating the solvent (freeze drying and rotary evaporation). The DESs formation by solvent evaporation took longer time than mixing, and heating and mixing took the least amount of time. The DESs synthesized by different methods varied the amount of water present which affected their freezing point. The freezing temperature of DESs was always found to be much lower than the melting point

of their starting materials, which is a characteristic feature of eutectic solvents. The freezing point of DESs depended on the composition and components of QASs and HBDs. The shifts in the representative peaks and broadening of the involved bonds in the FTIR spectra, and the shift in the resonance signal upfield in ^{1}H-NMR spectra confirmed the formation of eutectics and hydrogen bonds in DESs.

Comprehensive characterization of the synthesized DESs was carried out. DESs physicochemical and thermal properties including pH, density, refractive index (RI), surface tension, viscosity, octanol-water partition coefficient (K_{ow}), flammability, freezing temperature (T_f), and decomposition temperature (T_d) depended on their components and composition. Among the QASs and HBDs studied, the latter had the greater effect on most of the measured properties. A good agreement between the measured various properties of DESs was observed. Most of the measured physicochemical and thermal properties of DESs were comparable to the imidazolium ILs and lower or higher than organic solvents.

Miscibility and solvatochromic parameters, including polarity and Kamlet-Taft parameters, of synthesized DESs were determined. The studied DESs were miscible in polar protic solvents and immiscible in nonpolar solvents. Miscibility in polar aprotic solvents depend on the ability to form hydrogen bonds. The DESs studied fall into the category of polar hydrogen bond accepting (HBA) solvents due to their high E_T(NR) or E_T^N and β values. DESs E_T(NR) and E_N^T polarities were comparable to short-chain alcohols and imidazolium ILs, but Kamlet-Taft parameters α, β and π^* were quite different. DESs E_T(NR) and E_N^T polarities and hydrogen bond donating (HBD) acidity parameter α decreased, and HBA basicity parameter β and dipolarity/polarizability parameter π^* remain unchanged with an increase in temperature from 25 to 45 °C. DESs $E_T(NR)$ and E_N^T polarities, HBD acidity parameter α and dipolarity/polarizability parameter π^* increased, and HBA basicity parameter β remain unchanged with an increase in water content from zero to 20 wt %. The equation of lines derived from the studies of effect of temperature and added water content to predict the DESs polarity and Kamlet-Taft parameters.

Genotoxicity and cytotoxicity of DESs and their individual components were evaluated using the Ames test and LDH, and MTT assays. A synergetic effect was observed because the toxicity of DESs was higher than their individual components. Therefore careful use of the term nontoxic must be considered. The genotoxicity and cytotoxicity of the DESs under study are far less toxic than imidazolium ILs at studied concentration, time and cell lines.

The utilization of DESs for the dissolution of biopolymers such as cellulose, hemicellulose, and lignin was evaluated. The DESs selective dissolution of lignin was further explored and an extraction method optimized to selectively extract lignin from lignocellulosic biomass. The DESs extracted up to 80 % of the lignin from PCG and SWG biomass was achieved. The extracted lignin was confirmed by FTIR and ^1H NMR spectroscopy studies. A new source of DESs-extracted lignin was produced because the extraction was carried out with less harsh solvents at moderate temperature. The DESs were recovered simply by adding water and evaporating, which allowed reuse up to three times without losing significant activity. The selective extraction of lignin and ease of recovery of eutectic solvents demonstrated that DESs pretreatment is a promising green procedure in the biofuel production from lignocellulosic biomass. As DESs research for applications in this regard is still in its infancy, further investigations are still needed to successfully apply DESs as solvents in pretreatment processes at large-scale industrial applications.

CHAPTER 1

1 INTRODUCTION AND BACKGROUND

1.1 Problem Statement

There has been an increased interest in green solvents with the growing environmental awareness across the globe[1]. According to the twelve principles of green chemistry, green solvents should meet numerous criteria such as low toxicity, nonflammability, low volatility, rapid biodegradability, and widespread availability[2-3]. Moreover, these green solvents should be inexpensive, easy to handle, and recyclable[2-3]. A number of new solvents have been reported over the past several years, including sub- and supercritical water, supercritical fluids, fluorous solvents, liquid polymers, and room temperature ionic liquids (RTILs) as green solvent alternatives[4]. RTILs have attracted many researchers working in the field of green chemistry because of their nonvolatility and negligible vapor pressures[5]. Unfortunately, they have the disadvantages of high cost, complex synthetic and purification procedures, low moisture tolerance, toxicity, and biodegradability[4, 6-9]. Recently some studies even suggested further assessment of their applicability as green media[10].

Finding green solvents as replacements for solvents of any kind is the biggest challenge. Researchers are in the continued search for new and greener alternatives to molecular solvents and RTILs that may overcome their limitations. Among such new solvents, deep eutectic solvents (DESs) have emerged at the beginning of this century[10-14]. DESs have similar solvent properties to RTILs but are less expensive, more synthetically accessible, highly pure, nontoxic, and biodegradable[15].

1.2 Deep Eutectic Solvents

1.2.1 Definition

A eutectic system is a mixture of chemical compounds or elements that exhibit a single chemical composition that freezes at a lower temperature than any other composition[16]. As depicted in Figure 1.1, the eutectic point is given by the intersection of the eutectic temperature and the eutectic composition[16].

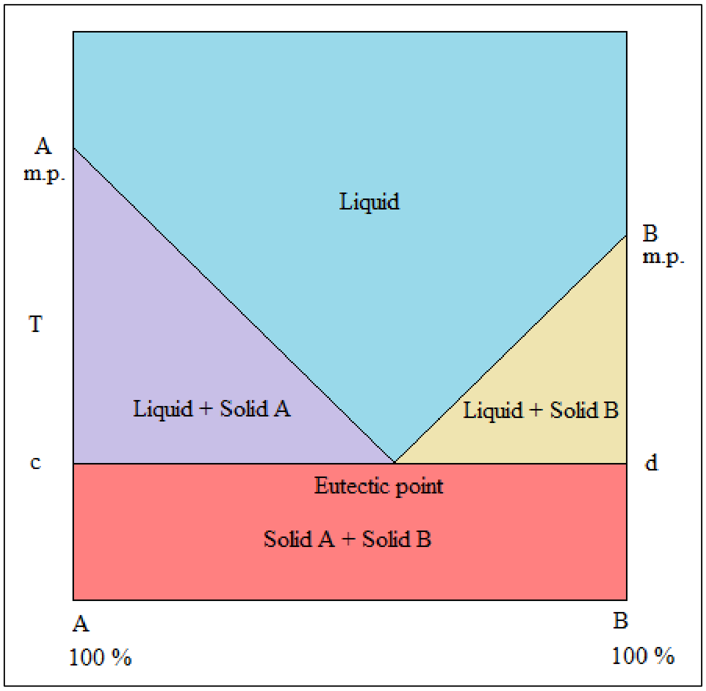

Figure 1.1. Schematic representation of a eutectic point on a two component phase diagram.

The term DESs has been coined mainly to differentiate them from true ionic liquids (ILs) (due to differences in some solvent properties and because some DES components are not ionic), and also to reflect the large depression in the freezing point of the eutectic mixture. The depression of the freezing point of the eutectic mixture can be as much as a few hundred degrees Celsius[12].

1.2.2 Classification

DESs are broadly classified into four categories depending on the nature of the complexing agent used[17-18]. Type I DESs are composed of quaternary ammonium salt (QAS) and metal chloride, type II are composed of QAS and metal chloride hydrate, type III are composed of QAS and hydrogen bond donor (HBD) and type IV are composed of metal chloride and HBD, as shown in Table 1.1.

Table 1.1. Types of DESs, their general formula, terms and examples.

Types	General formula	Terms	Example
Type I	$Cat^+X^- + zMCl_x$	M = Zn, In, Sn, Al, Fe	$ChCl + ZnCl_2$
Type II	$Cat^+X^- + zMCl_x.yH_2O$	M = Cr, Ni, Cu, Fe, Co	$ChCl + CoCl_2.6H_2O$
Type III	$Cat^+X^- + zRZ$	Z = OH, COOH, $CONH_2$	ChCl + Urea
Type IV	$MCl_x + zRZ$	M = Zn, Al and Z = OH, $CONH_2$	$ZnCl_2$ + Urea

The present work focuses on type III DESs because they are easy to prepare, inexpensive, tunable, relatively unreactive with water, and many are nontoxic and biodegradable[17].

1.2.3 Type III Deep Eutectic Solvents

Type III DESs are a type of ionic solvents comprising mixtures of quaternary ammonium, phosphonium or sulfonium salts with hydrogen bond donors (HBDs), such as amines, amides, alcohols, and carboxylic acids, which form a eutectic with a melting point significantly lower than that of its individual components[19]. Abbot first described the type III deep eutectic phenomenon in 2003 for a mixture of choline chloride (ChCl) and urea (U) in a 1:2 molar ratio[20]. ChCl is a QAS, and urea is an HBD, melting at 302 ºC and 133 ºC respectively. The mixture resulted in a eutectic that melts at 12 ºC. Thus, the depression in freezing point of the eutectic mixture is 178 ºC[20].

The reason for the large decrease in the freezing point of the mixture relative to the melting points of the individual components is the charge delocalization occurring through the hydrogen bonding between the halide anion of quaternary ammonium, phosphonium or sulfonium salt with the hydrogen donor group of HBD. The hydrogen bond donating group interacts with the anion of quaternary ammonium, phosphonium or sulfonium salt increasing its effective size. This, in turn, decreases the interaction with the cation and so decreases the freezing point of the mixture as illustrated in Figure 1.2.

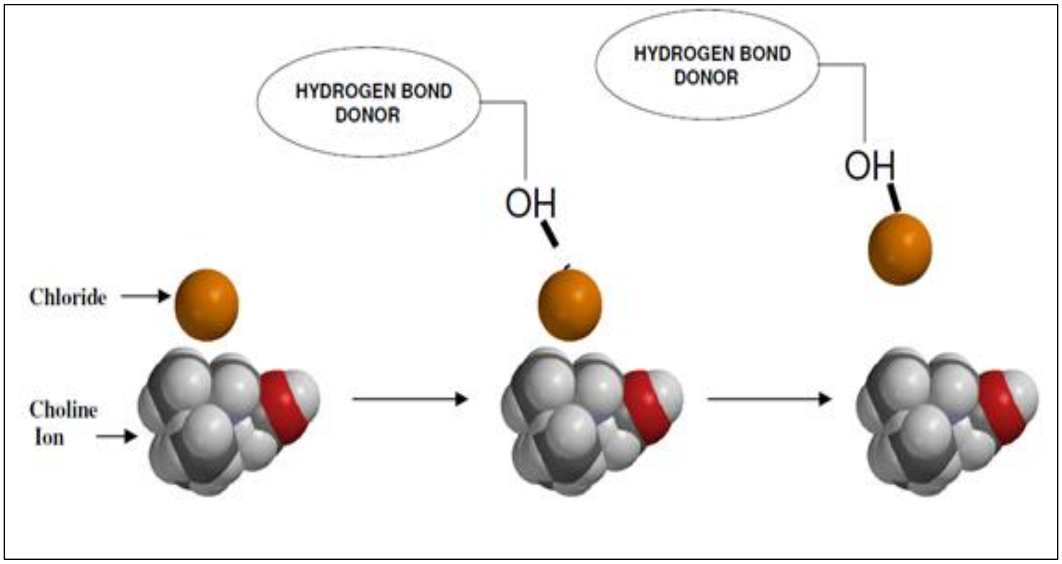

Figure 1.2. Schematic representation of the interaction between a hydrogen bond donating group of HBD and halide anion of a QAS[21].

The freezing point of any mixture containing a quaternary ammonium, phosphonium or sulfonium salt and an HBD is dependent on two major factors. The individual lattice energies of both quaternary ammonium, phosphonium or sulfonium salt and HBD, and the degree of interaction between the anion of the quaternary ammonium, phosphonium or sulfonium salt with the hydrogen bond donating group of HBD[12]. If the anion-hydrogen bond donor interaction is strong, the entropy of the system increases, thereby lowering the freezing point of the system. Thus to a certain degree, the depression in the freezing point can be a measure of the entropy change.

DESs general formula can be described by the following Equation 1.1[17, 21];

$(R_1R_2R_3R_4N^+)\ (X^-)\ (R_5)(YH)$ ……………………………………………...**Equation 1.1.**

Where,

$R_1R_2R_3R_4N^+$ = Quaternary ammonium, phosphonium or sulfonium cation,

X^- = Halide anion,

R_5 = R-group present in HBD, and

YH = Hydrogen-bond donating group.

There is no limit to the number of DESs that can be synthesized from the available chemicals because there is a large number of quaternary ammonium, phosphonium or sulfonium salts and HBDs which can be used to synthesize the DESs. Therefore, it is almost impossible to study all the variants. For this study, choline chloride (ChCl) and acetylcholine chloride (AcChCl) as quaternary ammonium salts (QASs) and urea (U) and glycerol (G) as HBDs have been selected because of their advantageous properties. Their environmental and economic benefits give them the potential to be used in large-scale industrial applications. The chemical structures of ChCl, AcChCl, U, and G are depicted in Figure 1.3.

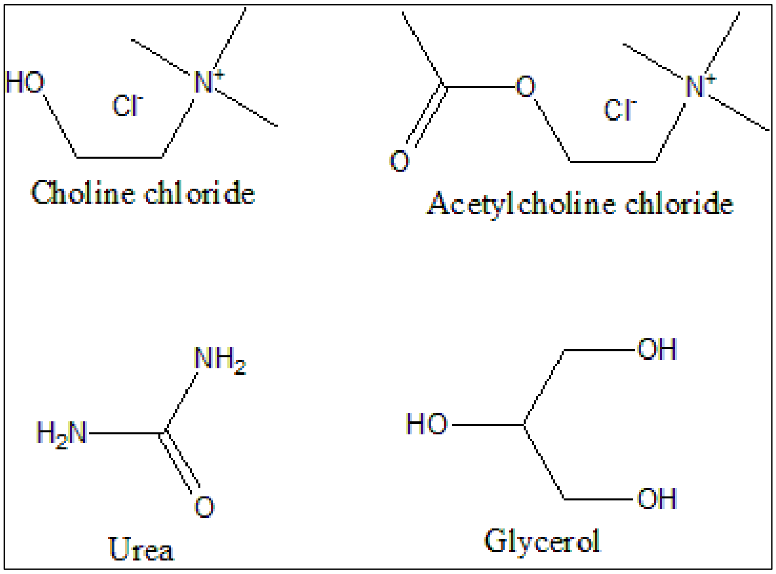

Figure 1.3. Chemical structure of components (ChCl, AcChCl, U, and G) used in this study.

1.2.3.1 Choline Chloride

Choline (2-hydroxyethyl-trimethylammonium) chloride is the most studied QAS since the introduction of the deep eutectic phenomenon in 2003[15]. It is produced on the metric ton (MT) per annum scale as an additive for chicken feed and a wide range of other applications. It is very cheap, about $2 per kilogram (kg) from Chinese chemical companies due to the current economic and industrial explosion occurring in China[21-22]. Its synthesis involves a gas-phase reaction between trimethylamine, ethylene oxide and hydrochloric acid as shown in Figure 1.4[23].

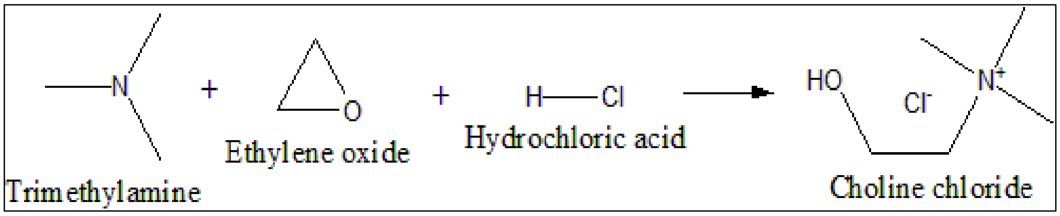

Figure 1.4. Synthesis of ChCl from gas phase reaction between trimethylamine, ethylene oxide, and hydrochloric acid.

The Sheldon E-factor for the ChCl synthesis is close to zero because almost no waste products are formed during this reaction[24]. The atom economy for this reaction is 100 %. ChCl is not only cheap and easy to make but also nontoxic and biodegradable[25]. Therefore, it is not hazardous even if it is released back into nature as ChCl or its deep eutectic mixture. Hence, DESs based on ChCl have the potential to be used economically in large-scale processes.

1.2.3.2 Acetylcholine Chloride

Acetylcholine (2-(acetyloxy)-N,N,N-trimethylethanamonium) chloride is an acetylated form of ChCl. It is biodegradable and more environmentally benign than ChCl, due to the incorporation of an acetyl group that significantly increases its biodegradability [26-27]. It is a naturally occurring neurotransmitter present in many organisms, including humans[28].

1.2.3.3 Urea

Urea (diaminomethanone) is a naturally occurring, biocompatible, environmentally safe compound present in all mammals[29]. It is the main nitrogen-containing substance in the urine of mammals. Urea is widely used in fertilizers as a source of nitrogen. The nitrogen in urea also makes it a good option for livestock feeds.

1.2.3.4 Glycerol

Glycerol (propane-1,2,3-triol) is an organic waste generated by the biodiesel industry and the main co-product of the vegetable oil industry[30]. It has been recently proposed as a valuable green solvent because it is available on a large scale, cheap, nontoxic, nonflammable, biodegradable, and highly inert[31].

In terms of the environmental health and safety (EHS) perspective, DESs are purely a eutectic composition of benign compounds. Table 1.2 lists the EHS impacts of the four DES components used in this study. Urea is considered as nonflammable whereas ChCl, AcChCl and G have high flash points (330, 288 and 176 ºC respectively). Therefore, it is safe to use them at elevated temperatures. The vapor pressures of all the four DES components are very small so the air pollution of these components caused by volatile organic carbon (VOC) emission can be considered as negligible. The lethal dose 50 % (LD_{50}) values of ChCl, AcChCl, U, and G, listed in Table 1.2, categorize them as nontoxic.

Table 1.2. EHS impacts of DESs components used in this study.

DES component	Flash point (°C)[32]	Vapor pressure at 25 °C (mm Hg)	LD_{50} oral rat (mg kg^{-1})
ChCl	330	4.93×10^{-10}	5000[33]
AcChCl	288	3.57×10^{-8}	4860[34]
U	Nonflammable	6.75×10^{-3}	8471[35]
G	176	1.05×10^{-7}	58400[36]

All of the properties mentioned above make DESs composed of ChCl, AcChCl, U, and G an interesting class of compounds to develop economically viable and environmentally safe industrial applications, even at elevated temperatures.

1.2.4 Advantages

DESs can be easily synthesized in high purity at low cost due to the low cost and high purity of the starting materials. Their synthesis procedure is very simple and straightforward, only requires the mixing of the quaternary ammonium, phosphonium or sulfonium salts and HBDs mechanically in an appropriate mole ratio and no waste products are formed during this reaction. Therefore according to the twelve principles of green chemistry, synthesis of DESs is green and environmentally benign because their reaction has zero emissions, zero E-factor value, and 100 % mass efficiency[37]. In terms of an economic perspective, DESs are inexpensive and approximately tenfold less expensive than the components of ILs[38].

The biggest advantageous of DESs are their potential as designer solvents because their physical, chemical and thermal properties, including pH, density, refractive index (RI), viscosity, surface tension, octanol-water partition ocefficent (K_{OW}), conductivity, freezing temperature (T_f), glass transition temperature (T_g), melting temperature (Tm), decomposition temperature (T_d), miscibility, and polarity, can be easily tuned by altering the components and their ratios[15, 17]. Moreover, a broader array of DESs can be obtained than with the conventional ILs, in terms of different quaternary ammonium, phosphonium or sulfonium salts and HBDs in various molar ratios, making this new type of designer solvents even more designable.

DESs have some beneficial properties in common with ILs such as nonvolatility and recyclability[20]. The starting materials of DESs can be more easily recoverable than ILs by disrupting the complex structures in their matrix, because no chemical reactions occur during their formation. In addition, DESs also possess several merits over conventional ILs such as nontoxicity and biodegradability that decreases the risk of exposure to humans and the environment. Moreover, these green solvents are easier to handle than most of the ILs because they doesn’t form toxic substances with air and water[39-40].

All of the advantages mentioned above make these solvents desirable as green alternatives for large-scale industrial applications, and some of them are already being processed on the tone scale and are available for commercial use. ChCl-U- and ChCl-G-based DESs are being used industrially for electrodeposition of metals[41] and biodiesel synthesis[42] and purification[43].

1.2.5 Number of Published References

The Abbott group at the University of Leicester, UK was the first to pioneer deep eutectic solvents systems in 2003 and, since then, there has been a growing number of publications in the literature related to DESs, revealing that they are gaining much attention by the scientific community[20]. At the end of 2016, a total of 1437 references were found to contain the keyword ‘‘deep eutectic solvent’’ in the SciFinder database. In recent years, DESs have attracted considerable attention, and the number of DES-related references has increased rapidly from 2009 to 2016. Before 2009, only 29 references noted the concept. Since 2009, more than 1400 references were published, as shown in Figure 1.5.

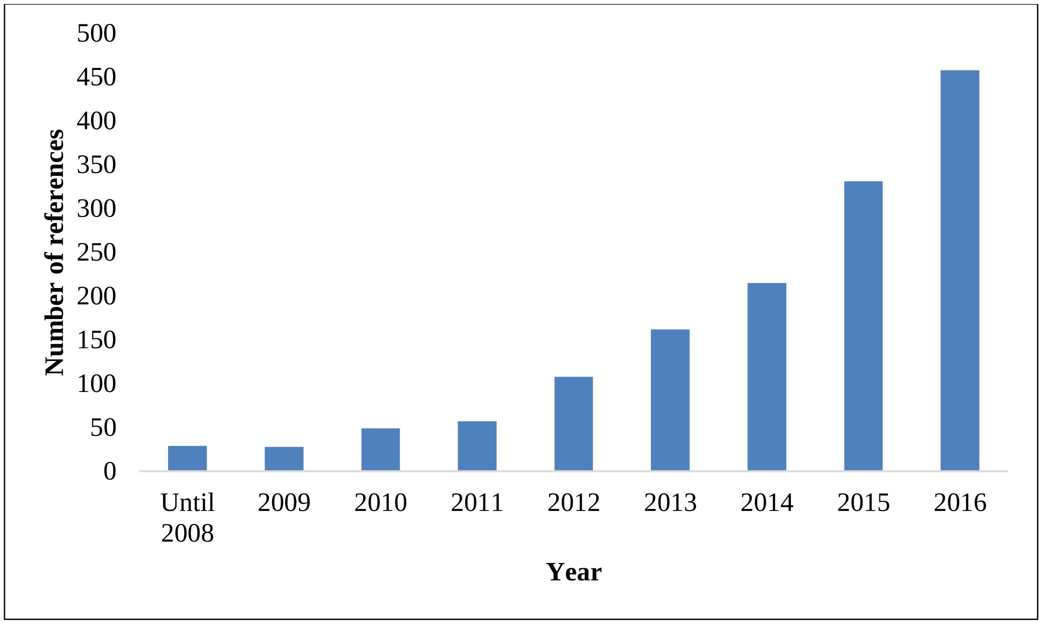

Figure 1.5. Annual number of references containing the concept ‘‘deep eutectic solvent’’ in the SciFinder database through 2016.

1.2.6 Distribution of the Research Field

Summarizing the distribution of the DESs research field in chemistry gives an idea of the general direction of this new concept. The results presented in Figure 1.6 shows that DES-related references distributed into different research fields of chemistry such as preparation, properties, synthesis, electrochemistry, nanomaterials, biochemistry, separation, and analysis. The result shows that approximately one-third of the references is related to synthesis, thus constituting the highest proportion of all fields. This is followed by electrochemistry, studies of their properties, and separation processes. The references focusing on the application of DESs to nanomaterials and biochemistry, as well as the preparation and application of DESs for analysis comprise less than 20 % of the total DES references. This shows that DESs are developing rapidly in the synthesis and electrochemistry fields, and more than half of the total DES references focused on these two fields. In other fields, there may be considerable room for the development of DESs applications.

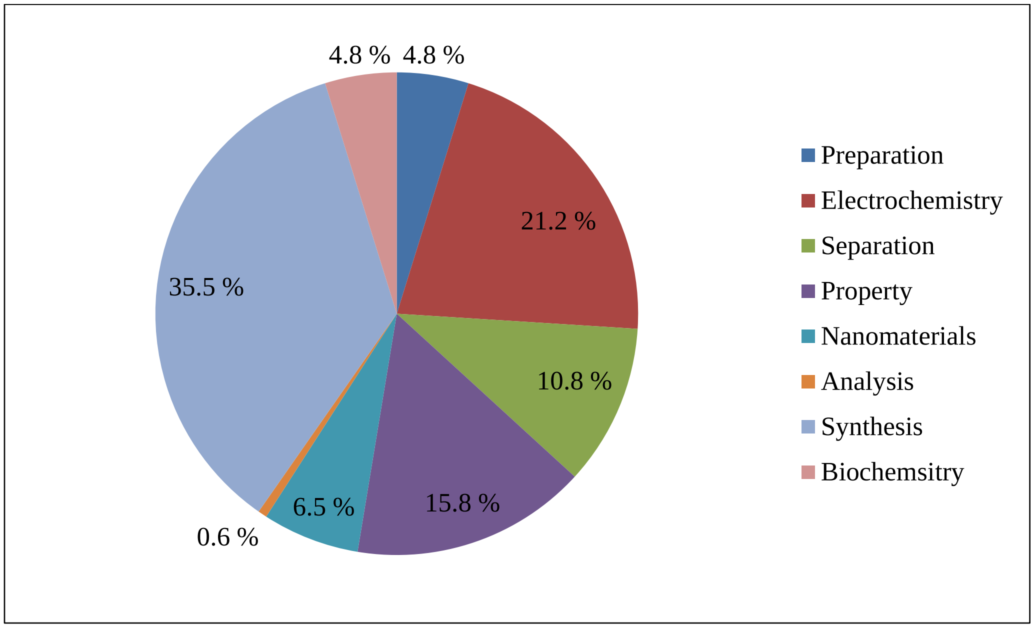

Figure 1.6. Percentage breakdown of references related to the DES research field among the total references assessed through 2016.

1.2.7 Applications

The applications of DESs are very promising. The literature reported the applicability of DESs as solvents in the bulk processing of metals[44-55], polymer synthesis[56], carbon-carbon nanotube composite preparation[57], biodiesel purification[43], drug solubilization[58], biological transformations[59-60], and CO_2 absorption[61-67]. DESs were also found to be viable solvents for the fabrication of novel metal surfaces and coatings[68-69] and thermochromic polyvinylidene fluoride composite film[70].

DESs have been attracting significant attention as an alternative and greener media for organic synthesis[71-73], materials synthesis and processing[74] and biotransformations[75-77]. The utility of DESs in more than fifty organic synthesis reactions is summarized in a recent review[78]. These solvents have been the choice for a number of enzyme-based biotransformation, because of their excellent properties for a wide variety of solutes, including enzymes and substrates[75-76].

DESs are introduced in many industrial applications as an alternative to ILs, such as the synthesis of zeolite analogs[79], nanoparticles[80], gold nanostars[81], extraction of aromatics from naphtha[82], removal of excess glycerol from biodiesel fuel[83], and electrochemical applications[14].

The recent applications of DESs in nanotechnology[84], as high-performance size exclusion chromatography packing materials[85], DES-modified cotton as a sorbent for solid-phase extraction[86], pretreatment for nanofibrillation of wood cellulose[87], dissolution of biological samples[88], biomass valorization[89], processing of leather[90], pulp fiber yarn[91], and killing bacteria[92] attracted the interest of the many researchers working in the field of green chemistry.

1.3 Lignocellulosic Biomass

The replacement of gasoline by biofuels produced from alternative sources is the primary goal of many countries across the globe[93]. Bioethanol produced from cellulose is the most investigated and explored among the second-generation biofuels[94]. Bioethanol provides a nontoxic, sustainable, biodegradable, and readily available source of biofuel. It is an ideal source of energy produced from lignocellulosic materials, which helps to drastically reduce the dependence on petroleum and the corresponding release of greenhouse gases[95].

Lignocellulosic materials are defined as plant-derived materials that contain both cellulose and lignin. The fundamental unit of lignocellulosic materials is the plant cell. Plant cell walls have three-dimensional polymeric composites comprised mainly of cellulose, hemicellulose, and lignin. Plant matter is made up of about 30 to 50 % cellulose, 20 to 40 % hemicellulose, 15 to 25 % lignin, and 5 to 25 % other extractives as shown in Figure 1.7[96].

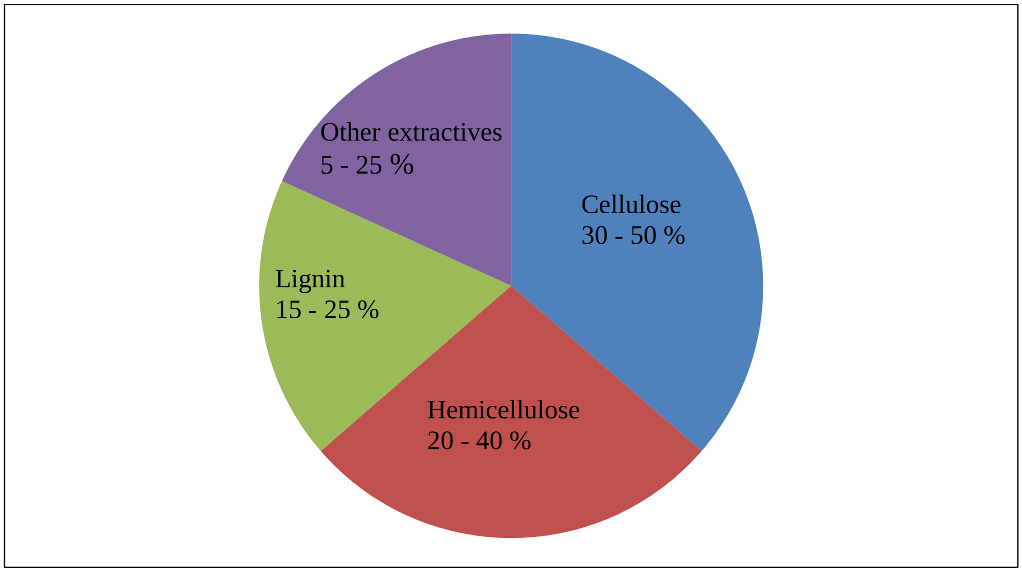

Figure 1.7. Lignocellulosic biomass components distribution.

Cellulose is the largest group of organic compounds in dry plant-cell matter. It is comprised of linear polymers of D-glucopyranose monomer units linked through β (1→4) linkages. Hemicellulose is the second largest group, made up of D-xylose, L-arabinose, 4-O-methylglucuronic acid, D-glucose, and D-galactose. Lignin, which makes up the third largest component, is made up of cross-linked phenylpropanoid subunits comprised of sinapyl, coniferyl, and p-coumaryl alcohols[97]. The complex arrangement of cellulose, hemicellulose, and lignin is shown in Figure 1.8.

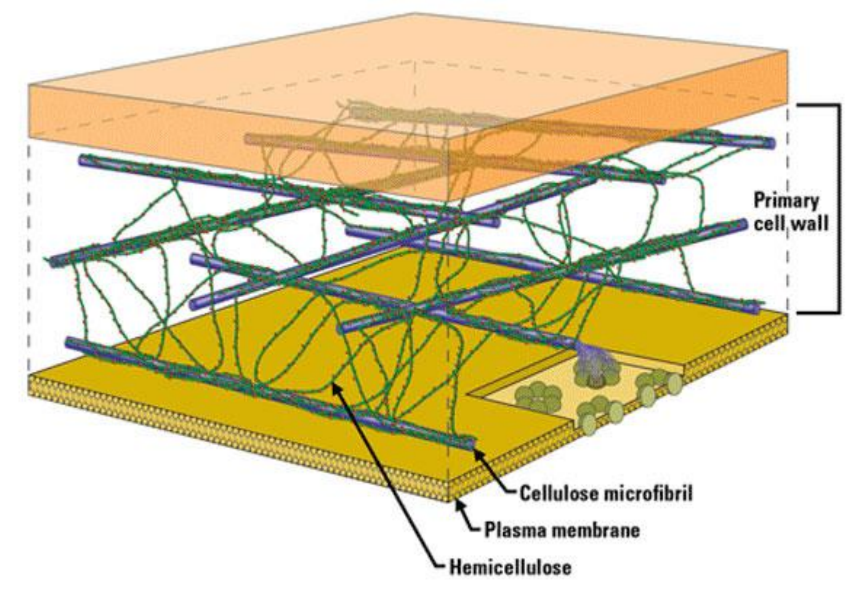

Figure 1.8. Simplified cell wall structure of lignocellulosic materials showing complex arrangement of cellulose, hemicellulose, and lignin [96].

The conversion of lignocellulosic biomass into biofuels typically includes three main steps. The first step is the pretreatment of lignocellulosic biomass to liberate cellulose and hemicellulose from their complex with lignin, the second step is the depolymerization of the carbohydrates to produce fermentable reducing sugars, and the last step is the fermentation of the sugars to ethanol or other products[98]. The present work is limited to pretreatment of lignocellulosic materials that is the fractionation of the biopolymer components.

1.3.1 Biomass Pretreatment

Biomass pretreatment is the crucial and limiting step in the production of biofuel from biomass. It is defined as the application of an external force to biomass to disrupt the hemicellulose and lignin components[99]. Biomass pretreatment, as shown in Figure 1.9, makes cellulose and hemicellulose available for ethanol production.

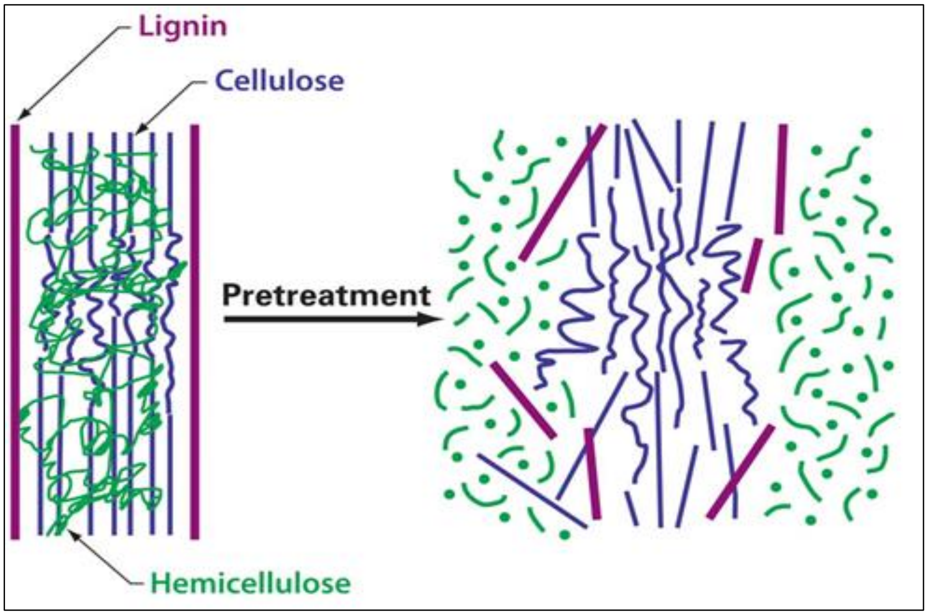

Figure 1.9. The effect of pretreatment on lignocellulosic biomass to liberate cellulose, and hemicellulose[96].

Various techniques, such as physical, chemical and biological approaches, are employed in pretreatment for the removal of hemicellulose and/or lignin from the biomass feedstock[100]. The chemical pretreatment techniques include organosolv processes, oxidative pretreatment, ozonolysis, acid hydrolysis, and alkaline hydrolysis, which involve the use of large quantities of molecular solvents or ILs[101].

The bioethanol produced from lignocellulosic materials is considered green energy, even though the process involves molecular solvents and ILs. The term green energy should be used carefully and only be applied if both the bioethanol and the process used to produce it are completely green. Here, we are proposing a new generation of novel green solvents called DESs made from biodegradable and nontoxic components, to replace molecular solvents and ILs for pretreatment of biomass, to make the process greener.

1.4 Justification of Study

Several researchers have considered DESs as the next-generation green solvents[29, 102-103]. These solvents have the potential as a green alternative to molecular solvents and ILs for the pretreatment of lignocellulosic biomass due to their advantages, including ease of preparation in high purity at low cost, nontoxicity, nonreactivity with water, biodegradability, and tunable-solvent properties that can be customized to a particular type of chemistry.

Synthesis of DESs from inexpensive, nontoxic and biodegradable components and investigating their physicochemical and thermal properties are vital to developing the sustainable processes to replace the toxic molecular solvents and ILs for pretreatment of lignocellulosic biomass to make the process greener.

The objectives of this research are;

1) Synthesizing DESs by various methods,
2) Physicochemical and thermal characterization, including pH, density, refractive index, surface tension, viscosity, octanol-water partition coefficient, flammability, freezing temperature, and decomposition temperature of synthesized DESs,
3) Determining miscibility and solvatochromic parameters, including polarity and Kamlet-Taft parameters of synthesized DESs,
4) Evaluating the toxicity of synthesized DESs, and
5) Exploring the applications of DESs in the pretreatment of lignocellulosic biomass to replace toxic molecular solvents and ILs.

CHAPTER 2

2 SYNTHESIS

2.1 Introduction

The preparation of molecular solvents and ILs involves a lot of synthetic and purification steps[8, 104-105]. These synthesis and purification steps can be complicated and increase the cost of the solvents[104]. In contrast, preparation of DESs requires only mixing their components with gentle warming[20] or dissolving the components in a suitable solvent and then evaporating the solvent[59]. The DESs formation depends only on intimate mixing at a molecular level. The purity of the starting materials determines the purity of the synthesized DESs and no further purification steps are required. Thus DESs can be synthesized from a number of inexpensive quaternary ammonium, phosphonium or sulfonium cation salts and HBDs with simple synthetic procedures, with no purification steps, to make these solvents less expensive, and their cost is similar to molecular solvents and about 20 % of comparable ILs[106].

DESs synthesis has not been as widely explored as molecular solvents and ILs. Thermal treatment and freeze-drying are the only two different methods have been reported so far for their synthesis[12, 60]. Exploring the freeze-drying synthesis of DESs led to the application of incorporating bacteria in DESs in its pure state with outstanding preservation of bacteria integrity and viability, and it opened up the interesting perspectives for the use of whole microorganisms in biocatalytic processes to synthesize fine chemicals[60]. This lead us to explore the possibility of different synthesis processes of DESs that might be useful to gain a clear insight into the feasible mechanisms of formation, intermolecular interactions, building principles and to explore their applications in various fields.

We have explored several feasible processes of DESs synthesis such as mixing (vortex), heating and mixing (thermal treatment, microwave irradiation, sonication, and shaking incubation) and solvent evaporation (freeze drying and rotary evaporation) by varying the components ChCl, AcChCl, U and G and their composition. Thermal treatment, microwave irradiation, sonication, vortex mixing, and shaking incubation processes for DESs synthesis might be helpful in developing one-step DESs synthesis and extraction applications. Freeze drying and rotary evaporation processes of DESs synthesis might be useful in recovery and recycling of DESs in various applications.

The DESs formation can be studied by freezing point (T_f) determination, Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopies. Freezing point depression (ΔT_f) is the characteristic feature of the DESs formation[107]. FTIR spectroscopy is an interesting tool to obtain hydrogen bonding information and presence of functional groups of DESs [108]. ^{1}H NMR spectroscopy can be used to study the hydrogen-bonding interactions and the formation of halide ion-hydrogen bond-donor supramolecular complexes, which are a characteristic of DESs[109].

2.2 Experimental

2.2.1 Materials

ChCl (99 %), AcChCl (≥ 99 %), U (99.5 %), G (certified ACS) and ethanol (200-proof) were purchased from Thermo-Fisher Scientific (Dubuque, IA). Deuterium oxide (D_2O) (99.8 atom % D) was purchased from Sigma-Aldrich (St Louis, MO). Dry ice was purchased from Hy-Vee (Brookings, SD). Ultrapure water was obtained from a Thermo-Fisher Scientific Barnstead E-Pure ultrapure water purifier system (Waltham, MA) set at 18.2 ΩM cm^{-1}. ChCl, AcChCl and U were recrystallized from absolute ethanol, filtered and dried under vacuum. G, D_2O, and dry ice were used as received.

2.2.2 Methods

2.2.2.1 Thermal Treatment

Sixteen different DESs were synthesized by adding the ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1. The components were heated at 80 ºC on top of a hot plate with constant stirring at 600 rpm until clear liquids were formed. The synthesis was carried out in a fume hood and the temperature was controlled with an oil bath.

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

Components	Mole ratio	Abbreviation
Choline chloride:Urea	1:2	DES01
Choline chloride:Glycerol	1:2	DES02
Acetylcholine chloride:Urea	1:2	DES03
Acetylcholine chloride:Glycerol	1:2	DES04
Choline chloride:Urea:Glycerol	1:1:1	DES05
Choline chloride:Urea:Glycerol	2:3:1	DES06
Choline chloride:Urea:Glycerol	2:1:3	DES07
Acetylcholine chloride:Urea:Glycerol	1:1:1	DES08
Acetylcholine chloride:Urea:Glycerol	2:3:1	DES09
Acetylcholine chloride:Urea:Glycerol	2:1:3	DES10
Choline chloride:Acetylcholine chloride:Urea	1:1:4	DES11
Choline chloride:Acetylcholine chloride:Urea	2:1:6	DES12
Choline chloride:Acetylcholine chloride:Urea	1:2:6	DES13
Choline chloride:Acetylcholine chloride:Glycerol	1:1:4	DES14
Choline chloride:Acetylcholine chloride:Glycerol	2:1:6	DES15
Choline chloride:Acetylcholine chloride:Glycerol	1:2:6	DES16

2.2.2.2 Microwave Irradiation

The sixteen different DESs were synthesized by adding the ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 and heating in a CEM MARS5 microwave reaction system (Matthews, NC) at 80 °C with constant stirring at medium speed for 30 minutes.

2.2.2.3 Shaking Incubation

The sixteen different DESs were synthesized by adding the ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 and continuously shaking them using VWR International 50001 shaker incubator (Radnor, PA) at a mixing rate of 400 rpm and 80 ºC until clear liquids were formed.

2.2.2.4 Sonication

The sixteen different DESs were synthesized by adding the ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 and sonicating using a Fisher Scientific FS110D ultrasonic bath sonicator (Hampton, NH) at 80 ºC bath temperature until clear liquids were formed.

2.2.2.5 Vortex Mixing

The sixteen different DESs were synthesized by adding the ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 and vortexing using Fisher Scientific 02215370 digital vortex mixer (Hampton, NH) until clear liquids were formed.

2.2.2.6 Freeze Drying

The sixteen different DESs were synthesized by freezing the aqueous solutions of ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 and subsequently freeze-dried using a Labconco 7750020 freeze dryer (Kansas City, MO) for 72 hours.

2.2.2.7 Rotary Evaporator

The sixteen different DESs were synthesized by evaporating the solvent from aqueous solutions of ChCl, AcChCl, U, and G in the mole ratios as specified in Table 2.1 under reduced pressure using a Buchi R-200 rotary evaporator (New Castle, DE) at 70 ºC for an hour.

2.2.2.8 Karl Fisher Titration

The water content of the synthesized DESs was measured using a Metrohm 831 Karl Fischer coulometric titrator (Herisau, Switzerland) and the measurement accuracy of Karl Fischer titrator was checked with Hydranal CMR water standard 1.00 mg g^{-1} (St. Louis, MO). The water content measurements were performed in triplicate.

2.2.2.9 Freezing Point Determination

The freezing point of all the synthesized DESs was determined by adding them into 20-mL scintillation vials equipped with thermocouples. Then, they were gradually cooled by immersing into a dry ice cooling bath. The temperature at which they start freezing recorded as their freezing point using a Vernier LabQuest 2 (Beaverton, OR). Freezing point measurements were carried out in triplicate and the average values are reported.

2.2.2.10 FTIR Spectroscopy

The FTIR spectra of the synthesized DESs and their individual components were recorded with a Nicolet 380 Thermo Fischer-Scientific FTIR spectrometer (Waltham, MA). The spectra were collected in the range of 400 to 4000 cm^{-1} at a resolution of 8 cm^{-1}. The spectra were processed using EZ OMNIC software.

2.2.2.11 ^{1}H NMR Spectroscopy

The ^{1}H NMR spectra of the synthesized DESs and their individual components were recorded with a Brucker 400 MHz spectrometer (Billerica, MA) equipped with a QNP 5 mm probe at 400 MHz and 22 °C. Deuterated water (D_2O) was used as a solvent. DESs and their components (25 mg each) were dissolved in 0.5 mL of D_2O. ^{1}H NMR parameters were set 90^o pulse angle, 2-s pulse delay, and 512 scans.

The synthesized DESs were allowed to cool to room temperature. Those solvents were then stored in screw-capped vials, inside a moisture-controlled desiccator with silica gel until further analysis to avoid the moisture contamination.

2.3 Results and Discussion

Sixteen different DESs were synthesized through various means such as mixing (vortex), heating and mixing (thermal treatment, microwave irradiation, sonication, and shaking incubation), and solvent evaporation (freeze drying and rotary evaporation) by varying the components ChCl, AcChCl, U and G and their composition as specified in Table 2.1. The DES01 to DES04 are binary eutectic systems composed of one QAS and one HBD. DES05 to DES10 are ternary eutectic systems composed of one QAS and two different HBDs and DES11 to DES16 are also ternary eutectic systems but composed of two different QASs and one HBD.

The different methods of synthesis took varying times for the formation of DESs. The DESs formation by solvent evaporation took longer time than mixing, and heating and mixing took the least amount of time. The combination of heating and mixing increase the interaction between the molecules of QASs and HBDs followed by the mixing alone and then evaporating the solvents off that signifies the formation of DESs occurs through the molecular level interaction between the QASs and HBDs. Thus, the key to the formation of DESs is the temperature and the initial distribution of starting compounds. The freeze-drying method of DESs synthesis took longer than any other method employed but has the advantage of avoidance of thermal degradation. A dramatic decrease in DESs synthesis reaction time from hours to minutes and the avoidance of thermal degradation with their method of synthesis will represent a step change in the production of DESs.

According to the twelve principles of green chemistry, the DESs synthesis reaction can be considered as green because it has 100 % reaction mass efficiency and there were no byproducts produced, that means that the purity of the DES is only dependent on the purity of the starting materials[3, 12, 20, 110].

The synthesized DESs were confirmed by their freezing point depression and analyzing their FTIR and ^{1}H NMR spectra for hydrogen bonding because freezing point depression and the presence hydrogen bonding are the two main characteristic features of the DESs formation[12, 17, 20].

2.3.1 Freezing Temperature (T_f)

The individual components ChCl, AcChCl, U, and G of the synthesized DESs melt at 302[33], 150[34], 133[35] and 18 °C[36] respectively. The measured freezing temperature (T_f) of the synthesized DESs by the various methods were lower than that of their individual components as presented in Table 2.2. The large depression in freezing temperature of DESs compared to their individual components confirms the formation of DESs. It is also important to note that the freezing temperatures are lower than room temperature, signifying that they are liquids at room temperature.

Table 2.2. Freezing temperature of DESs synthesized by thermal treatment, microwave irradiation, shaking incubation, sonication, vortex mixing, freeze drying, and rotary evaporation.

	Freezing temperature (ºC)						
DES	Thermal treatment	Microwave irradiation	Shaking incubation	Sonication	Vortex mixing	Freeze drying	Rotary evaporation
DES01	12.2	12.1	12.2	12.0	12.0	12.3	12.2
DES02	-40.4	-40.2	-40.2	-40.5	-40.2	-40.3	-40.0
DES03	19.9	19.4	19.7	19.4	19.5	19.6	19.3
DES04	-17.1	-16.9	-16.8	-17.0	-17.0	-17.1	-16.8
DES05	-34.1	-34.1	-34.3	-34.4	-34.5	-33.9	-33.9
DES06	-28.8	-28.2	-27.7	-27.9	-26.9	-26.8	-27.0
DES07	-40.8	-40.6	-40.2	-40.0	-39.8	-39.6	-39.8
DES08	-24.3	-24.0	-23.7	-23.6	-23.8	-23.8	-23.9
DES09	-18.5	-18.5	-17.9	-18.1	-18.2	-18.0	-17.8
DES10	-25.6	-25.3	-25.2	-25.4	-25.3	-24.6	-24.8
DES11	3.9	3.8	3.8	4.2	4.0	3.7	3.1
DES12	-5.2	-5.2	-4.9	-4.7	-4.7	-4.9	-4.2
DES13	8.8	9.1	9.3	8.7	8.7	8.9	9.0
DES14	-52.3	-51.3	-54.0	-53.8	-52.9	-54.4	-48.2
DES15	-57.6	-57.2	-57.8	-59.4	-59.0	-61.2	-53.7
DES16	-31.3	-31.0	-32.4	-32.9	-33.2	-30.0	-26.3

These results disclose freezing temperatures over the range from -61.2 to 19.9 °C. DES15 (ChCl:AcChCl:G (2:1:6)) had the lowest freezing temperature, -61.2 °C, while DES03 (AcChCl:U (1:2)) had the highest freezing temperature, 19.9 °C. The reported freezing temperatures of 12[111] and -40.0 °C[112] of DES01 and DES02 respectively agreed well with the measured values. The freezing temperature of the other DESs is reported for the first time, so no reference can be made to the literature.

The freezing point of DESs depends on the composition and components of QASs and HBDs. Binary DESs composed of ChCl and G had lower freezing temperature than corresponding eutectics of AcChCl and U, and ternary eutectic systems composed of two QASs (ChCl and AcChCl) and one HBD (G) had lower freezing temperatures than eutectics composed of one QAS (ChCl or AcChCl) and two HBDs (U and G) that can be explained in terms of strength of interaction between the components. The greater the interaction between the components of the mixture, the larger the freezing point depression[17]. Therefore we can conveniently conclude that binary DESs composed of ChCl and G had stronger interaction between their components than corresponding eutectics of AcChCl and U, and ternary eutectic systems composed of two QASs (ChCl and AcChCl) and one HBD (G) had stronger interaction than eutectics composed of one QAS (ChCl or AcChCl) and two HBDs (U and G).

The DESs synthesized through the various methods differs slightly in their freezing temperature, due to the difference in the amount of residual water, as shown in Table 2.3.

Table 2.3. Amount of water present in DESs synthesized by thermal treatment, microwave irradiation, shaking incubation, sonication, vortex mixing, freeze drying, and rotary evaporation.

	Water content (wt %)						
DES	Thermal treatment	Microwave irradiation	Shaking incubation	Sonication	Vortex mixing	Freeze drying	Rotary evaporation
DES01	0.957	0.954	0.942	0.935	0.938	0.930	1.607
DES02	0.912	0.910	0.918	0.910	0.908	0.905	1.473
DES03	0.962	0.967	0.959	0.954	0.938	0.934	1.767
DES04	0.940	0.940	0.938	0.931	0.932	0.930	1.386
DES05	0.939	0.936	0.932	0.930	0.931	0.930	1.507
DES06	0.989	0.985	0.977	0.986	0.977	0.958	1.600
DES07	0.931	0.929	0.927	0.927	0.928	0.930	1.499
DES08	0.952	0.950	0.948	0.948	0.942	0.920	1.570
DES09	0.995	0.985	0.975	0.972	0.978	0.960	1.682
DES10	0.975	0.972	0.973	0.973	0.970	0.952	1.633
DES11	1.214	1.196	1.182	1.180	1.172	0.982	1.949
DES12	1.128	1.118	1.098	1.090	1.093	0.980	2.014
DES13	1.420	1.342	1.380	1.296	1.260	0.987	2.322
DES14	1.009	1.000	1.003	1.100	1.107	0.971	1.890
DES15	1.004	0.997	0.994	0.992	0.994	0.982	1.732
DES16	1.134	1.360	1.290	1.294	1.190	0.970	1.838

It was impossible to synthesize the DESs with no water present even with the utmost care because of the highly hygroscopic nature of the QASs and HBDs involved in their synthesis. The results are shown in Table 2.3 clearly reveals that the quantity of water present in DESs varies with their methods of synthesis. Thermal treatment-, microwave irradiation-, shaking incubation-, sonication-, vortex mixing-, and freeze-dry-synthesized DESs did not vary noticeably in their amount of water present. Rotary evaporation-synthesized DESs had a higher amount of water present than other synthesis methods employed this may be due to rotary evaporation was not efficient enough to remove the water.

Combining the results from Tables 2.2 and 2.3 indicates that the freezing temperature of synthesized DESs depends on their method of synthesis to certain extent, due to the amount of residual water. The amount of water present in DESs composed of U varied to a greater extent than eutectics of G because U is highly hygroscopic. Therefore, the freezing temperature of DESs composed of U varied to a larger extent with the different methods of synthesis than DESs of G. The freezing temperature of DESs prepared by the rotary-evaporation is different than the DESs synthesized by other methods since the moisture content is greater with rotary-evaporation synthesis method. The freezing temperature of freeze-drying synthesized DESs is similar to various methods employed except rotary-evaporation that signifies the freeze-drying process was highly efficient at removing the water. Removal of water is a crucial step to obtain the DESs in their pure state. Otherwise, the components of DESs will be solvated by water molecules and do not form ion pairs. This is important because, depending on the amount of water present, water molecules can be solvated by DESs or the DESs are solvated by water. In the case of DESs molecules solvated by water, DESs no longer exists, but rather a simple dissolution of the individual components occurs. Our preliminary FTIR and ^{1}H NMR study (spectra not showed here) results confirmed the DESs characteristic halide ion-hydrogen-bond-donor supramolecular complexes up to 40 wt % water addition. Upon more than 40 wt % dilution, DESs became a simple solution of their individual counterparts and the halide ion-hydrogen-bond-donor supramolecular complex structure is lost. On the basis of our preliminary results, the DESs understudy with more than 40 wt % water is not a valid approach if one desires to preserve the halide ion-hydrogen-bond-donor supramolecular complexes characteristic of DESs.

2.3.2 FTIR Spectroscopy Analysis

FTIR spectroscopy is an attractive tool to obtain the hydrogen bonding information and to observe the functional groups to analyze and identify the structure of a material[113]. During the synthesis of DESs from their components undergo a number of changes. Thus, comparison of FTIR spectrum of DESs with their individual components provides useful information about possible interactions, structural changes and hydrogen bond formation in DESs. A number of changes between the FTIR spectra of the DESs and their individual components are clearly visible in Figures 2.1 to 2.4.

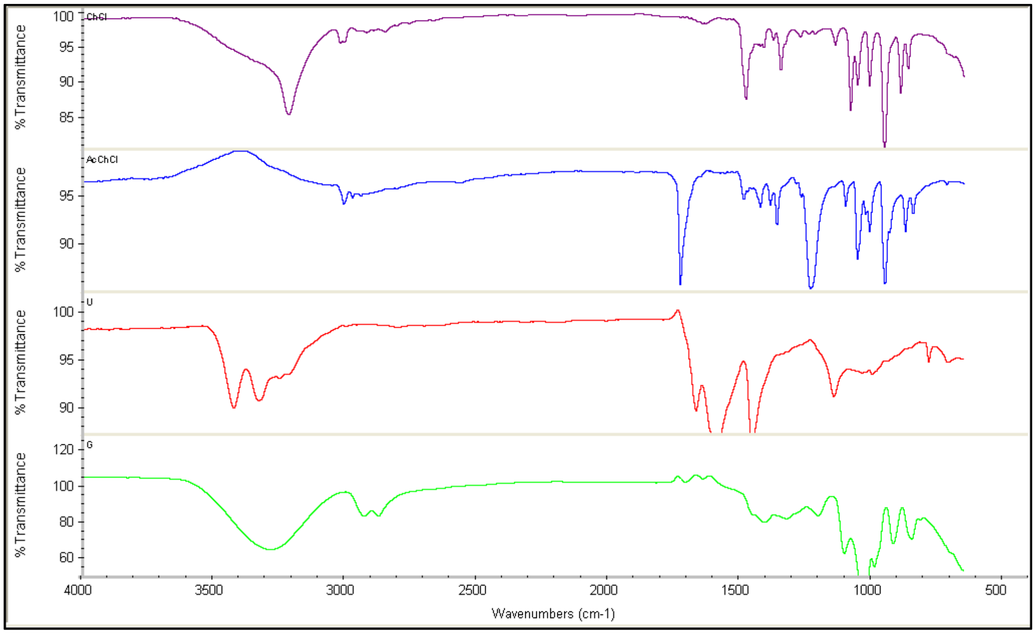

Figure 2.1. Overlaid FTIR spectra of DESs components ChCl, AcChCl, U, and G.

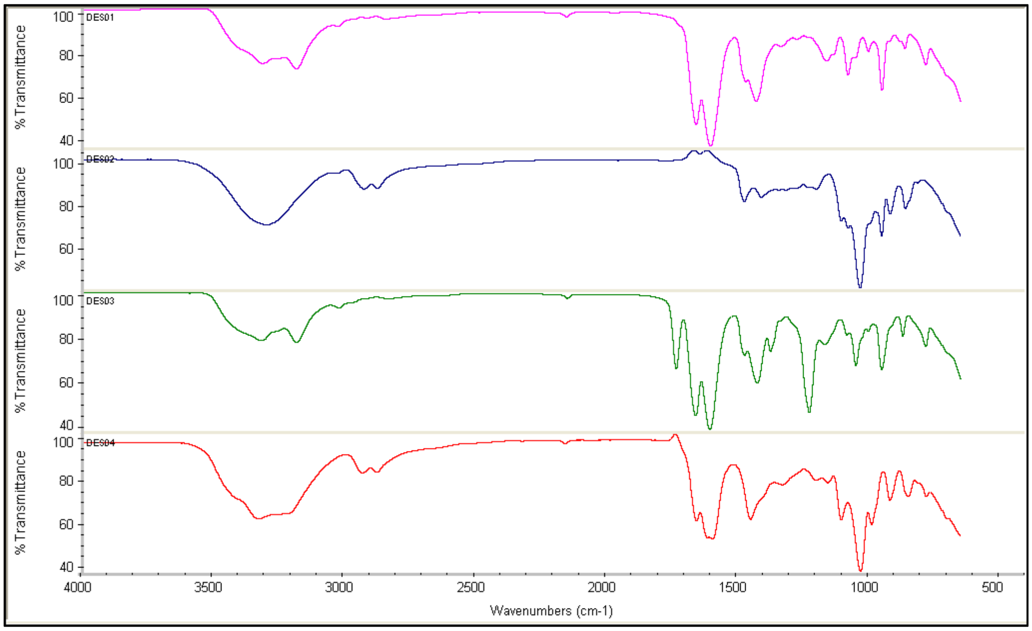

Figure 2.2. Overlaid FTIR spectra of thermal treatment synthesized DES01 to DES04.

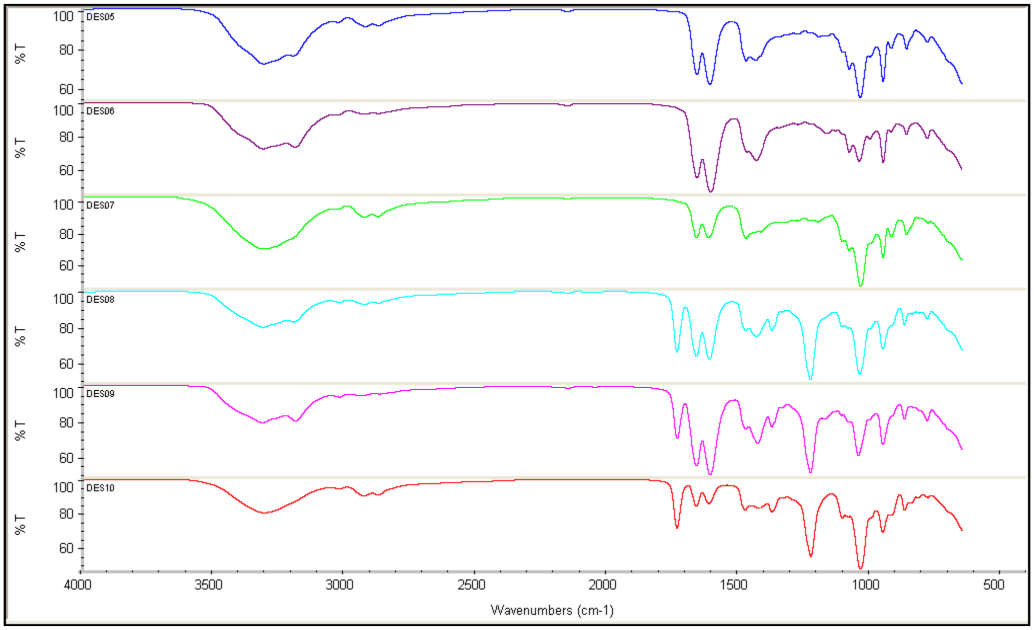

Figure 2.3. Overlaid FTIR spectra of thermal treatment synthesized DES05 to DES10.

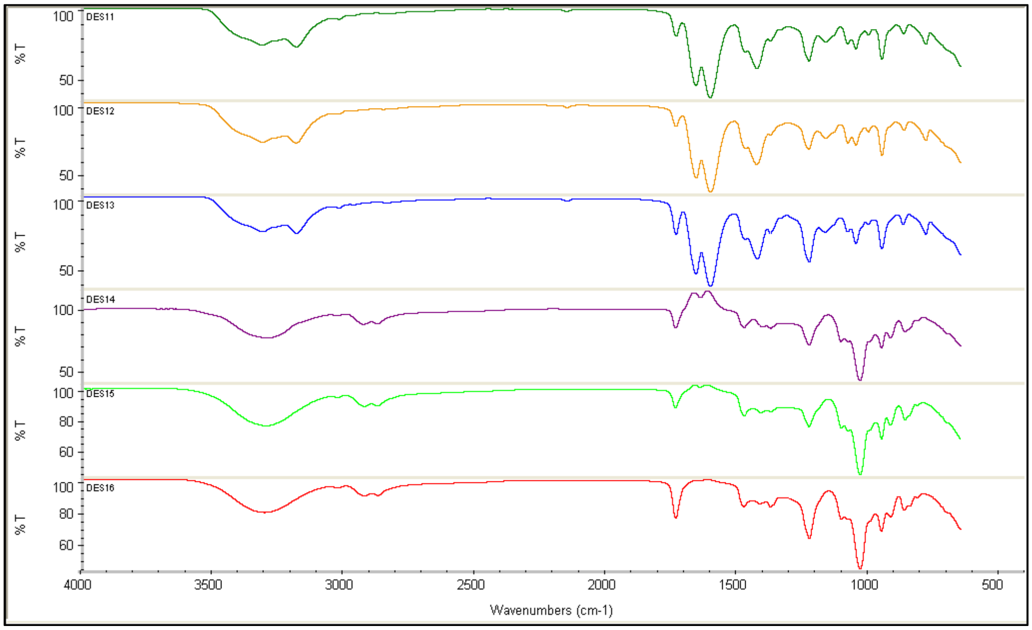

Figure 2.4. Overlaid FTIR spectra of thermal treatment synthesized DES11 to DES16.

The shifts in the representative peaks and band broadening of the involved bonds of DESs compared to individual components of ChCl ammonium ion at 1169 cm^{-1}, AcChCl ammonium ion at 1176 cm^{-1}, U amide at 3418 cm^{-1} and G hydroxyl 3318 cm^{-1} confirms the formation of hydrogen bond. The presence of a broad band in DESs at 3430 cm^{-1} and a band near 1620 cm^{-1} indicates the presence of water molecules in DESs.

Figures 2.2 to 2.4 shows very similar spectra for most of the DESs. This is due to the similar chemical structure of QASs, and the presence of water in all DESs gives them similar peaks in the same region. FTIR analysis of DESs functional groups was not reported often in literature[108, 114-124]. The shifts in peaks of DESs compared to their individual components and presence of water peak compliments the literature results.

2.3.3 ^{1}H NMR Spectroscopy Analysis

^{1}H NMR spectroscopy is also a useful technique to study the hydrogen-bonding interactions[125]. Very few NMR spectral studies of DESs are reported to confirm the presence of hydrogen bonds in eutectic systems[126-128]. The shifts in the resonance signal upfield in ^{1}H NMR signals of DESs compared to their individual components ChCl (δ 3.11, 3.44 and 3.98), AcChCl (δ 2.05, 3.13, 3.66 and 4.47), U (δ 5.78) and G (δ 3.44, 3.52, 3.55 and 3.66) signals are observed as shown in Figures 2.5 to 2.8 revealing the formation of halide ion-hydrogen bond-donor supramolecular complexes. The band at δ 4.70 is from solvent D_2O.

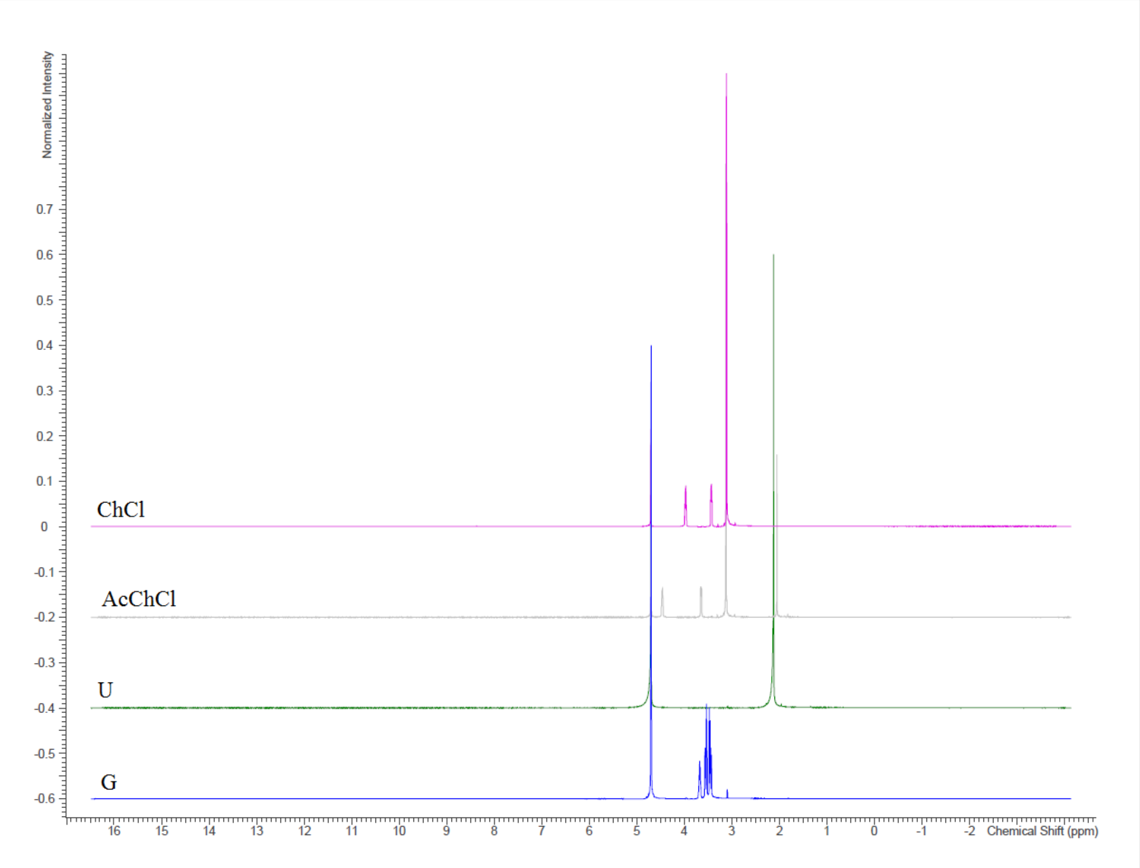

Figure 2.5. Overlaid ^{1}H NMR spectra of DESs components ChCl, AcChCl, U, and G.

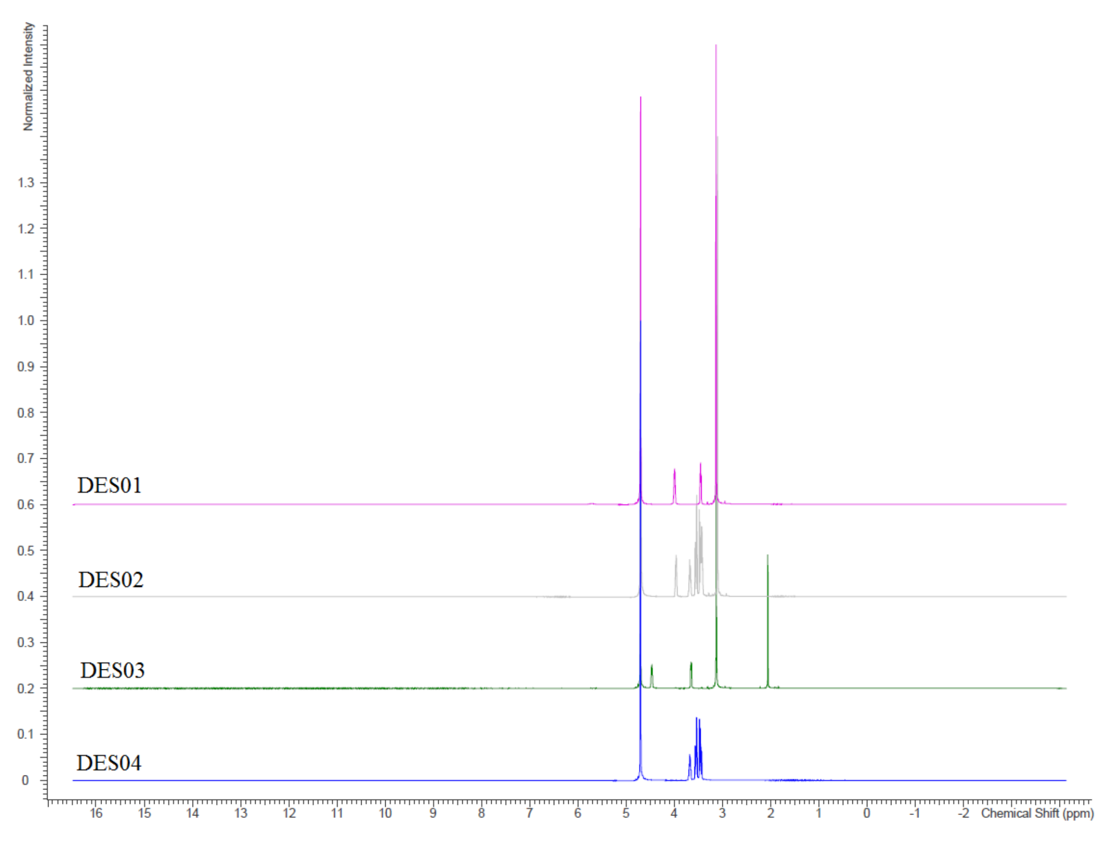

Figure 2.6. Overlaid ^{1}H NMR spectra of thermal treatment synthesized DES01 to DES04.

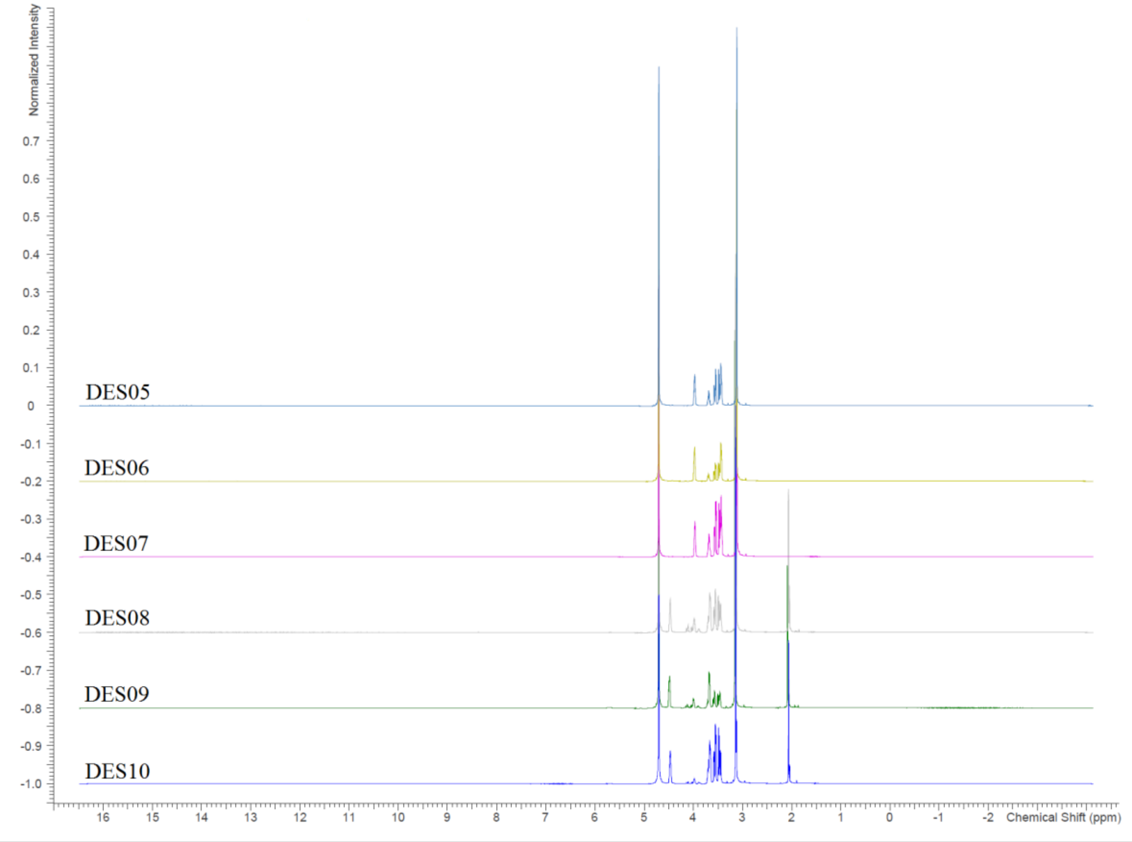

Figure 2.7. Overlaid ^{1}H NMR spectra of thermal treatment synthesized DES05 to DES10.

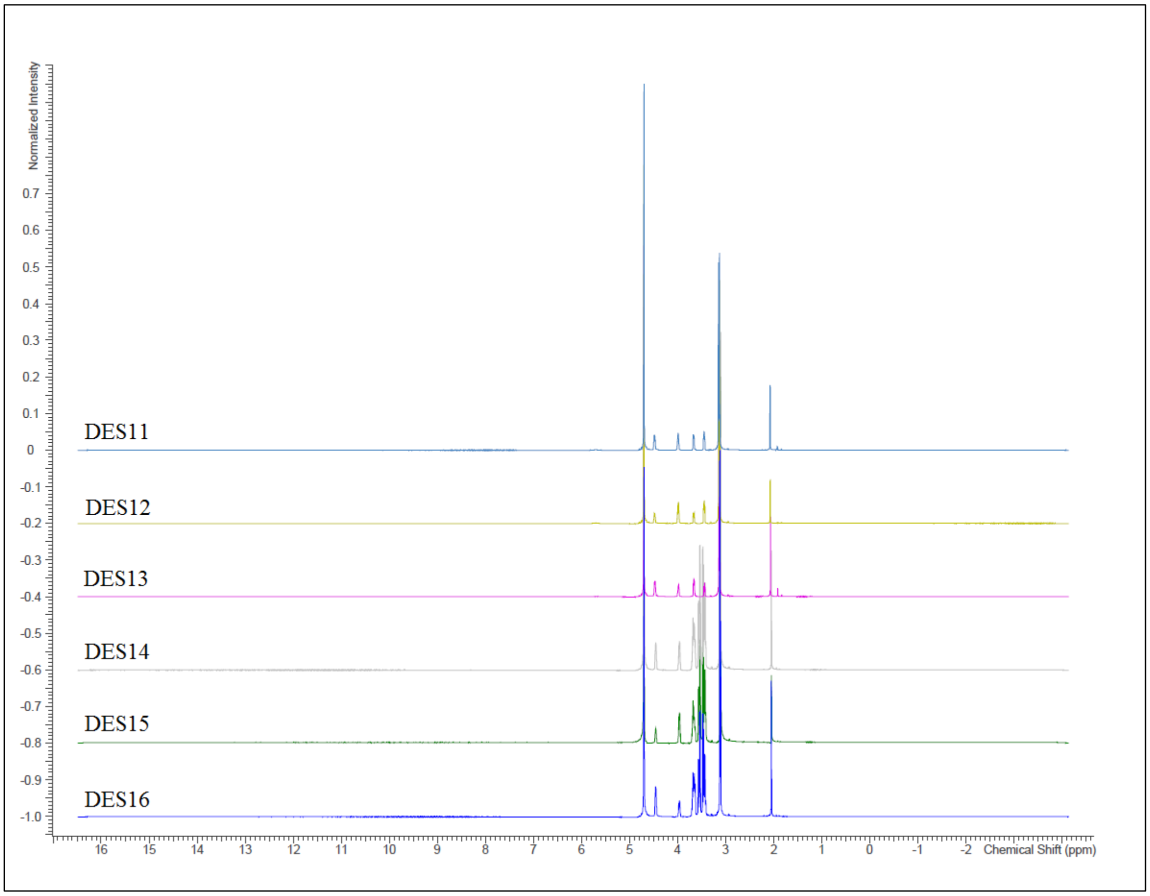

Figure 2.8. Overlaid ^{1}H NMR spectra of thermal treatment synthesized DES11 to DES16.

FTIR and ^{1}H NMR spectra of DESs prepared by different methods showed the same chemical profile for DESs with the same composition because there was little difference in moisture content. Therefore, FTIR and ^{1}H NMR spectra of DESs synthesized by different methods are not reported here, except the DESs synthesized by thermal treatment. FTIR and ^{1}H NMR spectra of DESs synthesized by thermal treatment explain the structural profile observed for all synthetic methods thermal treatment.

2.4 Conclusions

Sixteen different DESs composed of ChCl, AcChCl, U, and G were synthesized by various methods, and all the synthesis procedures required no subsequent purification steps. The different methods of synthesis took varying times for the formation of DESs. The freezing temperature of DESs was always found to be much lower than the melting point of their starting materials. The freezing point of DESs depends on the composition and components of QASs and HBDs. The DESs synthesized by different methods varied the amount of water present. The amount of water present had an effect on freezing point of DESs. The shifts in the representative peaks and band broadening of the involved bonds in the FTIR spectra and the shift in the resonance signal upfield in ^{1}H-NMR spectra confirmed the formation of eutectics and hydrogen bonds in DESs.

CHAPTER 3

3 CHARACTERIZATION

3.1 Introduction

The assessment of DESs as new generation solvents to replace molecular solvents and ILs for various applications requires knowledge about their physical, chemical and thermal properties. Regardless of the numerous possible applications of DESs and the advantages of their use, reports of the fundamental properties of these solvents are still rather scarce in the current literature. Few research works have dealt with measuring the properties of DESs[15, 20-21, 129-136].

Investigating the properties of novel solvents will increase the possibility of many future applications. For instance, having a knowledge of viscosity may help in the proper selection of a solvent and temperature that is suitable for a particular application which consequently saves material and energy. This lead us to objective of studying physical, chemical and thermal properties such as pH, density, refractive index (RI), surface tension, octanol-water partition coefficient (K_{OW}), viscosity, flammability, freezing temperature (T_f), and decomposition temperature (T_d) of synthesized novel DESs.

pH defines the acidity or basicity of the substances. It is an important solvent property that influences extractions, reactions and corrosion-related design aspects.

Density measurements are essential to design chemical materials and processes that involve fluid mechanics and mass transfer calculations. Refractive index (RI) is a useful physical property that can be used to check purity and characterize new substances due to its strong association with the electrical and magnetic permeability of a substance. Therefore the density and RI provides important information on the purity of the DESs and the molecular interactions in these solvents[12, 13].

Surface tension is a measure of cohesive forces in a liquid on its surface so measuring this property is a measure of intrinsic energy between liquid molecules. It is an important physical property that is crucial for a number of applications such as wettability, capability of mass transfer for liquid-liquid or gas-liquid extraction, or in multi-phasic homogeneous catalysis[137].

The octanol-water partition coefficient is defined as the ratio of the concentration of a substance in *n*-octanol and water at equilibrium at a specified temperature[138]. It is an important property that measures the hydrophilic and lipophilic nature of the substances. K_{OW} is important in the proper selection of solvents for extractions[139]. It is also an important indicator of the environmental fate of the substance[140].

Viscosity is a measure of resistance to flow. It is an important property that affects the applications of solvents, especially for dissolution[141] and extraction[142]. Viscosity measurements are essential for equipment design and fluid flow calculations. Additionally, studying the variation of viscosity with temperature is important to reduce energy requirements for processing DESs.

The Occupational Safety and Health Administration (OSHA) defines a flammable substance as any substance having a flash point at or above 37.8 °C[143]. It is the ability of a substance to burn or ignite, causing fire or combustion. Flammability information helps in proper handling and storage of substances and their applications at elevated temperatures.

Freezing temperature is defined as the temperature at which a liquid turns into a solid and decomposition temperature is the temperature at which the substance chemically decomposes. Freezing and decomposition temperature data is important to optimize the operating temperatures of processes and in exploring applications as a function of temperature[141, 144].

We have synthesized sixteen different DESs by altering the components ChCl, AcChCl, U and G and their molar ratio to study the effect of the components and composition on physical, chemical and thermal properties of the selected DESs. Out of the sixteen DESs, four of them are binary and the rest are ternary, providing an opportunity to understand the properties of binary versus ternary eutectic systems. Among the twelve ternary eutectic systems, six of them are composed of one QAS and two different HBDs and another six of them are composed of two different QASs and one HBD. It is also noteworthy to mention that there is not much work reported in the literature related to ternary eutectic systems[145-148].

3.2 Experimental

3.2.1 Materials

ChCl (99 %), AcChCl (≥ 99 %), U (99.5 %), G (certified ACS), ethanol (200-proof), acetone (≥ 99.5 %) and *n*-octanol (99 %) were purchased from Thermo-Fischer Scientific (Dubuque, IA). Ultrapure water was obtained from a Thermo-Fisher Scientific Barnstead E-Pure ultrapure water purification system (Waltham, MA) set at 18.2 ΩM

cm^{-1}. ChCl, AcChCl, and U were recrystallized from absolute ethanol, filtered and dried under vacuum. Glycerol, ethanol, acetone and *n*-octanol were used as received.

3.2.2 Methods

3.2.2.1 Synthesis

Sixteen different DESs were synthesized as per the procedure described in the section 2.2.2.1 (thermal treatment). All of the synthesized solvents were kept in well-sealed vials after preparation to avoid humidity effects that may affect the physical, chemical and thermal properties of DESs.

3.2.2.2 Water Content

The amount of water present in the synthesized DESs was measured using Karl Fisher titration, as described in section 2.2.2.8.

3.2.2.3 pH

The pH of the synthesized DESs was measured using a Mettler Toledo FEP20 pH meter (Columbus, OH) as a function of temperature from 25 to 50 ºC for every 5 ºC interval. The temperature was controlled using an oil-bath. The pH meter was calibrated using standard pH 4, 7 and 10 buffers. The average uncertainty in measurements was estimated to be ± 0.05.

3.2.2.4 Density

The density of the synthesized DESs was determined by gravimetric analysis. After calibrating a 1.00 mL pipet to dispense 1.00 g mL^{-1} of water, that pipette was used to transfer 1.00 mL of each DES to determine the mass of that volume of liquid. Each measurement was repeated ten times and the average value is reported. All measurements were carried out at room temperature (25 ± 1 °C).

3.2.2.5 Refractive Index

Refractive indices of the synthesized DESs were measured using a Bausch & Lomb Abbe-3L refractometer (Rochester, NY). Deionized water was used for calibration before each experiment. The average uncertainty of the measurements was estimated to be ± 0.001. All measurements were carried out at room temperature (25 ± 1 °C).

3.2.2.6 Surface Tension

Surface tension of the synthesized DESs was measured using a Kruss K100 tensiometer (Hamburg, Germany) by the Du Nouy ring method[149]. The platinum-iridium ring was cleaned by flaming, and the glassware was rinsed consecutively with acetone and distilled water before beginning of each measurement. Tensiometer calibration was determined by measuring the surface tension of pure water. The standard uncertainties of the surface tension values were within the range of ± 0.2 dynes cm^{-1}. Surface tension measurements were carried out in triplicate at room temperature (25 ± 1 °C).

3.2.2.7 Octanol-Water Partition Coefficient

The octanol-water partition coefficient (K_{OW}) of the synthesized DESs was determined by the shake-flask method[150]. A known concentration of DES was placed in an *n*-octanol-water system and allowed to equilibrate, and then both aqueous and *n*-octanol phases were separated through a separatory funnel. Concentration in the aqueous phase was then determined by removing the water by freeze drying. Based on a simple mass balance, the concentration in the *n*-octanol phase was determined, and the K_{OW} was calculated as the ratio of the concentration in the *n*-octanol phase to the concentration in the aqueous phase, as in equation 3.1. All measurements were carried out in triplicates at room temperature (25 ± 1 °C).

$$K_{OW} = \frac{[DES]_O}{[DES]_W} \quad \text{……………………………………………………..............} \textbf{Equation 3.1.}$$

Where,

K_{OW} = Octanol-water partition coefficient of DES,

$[DES]_O$ = Concentration of DES in *n*-octanol phase at equilibrium, and

$[DES]_W$ = Concentration of DES in aqueous phase at equilibrium.

3.2.2.8 Viscosity

The viscosity of the synthesized DESs was measured as a function of temperature from 25 to 50 ºC using a Brookfield DV-III Ultra Rheometer (Toronto, Canada). The rheometer was calibrated by a zero-calibration method. The variation in the temperature was achieved by employing an external water bath and Brookfield TC-502 circulator (Toronto, Canada). The standard uncertainty in the viscosity and temperature measurements were ± 0.0005 Pa.s and ± 0.01 ºC respectively.

3.2.2.9 Flammability

Flammability tests of the synthesized DESs and their individual components were carried out under the fume hood by placing a pea-size amount of solid or five drops of liquid in an evaporating dish on an iron ring. An ignited splint was applied to the substance to observe any combustion.

3.2.2.10 Freezing Temperature

The freezing temperature of the synthesized DESs and G was measured as described in section 2.2.2.9.

3.2.2.11 Decomposition Temperature

The decomposition temperature of the synthesized DESs and their individual components was determined by thermogravimetric analysis (TGA) measurements using a Seiko 220 TG/DTA (Tokyo, Japan) instrument with an aluminum reference pan. 35 to 50 mg each of DESs and their individual components were placed into an open aluminum pan and heated from room temperature up to 575 ºC at a heating rate of 10 ºC min^{-1} under a nitrogen atmosphere.

3.3 Results and Discussion

3.3.1 Synthesis and Water Content

Sixteen different DESs were synthesized by varying the components (ChCl, AcChCl, U, and G) and their composition and abbreviated as DES01 to DES16, presented in Table 3.1, to study the effect of the components and their composition on various physical, chemical, and thermal properties. The amount of water present in DESs was measured prior to determining their properties because the water present may affect their physical, chemical and thermal properties. DESs synthesis was performed under the fume hood with utmost care to minimize the amount of water present in the DESs. The measured of water present in DESs was less than 1.5 % (wt %) as presented in Table 3.1. The presence of water in all of the synthesized DESs provided evidence that they were hygroscopic in nature due to their hygroscopic components.

Table 3.1. Components, molar composition, abbreviation and water content of DESs.

Components	Mole ratio	Abbrev -iation	Water content (wt %)
Choline chloride:Urea	1:2	DES01	0.957 ± 0.027
Choline chloride:Glycerol	1:2	DES02	0.912 ± 0.051
Acetylcholine chloride:Urea	1:2	DES03	0.962 ± 0.029
Acetylcholine chloride:Glycerol	1:2	DES04	0.940 ± 0.043
Choline chloride:Urea:Glycerol	1:1:1	DES05	0.939 ± 0.045
Choline chloride:Urea:Glycerol	2:3:1	DES06	0.989 ± 0.049
Choline chloride:Urea:Glycerol	2:1:3	DES07	0.931 ± 0.050
Acetylcholine chloride:Urea:Glycerol	1:1:1	DES08	0.952 ± 0.042
Acetylcholine chloride:Urea:Glycerol	2:3:1	DES09	0.995 ± 0.036
Acetylcholine chloride:Urea:Glycerol	2:1:3	DES10	0.975 ± 0.043
Choline chloride:Acetylcholine chloride:Urea	1:1:4	DES11	1.214 ± 0.041
Choline chloride:Acetylcholine chloride:Urea	2:1:6	DES12	1.128 ± 0.037
Choline chloride:Acetylcholine chloride:Urea	1:2:6	DES13	1.420 ± 0.052
Choline chloride:Acetylcholine chloride:Glycerol	1:1:4	DES14	1.009 ± 0.039
Choline chloride:Acetylcholine chloride:Glycerol	2:1:6	DES15	1.004 ± 0.041
Choline chloride:Acetylcholine chloride:Glycerol	1:2:6	DES16	1.173 ± 0.039

The amount of water present in DESs composed of U was higher than the corresponding DESs of G due to the highly hygroscopic nature of U compared to the G. It is also important to note that the ternary eutectic solvents composed of two QASs and one HBD have higher water present than the ternary eutectic solvents composed of one QAS and two HBDs.

3.3.2 pH

pH of the synthesized DESs at room temperature ranges from 5.26 to 9.44 as depicted in below Figure 3.1 and Table 9.1 of appendix indicates the acidic, neutral and basic nature of the DESs under study.

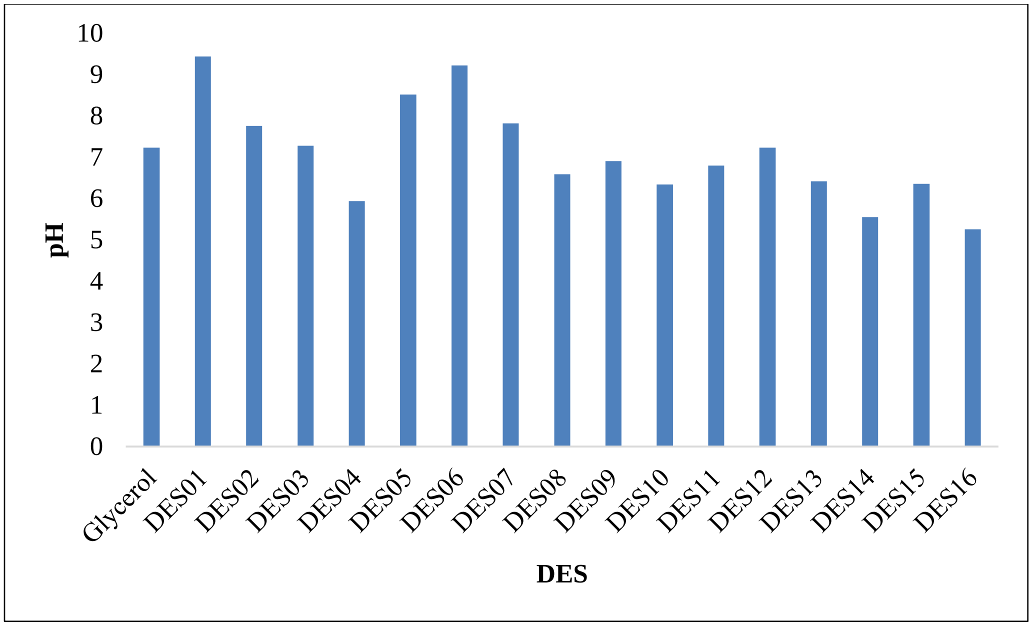

Figure 3.1. pH of glycerol and DESs at room temperature.

The results clearly show that the pH depends on the chemical nature of both the components (QASs and HBDs). The DESs composed of ChCl and U has higher pH values than the corresponding DESs composed of AcChCl and G. Glycerol measured pH of 7.23, falling in the range of literature reported pH values of 6.9 to 7.7[151]. The addition of ChCl to G did not vary the pH of G to a greater extent, whereas the addition of AcChCl significantly decreased pH.

The measured pH of 9.44 and 7.76 of DES01 and DES02 respectively close enough with their literature values of 10.39 and 7.54[152-153] respectively. pH of the rest of DESs under study is obtained for the first time. Hence no comparison with the literature made. pH of DESs is comparable to most of the ILs [154-155].

pH of DESs and G were also studied as a function of temperature from 25 to 50 ºC for every 5 ºC. pH values slightly decreased with increasing temperature but not significantly, as shown in Figure 3.2. The increase in temperature increases the kinetic energy of DES molecules, weakening the intermolecular hydrogen bonding strength. Hence, the collisions between these molecules lead to an equilibrium shift of the components and the dissociation of more H^+ in the system and the reduction in pH with temperature.

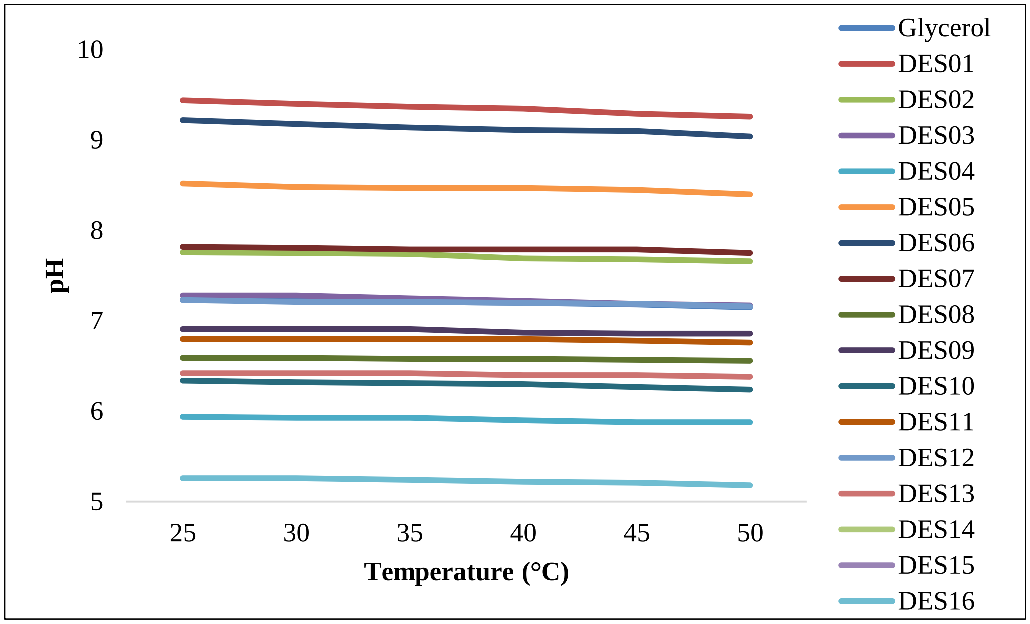

Figure 3.2. pH of glycerol and DESs as a function of temperature from 25 to 50 ºC.

The effect of temperature on pH was greatest for basic (DES01, DES02, DES03, DES05, DES06, DES07, and DES12) and minimal and acidic (DES04, DES08, DES09, DES10, DES11, DES13, DES14, DES15, and DES16) DESs. The effect of temperature on pH of DESs is obtained for the first time, so no comparison with the literature is made.

3.3.3 Density

The density of ChCl, AcChCl, U, and G collected from the literature are 1.10[156], 1.20[157], 1.33[158] and 1.26[159] g mL^{-1} respectively. The densities of the synthesized DESs were experimentally determined and the results are presented in below Figure 3.3 and Table 9.1 of appendix. The results clearly show that the densities of DESs depends on both QASs and HBDs.

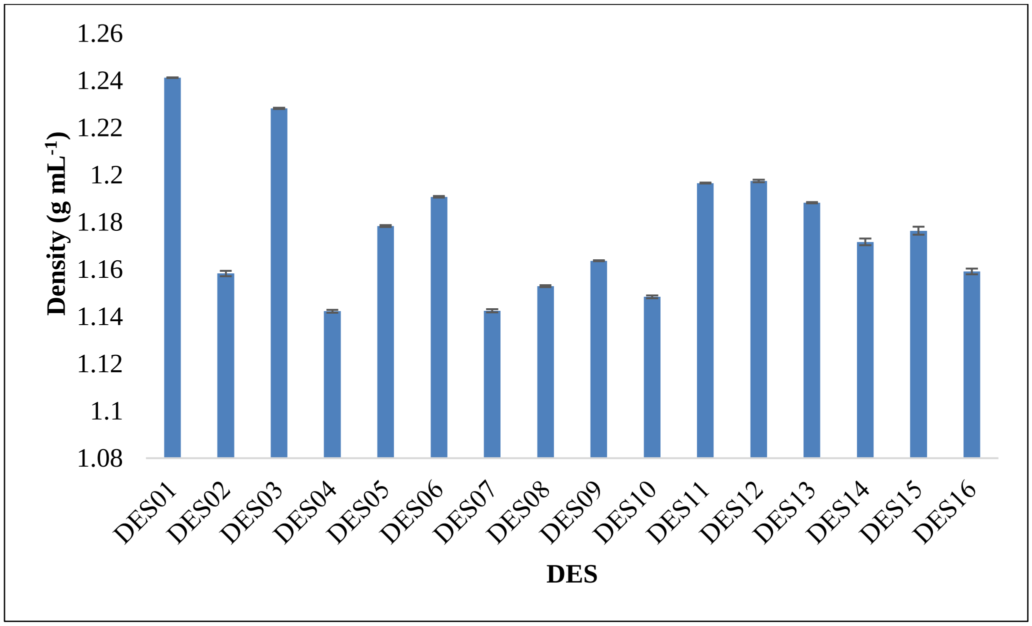

Figure 3.3. Densities of DESs determined gravimetrically at room temperature.

Urea and G density decreased significantly with the addition of QASs (ChCl and AcChCl) due to the lower density of ChCl and AcChCl. DESs composed of ChCl and U have higher density compared to the AcChCl and G eutectics. ChCl based eutectics having slightly higher density than the corresponding eutectics of AcChCl even though the individual density of ChCl lesser than AcChCl. The reason could be different molecular organization or packing of the DESs which in turn is related to the strength of hydrogen bonds in DESs.

The measured density of 1.2413 ± 0.001 and 1.1583 ± 0.0011 of DES01 and DES02 respectively slightly deviated from their literature reported densities of 1.25[127, 160] and 1.18[161] and predicted values of 1.2220[162] and 1.1920[163] respectively and the reasons could be the difference in the amount of water present. Even the literature densities of DES01 and DES02 were not in good agreement across the literature and they differed from each other because most of them did not consider or report the water present in the DESs[15, 161, 164-165].

It is also noteworthy to mention that all of the DESs under study exhibited comparable densities to that reported in the literature for other DESs[127, 160, 165-169] and imidazolium-based ILs[170-173], and higher than water and common organic solvents[174].

3.3.4 Refractive Index

The refractive indices of G and DESs ranged from 1.4824 to 1.5069, as shown in below Figure 3.4 and Table 9.1 of appendix. The addition of QASs decreased the RI of G. The results clearly show that RI values largely depends more on the HBDs than QASs because of the higher molar concentration of HBD. RI values decreased noticeably with the change of HBDs from U to G but with the change of QASs from ChCl to AcChCl decreased slightly. Measured RI values of 1.4607, 1.5069 and 1.4867 of G, DES01 and DES02, respectively correlates well (R^2 = 0.9969) with the literature their reported values of 1.4700[175], 1.5040[20] and 1.4868[161].

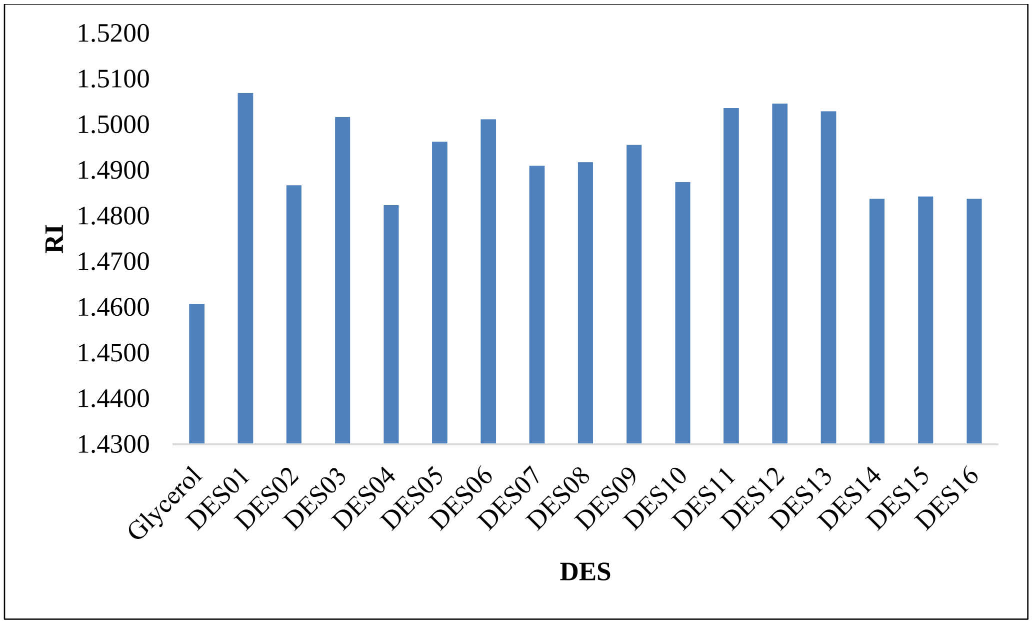

Figure 3.4. Refractive indices of glycerol and DESs at room temperature.

Refractive indices of the DESs are comparable to the literature for other deep eutectic systems[161, 167, 176], imidazolium ILs[172], and higher than water [177] and most of the common organic solvents[178-179]. RI is related to the electronic polarizability of the medium and hence results indicate that the DESs are more polarizable than G, water, common organic solvents and similar in polarizability to imidazolium ILs.

Refractive index is linearly proportional to the density of materials with a density less than 1.0 g mL^{-1}[178]. A plot is constructed to establish a relationship between the measured refractive indices versus densities of DESs as shown in Figure 3.5. An equation of line (y = 0.2804x + 1.1639) and R^2 (0.8839) derived from the graph clearly shows that the measured densities and refractive indices of DESs are not linearly proportional to each other as shown in Figure 3.5. This can be explained in terms of higher densities of DESs (>1.0 g mL^{-1}) because the linearity disappears for materials with a density greater than 1 g mL^{-1}. Closely looking at the measured densities and refractive indices of DESs, the results clearly shows that the density is still the primary determinant of the RI and follows a similar trend as shown in Table 9.1 of appendix.

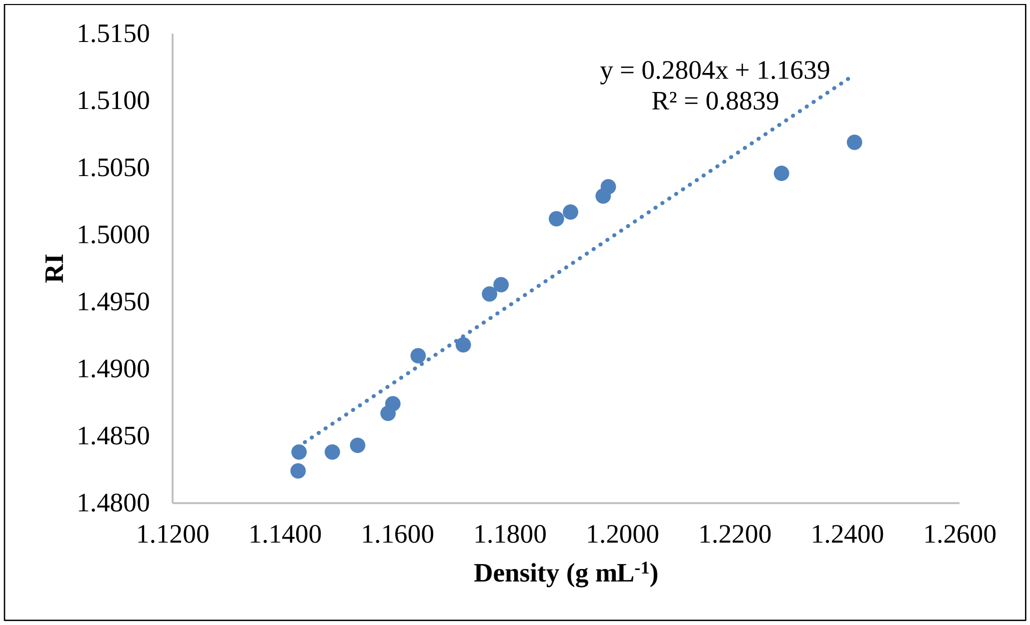

Figure 3.5. A plot of refractive indices versus density of DESs at room temperature.

3.3.5 Surface Tension

Surface tension of G and the DESs ranged from 57.10 to 78.1 dynes cm^{-1}, as shown in below Figure 3.6 and Table 9.1 of appendix. DESs surface tension values varied with their components and composition. Binary DESs composed of AcChCl and G has higher surface tension values compared to the corresponding eutectics of ChCl and U. Ternary eutectics composed of two QASs and one HBD have higher surface tension among the DESs under study.

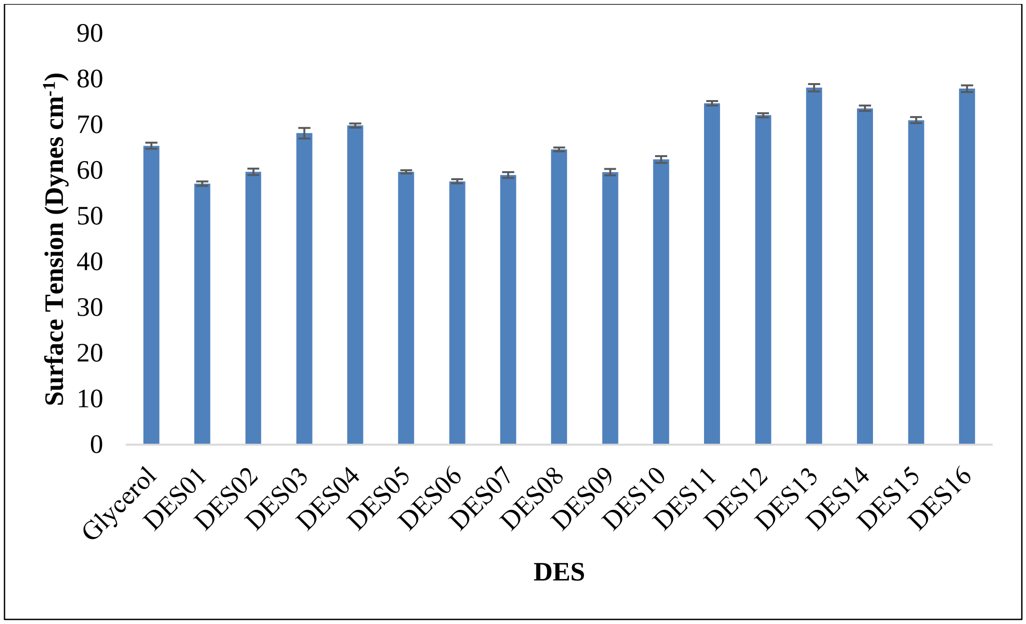

Figure 3.6. Surface tension of glycerol and DESs at room temperature.

Very few studies have been reported for the surface tension of DESs[12, 180-181]. DES01 and DES02 surface tension values of 57.10 and 59.70 dynes cm^{-1} were slightly higher than the literature values of 55.8 and 57.24 dynes cm^{-1} and the possible reason could be the difference in water present.

DESs surface tension values are higher than most common organic solvents[182] but are comparable to the imidazolium-based ILs[183]. The high surface tension values of DESs are indicative of the strong interactions between the ions and molecules at the liquid surface. DES011, DES13, DES14, and DES16 have higher surface tension values than water, 72.80 dyne cm^{-1}, while the rest of the DESs under study have lower surface tension than water[182].

3.3.6 Octanol-Water Partition Coefficient

K_{OW} is an extremely important quantity because it describes the lipophilicity or hydrophilicity of a compound and can be correlated to the bioaccumulation[184]. Measured K_{OW} values of DESs ranged from 0.0050 to 0.0481, shown in below Figure 3.7 and Table 9.1 of appendix. The DESs under study can be concluded as hydrophilic because of their very small K_{OW} values.

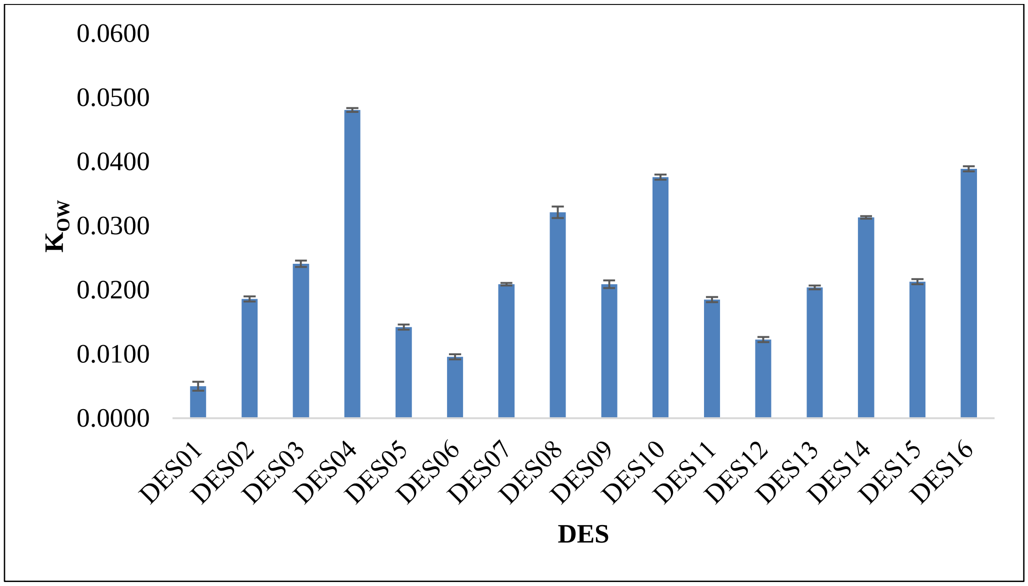

Figure 3.7. Octanol-water partition coefficient of DESs, determined by shake-flask method.

K_{OW} values of DESs under study varied with the components and their composition. DESs composed of AcChCl and G have higher K_{OW} values compared to the eutectics of ChCl and U, which can be explained by the higher solubility of ChCl (650 g L^{-1})[156] and U (1079 g L^{-1})[185] compared to AcChCl (100 g L^{-1})[157] and G (> 500 g L^{-1})[186] in water. The addition of QASs (ChCl and AcChCl) decreased the K_{OW} of G

(0.245[185]). The decrease in K_{OW} of G was higher with the addition of ChCl compared to AcChCl and is explained with the solubility of ChCl compared to AcChCl.

K_{OW} of DESs were lower than the lipophilic[187] and most of the hydrophilic solvents[187] but are comparable to imidazolium ILs[188]. Thus DESs will not accumulate or concentrate in the environment because of their extreme low K_{OW} values.

3.3.7 Viscosity

Viscosities measured for DESs range from 0.254 to 3.827 Pa.s at 25 ºC, shown in below Figure 3.8 and Table 9.1 of appendix. The high viscosity of DESs can be explained by the "hole theory"[160, 165]. It states the presence of an extensive hydrogen bonding network between the DESs components results in a lower mobility of free species within the DESs. The larger ion size and very small void volume of DESs, and other forces such as electrostatic or van der Waals interactions, may contribute to the high viscosity of DESs.

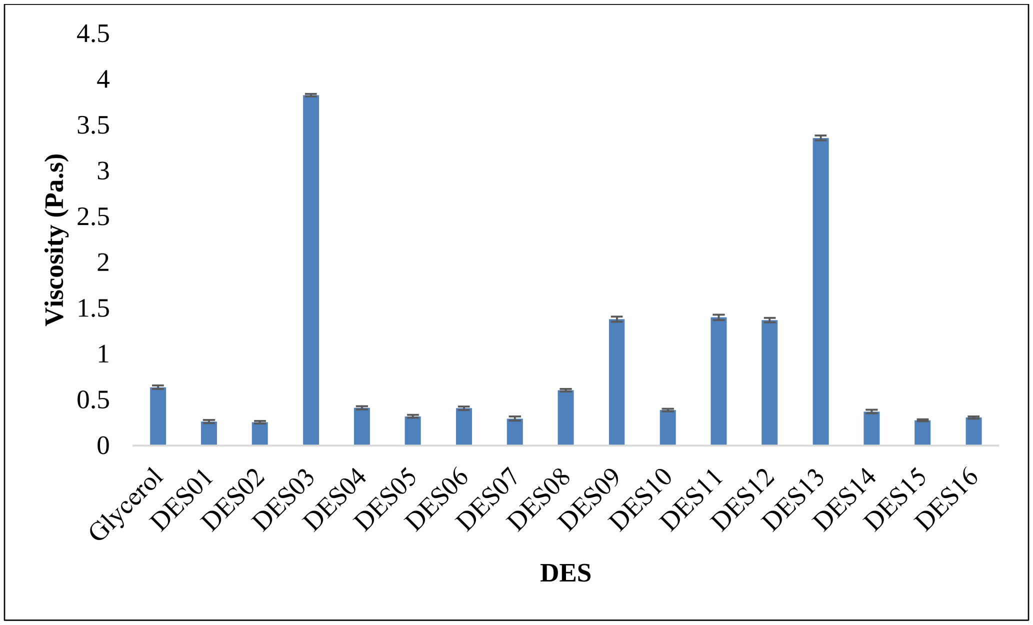

Figure 3.8. Viscosity of glycerol and DESs at 25 ºC.

Pure G was a viscous liquid. Comparison of G viscosity with DES02 and DES04 eutectic systems shows that the addition of 33 mole % ChCl and AcChCl salts to G, decreased its viscosity significantly. This is supported by the density measurements, which decreased as ChCl and AcChCl are added. The decrease in the density of DESs might cause an increase in free volume that results in a decrease in viscosity. DESs composed of ChCl and G have lower viscosities than comparable eutectics of AcChCl and U. Viscosity of DESs depends on QASs and HBS but to the larger extent on HBDs. The viscosity of DESs at 25 ºC was much higher than the viscosity of water (0.001 Pa.s[189]), common organic solvents[190], and typical for imidazolium ILs[173].

DESs viscosity was also studied as a function of temperature from 25 to 50 ºC because it is necessary to reduce energy requirements for processing these fluids at higher temperatures. As the temperature increases, viscosity decreased, as shown in Figure 3.9 and similar behaviour was reported in literaturature for various other DESs[191].

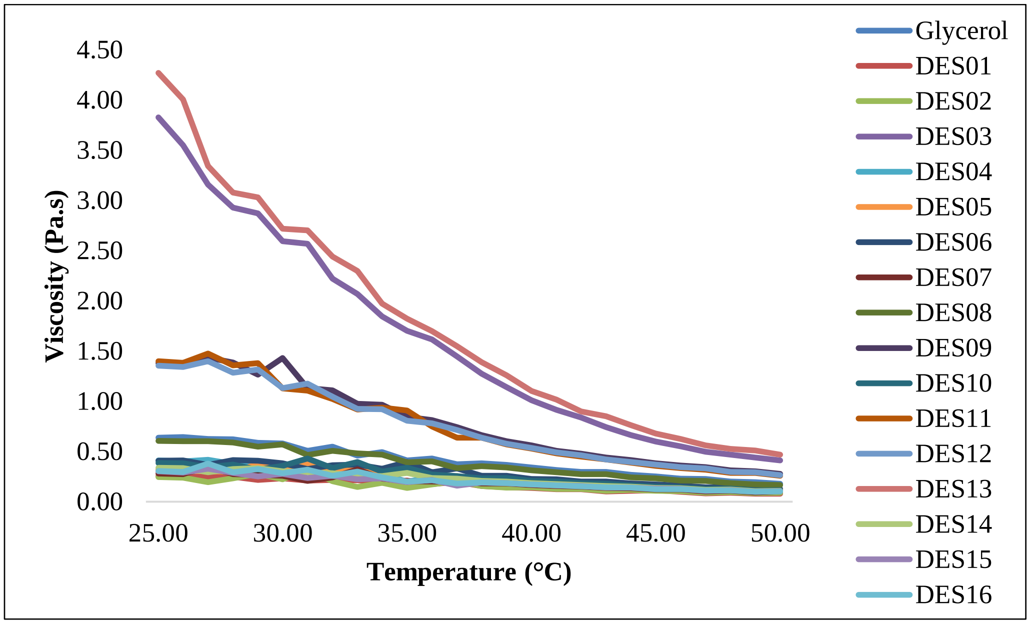

Figure 3.9. Viscosity of glycerol and DESs as a function of temperature from 25 to 50 ºC.

As temperature increases, viscosity decreased due to the weakening of van der Waals and hydrogen bond interactions attributed to the molecules obtaining enough kinetic energy to overcome the intermolecular forces and enable free movement. DESs viscosity as a function of temperature revealed that these mixtures exhibit a Newtonian behavior at high temperature, because high temperatures increase the mobility of the DES molecules that ultimately increases their fluidity. On the other hand, at the lower temperature the molecular mobility is less and consequently viscosity increases. At conditions close to ambient conditions, there was a clear reduction in the viscosity as a function of the shear rate that indicates some shear thinning behavior. Thus special precautions must be considered when using these fluids at conditions close to room temperature.

3.3.8 Flammability

The DESs and their individual components tested were nonflammable, which can be explained by the high flash point of individual components of eutectic solvents[32]. The non-flammable nature of DESs can be very helpful during transportation and storage of these solvents and developing applications at high temperatures. The nonflammable nature of DESs is comparable to water and ILs and in contrast to most organic solvents[6]. Nonflammable nature of DESs makes their storage convenient and easier.

3.3.9 Freezing Temperature

The freezing point of the eutectics was measured with a temperature probe by gradual cooling in dry ice. The freezing temperature (T_f) of the DESs ranged from -57.6 to 19.9 ºC, shown in Table 3.2. The T_f measured for DES01 and DES02, 12.2 and -40.4 ºC, agrees with the literature values of 12[20] and -40 ºC[112]. The T_f of the other DESs under study obtained for the first time, hence there was no reference to the literature made.

Table 3.2. Freezing temperature and freezing point difference of DESs determined by gradual cooling in dry ice.

DES	Freezing temperature (T_f) (ºC)	Freezing point difference (ΔT_f) (ºC)
DES01	12.2	120.8
DES02	-40.4	58.2
DES03	19.9	113.1
DES04	-17.1	34.9
DES05	-34.1	51.9
DES06	-28.8	46.6
DES07	-40.8	58.6
DES08	-24.3	42.1
DES09	-18.5	138.0
DES10	-25.6	158.6
DES11	3.9	129.1
DES12	-5.2	138.2
DES13	8.8	124.2
DES14	-52.3	70.1
DES15	-57.6	75.4
DES16	-31.3	49.1

The results in Table 3.2 clearly show that the T_f of DESs depends on the components (QASs and HBDs) and their composition. DESs composed of ChCl, G have lower freezing point, than the corresponding eutectics of AcChCl and U. Abbott et al.

reported that the freezing point depression of DESs depends on the lattice energies of the individual components and the interaction between the anion of QAS and the hydrogen bond donating group of HBD. They concluded that the compounds with greater ability to form hydrogen bonds display an enhanced freezing point depression when QASs and HBDs are mixed[17]. Thus we can conclude that the DESs composed of ChCl and G have stronger hydrogen bonds than the corresponding eutectics with AcChCl and U.

The individual components ChCl, AcChCl, U, and G melt (T_m) at 302[33], 150[34], 133[35], and 17.8 °C[36] respectively. Freezing point difference (ΔT_f) calculated by subtracting the DESs measured freezing temperature from the melting temperature (T_m) of lowest melting individual component[192-193]. The eutectic freezing point of DESs (T_f) and the melting temperature (T_m) of lowest melting individual component differed, suggesting that interactions existed between QASs and HBDs.

All of the synthesized DESs were liquids at room temperature. DESs existence as liquids near room temperature conditions will be helpful to explore these solvents for different applications. The freezing temperature of the DESs is comparable or lower than the literature reported other eutectic systems[12, 20, 112, 191, 194], higher than most of the common organic solvents[190], and comparable to imidazolium ILs[171].

3.3.10 Decomposition Temperature

Thermal stabilities of DESs and their individual components were quantified by measuring their decomposition temperature (T_d) using thermogravimetric analysis (TGA). T_d denotes the temperature at which 10 % of the initial mass had been lost during the TGA scanning experiment. The decomposition temperatures of the DESs under study range from 185.903 to 208. 218 °C, shown in Table 3.3.

Table 3.3. Thermogravimetrically determined decomposition temperature of DESs and their components.

DES	Decomposition Temperature (T_d) (°C)
ChCl	296.247
AcChCl	220.656
U	183.236
G	179.457
DES01	185.903
DES02	199.541
DES03	208.218
DES04	202.194
DES05	206.356
DES06	207.962
DES07	202.396
DES08	201.182
DES09	204.119
DES10	194.857
DES11	206.505
DES12	200.008
DES13	206.533
DES14	202.453
DES15	204.801
DES16	201.732

Decomposition temperatures for these DESs were lower than their QASs but higher than their HBD components as shown in Table 3.3. Binary eutectics composed of AcChCl have higher T_d than corresponding eutectics of ChCl even though ChCl decomposes at a higher temperature than AcChCl, implying that the thermal profile of DESs are entirely different than that of the individual components. T_d of ternary eutectic systems didn’t vary significantly with the composition, as shown in Table 3.3.

The thermograms of DESs shown in Figure 3.11 to 3.13 reveals that eutectic mixtures were stable up to about 200 °C, and there was a small mass loss on the TGA curve near 100 °C that resulted from the evaporation of water. Thermograms and T_d of ChCl, U, G, DES01, and DES02 well correlate with their literature thermal behavior[77, 195]. Thermograms of AcChCl and other DESs are obtained for the first time, so no reference is made to the literature.

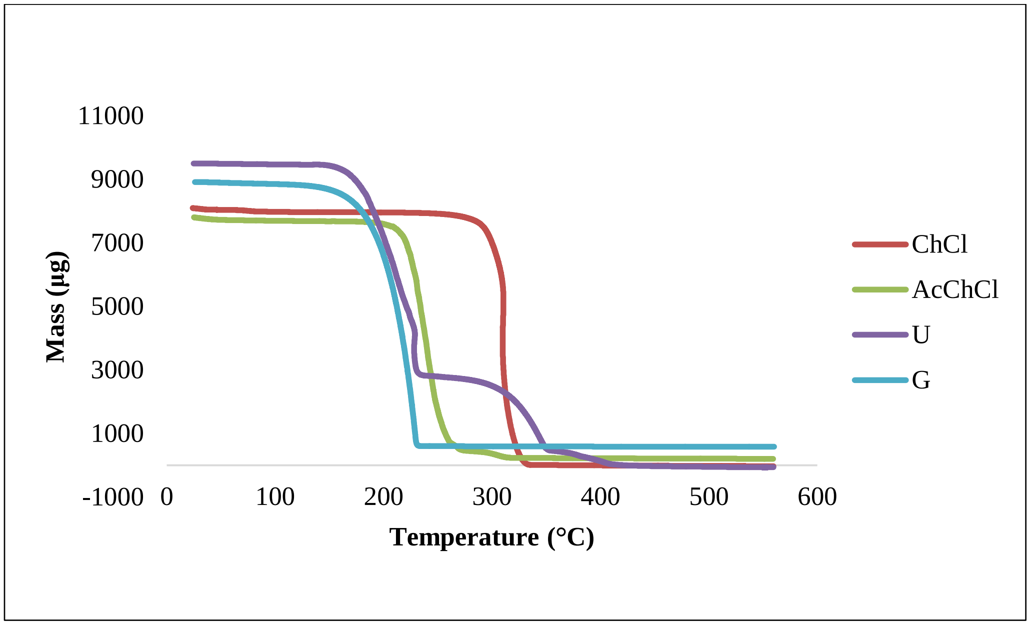

Figure 3.10. Thermogravimetric analysis thermographs of DESs components ChCl, AcChCl, U, and G.

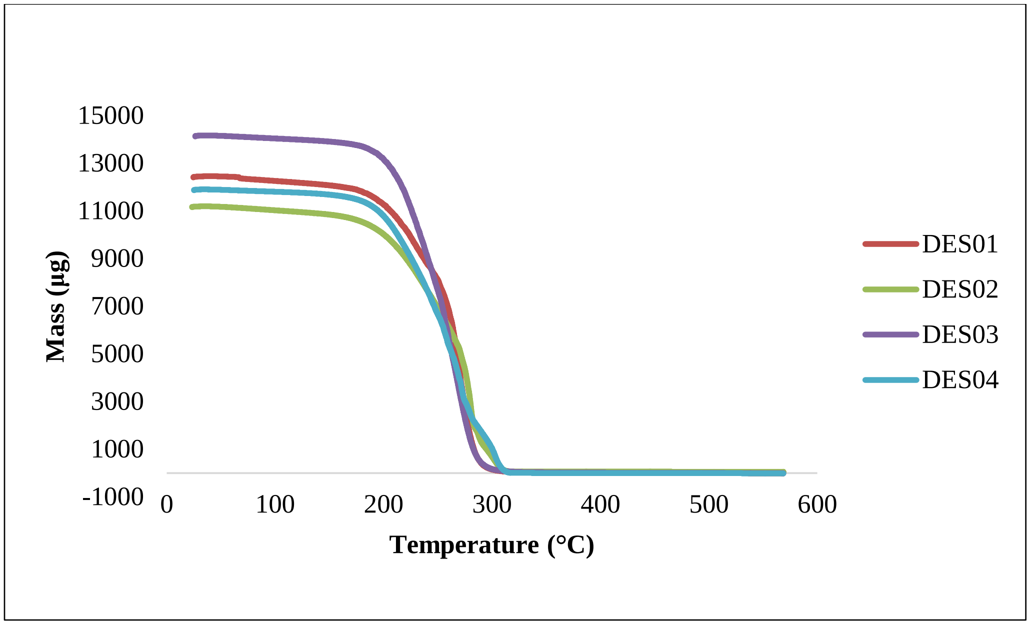

Figure 3.11. Thermogravimetric analysis thermographs of DES01 to DES04.

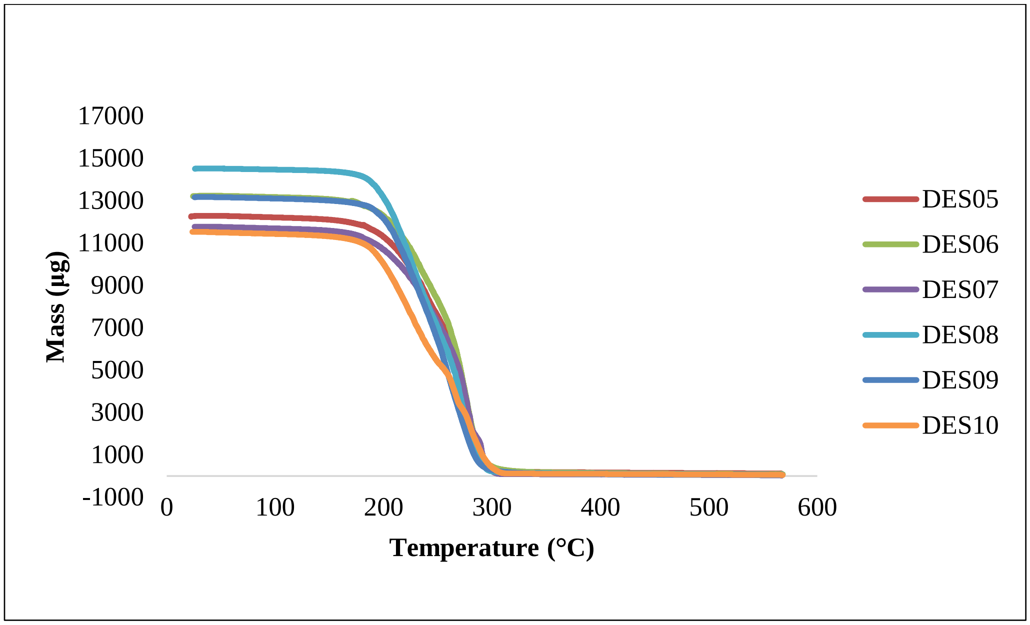

Figure 3.12. Thermogravetric analysis thermographs of DES05 to DES10.

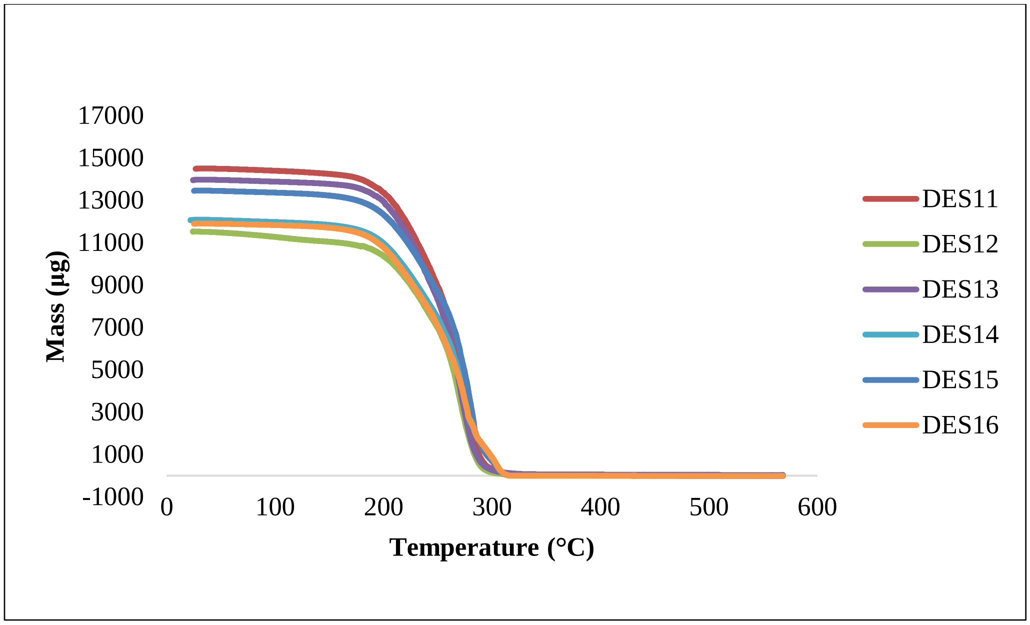

Figure 3.13. Thermogravimetric analysis thermographs of DES11 to DES16.

Freezing (T_f) and thermal decomposition (T_d) temperatures are very important to set the feasible operating range. Higher the difference between T_f and T_d, wider the operating range. Operating range (173.70 to 262.40 °C) of the DESs studied is higher than common organic solvents, comparable to the literature reported other eutectic systems and lower than imidazolium ILs. This can be explained by comparison of magnitude of difference between T_f and T_d of the DESs studied with other systems. The difference between T_f and T_d of the DESs studied is higher than common organic solvents (methanol (162 °C), isopropyl alcohol (171.6 °C) and hexane (164 °C))[190], comparable to the natural eutectic systems (malic acid: β-alanine: water (1:1:3) (234.88 °C), proline: malic acid: water (1:1:3) (217.29 °C) and lactic acid: glucose: water (5:1:3) (212.06 °C))[126] and lower than imidazolium ILs (EMIPF$_6$ (370 °C), EMIIm (555 °C) and EMIBeti (435 °C))[196].

3.4 Conclusions

A comprehensive investigation of physical, chemical and thermal properties of DESs and their individual component were carried out. The DESs components and their composition have a significant effect on most of the properties studied. Among the QASs and HBDs, the latter had the higher effect on most of the measured properties of DESs. A good agreement with each other measured properties of DESs was observed. The measured properties of DES01 and DES02 eutectic systems deviated slightly from the literature values due to the difference in the purity, the presence of water, and method of analysis. The properties of other DESs were obtained for the first time so no reference to the literature made. Most of the measured properties of DESs understudy was comparable to the literature reported other eutectic systems and imidazolium ILs and different than common organic solvents.

CHAPTER 4

4 MISCIBILITY AND SOLVATOCHROMIC PARAMETERS

4.1 Introduction

Growing environmental awareness across the globe has increased interest in green solvents[197]. DESs have been shown tremendous promise as alternative green solvents to molecular solvents and ILs[17]. DESs have the advantage of being nonflammable with negligible vapor pressure compared to molecular solvents and share many characteristics of ILs, but eutectics are cheaper to make, nontoxic and biodegradable[15]. Even though DESs are environmentally benign and touted as one of the most versatile alternative media with enormous potential in a variety of applications, the detailed knowledge of their miscibility and polarity is lacking.

It is important to know the miscibility of newly developed DESs in a range of common polar and nonpolar solvents to develop chemical processes involving synthesis, extraction, and separations. DESs are expected to exhibit unusual solvent properties due to the presence of hydrogen bonding. Therefore it is interesting and important to know the miscibility of these eutectic solvents in a range of common polar and nonpolar solvents.

Solvent polarity is an important property that plays a major role in the proper selection of greener alternative solvents. Understanding the polarity of eutectic solvents is essential to use them as green alternatives to molecular solvents and ILs in various applications. The knowledge of DESs polarity will help to understand solvent effects on chemical processes that will ultimately enhance their utilization in new applications.

Thus, determination of DESs miscibility and solvatochromic parameters and comparison with common molecular solvents and ILs is essential to develop new applications and find alternatives for solvents of concern.

Solvent polarity is a term commonly used in the context of the capacity of a solvent for solvating charged or neutral (dipolar or apolar) species[198]. Solvents are broadly divided into three main categories of nonpolar solvents (e.g. hydrocarbons), polar protic solvents (e.g. water and alcohols), and polar aprotic solvents (e.g. acetone and dimethyl sulfoxide), according to their behavior as a medium[199].

Solvent polarity cannot be quantitatively described by a single physical solvent parameter such as dielectric constant or dipole moment due to many different interactions such as hydrogen bonding, π-interactions or van der Waals forces [200]. ILs or DESs are even more complicated because both cations and anions can have their distinct interactions[201]. Most probes allow examination of only one major type of interaction with a solvent, so a range of different probes is required to get a clear insight of the strength of different types of solute-solvent interaction. Thus the solvatochromic behavior of DESs will be estimated collectively from polarity (E_T(NR) and E_T^N) and Kamlet-Taft parameters (hydrogen-bond acidity (α), hydrogen-bond basicity (β) and dipolarity/polarizability (π*)) through measuring the solvatochromic responses of several ultraviolet-visible (UV-Vis) absorbance probes[202-204].

In the present work we have used three widely used Kamlet-Taft dyes, Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline, to determine solvatochromic parameters. Their structures are presented in Figure 4.1[205]. The ability of a chemical substance to change color in a variety of solvent polarities is termed as solvatochromism[206]. The change in color is due to changes in the electronic absorption and emission spectra of the chemical substance and is related to the solute-solvent intermolecular attractions.

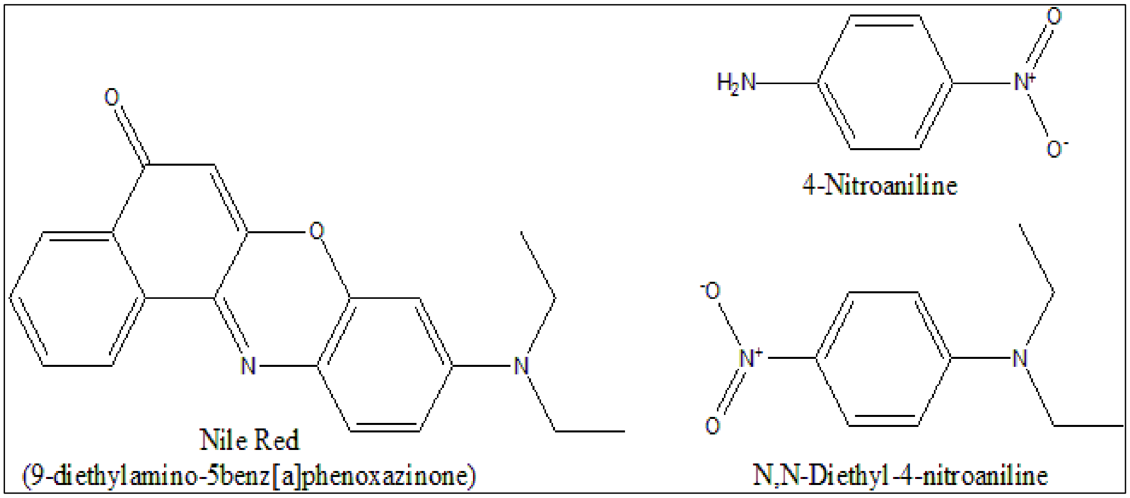

Figure 4.1. Chemical structures of Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes used in solvatochromic measurements.

4.2 Experimental

4.2.1 Materials

ChCl (99 %), AcChCl (99 %), U (99.5 %), G (certified ACS), acetone (certified ACS), acetonitrile (HPLC grade), chloroform (certified ACS), dichloromethane (99.5 %), methanol (HPLC grade), ethanol (200-proof), pentane (98 %), hexane (95 %), tetrahydrofuran (99.9 %), 1,4-dioxane (certified ACS), petroleum ether (certified ACS), Nile Red (99 %), 4-nitroaniline (99 %), N, N-diethyl-4-nitroaniline (99 %) were purchased from Thermo-Fischer Scientific (Dubuque, IA). Ultrapure water was obtained from a Thermo-Fisher Scientific Barnstead Ultrapure water purifier system set at 18 ΩM

cm^{-1}. ChCl, AcChCl, and U was recrystallized from absolute ethanol, filtered and dried under vacuum and the rest of the chemicals were used as received.

4.2.2 Methods

4.2.2.1 Synthesis

Sixteen different DESs were synthesized by thermal treatment as described in Section 2.2.2.1. All of the synthesized solvents were kept in well-sealed vials after preparation to avoid humidity effects that may affect the miscibility and solvatochromic parameters of DESs. Table 3.1 represents the compositions of synthesized DESs in this study together with their mole ratio.

4.2.2.2 Water Content

The synthesized DESs were considered as dry or zero wt % water-containing solvents even though they contain a small amount of residual water (0.9 to 1.4 wt %) because it was not possible to get completely dry them due to their highly hygroscopic nature. Five, ten, 15, and 20 wt % water-containing DESs were prepared by adding the appropriate amount of deionized water to the synthesized DESs. The amount of water present in the synthesized DESs was measured by Karl Fisher titration as described in the section 2.2.2.9.

4.2.2.3 Miscibility Measurements

200 µL each of the sixteen synthesized DESs were dissolved in 1 mL each of a range of common polar (water, acetone, acetonitrile, methanol, ethanol, and tetrahydrofuran) and nonpolar (chloroform, dichloromethane, pentane, hexane, 1, 4-dioxane, and petroleum ether) solvents at room temperature (25 ± 1 °C) and mixed well.

4.2.2.4 Solvatochromic Measurements

Solvatochromic measurements were made with three widely used dyes, Nile Red, 4-nitroaniline and N, N-diethyl-4-nitroaniline. Individual stock solutions of 10^{-3} molar (M) of the three dyes were prepared in dichloromethane (DCM). An appropriate amount of the dye stock solutions was micropipetted into clean, dry glass vials to prepare 10^{-5} M dye solutions. Then the residual DCM was evaporated under a gentle stream of nitrogen gas. DESs were then added to the glass vials containing the dyes. The glass vials were then capped and the samples were vortexed for one minute before the experimental measurements. A 200 µL aliquot of each sample was loaded into a microplate as shown in Figure 4.2.

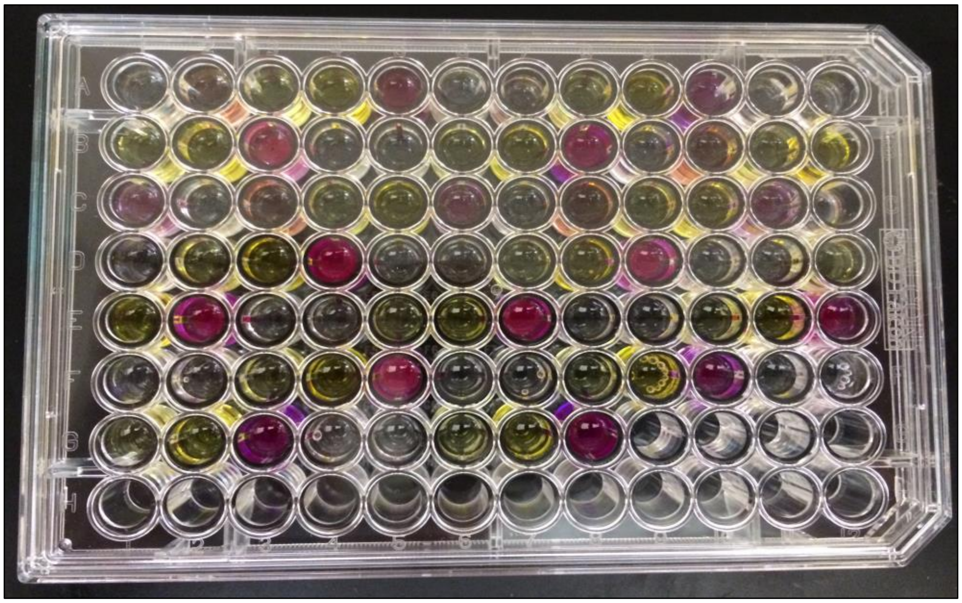

Figure 4.2. DESs samples of dissolved Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes loaded into a 96-well plate.

The absorbance maximum (λ_{max}) of the dyes dissolved in samples was measured by a SpectraMax 190 microplate reader equipped with a Cary Peltier temperature controller (± 0.1 ºC) (Molecular Devices, Sunnyvale, CA) with UVProbe software. The λ_{max} of the dyes was also recorded as a function of water content (5 to 20 % (wt %)) for every 5 wt % and temperature (25 to 45 °C for every 5 °C). All the measurements were made in triplicates and average values considered for further calculations.

4.3 Results and Discussion

4.3.1 Miscibility

Miscibility defined as the ability of a liquid to dissolve completely in another liquid solution. Miscible solutions don't form a distinct layer between two liquids whereas immiscible solutions form a clear, distinct layer[207]. All of the DESs under study were miscible in polar protic solvents such as water, methanol, ethanol and formed two phases with non-polar solvents such as chloroform, dichloromethane, pentane, hexane, 1, 4-dioxane and petroleum ether, as presented in Tables 4.1 and 4.2. DESs were miscible in the polar aprotic solvent tetrahydrofuran and immiscible in acetone and acetonitrile. The miscibility of these solvents in polar aprotic solvents strongly depends on their hydrogen bonding affinity. Therefore it can be hypothesized that acetone and acetonitrile do not hydrogen bond with DESs.

Table 4.1. Miscibility of DESs in common polar solvents including water, acetone, acetonitrile, methanol, ethanol, and tetrahydrofuran.

Sample	Water	Acetone	Acetonitrile	Methanol	Ethanol	Tetrahydrofuran
DES01	M	IM	IM	M	M	M
DES02	M	IM	IM	M	M	M
DES03	M	IM	IM	M	M	M
DES04	M	IM	IM	M	M	M
DES05	M	IM	IM	M	M	M

DES06	M	IM	IM	M	M	M
DES07	M	IM	IM	M	M	M
DES08	M	IM	IM	M	M	M
DES09	M	IM	IM	M	M	M
DES10	M	IM	IM	M	M	M
DES11	M	IM	IM	M	M	M
DES12	M	IM	IM	M	M	M
DES13	M	IM	IM	M	M	M
DES14	M	IM	IM	M	M	M
DES15	M	IM	IM	M	M	M
DES16	M	IM	IM	M	M	M

Table 4.2. Miscibility of DESs in common nonpolar solvents including chloroform, dichloromethane, pentane, hexane, 1,4-dioxane, and petroleum ether.

Sample	Chloroform	Dichloro-methane	Pentane	Hexane	1,4-dioxane	Petroleum-ether
DES01	IM	IM	IM	IM	IM	IM
DES02	IM	IM	IM	IM	IM	IM
DES03	IM	IM	IM	IM	IM	IM
DES04	IM	IM	IM	IM	IM	IM
DES05	IM	IM	IM	IM	IM	IM
DES06	IM	IM	IM	IM	IM	IM
DES07	IM	IM	IM	IM	IM	IM
DES08	IM	IM	IM	IM	IM	IM
DES09	IM	IM	IM	IM	IM	IM
DES10	IM	IM	IM	IM	IM	IM
DES11	IM	IM	IM	IM	IM	IM
DES12	IM	IM	IM	IM	IM	IM
DES13	IM	IM	IM	IM	IM	IM
DES14	IM	IM	IM	IM	IM	IM
DES15	IM	IM	IM	IM	IM	IM
DES16	IM	IM	IM	IM	IM	IM

Where,

M = Miscible, and

IM = Immiscible.

There was not noticeable difference observed between the miscibility of DESs studied in a range of common polar and nonpolar solvents, as presented in Tables 4.1 and 4.2. The miscibility of DESs does not vary with a change in their components and composition, and a possible reason might be the DESs molecules solvated by the solvents. In the instance of DESs molecules solvated by solvents, DESs may no longer exist but rather a simple dissolution of the individual molecules of DESs in solvent exists.

4.3.2 Solvatochromic Parameters

4.3.2.1 Nile Red E_T(NR) and Normalized E_T^N

The molar electronic transition energies of dissolved Nile Red (E_T(NR)) dye was calculated from its absorbance maximum according to Equation 4.1[200].

$$hcV_{max}N_A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{Equation 4.1.}$$

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

$$= \frac{28591}{\lambda_{max}(nm)}$$

Where,

h = Plank’s constant,

c = Speed of light,

V_{max} = Wavenumber,

N_A = Avogadro’s constant, and

λ_{max} = Wavelength of maximum absorbance.

The E_T(NR) scale ranges from 30.7 kcal mole^{-1} for tetramethylsilane (TMS), the least polar solvent, to 63.1 kcal mole^{-1} for water, as the most polar solvent[200].

In 1983, a normalized E scale (E_T^N) was introduced to avoid the non-SI unit kcal mol^{-1} and the conversion of the E_T(NR) values into kJ mole^{-1}. Normalized E_T^N values were calculated according to Equation 4.2 from Nile Red E_T(NR) values using water and tetramethylsilane (TMS) as the extreme polar and nonpolar reference solvents respectively according to Equation 4.2[208].

$$E_T^N = \frac{E_T(\text{solvent}) - E_T(\text{TMS})}{E_T(\text{water}) - E_T(\text{TMS})} \quad \text{…..……………………………….. Equation 4.2.}$$

$$= \frac{E_T(\text{solvent}) - 30.7}{32.4}$$

E_T^N scale ranges from 0.00 for TMS, as the least polar solvent, to 1.00 for water, the most polar solvent[209].

4.3.2.2 Kamlet-Taft Parameters

Kamlet-Taft parameters α, β and π* of DESs were calculated according to Equations 4.3 to 4.6.

$$\nu\,(\text{dye}) = \frac{1}{\lambda_{\max}(\text{dye})} \times 10^{-4} \quad \text{…………………..………………… Equation 4.3.}$$

$$\alpha = 0.00649\ E_T(\text{NR}) - 2.03 - 0.72\,\pi^* \quad \text{……………………………... Equation 4.4.}$$

$$\beta = \frac{(1.035\ \nu_{\text{N,N-diethyl-4-nitroaniline}} + 2.64 - \nu_{\text{4-nitroaniline}})}{2.80} \quad \text{..……………………. Equation 4.5.}$$

$$\pi^* = 0.314\ (27.52 - \nu_{\text{N, N-diethyl-4-nitroaniline}}) \quad \text{..……………………. Equation 4.6.}$$

The hydrogen bond-donating (HBD) acidity parameter α ranges from 0.00 for non-hydrogen bond-donating solvents such as aliphatic and aromatic hydrocarbons to

1.96 for hexafluoroisopropyl alcohol. The hydrogen bond-acceptor (HBA) basicity parameter β scale ranges from 0.00 for cyclohexane to 1.00 for hexamethylphosphoric acid triamide. The dipolarity/polarizability parameter π* scale ranges from 0.00 for cyclohexane to 1.00 for dimethyl sulfoxide[209].

Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes were completely dissolved in all of the DESs, and there was no aggregation observed as shown in Figure 4.3. The violet, light yellow and yellow colors were observed for Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes respectively in DES01 as shown in Figure 4.3. The color of the dyes doesn’t vary significantly among the eutectic solvents under study but intensity of the color varied depending on the polarity of the solvent. Thus the selected dye molecules displayed enough sensitivity to measure the polarity of the DESs under study.

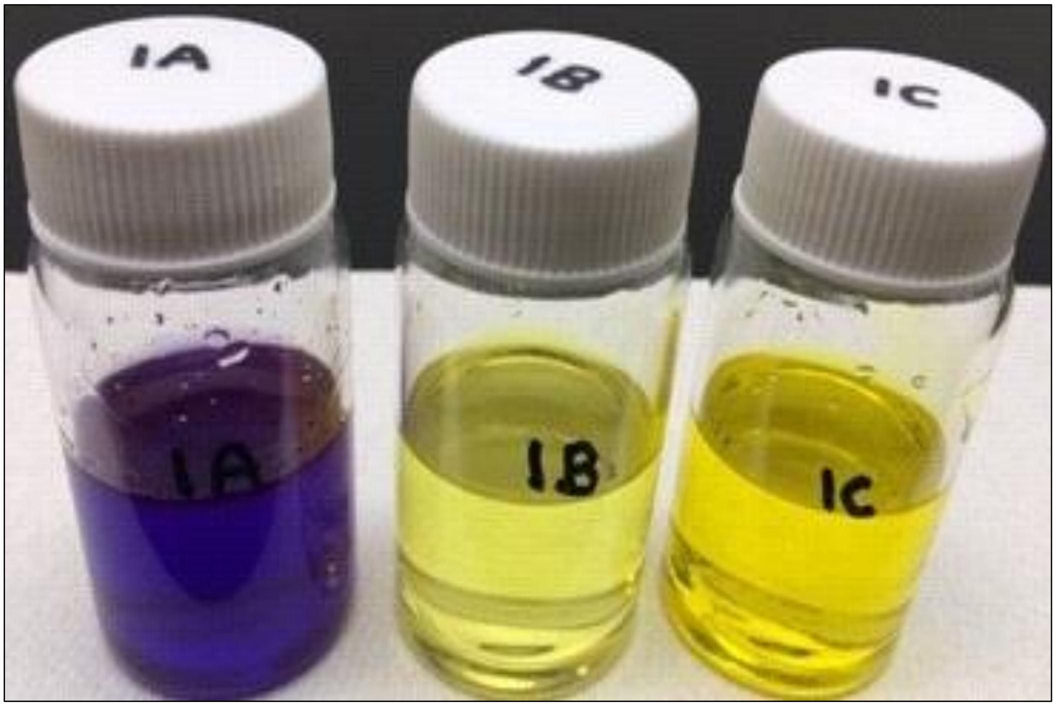

Figure 4.3. Violet, light yellow and yellow colors of Nile Red, 4-nitroaniline, and N, N-diethyl-4-nitroaniline dyes respectively (left to right) dissolved in DES01.

Solvatochromic parameters E_T(NR), E_T^N, α, β, and π* of all the synthesized DESs were determined. Measured E_T(NR) values range from 47.34 to 56.95 kcal mol^{-1} and E_T^N from 0.51 to 0.81, as shown in Table 4.3. Higher E_T(NR) or E_T^N values indicates the studied DESs as high polar solvents. Kamlet-Taft parameters α, β and π* range from 0.32 to 0.99, 0.78 to 1.10 and 0.67 to 0.85 respectively, as shown in Table 4.3. Higher β and lower α parameter values of these DESs classify them as hydrogen bond-acceptor basic solvents. Higher π* values indicate the studied DESs are polarizable solvents.

Table 4.3. Solvatochromic measured polarity (E_T(NR) and E_T^N) and Kamlet-Taft parameters (α, β and π*) of DESs at 25 °C.

DES	E_T(NR) kcal mol^{-1}	E_T^N	α	β	Π*
DES01	49.55	0.58	0.41	0.87	0.82
DES02	47.81	0.53	0.32	0.90	0.74
DES03	49.64	0.58	0.46	0.99	0.73
DES04	56.17	0.79	0.99	1.10	0.85
DES05	50.16	0.60	0.33	0.86	0.76
DES06	49.64	0.58	0.50	1.07	0.80
DES07	49.47	0.58	0.39	0.86	0.82
DES08	47.34	0.51	0.33	1.03	0.79
DES09	49.98	0.60	0.43	0.88	0.68
DES10	49.90	0.59	0.45	0.91	0.82
DES11	50.16	0.60	0.49	0.96	0.73
DES12	50.07	0.60	0.42	0.84	0.68
DES13	50.16	0.60	0.39	0.78	0.67
DES14	49.98	0.60	0.48	0.98	0.82
DES15	52.36	0.67	0.57	0.88	0.79
DES16	56.95	0.81	0.83	0.88	0.80

Measured solvatochromic parameters E_T(NR), E_T^N, α, β and π* values of DES01 and DES02 slightly deviated from the literature reported values and the possible reasons could be the dissolved gasses, the difference in water amount present or the dyes used for solvatochromic measurements[210-213]. Solvatochromic parameters of rest of the DESs under study are obtained for the first time, hence no direct reference is made to the literature.

Solvent polarity (E_T(NR) or E_T^N) and Kamlet-Taft parameters (α, β and π*) did not follow any trend to make conclusions. This is likely due to the complex interactions involved in the eutectic systems and the dye molecules. Hydrogen bonding, van der Waal interactions, electrostatic interactions, the lone pair of electrons present on the NH_2

group of U or the OH group of G might have contributed to these complex interactions. The polarizability parameter π* values of most of the DESs under study were similar, suggesting that this parameter is controlled by the cation present, which is almost similar for all of the solvents.

4.3.2.3 Comparison of Solvatochromic Parameters of DESs with Organic Solvents and Imidazolium ILs

To compare the measured solvatochromic parameters (E_T^N, α, β, and π*) of DESs with organic solvents and imidazolium ILs, data from selected organic solvents and imidazolium ILs are collected from the literature and presented in Table 4.4. This data is very useful in assessing the relative polarities of these DESs with the selected organic solvents and imidazolium ILs.

Table 4.4. E_T^N, α, β and π* of selected organic solvents and imidazolium-based ILs collected from the literature.

Solvent	E_T^N	α	β	Π*
Methanol	0.76[200]	1.05[214]	0.61[214]	0.73[214]
Ethanol	0.65[200]	0.83[214]	0.75[214]	0.51[214]
1-Propanol	0.62[200]	0.78[215]	0.90[216]	0.52[215]
2-proponal	0.55[200]	0.76[215]	0.84[216]	0.48[215]
1-Butanol	0.59[200]	0.79[215]	0.84[216]	0.47[215]
t-Butanol	0.39[200]	0.68[215]	0.93[216]	0.41[215]
Acetone	0.35[200]	0.20[215]	0.54[215]	0.70[215]
Acetonitrile	0.46[217]	0.35[217]	0.37[217]	0.80[217]
Dichloromethane	0.31[218]	0.13[215]	0.10[215]	0.82[215]
Chloroform	0.37[218]	0.20[215]	0.10[215]	0.58[215]
Dimethyl sulfoxide	0.44[200]	0.00[204]	0.76[219]	1.00[219]
Ethyl acetate	0.23[200]	0.00[215]	0.45[215]	0.55[215]
Cyclopentyl methyl ether	0.12[220]	0.00[220]	0.53[220]	0.42[220]
[bmim]OTf	0.66[221]	0.63[222]	0.46[222]	1.00[201]
[bmim]Cl	0.56[223]	0.47[223]	0.87[223]	1.10[223]

[bmim]OAc	0.61[224]	0.43[224]	1.05[224]	0.96[224]
[bmim]NTf$_2$	0.55[221]	0.38[221]	0.24[221]	1.01[221]
[bmim]N(CN)$_2$	0.64[225]	0.54[225]	0.71[225]	1.05[225]
[bmim]BF$_4$	0.66[226]	0.77[221]	0.38[221]	1.05[221]
[bmim]PF$_6$	0.67[221]	0.63[221]	0.19[201]	1.04[201]
[bmim]O$_2$CEt	0.57[224]	0.48[224]	1.10[223]	0.94[224]
[bmim]O$_2$CCH$_2$OH	0.61[224]	0.44[224]	0.87[224]	1.12[224]
[bmim][H-maleate]	0.52[224]	0.32[224]	0.62[224]	1.08[224]
[emim]ClO$_4$	0.67[227]	0.56[227]	0.41[227]	1.11[227]
[emim]N(CN)$_2$	0.65[227]	0.53[227]	0.35[227]	1.08[227]
[emim]NO$_3$	0.64[227]	0.48[227]	0.66[227]	1.13[227]
[emim]NTf$_2$	0.69[228]	0.66[226]	0.28[227]	0.90[227]
[emim]O$_2$CMe	0.59[227]	0.40[227]	0.95[227]	1.09[227]
[emim]PF$_6$	0.68[227]	0.66[227]	0.20[227]	0.99[227]
[hmim]Cl	0.59[229]	0.48[229]	0.94[229]	1.02[229]
[hmim]NTf$_2$	0.65[230]	0.65[230]	0.25[230]	0.98[230]
[hmim]PF$_6$	0.66[229]	0.57[231]	0.58[231]	1.02[229]
[hmim]OTf	0.67[230]	0.67[230]	0.52[230]	0.98[230]
[HO(CH$_2$)$_2$mim]O$_2$CMe	0.63[227]	0.53[227]	0.90[227]	1.04[227]

The results presented in Table 4.4 clearly shows the E_T^N of DESs are comparable to the short-chain alcohols (1-propanol and 2-propanol) and imidazolium ILs, which is not surprising given the similarity in the structures of ChCl and AcChCl with imidazolium ILs, because imidazole is also a quaternary ammonium compound. The HBD acidity parameter α of DESs range from 0.33 to 0.99. The parameter α of DESs studied (excluding DES04 and DES16) are less than methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, and t-butanol, higher than acetone, acetonitrile, ethyl acetate, dimethyl sulfoxide, ethyl acetate, cyclopentyl methyl ether, chloroform and dichloromethane, and similar to the imidazolium ILs[220, 222, 232-233]. The HBA basicity parameter β of DESs range from 0.66 to 1.10, which are similar to the methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, t-butanol and dimethyl sulfoxide and higher than ethyl acetate, cyclopentyl methyl ether, chloroform and dichloromethane [220, 222, 232-233]. Most of the imidazolium ILs had smaller β values, only a few of which were similar to the DESs. The dipolarity/polarizability parameter π* of DESs range from 0.67 to 0.85, which are higher than ethanol, 1-propanol, 2-propanol, 1-butanol, t-butanol, chloroform, and ethyl acetate, and lower than dimethyl sulfoxide and imidazolium ILs[223-230, 234-235].

Comparison of the polarities and Kamlet-Taft parameters of DESs with the selected organic solvents and imidazolium ILs indicates the care must be taken in claims of similarities between DESs and such solvents, because the E_T^N of DESs is similar to short-chain alcohols and imidazolium ILs but the parameters α, β, and π * are quite different. To visualize the parameters α, β, and π* of DESs with selected organic solvents and imidazolium ILs, a triangle plot is constructed using the OriginPro software presented Figure 4.4. Snyder introduced these type of solvent selectivity triangle (SST) to characterize the solvents and grouping them by plotting α, β, and π* of solvents[236-238]. Snyder SST grouped the solvents in to eight different groups and DESs falls in to the category of group IV solvents along with acetic acid, benzyl alcohol and ethylene glycol.

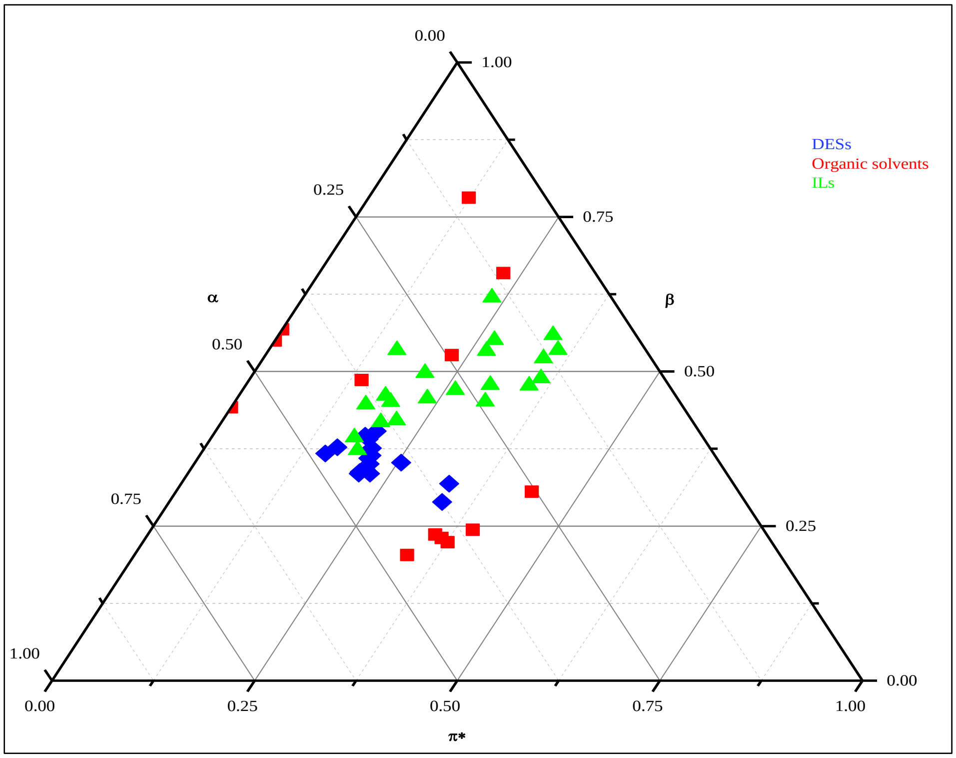

Figure 4.4. Comparison of DESs measured parameters α, β, and π* with selected organic solvents and imidazolium ILs collected from the literature.

Figure 4.4 clearly shows the parameters α, β and π* of DESs, selected organic solvents, and imidazolium ILs are quite different from each other. Therefore, it would not be appropriate to state the DESs are like short-chain alcohols and imidazolium ILs without specifying the respective property in which this might be considered to be true.

4.3.2.4 Effect of Temperature on Polarity and Kamlet-Taft parameters of DESs

Polarity and Kamlet-Taft parameters of DESs were measured as a function of temperature from 25 to 45 °C for every 5 °C. A slight decrease in polarity (E_T(NR), and E_T^N) of the DESs with rising temperature was observed, as shown in Figures 4.5 and 4.6 respectively, due to the positive solvatochromism A bathochromic or red shift of UV-Vis absorption band with decreasing solvent polarity is called as positive solvatochromism [216]. Solvent polarity usually decreases with increasing temperature due to the thermal reorientation of dipoles which ultimately decreases the dielectric constant[239]. Polarity of DESs composed of G were more sensitive to temperature than the corresponding eutectics composed of U because alcohol groups are more labile with the change in temperature than amide groups[120]. It is also important to note that the decrease in E_T(NR), and E_T^N of the DESs investigated with increasing temperature followed the trend as reported for other DESs and imidazolium ILs[120, 213, 232, 240-242].

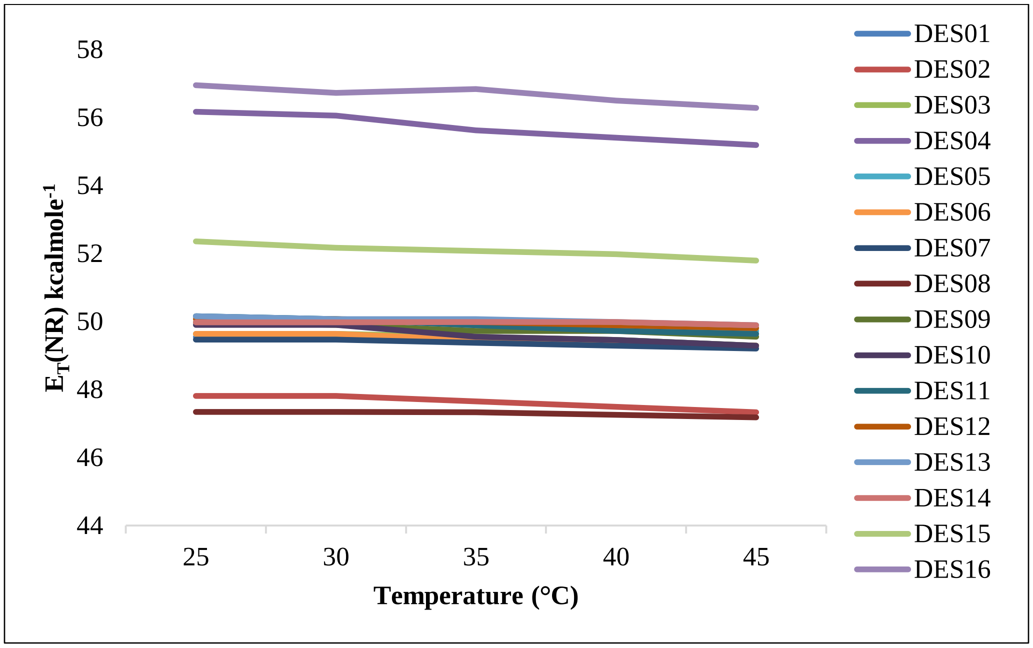

Figure 4.5. DESs polarity parameter E_T(NR) as a function of temperature from 25 to 45 °C.

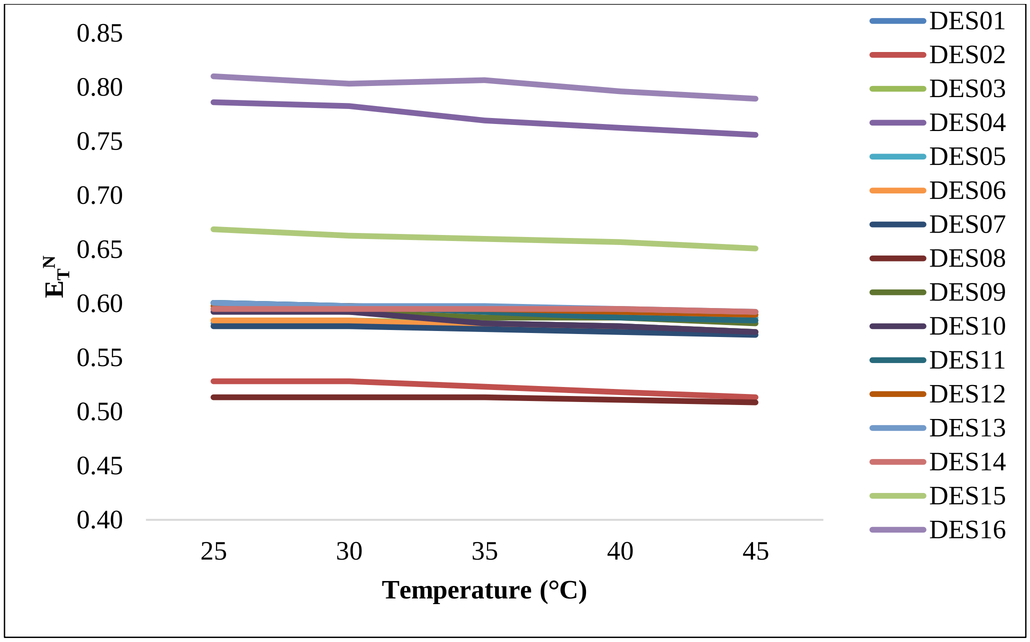

Figure 4.6. DESs polarity parameter E_T^N as a function of temperature from 25 to 45 °C.

The HBD acidity parameter α of the DESs under study slightly decreases with increasing temperature from 25 to 45 °C as shown in Figure 4.7 due to the weakening of the hydrogen bonding with increasing temperature. The decrease in α for the DESs under study is similar to those reported in the literature and water, and significantly higher than imidazolium ILs[242-244].

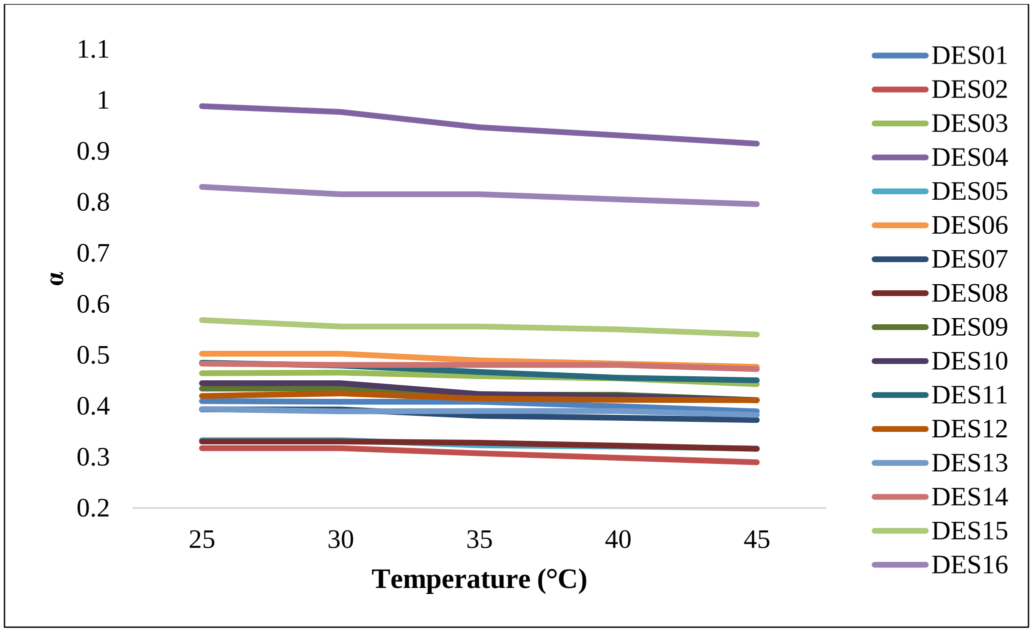

Figure 4.7. HBD acidity parameter α of DESs as a function of temperature from 25 to 45 °C.

There was not noticeable effect on the Kamlet-Taft parameters β and π* of DESs with increasing temperature from 25 to 45 °C as shown in Figures 4.8 and 4.9. This behavior of the eutectic solvents studied is similar to the literature-reported DESs and different to the imidazolium ILs, where β and π* decreased with increasing temperature[120, 243].

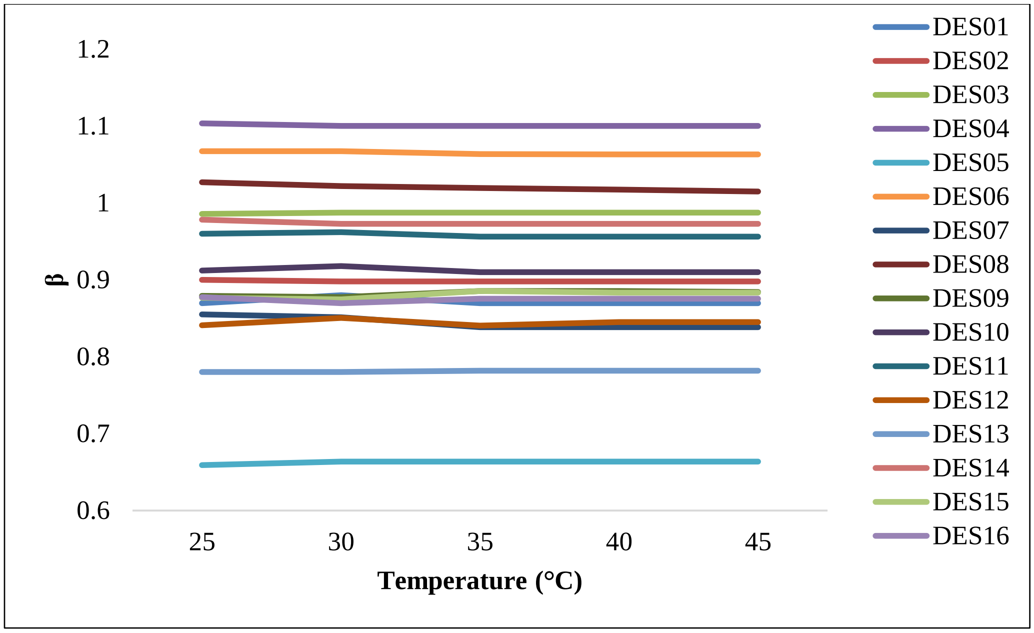

Figure 4.8. HBA basicity parameter β of DESs as a function of temperature from 25 to 45 °C.

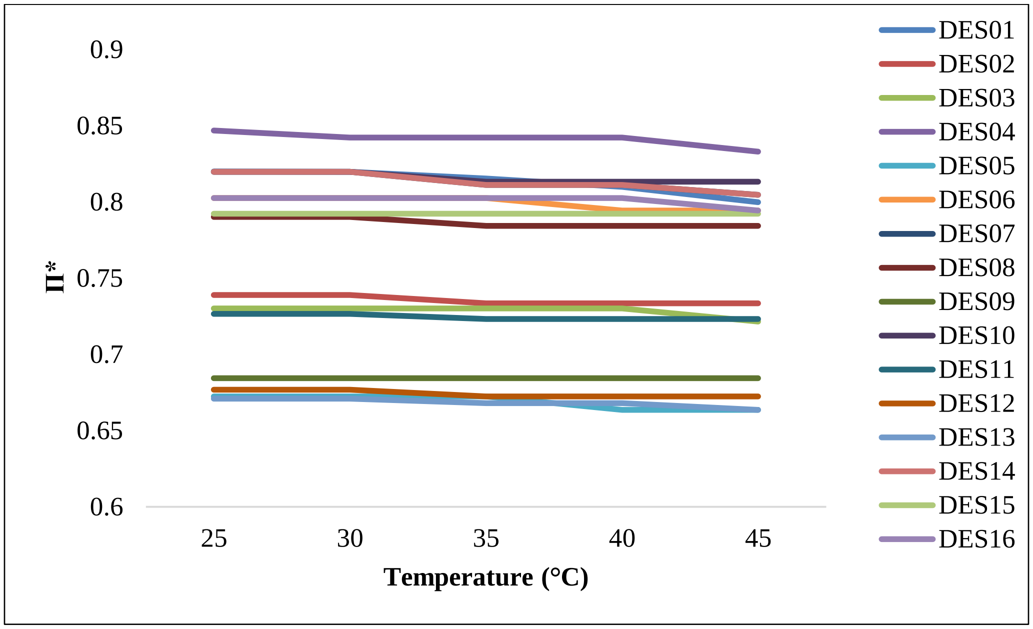

Figure 4.9. Polarizability/dipolarity parameter π* of DESs as a function of temperature from 25 to 45 °C.

The polarity and Kamlet-Taft parameters of DESs studied as a function of temperature clearly showed the decrease in polarity parameters E_T(NR), E_T^N, α and no noticeable effect on β and π*as shown in Figures 4.5 to 4.9. Hence, the equation of lines derived to predict the DESs polarity parameters E_T(NR), E_T^N and α as a function of temperature from the Figures 4.5 to 4.7 and presented in Table 4.5.

Table 4.5. The derived equation of lines to predict DESs polarity parameters E_T(NR), E_T^N and α as a function of temperature.

DES	E_T(NR) kcal mol^{-1}	E_T^N	α
DES01	y = -0.0605x + 49.662	y = -0.0018x + 0.5853	y = -0.0049x + 0.418
DES02	y = -0.1264x + 47.999	y = -0.0039x + 0.534	y = -0.0075x + 0.3288
DES03	y = -0.0865x + 49.778	y = -0.0026x + 0.5887	y = -0.0054x + 0.4737
DES04	y = -0.2601x + 56.472	y = -0.008x + 0.7955	y = -0.0193x + 1.0098
DES05	y = -0.1133x + 50.272	y = -0.0035x + 0.6041	y = -0.0044x + 0.3387
DES06	y = -0.095x + 49.769	y = -0.0029x + 0.5884	y = -0.0072x + 0.5129
DES07	y = -0.0695x + 49.574	y = -0.0021x + 0.5823	y = -0.0058x + 0.4011
DES08	y = -0.0402x + 47.411	y = -0.0012x + 0.5156	y = -0.0037x + 0.3368
DES09	y = -0.1034x + 50.086	y = -0.0032x + 0.5984	y = -0.0056x + 0.442
DES10	y = -0.1645x + 50.116	y = -0.005x + 0.5991	y = -0.0092x + 0.457
DES11	y = -0.1392x + 50.315	y = -0.0043x + 0.6054	y = -0.0093x + 0.4957
DES12	y = -0.0693x + 50.174	y = -0.0022x + 0.6011	y = -0.0029x + 0.4257
DES13	y = -0.0612x + 50.22	y = -0.0019x + 0.6025	y = -0.0022x + 0.3962
DES14	y = -0.0162x + 50.014	y = -0.0005x + 0.5963	y = -0.0021x + 0.486
DES15	y = -0.1316x + 52.472	y = -0.0041x + 0.6721	y = -0.0062x + 0.5732
DES16	y = -0.1563x + 57.13	y = -0.0048x + 0.8158	y = -0.0078x + 0.8363

4.3.2.5 Effect of Water Content on Polarity and Kamlet-Taft parameters of DESs

Polarity and Kamlet-Taft parameters of DESs were measured as a function of water content from 0 to 20 % (wt %) for every 5 % (wt %). The addition of water significantly increased the polarity (E_T(NR), and E_T^N) of the DESs under study as shown in Figures 4.10 and 4.11 due to the negative solvatochromism. A hypsochromic or blue shift of the UV-Vis absorption band with increasing solvent polarity is called negative solvatochromism [216]. It is important to note that the increase in E_T(NR) and E_T^N of the DESs with increasing water content followed the similar trend as literature-reported DESs and imidazolium ILs[120, 242].

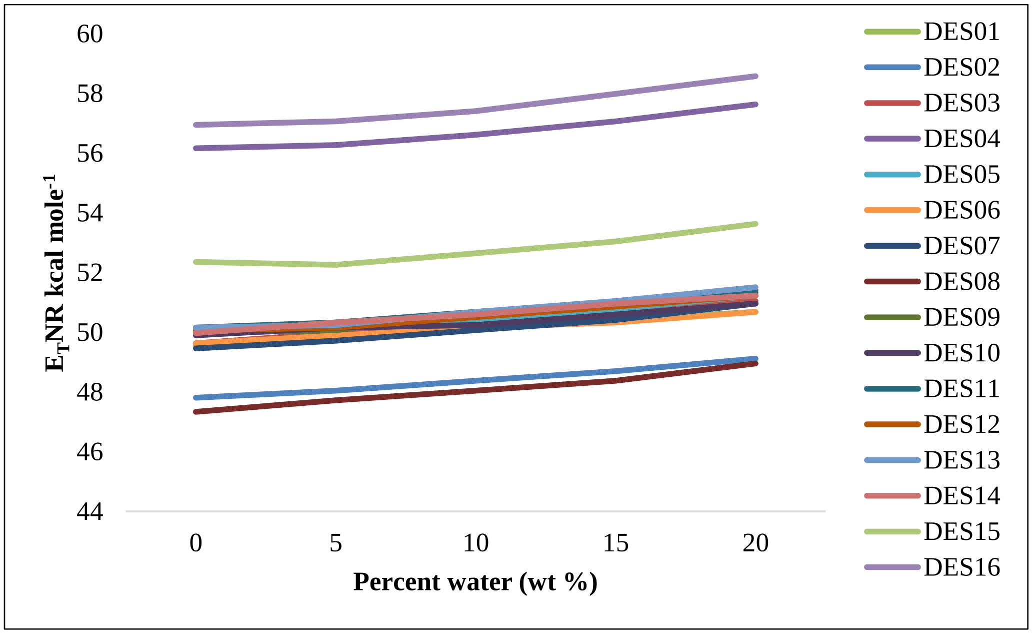

Figure 4.10. Polarity parameter E_T(NR) of DESs as a function of percent water present from zero to 20 wt %.

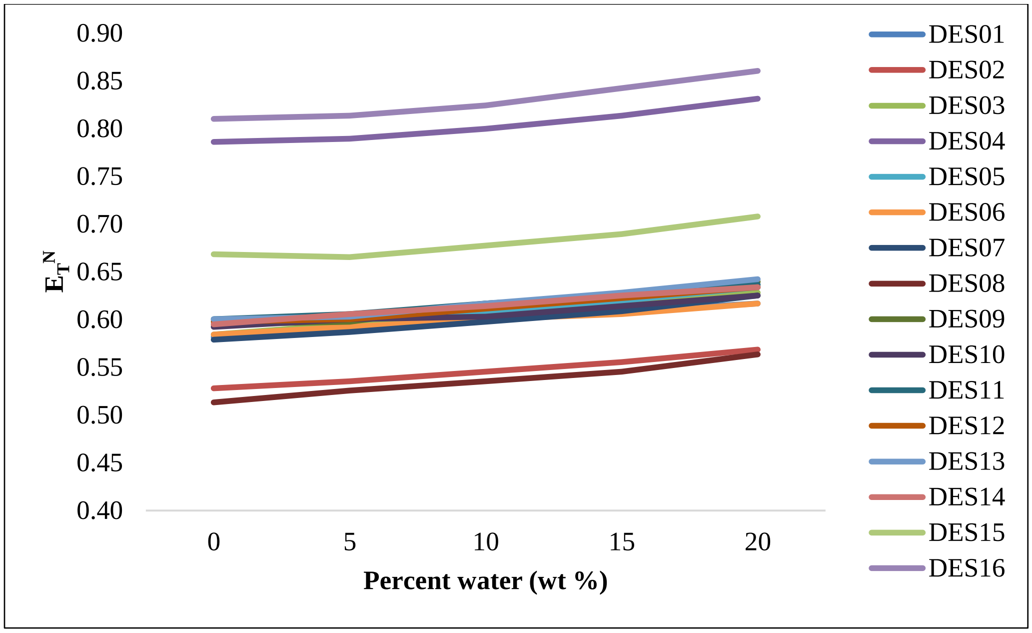

Figure 4.11. Polarity parameter E_T^N of DESs as a function of percent water present from zero to 20 wt %.

The HBD acidity parameter α and dipolarity/polarizability parameter π* of the DESs under exploration increased with increasing water content from 0 to 20 % (wt %) as shown in Figures 4.12 and 4.13 may be due to the individual hydrogen bonding ability and polarizability of water which is higher than in eutectic solvents. The increase in Kamlet-Taft parameters α and π* of these DESs with an increase in water content is similar to the literature-reported DESs and lower than imidazolium ILs, due to interspecies hydrogen bonding between DESs and water[120, 242, 245-246].

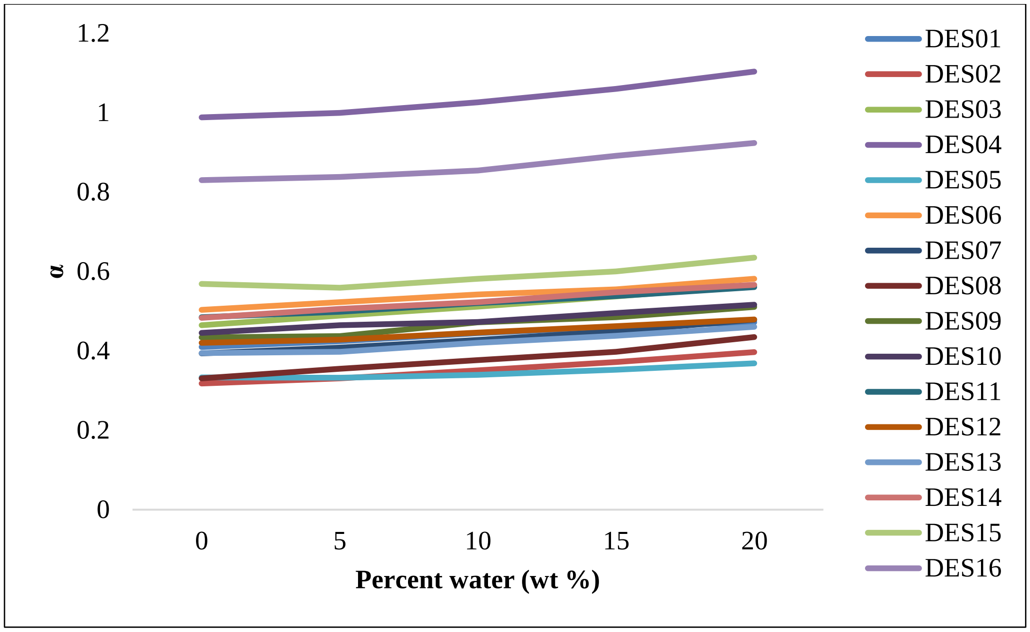

Figure 4.12. HBD acidity parameter α of DESs as a function of percent water present from zero to 20 wt %.

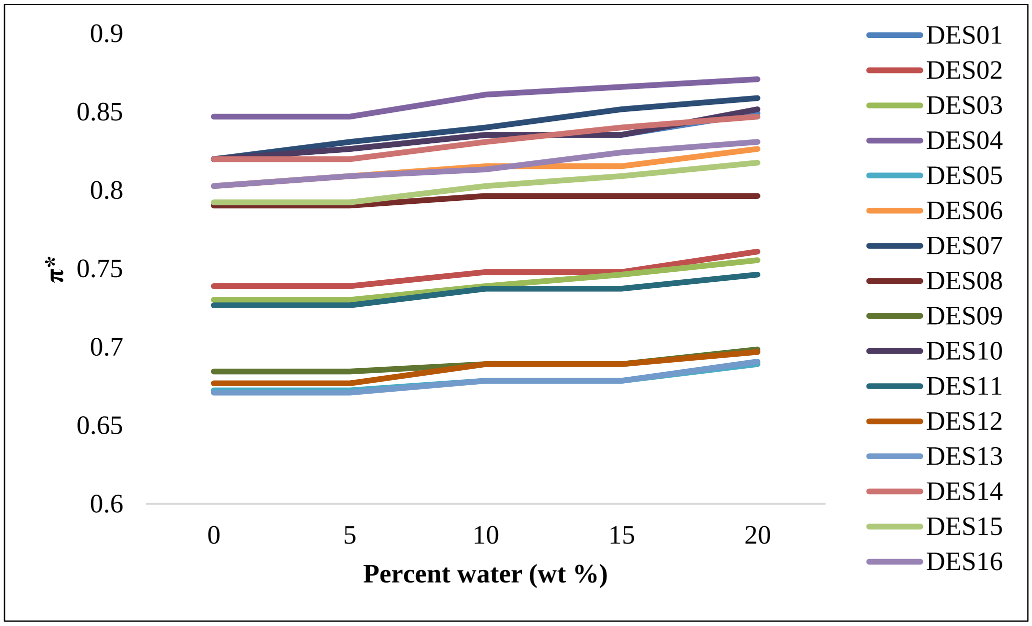

Figure 4.13. Dipolarity/polarizability parameter π* of DESs as a function of percent water present from zero to 20 wt %.

The HBA basicity parameter β of the DESs under study remained almost unchanged or slightly decreased with increasing water content from 0 to 20 % (wt %) as shown in Figure 4.14, due to the individual hydrogen bonding ability of water, which is higher than in eutectic solvents. The decrease in Kamlet-Taft parameters β of these DESs with an increase in water content is similar to the literature-reported DESs and lower than imidazolium ILs due to interspecies hydrogen bonding between DESs and water[120, 242, 245-246].

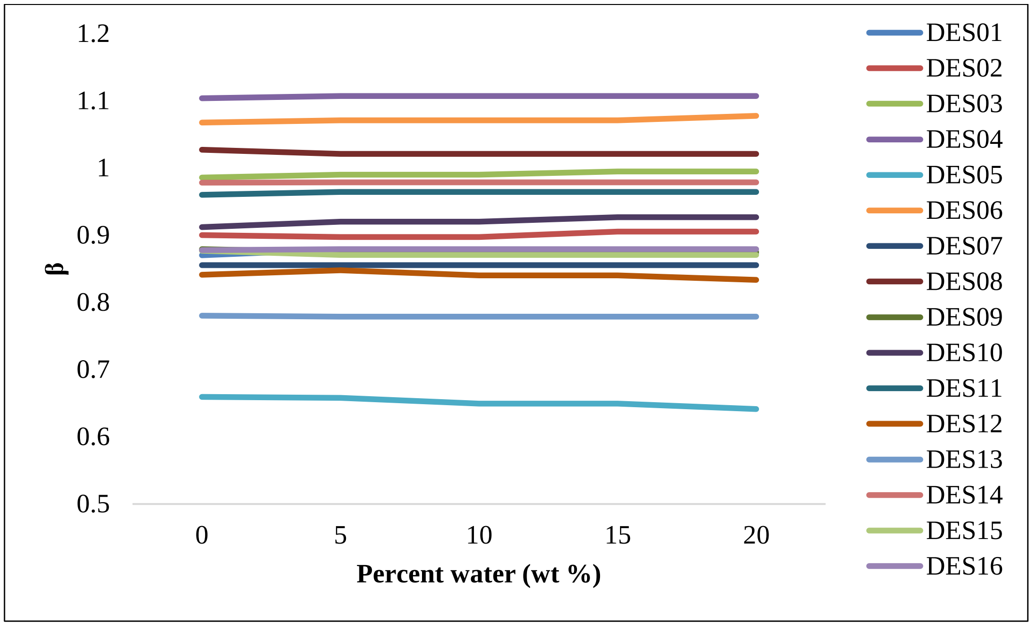

Figure 4.14. HBA basicity parameter β of DESs as a function of percent water present from zero to 20 wt %.

The polarity and Kamlet-Taft parameters of DESs studied as a function of added water content clearly showed an increase polarity parameters E_T(NR), E_T^N, α, π* and no noticeable effect on β as shown in Figures 4.10 to 4.14. Hence, the equation of lines derived to predict the DESs polarity parameters E_T(NR), E_T^N, α and π* and as a function of water content from the Figures 4.10 to 4.13 and presented in Table 4.6.

Table 4.6. The derived equation of lines to predict DESs polarity parameters E_T(NR), E_T^N, α and π* as a function of water content.

DES	E_T(NR) kcal mol^{-1}	E_T^N	α	Π*
DES01	y = 0.2901x + 49.257	y = 0.0089x + 0.5728	y = 0.016x + 0.3942	y = 0.0068x + 0.8131
DES02	y = 0.3286x + 47.429	y = 0.0101x + 0.5163	y = 0.0201x + 0.2936	y = 0.0053x + 0.7311
DES03	y = 0.354x + 49.28	y = 0.0109x + 0.5734	y = 0.0248x + 0.4392	y = 0.0066x + 0.7203
DES04	y = 0.3733x + 55.636	y = 0.0115x + 0.7696	y = 0.0291x + 0.9483	y = 0.0067x + 0.8383
DES05	y = 0.278x + 49.719	y = 0.0086x + 0.587	y = 0.0092x + 0.3177	y = 0.0039x + 0.6664
DES06	y = 0.2546x + 49.382	y = 0.0079x + 0.5765	y = 0.0189x + 0.4843	y = 0.0054x + 0.7977
DES07	y = 0.369x + 49.024	y = 0.0114x + 0.5654	y = 0.0211x + 0.3682	y = 0.0099x + 0.8107
DES08	y = 0.388x + 46.927	y = 0.012x + 0.5007	y = 0.0251x + 0.3036	y = 0.0018x + 0.7885
DES09	y = 0.3778x + 49.493	y = 0.0116x + 0.5801	y = 0.02x + 0.4077	y = 0.0033x + 0.6794
DES10	y = 0.2573x + 49.603	y = 0.008x + 0.5834	y = 0.0172x + 0.4272	y = 0.0073x + 0.812
DES11	y = 0.2969x + 49.806	y = 0.0092x + 0.5897	y = 0.019x + 0.4635	y = 0.0049x + 0.72
DES12	y = 0.3051x + 49.656	y = 0.0094x + 0.5851	y = 0.0153x + 0.4015	y = 0.0052x + 0.6702
DES13	y = 0.3518x + 49.679	y = 0.0109x + 0.5858	y = 0.0173x + 0.3703	y = 0.0047x + 0.6639
DES14	y = 0.3145x + 49.681	y = 0.0097x + 0.5859	y = 0.0208x + 0.4627	y = 0.0074x + 0.8092
DES15	y = 0.3339x + 51.792	y = 0.0103x + 0.6511	y = 0.0174x + 0.5368	y = 0.0067x + 0.7827
DES16	y = 0.4202x + 56.342	y = 0.0129x + 0.7915	y = 0.0239x + 0.7959	y = 0.0072x + 0.7946

4.4 Conclusions

Miscibility and solvatochromic parameters of sixteen different DESs were studied. The studied DESs were miscible in polar protic solvents and immiscible in nonpolar solvents. Miscibility in polar aprotic solvents depend on the ability to form hydrogen bonds. The studied DESs can be classified as polar HBA solvents due to their high E_T(NR) or E_T^N and β values. DESs polarity and Kamlet-Taft agreed well with their miscibility results. DESs polarities E_T(NR) and E_N^T were comparable to short-chain alcohols and imidazolium ILs, but the Kamlet-Taft parameters α, β and π* were quite different. DESs polarities E_T(NR) and E_N^T and HBD acidity parameter α decreased, and

HBA basicity parameter β, and dipolarity/polarizability parameter π* remain unchanged with an increase in temperature over the range studied. DESs polarities E_T(NR) and E_N^T, HBD acidity parameter α and dipolarity/polarizability parameter π* increased and HBA basicity parameter β remain unchanged with an increase in water content over the range studied. The equation of lines derived from the studies of effect of temperature and added water content to predict the DESs polarity and Kamlet-Taft parameters.

CHAPTER 5

5 TOXICITY

5.1 Introduction

Lately DESs have attracted widespread academic and industrial interest as an alternative to molecular solvents and ILs because of their environmental advantages, physicochemical and thermal properties and a broad range of possible applications[111, 133, 247]. Several researchers described DESs as green solvents even though their toxicological profiles are poorly known[12, 14, 88, 102, 248-249]. Their claims as green solvents were based on the properties of individual components of the DESs rather as eutectic mixtures. This hypothesis may or may not be correct since it did not take into consideration of the possible synergetic effect of combining the components of DESs. Therefore the use of terminology as “green” in the case of DESs should be considered carefully since their toxicity studies are not yet studied.

There is not a whole lot of work carried out in regards to DESs toxicity because they are considered as green based on the information of individual components of the DESs. DESs do not need registration at REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation, since their registry contains the components of DESs only. DESs are pharmaceutically acceptable since their safety data sheets (SDS) indicate the toxicity profile of the individual components rather eutectic mixtures. Thus there is less amount of work devoted to literature dealing with the toxicity of DESs, but the lack of toxicological data limits their biological and industrial applications. Hayyan tried to assess the toxic effects of both cholinium and phosphonium-based DESs on four bacteria and brine shrimps, and discovered that the DESs tested did not exhibit toxic effects on the bacteria but showed higher potent cytoxicity than their individual components[248, 250]. Radosevic evaluated three choline chloride-based DESs for their cytotoxicity towards fish and human cell lines, phytotoxicity on wheat, and biodegradability using closed bottle tests, indicate that the three studied ChCl-based DESs possess low cytotoxicity to moderate cytotoxicity[251]. Paulo de Morais studied the toxicity of some cholinium and organic acid based DESs with the Microtox toxicity test and concluded that the intermediate toxicity of DESs studied compared with their respective starting materials[252]. Qing Wen investigated the toxic effects of the cholinium based DESs on diverse living organisms such as E. coli (a bacterium), garlic (a plant) and hydra (an invertebrate), observed that the toxic effects on the selected living organisms, depending on the composition and concentration of the DESs studied[253].

The goal of the present study is to assess the genotoxicity and cytotoxicity of DESs and their individual components to extend the knowledge of their toxicological profile thus providing useful information to design less toxic DESs. Understanding the DESs toxicity profile will also increase the knowledge of their utility for in vivo use as well as the potential toxicological impacts due to their implementation in diverse applications, such as the pharmaceutical industry and food production. Therefore, the toxicity of DESs is a fundamental aspect that must be addressed before exploring their applications.

Genotoxicity of DESs and their individual components will be evaluated through the Ames' test that estimates their mutant-generating ability[254]. Cytotoxicity will be evaluated by the two popular enzyme based in-vitro assays LDH (lactate dehydrogenase) and MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)[255]. LDH measures the amount of LDH released after cell lysis, whereas MTT measures the redox activity[256].

5.2 Experimental

5.2.1 Materials

ChCl (99 %), AcChCl (99 %), U (99.5 %), G (certified ACS) and ethanol (200-proof) were purchased from Thermo-Fischer Scientific (Dubuque, IA). Ultrapure water was obtained from a Thermo-Fisher Scientific Barnstead Ultrapure water purifier system set at 18 ΩM cm^{-1}. ChCl, AcChCl, and U were recrystallized from absolute ethanol, filtered and dried under vacuum. Glycerol and ethanol was used as received.

The Ames test kit was purchased from Presque Isle Cultures (Erie, PA). LDH and MTT assay kits were purchased from Cayman Chemicals (Ann Arbor, MI). LDH diaphorase, LDH INT, and LDH standard from the LDH kit were stored at -20 ºC. LDH NAD+ and lactic acid were stored at 4 ºC. RPMI 1640 serum-free medium, sterile 96-well plates, and sterile pipette tips were purchased from Thermo-Fischer Scientific (Dubuque, IA). Absorbance was measured using SpectraMax 190 Absorbance Microplate Reader equipped with a Cary Peltier temperature controller (± 0.1 ºC) (Sunnyvale, CA).

5.2.2 Methods

5.2.2.1 Synthesis

Sixteen different DESs were synthesized as per the procedure described in section 2.2.2.1. All of the synthesized DESs were kept in well-sealed vials after preparation. Freshly prepared samples were used for toxicity studies to avoid humidity effects from the environment which might affect their structures. Table 3.1 represents the compositions of synthesized DESs in this study together with their mole ratio and abbreviation.

5.2.2.2 Water Content

The water content of all the DESs was measured as per the procedure described in section 2.2.2.10.

5.2.2.3 Ames Test

The *Salmonella typhimurium* culture from the Ames test kit was inoculated in sterile tryptic soy broth (TSB) and incubated at 37 ºC for 24 hours prior to the experiment. Each glucose minimal-salts agar tube was prepared by adding 0.3 mL of histidine/biotin stock per tube, 0.1 mL of *Salmonella typhimurium* and maintained at 50 to 55 °C. The components of each agar tube were poured into a sterile Petri plate each. Sterile paper discs were impregnated with a negative control (sterile water), a positive control (100 µg mL^{-1} of 4-nitro-o-phenylenediamine (4-NOPD)), DESs and their individual components (100 µg mL^{-1} each), and incubated for 48 hours at 37 ºC.

5.2.2.4 LDH Assay

Reagents were prepared from the kits by reconstituting or diluting with proper volumes of LDH assay buffer. The cell-based assay buffer from the kit was dissolved in 100-mL distilled water and stored at room temperature. The LDH diaphorase from the vial was reconstituted with 150 µL of assay buffer and stored at -20 ºC. The LDH stock was prepared immediately prior to use, reconstituting the contents of the LDH standard in 1.8 mL of assay buffer. Six LDH working standards ranging from 2000 to 12000 (µU mL^{-1}) were prepared through serial dilution from the stock. The blank was prepared in the same procedure as the working standards except that cell culture medium was used in place of LDH stock.

Cell culture was prepared by seeding the monkey-kidney cells in a 96-well plate at a density of 10^5 cells per well in 120-µL culture medium (Roswell Park Memorial Institute (RPMI) medium without fetal bovine serum). The cells were cultured in a CO_2 incubator at 37 ºC for 24 hours with DESs and their individual components at a concentration of 100 µg mL^{-1}.

The LDH assay was performed by centrifuging the 96-well tissue culture plate at 400 x g for five minutes. From each well of the cultured cells 100 µL of each supernatant was transferred to the corresponding wells on the new 96-well plate. Into the appropriate wells in the new plate, 100 µL each of the working standards was transferred. To all of the wells, 100 µL of the reaction solution was added. The plate was incubated with gentle shaking on an orbital shaker for 30 minutes at room temperature and the absorbance read at 490 nm with a plate reader. The standards were run in duplicate and samples were run in triplicate.

5.2.2.5 MTT Assay

MTT stock solution of 5 mg mL^{-1} was prepared in sterile Milli-Q water. MTT working solution for cell determination was prepared by diluting the MTT stock solution ten times with RPMI 1640 medium without fetal bovine serum (FBS). The MTT solutions are light sensitive, so they are covered with aluminum foil to protect from light.

Cell culture was prepared by seeding the monkey kidney cells in a 96-well plate at a density of 10^5 cells per well in 120-µL culture medium (RPMI without FBS). The cells were cultured in a CO_2 incubator at 37 ºC for 24 hours with DESs and their individual components at a concentration of 100 µg mL^{-1}.

The fluid covering the cells was aspirated using 200 µL multichannel pipets set at 160 to 170 µL. Once the covering fluid has been removed the cells were then incubated with 50 µL of MTT working solution. The plate was then incubated at 37 ºC for 4 hours. After 4 hours, 150 µL of DMSO was added, the plate was allowed to stand for one hour in the incubator (37 ºC, 5 % CO_2) and then mixed using the microplate shaker for 5 minutes and the absorbance was read at 570 nm with a plate reader.

5.3 Results and Discussion

5.3.1 Ames Test

The Ames test results were analyzed by counting the number of reverbants colonies per plate (RCP) present after incubation. The higher the number indicates more the mutagenic and vice versa[257]. The negative control plate barely had any reverbants colonies because it had sterile water, whereas the positive control had 28 ± 1.9 RCP because it had carcinogenic NADP as shown in Figure 5.1.

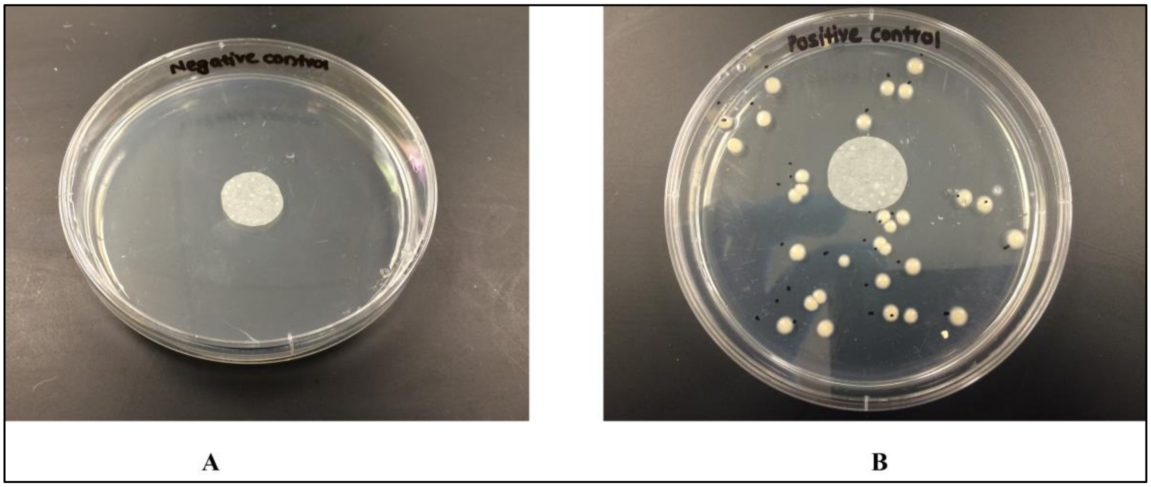

Figure 5.1. Ames test results: (A) Negative (sterile water) and (B) Positive control (4-NOPD).

A higher number of reverbants colonies were observed for all of the DESs than their individual components as shown in Figure 5.2 that indicates the occurrence of a synergistic additivity effect. A significant difference between the inhibition diameters of DESs and their individual components was observed, which also proves the synergistic effects of DESs. This synergetic toxicological behavior of DESs under study complements the literature reported toxicity studies other eutectic systems[27, 248, 250-251, 258]. Hydrogen bonding present in eutectics a mixture may be the reason for toxicity compared their individual components because the chemicals with delocalized charges are more toxic than those with localized charges[250, 259]. Glycerol has the least number of RCP followed by U, AcChCl and ChCl among the individual components of eutectics as shown in Figure 5.2, which can explained in terms of their LD_{50} values. Higher the LD_{50} (ChCl-5000, AcChCl-4860, U-8471 and G-58400 mm kg^{-1}, rat oral)[33-36], lower the number of RCP were observed. It was interesting to note that the G has the same number of RCP as sterile water (negative control).

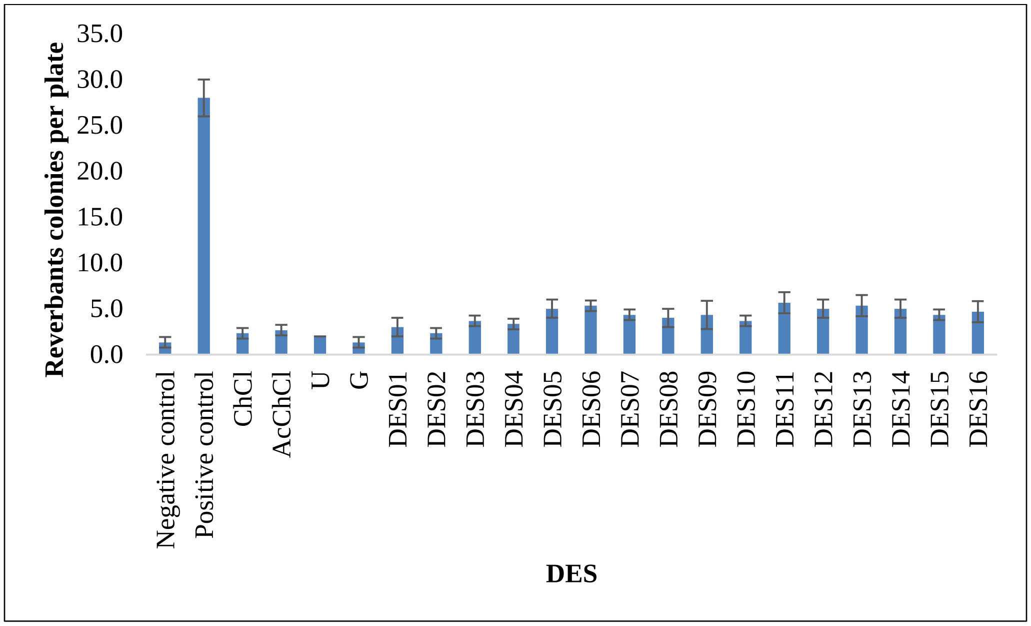

Figure 5.2. Number of reverbants colonies present per plate of a negative control, positive control, DESs, and their individual components.

The genotoxicity of studied DESs depends on both the components QASs and HBDs. Binary DESs composed of ChCl and G has less number of RCP than the corresponding eutectics composed of AcChCl and U. Ternary eutectic solvents has more RCP than the binary eutectics. Ternary eutectics with two QASs (ChCl and AcChCl) and one HBD (U) had a high number of RCP among the ternary eutectic systems studied. Careful examination of the results clearly shows the DESs genotoxicity depends on their individual components even though the synergetic effect was observed.

To the best of our knowledge, the mutagenicity and genotoxicity (Ames test results) of DESs is obtained for the first time. Hence, no composition direct comparison with the eutectics made. Comparison of Ames test results of DESs under study with imidazolium ILs at similar conditions (concentration and incubation time) clearly implies the eutectics are less mutagenic than ILs[257, 260]. DESs under study has 2.3 ± 0.6 to 5.7 ± 1.2 RCP whereas EMIM benzene sulfonate (EBS), EMIM labs (EEBS), EMIM toluene sulfonate (ETS) and EMIM xylene sulfonate (EXS) ionic liquids has 10.7 ± 0.6, 11.0 ± 1.0, 10.3 ± 0.6 10.0 ± 1.0 RCP respectively at the similar conditions[260].

5.3.2 LDH Assay

The average absorbance of blanks was subtracted from the absorbance of each of the other wells. A standard curve of absorbance at 490 nm as a function of LDH concentration was constructed and the equation of the line was determined. The cytotoxicity of the DESs and their individual components were determined by calculating total LDH activity based on LDH activity following Equations 5.1 and 5.2. A single LDH unit (U) is defined as the amount of LDH required to catalyze one mole of substrate per minute.

$$\text{LDH activity } (\mu\text{U}) = \frac{(A_{490} - Y_{\text{intercept}})}{\text{Slope}} \quad \text{……………………………………...}\textbf{Equation 5.1.}$$

$$\text{Total LDH activity } \left(\mu\text{U mL}^{-1}\right) = \frac{\text{Volume from LDH activity assay } (\mu\text{U})}{\text{X sample volume assay (usually 0.1 mL)}} \quad \text{…..….}\textbf{Equation 5.2.}$$

The LDH assay results of DESs presented in Figure 5.2 shows 94.33 ± 1.03 to 97.67 ± 0.96 % cell viability after 24 hours of treatment which implies that they can be considered as nontoxic at the tested concentration, time period and cell lines. LDH assay results of DESs and their individual components supports the Ames test results and follows the similar trend as shown in Figures 5.2 and 5.3.

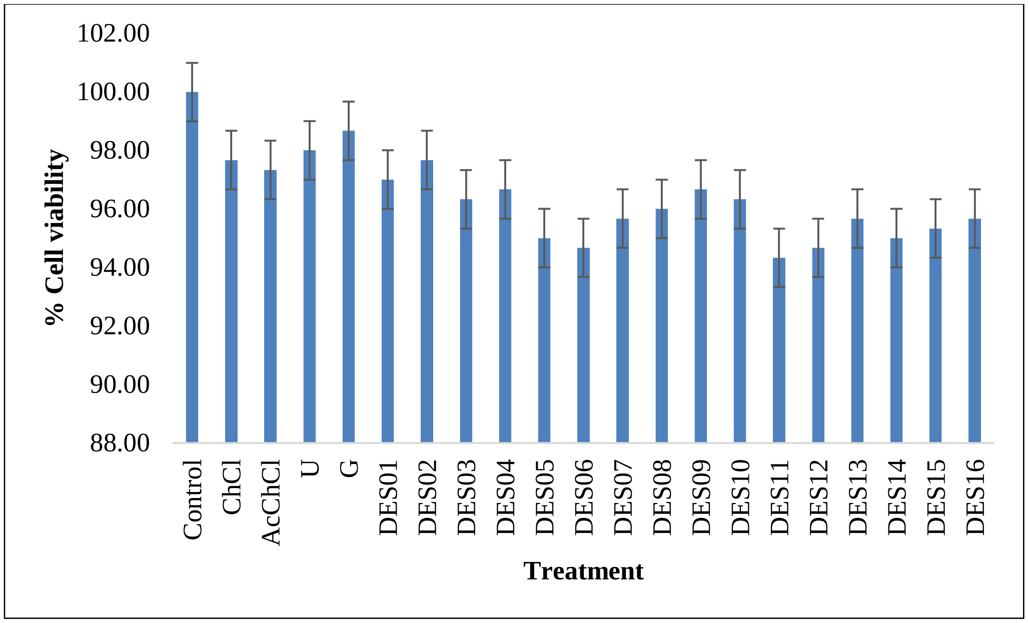

Figure 5.3. LDH assay percent cell viability of DESs and their individual components at 100 µg mL^{-1} concentration and 24 hours incubation.

5.3.3 MTT Assay

The average absorbance of blanks was subtracted from the absorbance of each of the other wells and a standard curve of absorbance at 570 nm as a function of percent cell viability. The MTT assay results presented in Figure 5.3 shows 95.19 ± 0.49 to 98.54 ± 0.98 % cell viability after 24 hours of treatment which meaning that they can be considered as nontoxic at the tested concentration, time period and cell lines. MTT assay results of DESs and their individual components supports the Ames test and LDH results and follows the similar trend of synergetic effect as shown in Figures 5.2, 5.3 and 5.4. The reason for the slight difference in percent cell viability of DESs with LDH and MTT assays is the difference in the method of testing. LDH tests levels of lactate dehydrogenase released into surrounding medium when cell membrane damages as a marker of dead cells, whereas, MTT tests mitochondrial dehydrogenase enzymes of living cells as a marker of living cells[261].

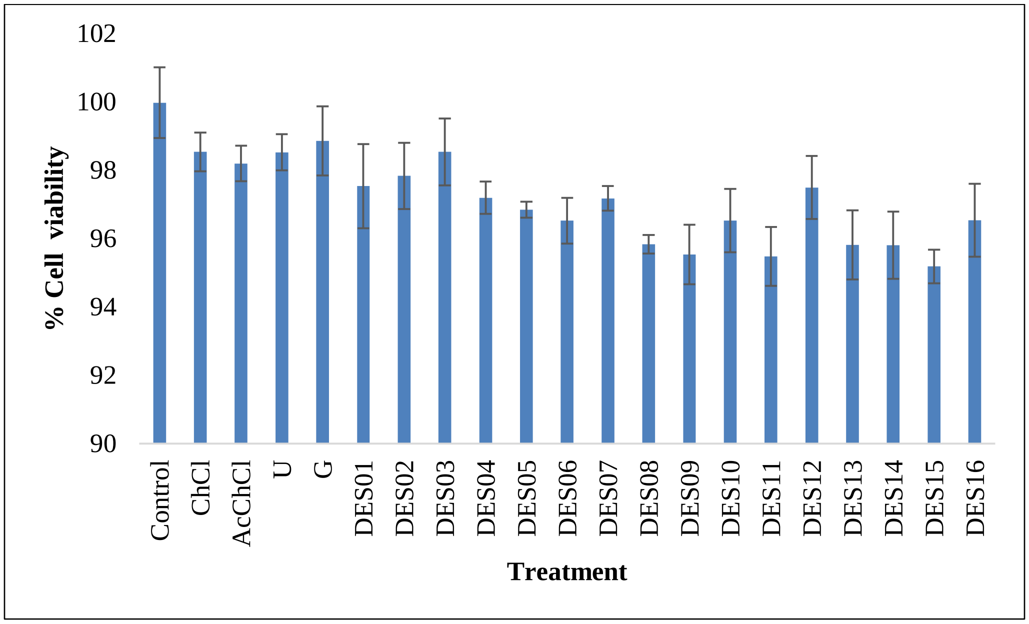

Figure 5.4. MTT assay percent cell viability of DESs and their individual components at 100 µg mL^{-1} concentration and 24 hours incubation.

Cytotoxicity mechanism of DESs may be related to their interactions with monkey-kidney cellular membranes. The possible reason for higher cytotoxicity of DESs compared to their individual components may be due to the difference in action on the monkey-kidney cell walls. The charge delocalization occurring through the hydrogen bonding between the QASs anion and the HBDs may increase their cytotoxicity to the monkey-kidney cells. The synergetic effect observed might be from the mixture in dilute systems because DESs doesn't exist as eutectic (at least partially) in highly diluted aqueous solutions (100 µg mL^{-1}). Our preliminary studies (^{1}H NMR and FTIR) of diluted DESs shows that the eutectic interaction disappeared around 40 wt % dilution with water. Further investigation of the cytotoxicity mechanism of DESs is essential to understand this synergetic behavior, but unfortunately, it was out of the scope of our dissertation and strongly suggested for future studies.

Cytotoxicity results of the DESs studied cannot be directly compared with the literature reported toxicity studies of DESs due to differences in cell lines, experimental conditions, DESs components and composition[27, 247-248, 250-251, 258]. Comparison of cytotoxicity results of the DESs under study with imidazolium ILs under similar conditions (concentration, time and cell lines) shows that eutectics are far less cytotoxic than imidazolium ILs[260]. DESs under study has more than 94 % cell viability at 10 mM concentration whereas ILs (EMIMAc, EBS, EEBS, ETS and EXS) has less than 50 % cell viability at 5 mM concentration at the similar incubation time and cell lines[260].

LD_{50} values of the DESs under investigation were not calculated because they did not cause 50 % growth inhibition at the tested concentration and time, but can be considered as compounds of fairly low cytotoxicity (> 94 % cell viability after 48 hours at 100 µg mL^{-1}). Further studies are recommended at higher concentration, time to measure their LD_{50}, but preliminary results show that the LD_{50} for DESs is lesser than their individual components. Therefore, the investigation of cytotoxicity of DESs at different concentration, time and cell lines is an essential future study.

5.4 Conclusions

The genotoxicity and cytotoxicity of DESs and their individual components were investigated using *Salmonella typhimurium* and monkey-kidney cells respectively. It was found that the toxicity of DESs was higher than their individual components indicating the occurrence of a synergetic effect. Therefore the careful use of the terminology nontoxicity must be considered. Ames test, LDH and MTT results of DESs and their individual components correlate well with each other. The genotoxicity and cytotoxicity of the DESs under study are far less toxic than imidazolium ILs at concentration, time and cell lines. Further studies at higher concentrations, longer times and with different cell lines are recommended to understand their toxicological behavior more fully.

CHAPTER 6

6 APPLICATIONS IN BIOMASS PROCESSING

6.1 Introduction

The diminishing availability of petrochemical resources is forcing us to look for sustainable resources[262]. Biomass, as an abundant and widely distributed sustainable resource, is a promising substitute for fossil-based resources [263]. The conversion of biomass into biofuels typically involves three major steps. The first step is the pretreatment of lignocellulose to liberate cellulose and hemicellulose from their complex with lignin; the second step is the depolymerization of the carbohydrates to produce fermentable reducing sugars; and the last step is the fermentation of the sugars to ethanol or other conversion processes[98].

The first step of biomass pretreatment is the crucial and unavoidable liberation of cellulose and hemicellulose available for biofuels (cellulosic ethanol) production[100]. Conventional methods of biomass pretreatment involve extreme and expensive techniques such as steam explosion, high temperatures, and the addition of strong acids and or bases that will pose important concerns over safety, health, and environmental pollution[264-267]. Ever since ILs found applications in the dissolution of cellulose, they have been explored extensively for the pretreatment of biomass[268]. They have a lot advantages compared to the conventional methods of pretreatment[269]. Thus, ILs pretreatment of biomass generated intense worldwide academic and industrial interest in a relatively short period, but their commercial applications are limited even today because of their setbacks. They have the limitations mainly in terms of recoverability, cost, and toxicity[270]. ILs toxicity remained unclear because most of them are synthesized from petrochemical sources[271]. Recently some studies even suggested further assessment of ILs toxicity and applicability as green media[272]. Finding a suitable green solvent for pretreatment of lignocellulosic biomass is one of the biggest challenges the biofuel industry is facing. Recently DESs have been proposed as a new generation of alternatives for biomass processing because they are inexpensive, nontoxic, recyclable, and have the physicochemical properties comparable to ILs.[42, 89, 98, 106, 270, 273-277]. This led us to explore our novel eutectic solvent systems for the pretreatment of lignocellulosic biomass.

We choose prairie cordgrass (PCG) and switchgrass (SWG) as lignocellulosic biomass samples because they are highly used for biofuel production[278-279]. The significance of these grasses is high energy production per acre, good resistance to diseases and pests, large geographical adoption, avoidance of the use of food crops, and native to Midwestern states and throughout the USA[278].

6.2 Experimental

6.2.1 Materials

ChCl (99 %), AcChCl (99 %), U (99.5 %), G (certified ACS) and ethanol (200-proof) was purchased from Thermo-Fischer Scientific (Dubuque, IA). Kraft pine lignin (Indulin AT) was purchased from Mead Westvaco (Charleston, SC). α-Cellulose (99 %), hemicellulose (98 %), D_2O (99.9 atom % D), and acetone-d6 (99.9 atom % D) were purchased from Sigma-Aldrich (St Louis, MO). PCG and SWG used in this study were harvested from Brookings, South Dakota in 2010. These grasses were dried, milled to 1 mm using a Wiley mill and were stored at room temperature until the analysis. Ultrapure water was obtained from a Thermo-Fisher Scientific Barnstead Ultrapure water purifier system set at 18 ΩM cm^{-1}. ChCl, AcChCl, and U was recrystallized from absolute ethanol, filtered and dried under vacuum and the rest of the chemicals were used as received.

6.2.2 Methods

6.2.2.1 Synthesis

DESs were synthesized as per the procedure described in section 2.2.2.1. The synthesized samples were kept in well-sealed vials and fresh samples were used to avoid any structural changes and to avoid humidity effects from the environment. Table 3.1 represents the compositions of synthesized DESs in this study together with their mole ratio and abbreviation.

6.2.2.2 Water Content

The water content of all the DESs was measured as per the procedure described in section 2.2.2.10.

6.2.2.3 Screening of Biopolymers Solubility

The solubility of the biopolymers such as cellulose, hemicellulose and lignin were determined by the cloud point method as reported earlier[273]. It is the most accurate method to determine solubility even though it is time-consuming. The vials containing 2 g of DES each was placed into a temperature-controlled oil bath at a constant temperature of 100 °C. Consecutive additions of 0.2 to 1 mg of solute were made while vigorously stirring. Once turbidity was noticeable, the samples were equilibrated for 24 hours. If the sample did not become clear, the cloud point was registered and at less below 0.1 wt % no solubility was considered. Every experiment was repeated at least three times to ensure the reproducibility of the results and the average value is reported.

6.2.2.4 Extraction of Lignin from Grasses

Lignin extraction from PCG/SWG biomass was carried out by series of steps as described in the flow diagram (Figure 6.1).

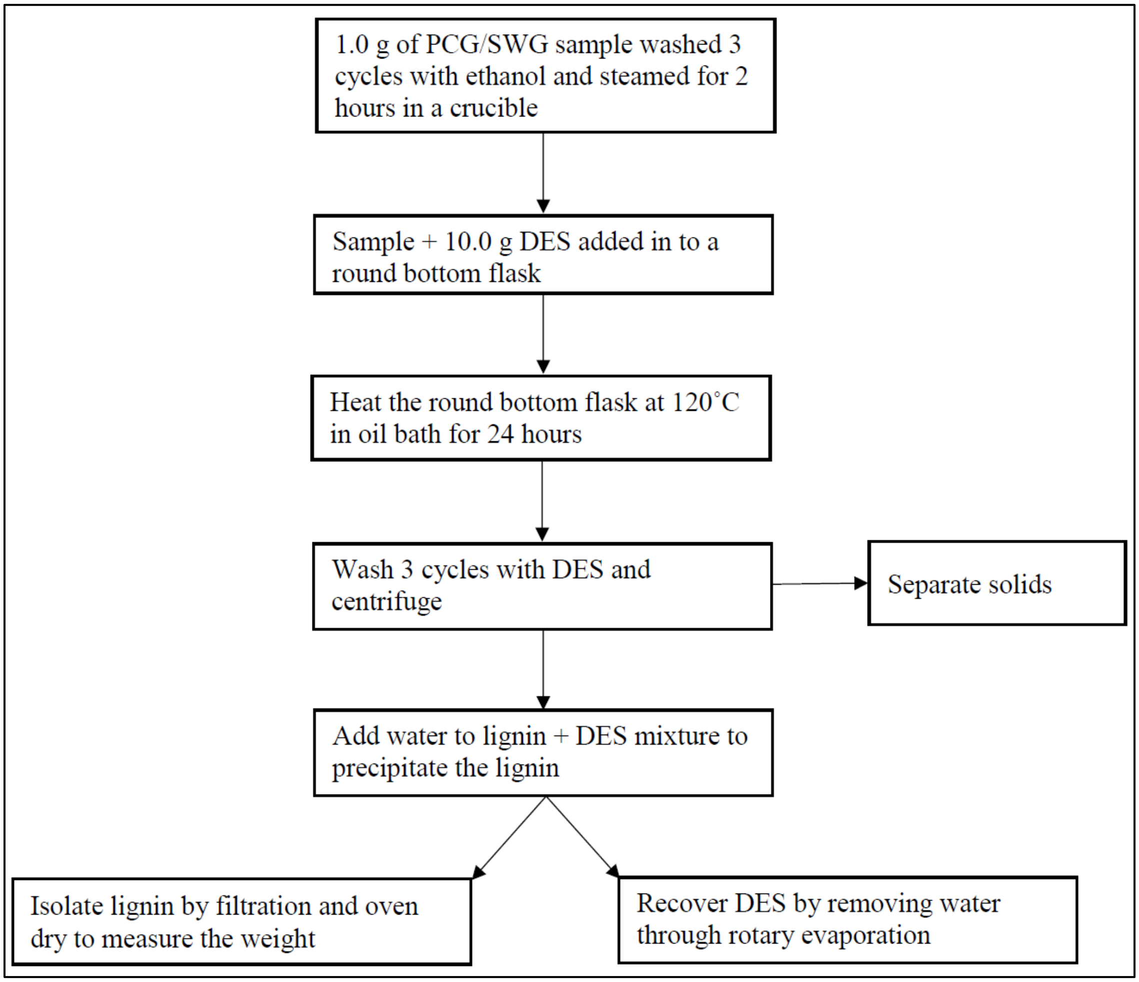

Figure 6.1. Flow diagram of DESs process of lignin extraction from grasses.

One gram of PCG/SWG sample was washed three cycles with ethanol, then placed in a crucible and steamed for two hours. The dried biomass sample transferred into a round-bottom flask containing 10 g of DES and a magnetic stir bar was added. The biomass suspension was heated overnight with stirring in an oil bath with a reflux condenser. Although biomass particles or fibers were still present, the solution changed to brown color. Those biomass fibers were washed three cycles with DES. Direct separation of the suspended and dissolved biomass was carried out by centrifuging the DES with biomass. The dissolved lignin recovered from the DES phase by water addition. Water acts as an anti-solvent and precipitates lignin out of the DES solution. The precipitated lignin and DES aqueous mixture separated by vacuum filtration using a Buchner funnel. The separated precipitate which was entirely composed of lignin oven dried and let it cool to room temperature to measure the weight of extracted lignin.

6.2.2.5 FTIR Spectroscopy

The FTIR absorption spectra were recorded using a Nicolet 380 FTIR spectrometer from Thermo-Fisher Scientific (Waltham, MA) with 8 cm^{-1} resolution with 100 scans in the range 400 to 4000 cm^{-1}. The spectra were processed using EZ OMNIC software.

6.2.2.6 ^{1}H NMR Spectroscopy

The ^{1}H NMR spectra were recorded at 400 MHz on a Bruker 400 spectrometer (Billerica, MA) equipped with a QNP 5-mm probe at 22 °C. D_2O and acetone-d6 were used as solvents. 25 mg each of DESs and lignin samples were dissolved in 0.5 mL D_2O and acetone-d6 respectively. The conditions for recording ^{1}H NMR spectra were a 90º pulse angle, 2-s pulse delay, and 512 scans. The spectra were processed using TopSpin and ACD spectrus processor software.

6.3 Results and Discussion

6.3.1 Screening of Biopolymers Solubility

It is difficult to estimate the suitability of a particular DES for pretreatment of lignocellulosic biomass because multiple factors such as pH, density, viscosity, surface tension, polarity, and HBD interaction affects the pretreatment. Therefore, all of the DESs studied were tested for dissolution of biopolymers. Cellulose, hemicellulose and lignin biopolymers were chosen because they are the most abundant renewable polymers in lignocellulosic biomass. The solubility of cellulose, hemicellulose and lignin were experimentally determined by the cloud point method and the results of the biopolymers solubility in DESs are presented in Table 6.1 as weight percent solubility.

Table 6.1. Cloud point solubility of the biopolymers cellulose, hemicellulose and lignin in DESs (wt %).

DES	Cellulose solubility (wt %)	Hemicellulose solubility (wt %)	Lignin solubility (wt %)
DES01	ND	ND	12.92
DES02	ND	ND	10.23
DES03	ND	ND	8.56
DES04	ND	0.18	6.38
DES05	ND	ND	11.26
DES06	ND	ND	14.25
DES07	ND	0.42	8.21
DES08	ND	ND	8.02
DES09	ND	ND	7.77
DES10	ND	0.14	6.80
DES11	ND	ND	6.98

DES12	ND	ND	7.86
DES13	ND	ND	6.27
DES14	ND	0.13	7.14
DES15	ND	0.14	6.90
DES16	ND	0.16	6.86

Where,

ND = Not dissolved.

The results presented in Table 6.1 shows that the DESs preferentially dissolves lignin over cellulose or hemicellulose. Thus, these DESs may be attractive solvents for selective extraction of lignin from lignocellulose. Our study results support previous studies of eutectics for lignin dissolution[270, 276, 280-281]. Recently some studies have been reported for DESs extraction and valorization of lignin because of selective dissolution of lignin in eutectic systems[282-285].

It is tough to estimate the properties governing the dissolution of lignin biopolymer in DESs because it involves complex interactions. Careful examination of DESs properties and the lignin solubility results revealed an interesting correlation between the physical properties (pH, viscosity, surface tension, and HBA basicity parameter (β)) and lignin solubility. DESs with high pH has high lignin solubility because lignin dissolves to a greater extent in a basic medium[280]. Lower viscosity eutectic solvents have the high dissolution of lignin because of the mass transfer that leads to higher solubility. DESs with lower surface tension has higher lignin solubility because the sample matrix can be infiltrated more easily by DESs with low surface tension. DESs with higher HBA basicity parameter (β) has higher lignin dissolution. It is important to note that the above-mentioned correlations were applicable in most cases but not in all the situations since the dissolution of lignin is the result of complex interactions.

Proper selection of solvent is very important for developing the extractions of target compounds from a sample matrix. After careful examination of lignin solubility results, physicochemical and thermal properties of eutectics, DES01, DES02, DES05, and DES06 were selected to further explore their utilization in selective extraction of lignin from lignocellulosic biomass.

6.3.2 Extraction of Lignin from Grasses

Extraction or separation of lignin from cellulose and hemicellulose is the crucial step for the bioethanol production[264]. The selective extraction of lignin from PCG and SWG was carried out with DES01, DES02, DES05, and DES06 as per the flowchart described in section 6.2.3. The change in color of DESs after overnight pretreatment was observed as shown in Figure 6.2 (B), reflecting the lignin extraction.

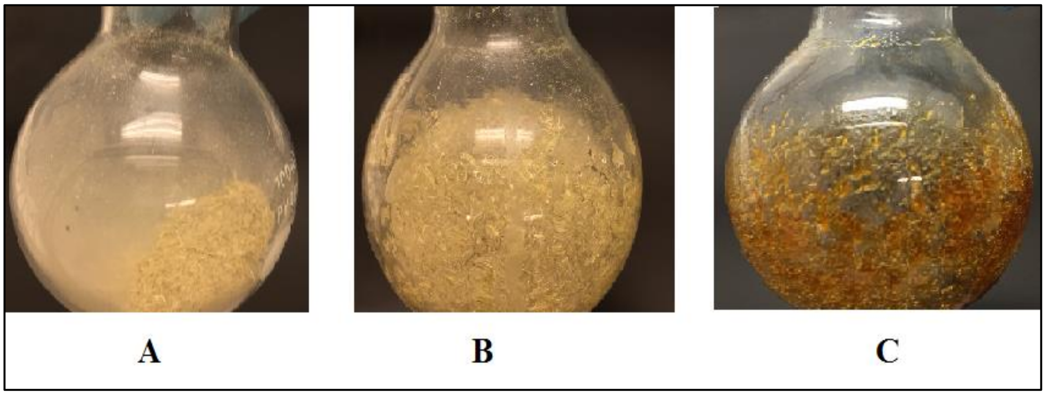

Figure 6.2. PCG biomass processing with DES01: (A) PCG biomass sample, (B) PCG biomass and DES01, and (C) After pretreatment with DES01 overnight at 120 °C.

The exact mechanism by which the eutectic solvents extracts the lignin selectively from PCG or SWG is not clear. It can be hypothesized that eutectics may have provided a mild acid-base catalysis mechanism which might cause controlled cleavage of labile ether linkages among the lignin phenylpropane units and thus lead to lignin separation from the biomass as reported in literature[280, 285-286].

6.3.2.1 Optimization of Extraction Temperature

The exaction of lignin from PCG was carried out at as per the flowchart described in section 6.2.3 at 80, 100, 120 and 150 °C to optimize the DESs lignin extraction temperature, and the results are presented in Figure 6.3.

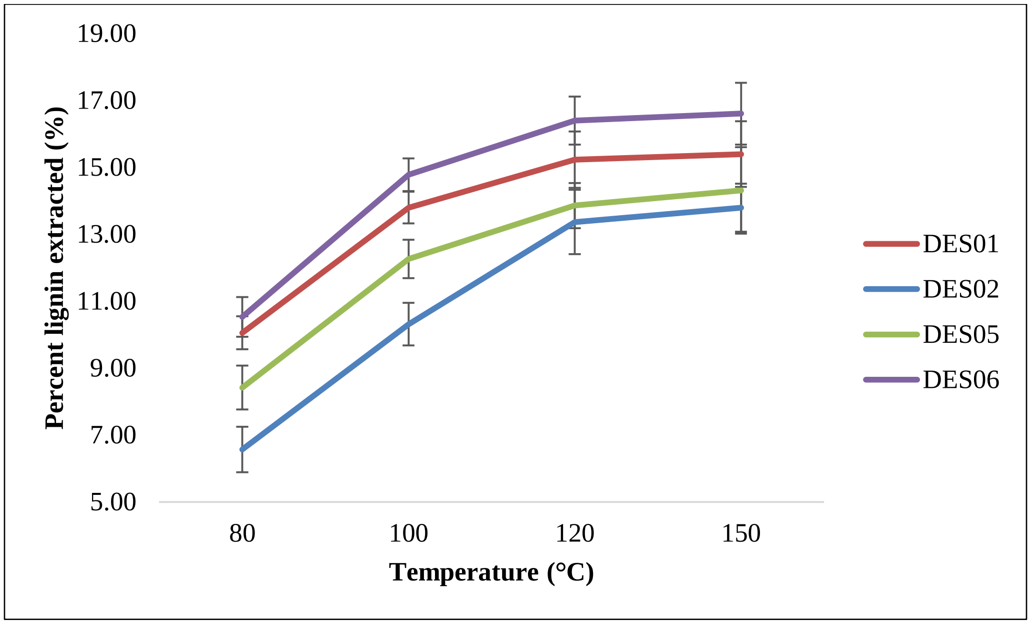

Figure 6.3. Optimization of DESs extraction temperature of PCG lignin at 80, 100, 120 and 150 °C.

It is clearly evident from the results in Figure 6.3 that with an increase in temperature from 80 to 120 °C, an increase in percent lignin extracted was observed. In general, the solubility of solids in a liquid solvent increases with increasing temperature. This may be due to decreasing viscosity of eutectics with an increase in temperature, which might have facilitated high diffusion and mass transfer. The glass transition temperature of grass lignin is around 120 ºC[287], hence no significant difference between the percent lignin extracted at 120 and 150 ºC hours was observed. The extraction was not carried out beyond 150 ºC due to the increased risk of eutectic decomposition. Hence, the extraction temperature optimized as 120 ºC.

6.3.2.2 Optimization of Extraction Time

The exaction of lignin from PCG was carried out at as per the flowchart described in section 6.2.3 at 12, 18, 24 and 36 hours to optimize the DESs lignin extraction time and the results are presented in Figure 6.4.

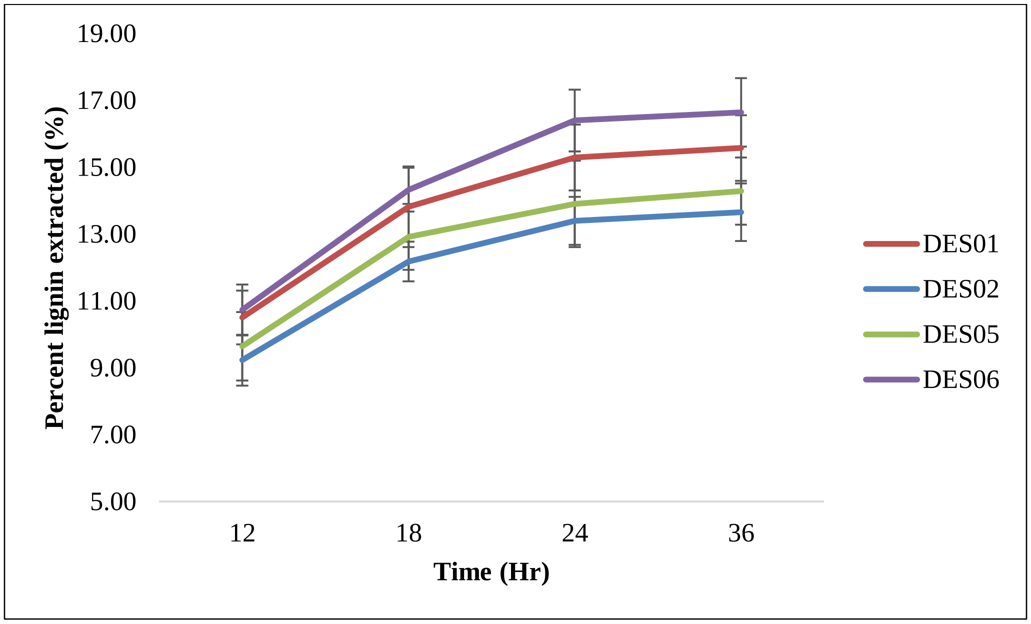

Figure 6.4. Optimization of DESs extraction time of PCG lignin at 12, 18, 24 and 36 hours.

The results presented in Figure 6.4 shows that the average percent lignin extracted increased noticeably from 12 to 24 hours but there was almost no difference in percent lignin extracted at 24 and 36 hours was observed. With increasing time, more lignin is solubilized and extracted until approximately 24 hours where the lignin isolation follows more of a diffusion mechanism. The extraction was not carried out beyond 36 hours because there was little difference between the percent lignin extracted at 24 and 36 hours and prolonged extraction times might result in high energy requirements with large-scale industrial applications. Hence, the extraction time optimized as 24 hours.

The exaction of lignin from PCG and SWG was carried out at as per the flowchart described in section 6.2.3 at the optimized conditions of 120 ºC and 24 hours. The percent lignin extracted varied from 13.8 ± 0.7 to 16.6 ± 0.9, and 15.4 ± 0.7 to 18.7 ± 0.5 for PCG and SWG respectively as shown in Figure 6.5.

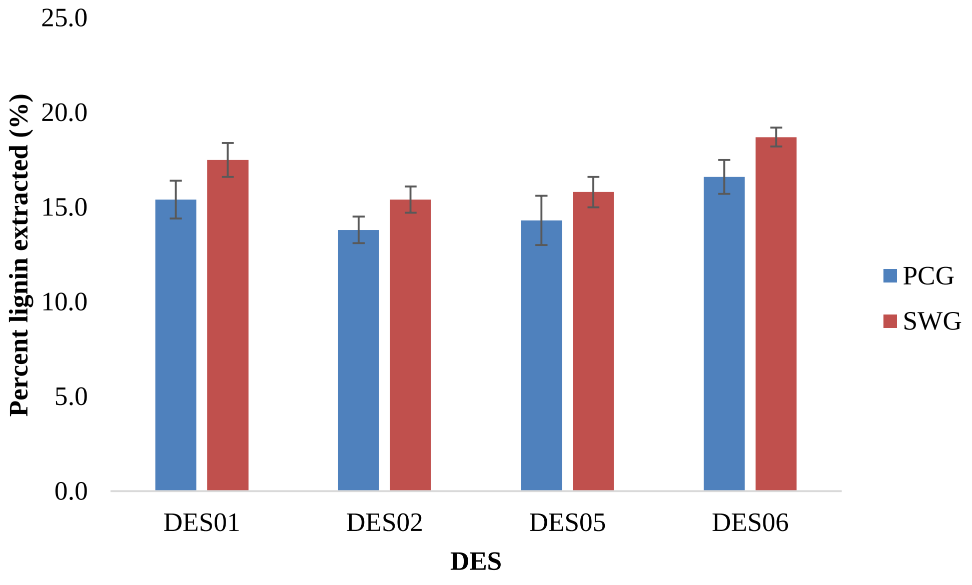

Figure 6.5. DESs percent lignin extracted from PCG and SWG at optimized conditions of 120 ºC and 24 hours.

Lignin extraction efficiency with DES06 was found be higher compared to DES01, followed by DES05 and DES02, as shown in Figure 6.5. Closely looking at DES01, DES02, DES05 and DES06 properties clearly indicates the lignin extraction abilities of DESs rely mainly on their pH and HBA basicity parameter β.

The purity of the extracted lignin was checked by comparing the IR spectra of extracted lignin and the standard. Figures 6.6 and 6.7 shows the similarities in the spectral profiles and lignin characteristic peaks at 1590/1509 cm^{-1} (C=C stretching vibration), 1459 cm^{-1} (asymmetric bending in CH_3), 1419 cm^{-1} (C-H deformation), 1320 cm^{-1} (C-O vibration), and 1261/1231 cm^{-1} (guaiacyl/syringyl ring and C-O stretching vibration) [43, 50] confirms the DESs-extracted PCG and SWG lignin purity.

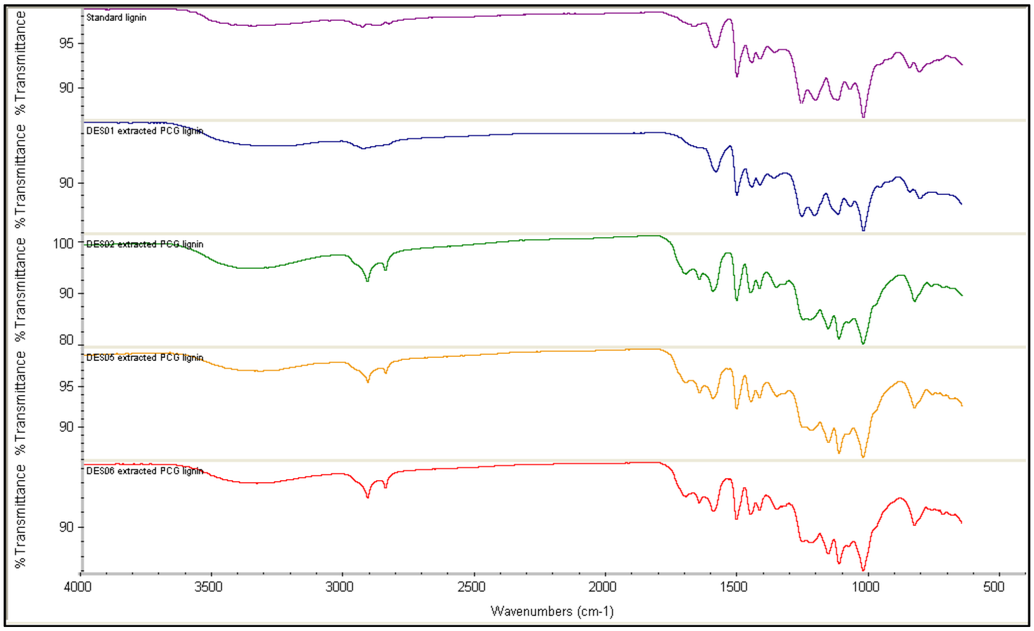

Figure 6.6. Overlaid FTIR spectra of standard lignin and DESs-extracted lignin from PCG.

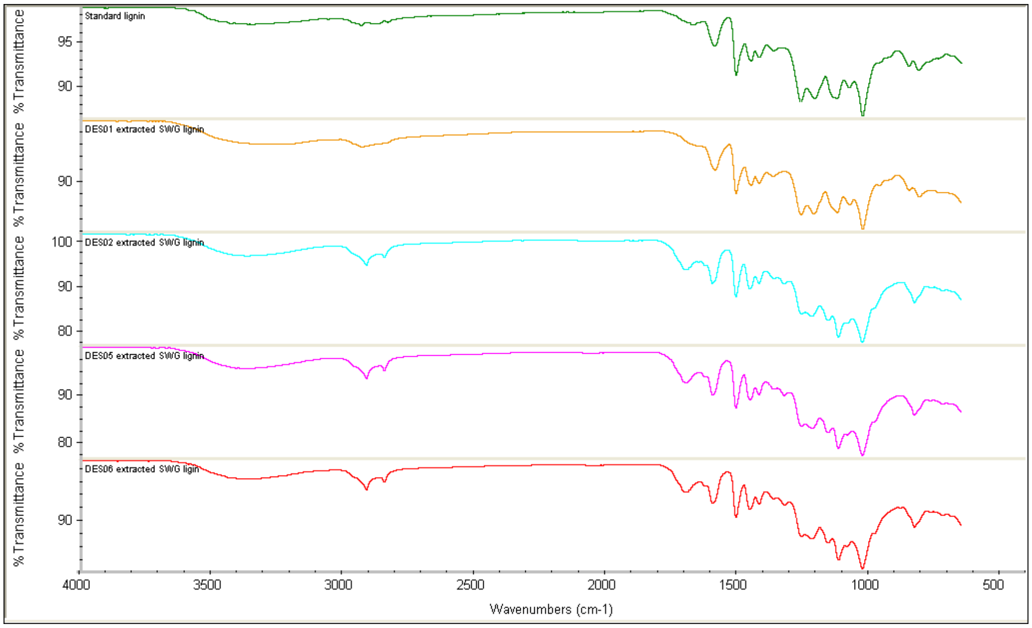

Figure 6.7. Overlaid FTIR spectra of standard lignin and DESs-extracted lignin from SWG.

^{1}H NMR spectroscopy is one of the best tools to check the structural similarities and difference between DESs-extracted lignin and the lignin standard. Figures 6.8 and 6.9 shows the similarities in the spectral profiles and lignin characteristic peaks at δ 1.26 (hydrocarbon contaminant), 2.62 (methylene protons), 3.78 (methoxy groups protons), 3.89 (hydroxyl groups protons), 6.27 (cinnayl alcohol protons) and 7.00 (aromatic protons) confirms the DESs-extracted PCG and SWG lignin. The band at δ 2.05 is from solvent acetone-d6.

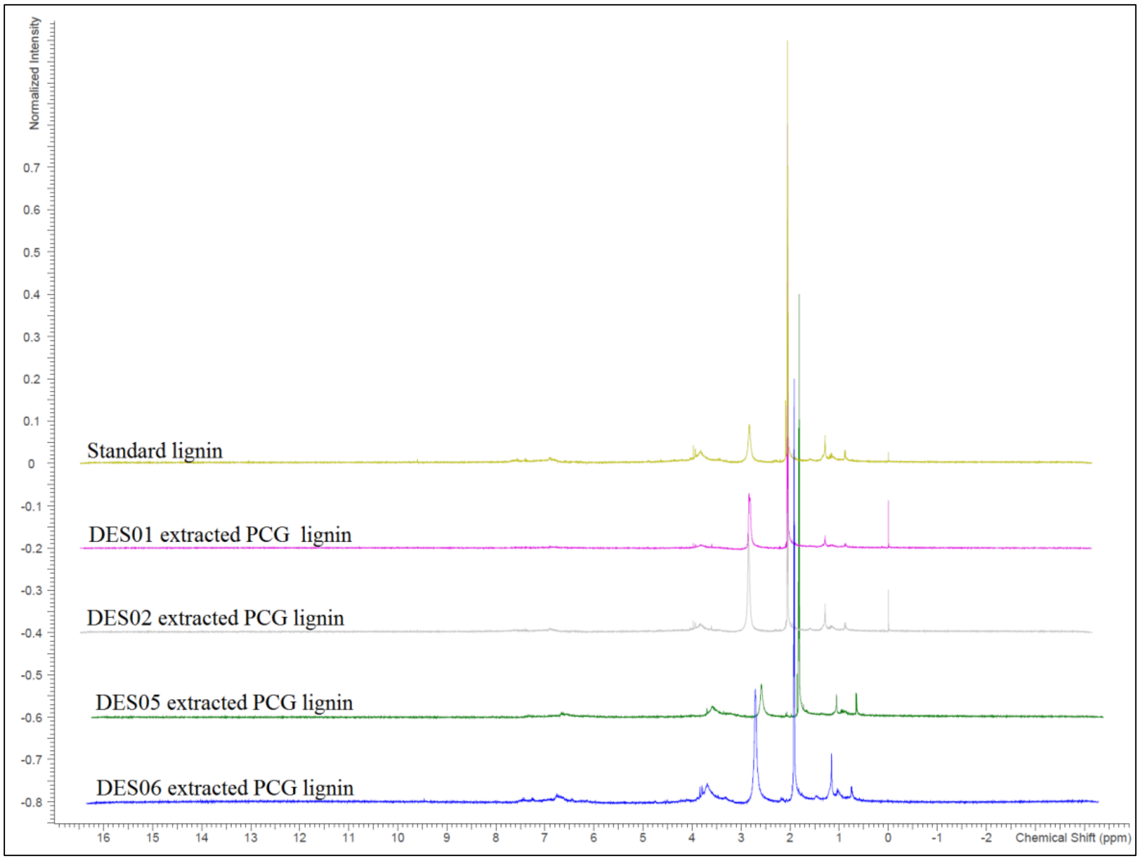

Figure 6.8. Overlaid ^{1}H NMR spectra of standard lignin and DESs-extracted lignin from PCG.

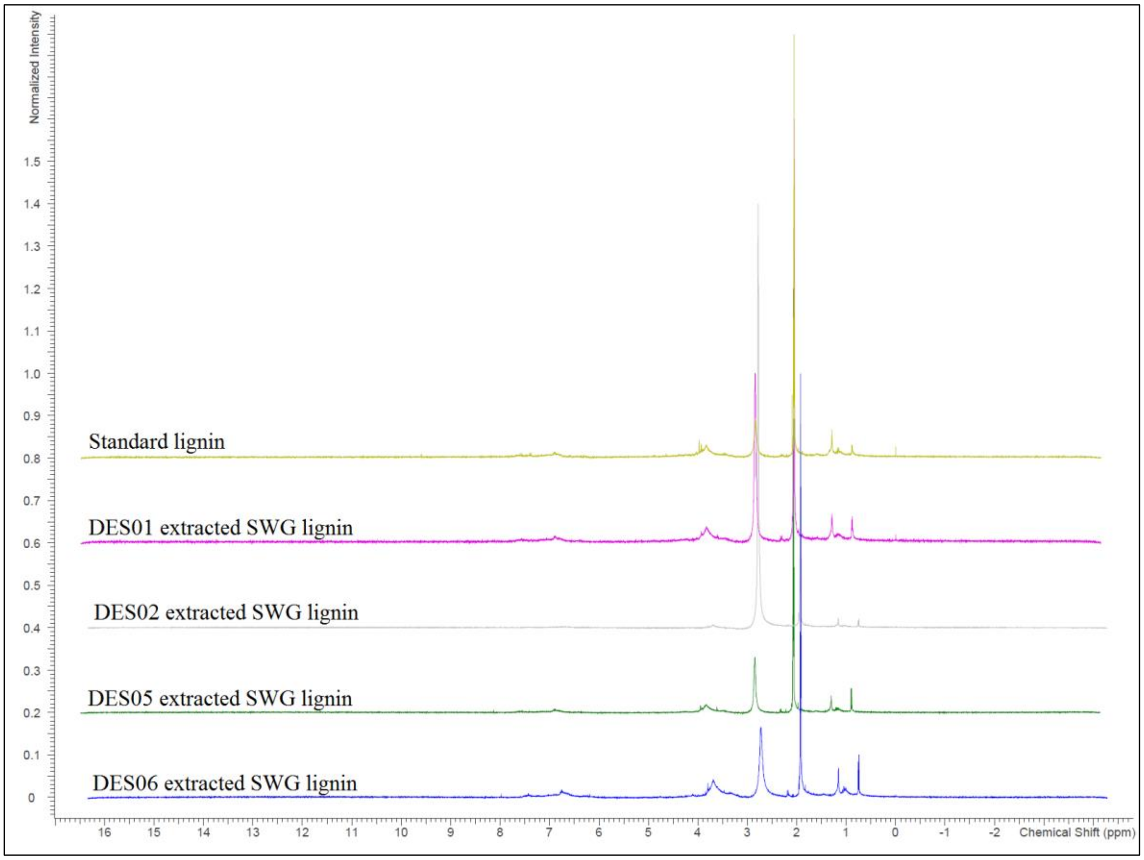

Figure 6.9. Overlaid ^{1}H NMR spectra of standard lignin and DESs-extracted lignin from SWG.

The DESs-extracted lignin FTIR and ^{1}H NMR spectra found to be slightly different compared to the spectra of lignin extracted in other conventional processes[288-290]. This may be due to the eutectic-solvent extraction being carried at a lower temperature with less harsh solvents compared to conventional methods. Thus, the lignin extracted using current method produced a new source of lignin that may convert to more valuable chemicals.

The percent lignin extraction of eutectics compared to the ionic liquids (ILs) extraction method to know the efficiency of the presently developed extraction method.

The percent lignin extraction of our method varied from 13.8 ± 0.7 to 16.6 ± 0.9, and 15.4 ± 0.7 to 18.7 ± 0.5 for PCG and SWG respectively at optimized conditions as shown in Figure 6.5. The percent lignin extraction of ILs method using the same source of PCG and SWG carried out in our lab found to be 20.5 ± 1.3 and 22.7 ± 1.8 respectively[290]. The percent lignin extraction with our method relative to the ILs method is presented in Figure 6.10. The results presented in Figure 6.10 indicates lower lignin extraction efficiency for eutectic systems (67.8 ± 3.21 to 81.0 ± 4.4 %) compared to the ILs method (100 %), but the former systems have the advantages of higher lignin selectivity, less harsh solvents and moderate extraction conditions.

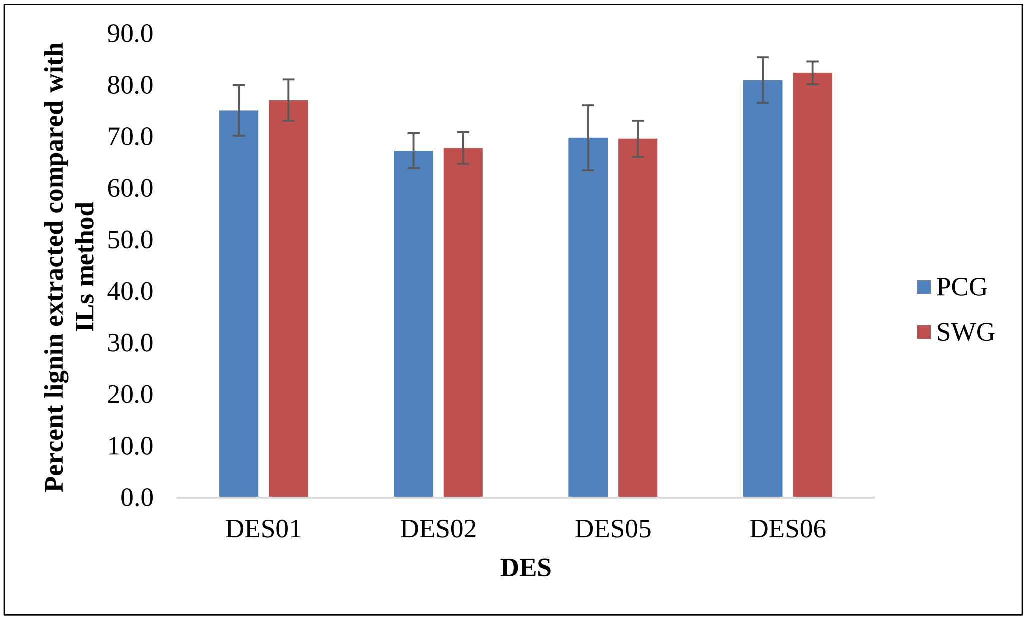

Figure 6.10. DESs lignin extraction efficiency comparison with ILs method from PCG and SWG biomass.

DESs biomass pretreatment differs in many ways with ILs as shown in Table 6.2. ILs dissolves both the lignin and cellulose of the biomass. After the use of ILs, the process involves precipitation of cellulose so it can be filtered off. The lignin is then recovered by acetone evaporation[291]. As explained in the flow chart in section 6.2.3, DESs selectively dissolves lignin and that avoids the extra step of cellulose precipitation and separation. The extracted lignin can be easily precipitated by adding an antisolvent.

Table 6.2. Differences between DESs and ILs pretreatment of lignocellulosic biomass.

Solvents	DESs	ILs
Lignin	Soluble	Soluble
Cellulose	Insoluble	Soluble
Recovery order	Lignin first	Cellulose first
Lignin recovery	Precipitation followed by solid-liquid separation	Evaporation of acetone

6.3.3 Recovery/Recycling of DESs

Recycling and reuse of the solvents used in the processes is an important aspect of green chemistry[8]. The biggest limitation of ILs in biofuel industry in terms of cost effectiveness is the inability to recycle and reuse them[292]. Therefore, in the present study, we have evaluated the recyclability and reusability of these deep eutectic solvents.

The important characteristic feature of the DESs under study is that their individual components are water soluble. The solvent power of the DESs will be reduced if water is added. Thus the water acts as a good antisolvent to precipitate the solutes out from eutectic solutions. Lignin was precipitated by adding 3:1 (v/v) ratio water to DESs. The precipitated lignin was removed by a simple solid/liquid separation. The eutectic mixtures were recovered by evaporating the water by using rotary evaporation until the mixture reached a constant weight, indicating the regeneration of the DESs.

The composition of the synthesized and the recycled DESs were checked by comparing the FTIR and ^{1}H NMR as shown in Figure 6.11 to 6.14, and the results confirm the purity of the recovered DESs due to their similar spectral profile.

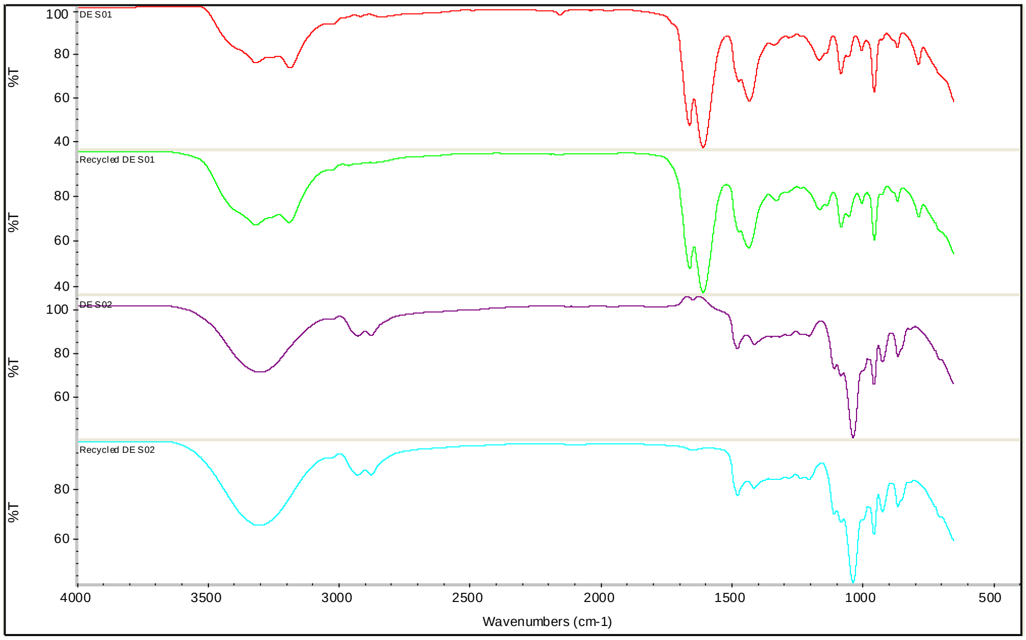

Figure 6.11. Comparison of FTIR spectra of freshly synthesized and recovered DES01 and DES02.

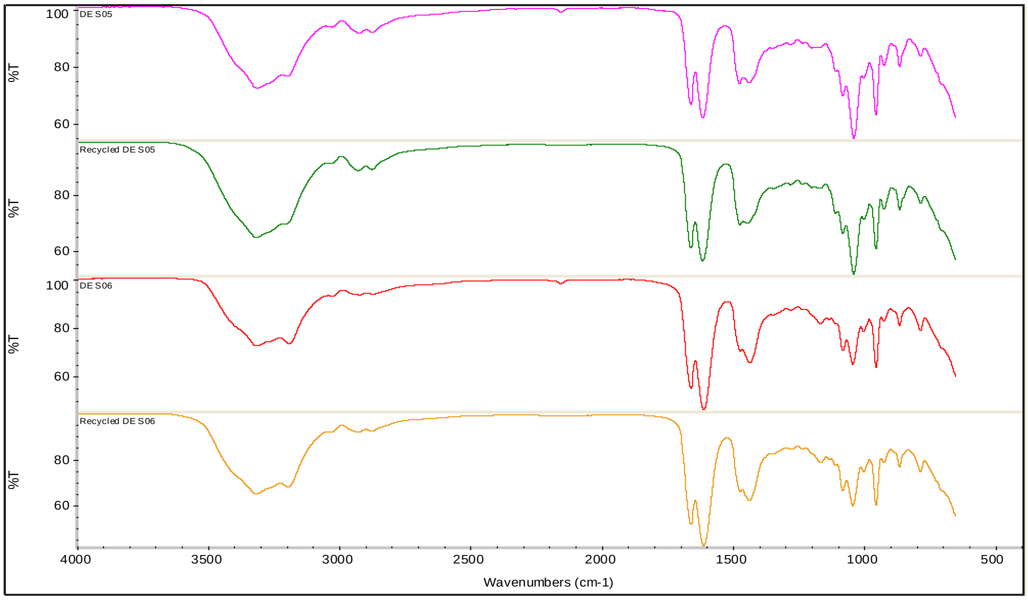

Figure 6.12. Comparison of FTIR spectra of freshly synthesized and recovered DES05 and DES06.

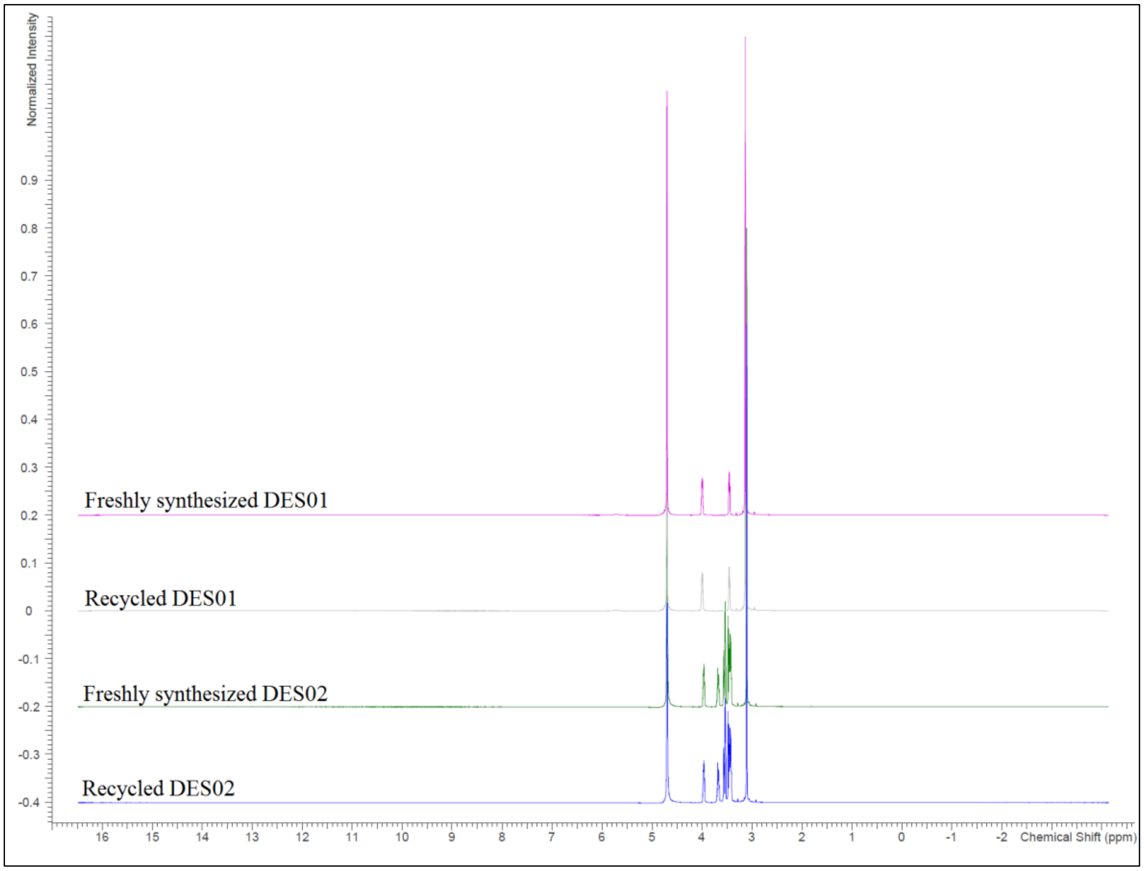

Figure 6.13. Comparison of ^{1}H NMR spectra of freshly synthesized and the recovered DES01 and DES02.

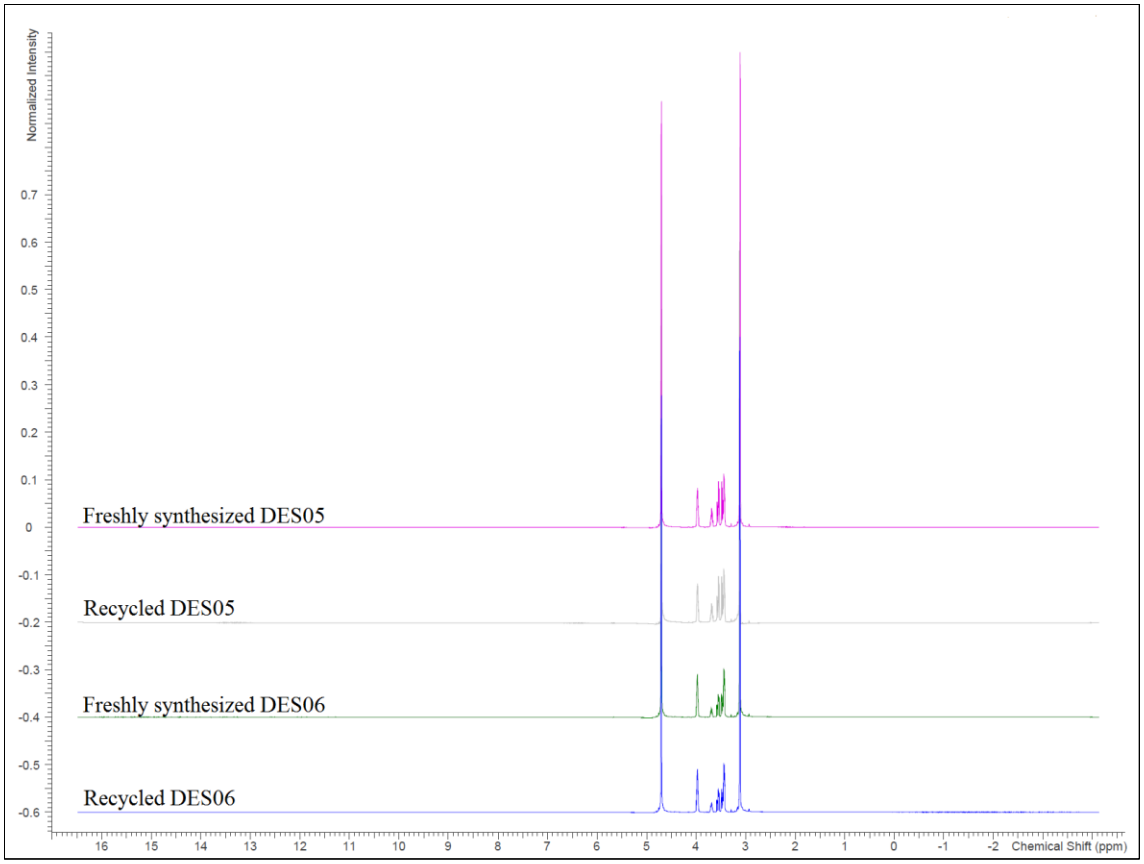

Figure 6.14. Comparison of ^{1}H NMR spectra of freshly synthesized and the recovered DES05 and DES06.

We have recycled the DESs three times and they continued to produce excellent results for lignin extraction as shown in Figure 6.15. The extraction efficiencies of the thrice recycled DESs was not less than 80 % of that of the freshly synthesized solvent which confirms the DESs can be recycled for three consecutive batches with acceptable loss of lignin extraction efficiency.

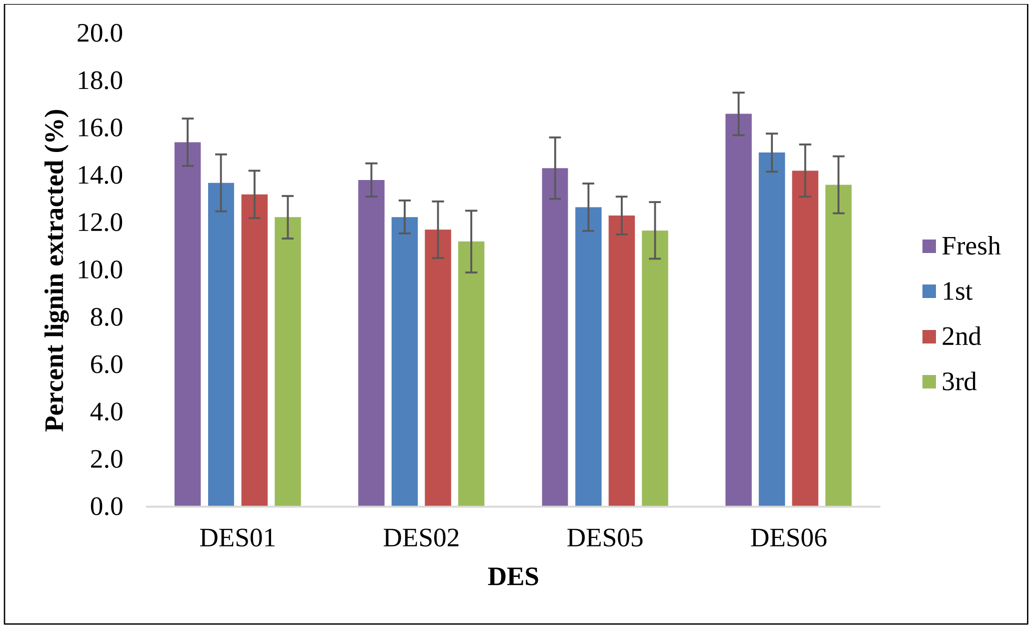

Figure 6.15. Percent lignin extraction of freshly synthesized, first, second and thrice recycled DESs.

6.4 Conclusions

The utilization of DESs for the dissolution of biopolymers such as cellulose, hemicellulose, and lignin was evaluated. The DESs selective dissolution of lignin further explored and an extraction method optimized to selectively extract lignin out of lignocellulosic biomass. The DESs lignin extraction up to 80 % from PCG and SWG biomass was achieved. The extracted lignin was confirmed by FTIR and ^{1}H NMR spectroscopy studies. A new source of DESs-extracted lignin was produced because the extraction was carried out with less harsh solvents at moderate temperature. The DESs were recovered simply by adding water and evaporation and reuse up to three times without losing much activity. The selective extraction of lignin and ease of recovery of eutectic solvents demonstrated that DESs pretreatment is a promising green procedure in the biofuel production from lignocellulosic biomass. As DESs research for this application is still in its infancy, further investigations are still needed to successfully apply DESs as solvents in pretreatment processes at large-scale industrial applications.

CHAPTER 7

7 CONCLUSIONS AND RECOMMENDATIONS

The overall goal of this dissertation was to design environmentally sustainable procedures in the production of biofuels. To achieve this goal, DESs made from inexpensive, non-toxic, and biodegradable components were selected, synthesized and studied. Their applicability for processing lignocellulosic biomass was explored.

The first part of the dissertation dealt with synthesizing sixteen different novel DESs by varying the components (ChCl, AcChCl, U, and G) and their composition, employing various methods such as such as mixing (vortex), heating and mixing (thermal treatment, microwave irradiation, sonication, and shaking incubation), and solvent evaporation (freeze drying and rotary evaporation). The DESs formation by solvent evaporation took longer than mixing, and heating and mixing took the least amount of time. The DESs synthesized by the different methods varied in the amount of water present, which affected their freezing point. All of the methods developed for DESs synthesis are considered “green” according to twelve principles of green chemistry because they required no prior or subsequent purification steps and no by-products were formed.

The freezing temperature of DESs was always found to be much lower than the melting point of their starting materials which is the characteristic feature of eutectic solvents. The freezing point of DESs depends on the composition and components of QASs and HBDs. Binary DESs composed of ChCl and G had lower freezing temperature than corresponding eutectics of AcChCl and U, and ternary eutectic systems composed of two QASs (ChCl and AcChCl) and one HBD (G) had lower freezing temperatures than eutectics composed of one QAS (ChCl or AcChCl) and two HBDs (U and G).

The shifts in the representative peaks and band broadening of the involved bonds in the FTIR spectra and the shift in the resonance signal upfield in ^{1}H-NMR spectra confirmed the formation of eutectics and hydrogen bonds in DESs. Further studies such as crystallography, Raman spectroscopy, and two-dimensional (2D) NMR are required to get a deeper understanding of hydrogen bond interactions and clearer insight into the building principles of formation of DESs.

The second part of the dissertation dealt with a comprehensive characterization of the synthesized DESs. DESs physicochemical and thermal properties including pH, density, refractive index (RI), surface tension, viscosity, octanol-water partition coefficient (K_{ow}), flammability, freezing temperature (T_f), and decomposition temperature (T_d) depended on their components and composition. Among the studied QASs and HBDs, the latter had the higher effect on most of the measured properties. A good agreement between the measured various properties of DESs was observed. Most of the measured physicochemical and thermal properties of DESs studied was comparable to the imidazolium ILs.

Miscibility and solvatochromic parameters including polarity and Kamlet-Taft parameters of synthesized DESs were determined. The studied DESs were miscible in polar protic solvents and immiscible in nonpolar solvents. Miscibility in polar aprotic solvents depend on the ability to form hydrogen bonds. These DESs fall into the category of polar HBA solvents due to their high E_T(NR) or E_T^N and β values. It is expected to application these DESs in many fields, such as organic synthesis, catalysis, electrochemistry, and extractive process in near future, due to their high polarity. DESs polarities E_T(NR) and E_N^T were comparable to short-chain alcohols and imidazolium ILs but Kamlet-Taft parameters α, β and π* were quite different. DESs polarities E_T(NR) and E_N^T and HBD acidity parameter α decreased, and HBA basicity parameter β, and dipolarity/polarizability parameter π* remain unchanged with an increase in temperature from 25 to 45 °C. DESs polarities E_T(NR) and E_N^T, HBD acidity parameter α and dipolarity/polarizability parameter π* are increased, and HBA basicity parameter β remain unchanged with an increase in water content from zero to 20 wt %. The equation of lines derived from the studies of effect of temperature and added water content to predict the DESs polarity and Kamlet-Taft parameters at higher temperature and water content.

Genotoxicity and cytotoxicity of DESs and their individual components were evaluated using the Ames test and LDH and MTT assays. A synergetic effect was observed because the toxicity of DESs was higher than their individual components. This has given a warning that attention has to be paid to the potential toxicity of both DESs and their components. Therefore the careful use of the terminology nontoxicity must be considered. The toxicity of these DESs was far less compared to imidazolium ILs at the studied conditions[257, 260].

For further studies of DESs characterization, the effect of variables such as temperature, water content, and dissolved gasses on different physicochemical and thermal properties needs to be investigated. DESs vapor pressure, biodegradability, and corrosion studies need to be carried out to classify them as sustainable solvents and for use in large-scale industrial applications. Developing computational models to predict the properties of eutectics solvents and their combinations and the effect of variables is suggested to save the time and resources. Toxicity studies at higher concentration of DESs, time, and different cell lines are strongly recommended to understand their toxicological behavior fully.

The last part of the dissertation deals with exploring the applications of DESs in the pretreatment of lignocellulosic biomass to develop sustainable processes by replacing molecular solvents and ILs. The DESs explored have preferential dissolution of lignin compared to cellulose and hemicellulose. The DESs extraction method to selectively extract lignin from lignocellulosic biomass was optimized. The DESs lignin extraction up to 80 % from PCG and SWG biomass was achieved. The extracted lignin was confirmed by FTIR and ^{1}H NMR spectroscopy studies. A new source of DESs-extracted lignin was produced because the extraction was carried out with less harsh solvents at moderate temperature. Comprehensive characterization of this new source of lignin is needed to convert it into value-added products. Selective lignin dissolution and extraction with DESs may have the potential to use these solvents in various applications, such as lignin valorization, pulp and paper making, cardboard industry, refining inedible crops, and cellulose hydrolysis. The DESs were recovered and reused up to three times while retaining more than 80 % of their activity. The selective extraction of lignin and ease of recovery of eutectic solvents demonstrated that DESs pretreatment is a promising sustainable procedure in the production of biofuels from lignocellulosic biomass. Lignin extraction efficiency with the studied DESs was less than previously reported methods using molecular solvents and ILs, but the eutectics have the advantage of selectivity, less harsh solvents and moderate extraction conditions.

For future pretreatment studies, combination of DESs with other technologies such ultrasonic or microwave irradiation are suggested to improve the lignin extraction efficiencies. The use of CO_2 is suggested to precipitate the lignin out of DESs in place of water even though water is a safe solvent, CO_2 stands out in terms of sustainability point of view. The search for novel DES compositions capable of dissolving cellulose will be undoubtedly a major research topic in the future. As DESs research for applications in this regard is still infancy, further investigations are still needed to successfully apply DESs in pretreatment processes for large-scale industrial applications. After fully developing the DESs pretreatment for the production of biofuels the economics needs to be evaluated because this information will be very vital in the scaling up of a process.

It is important to note that DESs not only offer undeniable advantages over other solvents regarding the sustainability point of view, but they also offer scientific advantages. We believe that DESs have a promising future and that their application potential is far from being thoroughly studied. We believe that conversion of renewable raw materials in deep eutectic solvent media is going to be a topic of investigation in our group for quite a long period.

8 REFERENCES

1. Nelson, W. M., Green solvents for chemistry. *Perspective and practice. New York, NY: Oxford University Press* **2003**.
2. Capello, C.; Fischer, U.; Hungerbuhler, K., What is a green solvent? A comprehensive framework for the environmental assessment of solvents. *Green Chemistry* **2007,** *9* (9), 927-934.
3. Anastas, P.; Warner, J., *Green Chemistry: Theory and Practice*. Oxford Univ Press: 1998; p 160 pp.
4. Welton, T., Room-temperature ionic liquids. Solvents for synthesis and catalysis. *Chemical reviews* **1999,** *99* (8), 2071-2084.
5. Wilkes, J. S., A short history of ionic liquids—from molten salts to neoteric solvents. *Green Chemistry* **2002,** *4* (2), 73-80.
6. Plechkova, N. V.; Seddon, K. R., Applications of ionic liquids in the chemical industry. *Chem Soc Rev* **2008,** *37* (1), 123-50.
7. Romero, A.; Santos, A.; Tojo, J.; Rodriguez, A., Toxicity and biodegradability of imidazolium ionic liquids. *Journal of Hazardous Materials* **2008,** *151* (1), 268-273.
8. Nockemann, P.; Binnemans, K.; Driesen, K., Purification of imidazolium ionic liquids for spectroscopic applications. *Chemical physics letters* **2005,** *415* (1), 131-136.
9. Seddon, K. R.; Stark, A.; Torres, M.-J., Influence of chloride, water, and organic solvents on the physical properties of ionic liquids. *Pure and Applied Chemistry* **2000,** *72* (12), 2275-2287.

10. Avalos, M.; Babiano, R.; Cintas, P.; Jimenez, J. L.; Palacios, J. C., Greener media in chemical synthesis and processing. *Angewandte Chemie International Edition* **2006,** *45* (24), 3904-3908.

11. Lobo, H. R.; Singh, B. S.; Shankarling, G. S., Deep eutectic solvents and glycerol: a simple, environmentally benign and efficient catalyst/reaction media for synthesis ofN-aryl phthalimide derivatives. *Green Chemistry Letters and Reviews* **2012,** *5* (4), 487-533.

12. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. *Journal of the American Chemical Society* **2004,** *126* (29), 9142-9147.

13. Mamajanov, I.; Engelhart, A. E.; Bean, H. D.; Hud, N. V., DNA and RNA in anhydrous media: duplex, triplex, and G-quadruplex secondary structures in a deep eutectic solvent. *Angew Chem Int Ed Engl* **2010,** *49* (36), 6310-4.

14. Abbott, A. The electrodeposition of composite materials using deep eutectic solvents. Ph.D. Dissertation, University of Leicester, Leicester, 2011.

15. Zhang, Q.; Vigier, K. D. O.; Royer, S.; Jérôme, F., Deep eutectic solvents: syntheses, properties and applications. *Chemical Society Reviews* **2012,** *41* (21), 7108-7146.

16. Atkins, P.; De Paula, J., Atkins' physical chemistry. *Eight edition) WH Freeman and Company, New York* **2006**.

17. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep eutectic solvents (DESs) and their applications. *Chem Rev* **2014,** *114* (21), 11060-82.

18. Abbott, A. P.; Al-Barzinjy, A. A.; Abbott, P. D.; Frisch, G.; Harris, R. C.; Hartley, J.; Ryder, K. S., Speciation, physical and electrolytic properties of eutectic mixtures based on CrCl 3· 6H 2 O and urea. *Physical Chemistry Chemical Physics* **2014,** *16* (19), 9047-9055.

19. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Shikotra, P., Selective extraction of metals from mixed oxide matrixes using choline-based ionic liquids. *Inorganic chemistry* **2005,** *44* (19), 6497-6499.

20. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications* **2003,** (1), 70-71.

21. Harris, R. C. Physical Properties of Alcohol Based Deep Eutectic Solvents. Ph.D Dissertation, University of Leicester, Leicester, 2009.

22. https://wet.kuleuven.be/english/summerschools/ionicliquids/lectures/abbott.pdf, Accessed July 15, 2015.

23. http://davyprotech.com/what-we-do/licensed-processes-and-core-technologies/licensed-processes/choline-chloride/specification/, Accessed July 15, 2015.

24. Sheldon, R. A., E factors, green chemistry and catalysis: an odyssey. *Chemical Communications* **2008,** (29), 3352-3365.

25. Abbott, A.; Davies, D., Salty solvents--Ionic really. *Education in Chemistry* **2005,** *42* (1), 12-13.

26. Allen, D. T.; Shonnard, D. R., *Green engineering: environmentally conscious design of chemical processes*. Pearson Education: 2001.

27. Petkovic, M.; Ferguson, J. L.; Gunaratne, H. Q. N.; Ferreira, R.; Leitão, M. C.; Seddon, K. R.; Rebelo, L. P. N.; Pereira, C. S., Novel biocompatible cholinium-based ionic liquids—toxicity and biodegradability. *Green Chemistry* **2010,** *12* (4), 643.

28. Himmelheber, A. M.; Sarter, M.; Bruno, J. P., Increases in cortical acetylcholine release during sustained attention performance in rats. *Cognitive Brain Research* **2000,** *9* (3), 313-325.

29. de María, P. D.; Maugeri, Z., Ionic liquids in biotransformations: from proof-of-concept to emerging deep-eutectic-solvents. *Current opinion in chemical biology* **2011,** *15* (2), 220-225.

30. Tan, H.; Aziz, A. A.; Aroua, M., Glycerol production and its applications as a raw material: A review. *Renewable and Sustainable Energy Reviews* **2013,** *27*, 118-127.

31. Pagliaro, M.; Ciriminna, R.; Kimura, H.; Rossi, M.; Della Pina, C., From Glycerol to Value-Added Products. *Angewandte Chemie International Edition* **2007,** *46* (24), 4434-4440.

32. Padmanabhan Ramalekshmi Thanu, D. Use of dilute hydrofluoric acid and deep eutectic solvent systems for back end of line cleaning in integrated circuit fabrication. Ph.D Dissertation, The University of Arizona, Ann Arbor, 2011.

33. https://www.fishersci.ca/viewmsds.do?catNo=O1972250, Accessed July 15, 2015.

34. https://www.fishersci.ca/viewmsds.do?catNo=AC159171000, Accessed July 15, 2015.

35. https://www.fishersci.com/shop/products/urea-99-acs-reagent-acros-organics-4/p-210696, Accessed July 15, 2015.

36. https://uwaterloo.ca/giga-to-nanoelectronics-centre/sites/ca.giga-to-nanoelectronics-centre/files/uploads/files/glycerine.pdf, Accessed July 15, 2015.

37. Deetlefs, M.; Seddon, K. R., Assessing the greenness of some typical laboratory ionic liquid preparations. *Green Chemistry* **2010,** *12* (1), 17-30.

38. GORKE, J. T.; SRIENC, F.; KAZLAUSKAS, R. J. In *Deep eutectic solvents for Candida antarctica lipase B-catalyzed reactions*, ACS symposium series, Oxford University Press: 2010; pp 169-180.

39. Shamsuri, A. A.; Abdullah, D. K., Ionic liquids: Preparations and limitations. *Makara, Sains* **2010,** *14*, 101-106.

40. Chen, Z.; Zhu, W.; Zheng, Z.; Zou, X., One-pot α-nucleophilic fluorination of acetophenones in a deep eutectic solvent. *Journal of Fluorine Chemistry* **2010,** *131* (3), 340-344.

41. Abbott, A. P.; McKenzie, K. J., Application of ionic liquids to the electrodeposition of metals. *Phys Chem Chem Phys* **2006,** *8* (37), 4265-79.

42. Zhao, H.; Zhang, C.; Crittle, T. D., Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil. *Journal of Molecular Catalysis B: Enzymatic* **2013,** *85-86*, 243-247.

43. Abbott, A. P.; Cullis, P. M.; Gibson, M. J.; Harris, R. C.; Raven, E., Extraction of glycerol from biodiesel into a eutectic based ionic liquid. *Green Chemistry* **2007,** *9* (8), 868-872.

44. Yanai, T.; Shiraishi, K.; Shimokawa, T.; Watanabe, Y.; Ohgai, T.; Nakano, M.; Suzuki, K.; Fukunaga, H., Electroplated Fe films prepared from a deep eutectic solvent. *Journal of Applied Physics* **2014,** *115* (17), 17A344.

45. Abbott, A. P.; Nandhra, S.; Postlethwaite, S.; Smith, E. L.; Ryder, K. S., Electroless deposition of metallic silver from a choline chloride-based ionic liquid: a study using acoustic impedance spectroscopy, SEM and atomic force microscopy. *Phys Chem Chem Phys* **2007,** *9* (28), 3735-43.

46. Ali, M. R.; Rahman, M. Z.; Saha, S. S., Electroless and electrolytic deposition of nickel from deep eutectic solvents based on choline chloride. *Indian J. Chem. Technol.* **2014,** *21* (2), 127-133.

47. Xu, C.; Wu, Q.; Hua, Y.; Li, J., The electrodeposition of Zn-Ti alloys from ZnCl2-urea deep eutectic solvent. *Journal of Solid State Electrochemistry* **2014,** *18* (8), 2149-2155.

48. Abbott, A. P.; Capper, G.; McKenzie, K. J.; Ryder, K. S., Electrodeposition of zinc–tin alloys from deep eutectic solvents based on choline chloride. *Journal of Electroanalytical Chemistry* **2007,** *599* (2), 288-294.

49. Abbott, A. P.; Barron, J. C.; Ryder, K. S.; Wilson, D., Eutectic-based ionic liquids with metal-containing anions and cations. *Chemistry* **2007,** *13* (22), 6495-501.

50. Pereira, N. M.; Fernandes, P. M.; Pereira, C. M.; Silva, A. F., Electrodeposition of zinc from choline chloride-ethylene glycol deep eutectic solvent: effect of the tartrate ion. *Journal of The Electrochemical Society* **2012,** *159* (9), D501-D506.

51. Gao, Y.; Hu, W.; Gao, X.; Duan, B., Electrodeposition of SnBi coatings based on deep eutectic solvent. *Surf. Eng.* **2014,** *30* (1), 59-63.

52. Abbott, A. P.; El Ttaib, K.; Frisch, G.; Ryder, K. S.; Weston, D., The electrodeposition of silver composites using deep eutectic solvents. *Phys Chem Chem Phys* **2012,** *14* (7), 2443-9.

53. You, Y.; Gu, C.; Wang, X.; Tu, J., Electrodeposition of Ni–Co alloys from a deep eutectic solvent. *Surface and Coatings Technology* **2012,** *206* (17), 3632-3638.

54. Niu, G.; Yang, S.; Li, H.; Yi, J.; Wang, M.; Lv, X.; Zhong, J., Electrodeposition of Cu-Ga Precursor Layer from Deep Eutectic Solvent for CuGaS2 Solar Energy Thin Film. *Journal of the Electrochemical Society* **2014,** *161* (6), D333-D338.

55. De Vreese, P.; Skoczylas, A.; Matthijs, E.; Fransaer, J.; Binnemans, K., Electrodeposition of copper–zinc alloys from an ionic liquid-like choline acetate electrolyte. *Electrochimica Acta* **2013,** *108*, 788-794.

56. Liao, J.-H.; Wu, P.-C.; Bai, Y.-H., Eutectic mixture of choline chloride/urea as a green solvent in synthesis of a coordination polymer: [Zn(O3PCH2CO2)]·NH4. *Inorganic Chemistry Communications* **2005,** *8* (4), 390-392.

57. Gutiérrez, M. a. C.; Rubio, F.; del Monte, F., Resorcinol-Formaldehyde Polycondensation in Deep Eutectic Solvents for the Preparation of Carbons and Carbon−Carbon Nanotube Composites. *Chemistry of Materials* **2010,** *22* (9), 2711-2719.

58. Morrison, H. G.; Sun, C. C.; Neervannan, S., Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. *Int. J. Pharm.* **2009,** *378* (1-2), 136-139.

59. Gutierrez, M. C.; Ferrer, M. L.; Mateo, C. R.; del Monte, F., Freeze-drying of aqueous solutions of deep eutectic solvents: a suitable approach to deep eutectic suspensions of self-assembled structures. *Langmuir* **2009,** *25* (10), 5509-15.

60. Gutierrez, M. C.; Ferrer, M. L.; Yuste, L.; Rojo, F.; del Monte, F., Bacteria incorporation in deep-eutectic solvents through freeze-drying. *Angew Chem Int Ed Engl* **2010,** *49* (12), 2158-62.

61. Li, X.; Hou, M.; Zhang, Z.; Han, B.; Yang, G.; Wang, X.; Zou, L., Absorption of CO 2 by ionic liquid/polyethylene glycol mixture and the thermodynamic parameters. *Green Chemistry* **2008,** *10* (8), 879-884.

62. Lu, M.; Han, G.; Jiang, Y.; Zhang, X.; Deng, D.; Ai, N., Solubilities of carbon dioxide in the eutectic mixture of levulinic acid (or furfuryl alcohol) and choline chloride. *The Journal of Chemical Thermodynamics* **2015,** *88*, 72-77.

63. Leron, R. B.; Li, M.-H., Solubility of carbon dioxide in a choline chloride–ethylene glycol based deep eutectic solvent. *Thermochimica Acta* **2013,** *551*, 14-19.

64. Leron, R. B.; Li, M.-H., Solubility of carbon dioxide in a eutectic mixture of choline chloride and glycerol at moderate pressures. *The Journal of Chemical Thermodynamics* **2013,** *57*, 131-136.

65. Li, X.; Hou, M.; Han, B.; Wang, X.; Zou, L., Solubility of CO2 in a choline chloride+ urea eutectic mixture. *Journal of Chemical & Engineering Data* **2008,** *53* (2), 548-550.

66. Ali, E.; Hadj-Kali, M. K.; Mulyono, S.; Alnashef, I.; Fakeeha, A.; Mjalli, F.; Hayyan, A., Solubility of CO2 in deep eutectic solvents: Experiments and modelling using the Peng–Robinson equation of state. *Chemical Engineering Research and Design* **2014,** *92* (10), 1898-1906.

67. Claes, P.; Thirion, B.; Glibert, J., Solubility of CO 2 in the molten NA 2 CO 3—K 2 CO 3 (42 mol%) eutectic mixture at 800° C. *Electrochimica acta* **1996,** *41* (1), 141-146.

68. Gu, C.; You, Y.; Yu, Y.; Qu, S.; Tu, J., Microstructure, nanoindentation, and electrochemical properties of the nanocrystalline nickel film electrodeposited from choline chloride–ethylene glycol. *Surface and Coatings Technology* **2011,** *205* (21), 4928-4933.

69. Gu, C.; Tu, J., One-step fabrication of nanostructured Ni film with lotus effect from deep eutectic solvent. *Langmuir* **2011,** *27* (16), 10132-40.

70. Gu, C.-D.; Tu, J.-P., Thermochromic behavior of chloro-nickel(II) in deep eutectic solvents and their application in thermochromic composite films. *RSC Advances* **2011,** *1* (7), 1220.

71. Serrano, M. C.; Gutierrez, M. C.; Jimenez, R.; Ferrer, M. L.; del Monte, F., Synthesis of novel lidocaine-releasing poly(diol-co-citrate) elastomers by using deep eutectic solvents. *Chem Commun (Camb)* **2012,** *48* (4), 579-81.

72. Borse, B. N.; Shukla, S. R.; Sonawane, Y. A.; Shankerling, G. S., Synthesis of Some Novel Pyrimidinedione and Pyrimidinetrione Derivatives by a Greener Method: Study of Their Antimicrobial Activity and Photophysical Properties. *Synthetic Communications* **2013,** *43* (6), 865-876.

73. Dai, D.-y.; Wang, L.; Chen, Q.; He, M.-Y., Selective oxidation of sulfides to sulfoxides catalysed by deep eutectic solvent with H2O2. *Journal of Chemical Research* **2014,** *38* (3), 183-185.

74. Carriazo, D.; Serrano, M. C.; Gutiérrez, M. C.; Ferrer, M. L.; del Monte, F., Deep-eutectic solvents playing multiple roles in the synthesis of polymers and related materials. *Chemical Society Reviews* **2012,** *41* (14), 4996-5014.

75. Krystof, M.; Perez-Sanchez, M.; Dominguez de Maria, P., Lipase-catalyzed (trans)esterification of 5-hydroxy- methylfurfural and separation from HMF esters using deep-eutectic solvents. *ChemSusChem* **2013,** *6* (4), 630-4.

76. Pöhnlein, M.; Ulrich, J.; Kirschhöfer, F.; Nusser, M.; Muhle-Goll, C.; Kannengiesser, B.; Brenner-Weiß, G.; Luy, B.; Liese, A.; Syldatk, C.; Hausmann, R., Lipase-catalyzed synthesis of glucose-6-O-hexanoate in deep eutectic solvents. *European Journal of Lipid Science and Technology* **2015,** *117* (2), 161-166.

77. Zhao, H.; Baker, G. A.; Holmes, S., Protease activation in glycerol-based deep eutectic solvents. *Journal of Molecular Catalysis B: Enzymatic* **2011,** *72* (3), 163-167.

78. Alonso, D. A.; Baeza, A.; Chinchilla, R.; Guillena, G.; Pastor, I. M.; Ramón, D. J., Deep Eutectic Solvents: The Organic Reaction Medium of the Century. *European Journal of Organic Chemistry* **2016**.

79. Cooper, E. R.; Andrews, C. D.; Wheatley, P. S.; Webb, P. B.; Wormald, P.; Morris, R. E., Ionic liquids and eutectic mixtures as solvent and template in synthesis of zeolite analogues. *Nature* **2004,** *430* (7003), 1012-1016.

80. Liao, H. G.; Jiang, Y. X.; Zhou, Z. Y.; Chen, S. P.; Sun, S. G., Shape-Controlled Synthesis of Gold Nanoparticles in Deep Eutectic Solvents for Studies of Structure–Functionality Relationships in Electrocatalysis. *Angewandte Chemie* **2008,** *120* (47), 9240-9243.

81. Stassi, S.; Cauda, V.; Canavese, G.; Manfredi, D.; Pirri, C. F., Synthesis and Characterization of Gold Nanostars as Filler of Tunneling Conductive Polymer Composites. *European Journal of Inorganic Chemistry* **2012,** *2012* (16), 2669-2673.

82. Kareem, M. A.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M., Liquid–liquid equilibria for the ternary system (phosphonium based deep eutectic solvent–benzene–hexane) at different temperatures: A new solvent introduced. *Fluid Phase Equilibria* **2012,** *314*, 52-59.

83. Hayyan, M.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M., A novel technique for separating glycerine from palm oil-based biodiesel using ionic liquids. *Fuel Processing Technology* **2010,** *91* (1), 116-120.

84. Abo-Hamad, A.; Hayyan, M.; AlSaadi, M. A.; Hashim, M. A., Potential applications of deep eutectic solvents in nanotechnology. *Chemical Engineering Journal* **2015,** *273*, 551-567.

85. Tang, B.; Row, K. H., Exploration of deep eutectic solvent-based mesoporous silica spheres as high-performance size exclusion chromatography packing materials. *Journal of Applied Polymer Science* **2015,** *132* (27), n/a-n/a.

86. Karimi, M.; Dadfarnia, S.; Shabani, A. M. H., Application of Deep Eutectic Solvent Modified Cotton as a Sorbent for Online Solid-Phase Extraction and Determination of Trace Amounts of Copper and Nickel in Water and Biological Samples. *Biological Trace Element Research* **2016**, 1-9.

87. Sirviö, J. A.; Visanko, M.; Liimatainen, H., Deep eutectic solvent system based on choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose. *Green Chem.* **2015**.

88. Helalat-Nezhad, Z.; Ghanemi, K.; Fallah-Mehrjardi, M., Dissolution of biological samples in deep eutectic solvents: An approach for extraction of polycyclic aromatic hydrocarbons followed by liquid chromatography-fluorescence detection. *J Chromatogr A* **2015,** *1394*, 46-53.

89. Vigier, K. D. O.; Chatel, G.; Jérôme, F., Contribution of Deep Eutectic Solvents for Biomass Processing: Opportunities, Challenges, and Limitations. *ChemCatChem* **2015,** *7* (8), 1250-1260.

90. Abbott, A. P.; Alaysuy, O.; Antunes, A. P. M.; Douglas, A. C.; Guthrie-Strachan, J.; Wise, W. R., Processing of Leather Using Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering* **2015**, 150427145725007.

91. Tenhunen, T.-M.; Hakalahti, M.; Kouko, J.; Salminen, A.; Härkäsalmi, T.; Pere, J.; Harlin, A.; Hänninen, T., Method for Forming Pulp Fibre Yarns Developed by a Design-driven Process. *BioResources* **2016,** *11* (1), 2492-2503.

92. Toennesen, H. H.; Wikene, K. O. Eutectic solvents for use in killing of bacteria. WO2016108083A1, 2016.

93. Demirbas, A., Progress and recent trends in biodiesel fuels. *Energy conversion and management* **2009,** *50* (1), 14-34.

94. Ziolkowska, J. R., Prospective technologies, feedstocks and market innovations for ethanol and biodiesel production in the US. *Biotechnology Reports* **2014,** *4*, 94-98.

95. Wyman, C., *Handbook on bioethanol: production and utilization*. CRC press: 1996.

96. http://genomicsgtl.energy.gov/biofuels/2005workshop/b2blowres63006.pdf, Accessed July 15, 2015.

97. DING, S., Overview of Lignocellulose: Structure and Chemistry. *Biological Concerstion of Biomass for Fuels and Chemicals: Exploration from Natural Utilization Systems* **2013,** *10*, 14.

98. Fu, D.; Mazza, G.; Tamaki, Y., Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. *J Agric Food Chem* **2010,** *58* (5), 2915-22.

99. Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P., Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel

production. *Industrial & engineering chemistry research* **2009,** *48* (8), 3713-3729.

100. Agbor, V. B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D. B., Biomass pretreatment: fundamentals toward application. *Biotechnology advances* **2011,** *29* (6), 675-685.

101. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.; Holtzapple, M.; Ladisch, M., Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology* **2005,** *96* (6), 673-686.

102. Gorke, J.; Srienc, F.; Kazlauskas, R., Toward advanced ionic liquids. Polar, enzyme-friendly solvents for biocatalysis. *Biotechnology and Bioprocess Engineering* **2010,** *15* (1), 40-53.

103. Dai, Y.; van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H., Natural deep eutectic solvents as new potential media for green technology. *Analytica chimica acta* **2013,** *766*, 61-68.

104. Burrell, A. K.; Del Sesto, R. E.; Baker, S. N.; McCleskey, T. M.; Baker, G. A., The large scale synthesis of pure imidazolium and pyrrolidinium ionic liquids. *Green Chemistry* **2007,** *9* (5), 449-454.

105. Duan, Z.; Gu, Y.; Zhang, J.; Zhu, L.; Deng, Y., Protic pyridinium ionic liquids: Synthesis, acidity determination and their performances for acid catalysis. *Journal of Molecular Catalysis A: Chemical* **2006,** *250* (1), 163-168.

106. Gorke, J. T.; Srienc, F.; Kazlauskas, R. J., Hydrolase-catalyzed biotransformations in deep eutectic solvents. *Chem Commun (Camb)* **2008,** (10), 1235-7.

107. Parnham, E. R.; Drylie, E. A.; Wheatley, P. S.; Slawin, A. M. Z.; Morris, R. E., Ionothermal Materials Synthesis Using Unstable Deep-Eutectic Solvents as Template-Delivery Agents. *Angewandte Chemie* **2006,** *118* (30), 5084-5088.

108. Aissaoui, T.; Al Nashef, I. M.; Hayyan, M.; Hashim, M. A., Neoteric FT-IR investigation on the functional groups of phosphonium-based deep eutectic solvents. *Spectrochim. Acta, Part A* **2015**, Ahead of Print.

109. Florindo, C.; Oliveira, F. S.; Rebelo, L. P. N.; Fernandes, A. M.; Marrucho, I. M., Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry & Engineering* **2014,** *2* (10), 2416-2425.

110. Ruß, C.; König, B., Low melting mixtures in organic synthesis–an alternative to ionic liquids? *Green Chemistry* **2012,** *14* (11), 2969-2982.

111. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixturesElectronic supplementary information (ESI) available: spectroscopic data. See http://www.rsc.org/suppdata/cc/b2/b210714g. *Chemical Communications* **2003,** (1), 70-71.

112. Abbott, A. P.; Harris, R. C.; Ryder, K. S.; D'Agostino, C.; Gladden, L. F.; Mantle, M. D., Glycerol eutectics as sustainable solvent systems. *Green Chem.* **2011,** *13* (1), 82-90.

113. Yue, D.; Jia, Y.; Yao, Y.; Sun, J.; Jing, Y., Structure and electrochemical behavior of ionic liquid analogue based on choline chloride and urea. *Electrochimica Acta* **2012,** *65*, 30-36.

114. Li, N.; Wang, Y.; Xu, K.; Huang, Y.; Wen, Q.; Ding, X., Development of green betaine-based deep eutectic solvent aqueous two-phase system for the extraction of protein. *Talanta* **2016,** *152*, 23-32.

115. Xu, G. C.; Ding, J. C.; Han, R. Z.; Dong, J. J.; Ni, Y., Enhancing cellulose accessibility of corn stover by deep eutectic solvent pretreatment for butanol fermentation. *Bioresour Technol* **2016,** *203*, 364-9.

116. Xu, G.-C.; Ding, J.-C.; Han, R.-Z.; Dong, J.-J.; Ni, Y., Enhancing cellulose accessibility of corn stover by deep eutectic solvent pretreatment for butanol fermentation. *Bioresour. Technol.* **2016,** *203*, 364-369.

117. Dobrică, I.; Bugheanu, P.; Stănculescu, I.; Ponta, C., FTIR spectral data of wood used in Romanian traditional village constructions. *Ann. Univ. Buc. Chim* **2008,** *17*, 33-37.

118. Hayyan, M.; Abo-Hamad, A.; AlSaadi, M. A.; Hashim, M. A., Functionalization of graphene using deep eutectic solvents. *Nanoscale Res Lett* **2015,** *10* (1), 1004.

119. AlOmar, M. K.; Hayyan, M.; Alsaadi, M. A.; Akib, S.; Hayyan, A.; Hashim, M. A., Glycerol-based deep eutectic solvents: Physical properties. *J. Mol. Liq.* **2016,** *215*, 98-103.

120. Pandey, A.; Pandey, S., Solvatochromic probe behavior within choline chloride-based deep eutectic solvents: effect of temperature and water. *J Phys Chem B* **2014,** *118* (50), 14652-61.

121. Ping, Z.; Nguyen, Q.; Chen, S.; Zhou, J.; Ding, Y., States of water in different hydrophilic polymers—DSC and FTIR studies. *Polymer* **2001,** *42* (20), 8461-8467.

122. Zeng, Q.; Wang, Y.; Huang, Y.; Ding, X.; Chen, J.; Xu, K., Deep eutectic solvents as novel extraction media for protein partitioning. *Analyst* **2014,** *139* (10), 2565-73.

123. Zeng, C.-X.; Qi, S.-J.; Xin, R.-P.; Yang, B.; Wang, Y.-H., Synergistic behavior of betaine-urea mixture: Formation of deep eutectic solvent. *J. Mol. Liq.* **2016,** *219*, 74-78.

124. Dai, Y.; Witkamp, G. J.; Verpoorte, R.; Choi, Y. H., Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chem* **2015,** *187*, 14-9.

125. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C., *Spectrometric Identification of Organic Compounds. 5th Ed*. John Wiley and Sons, Inc.: 1991; p 419 pp.

126. Dai, Y.; van Spronsen, J.; Witkamp, G. J.; Verpoorte, R.; Choi, Y. H., Natural deep eutectic solvents as new potential media for green technology. *Anal Chim Acta* **2013,** *766*, 61-8.

127. D'Agostino, C.; Harris, R. C.; Abbott, A. P.; Gladden, L. F.; Mantle, M. D., Molecular motion and ion diffusion in choline chloride based deep eutectic solvents studied by 1H pulsed field gradient NMR spectroscopy. *Phys Chem Chem Phys* **2011,** *13* (48), 21383-91.

128. Hadj-Kali, M. K.; Al-khidir, K. E.; Wazeer, I.; El-blidi, L.; Mulyono, S.; AlNashef, I. M., Application of deep eutectic solvents and their individual constituents as surfactants for enhanced oil recovery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2015,** *487*, 221-231.

129. Gorke, J. T. Application of deep eutectic solvents and ionic liquids to hydrolase-catalyzed reactions. Ph.D. Dissertation, University of Minnesota, Minneapolis, 2010.

130. Guo, W.; Hou, Y.; Ren, S.; Tian, S.; Wu, W., Formation of Deep Eutectic Solvents by Phenols and Choline Chloride and Their Physical Properties. *Journal of Chemical & Engineering Data* **2013,** *58* (4), 866-872.

131. Hayyan, A.; Mjalli, F. S.; AlNashef, I. M.; Al-Wahaibi, T.; Al-Wahaibi, Y. M.; Hashim, M. A., Fruit sugar-based deep eutectic solvents and their physical properties. *Thermochimica Acta* **2012,** *541*, 70-75.

132. Bahadori, L.; Chakrabarti, M. H.; Mjalli, F. S.; AlNashef, I. M.; Manan, N. S. A.; Hashim, M. A., Physicochemical properties of ammonium-based deep eutectic solvents and their electrochemical evaluation using organometallic reference redox systems. *Electrochimica Acta* **2013,** *113*, 205-211.

133. Zhang, Y.; Lu, X.; Feng, X.; Shi, Y.; Ji, X., Properties and applications of choline-based deep eutectic solvents. *Huaxue Jinzhan* **2013**.

134. Fischer, V. Properties and applications of deep eutectic solvents and low-melting mixtures. University of Regensburg, Regensburg, 2015.

135. Mjalli, F. S.; Naser, J.; Jibril, B.; Alizadeh, V.; Gano, Z., Tetrabutylammonium Chloride Based Ionic Liquid Analogues and Their Physical Properties. *Journal of Chemical & Engineering Data* **2014,** *59* (7), 2242-2251.

136. Hayyan, M.; Aissaoui, T.; Hashim, M. A.; AlSaadi, M. A.; Hayyan, A., Triethylene glycol based deep eutectic solvents and their physical properties. *Journal of the Taiwan Institute of Chemical Engineers* **2015**.

137. Aparicio, S.; Atilhan, M.; Karadas, F., Thermophysical properties of pure ionic liquids: review of present situation. *Industrial & Engineering Chemistry Research* **2010,** *49* (20), 9580-9595.

138. Pontolillo, J.; Eganhouse, R. P., *The search for reliable aqueous solubility (Sw) and octanol-water partition coefficient (Kow) data for hydrophobic organic compounds: DDT and DDE as a case study*. US Department of the Interior, US Geological Survey Reston, Virginia: 2001.

139. Sangster, J., *Octanol-water partition coefficients: fundamentals and physical chemistry*. John Wiley & Sons: 1997.

140. Leo, A.; Hansch, C.; Elkins, D., Partition coefficients and their uses. *Chemical reviews* **1971,** *71* (6), 525-616.

141. Kosan, B.; Michels, C.; Meister, F., Dissolution and forming of cellulose with ionic liquids. *Cellulose* **2008,** *15* (1), 59-66.

142. Huddleston, J.; Rogers, R., Room temperature ionic liquids as novel media for 'clean'liquid–liquid extraction. *Chemical Communications* **1998,** (16), 1765-1766.

143. https://www.osha.gov/dte/library/flammable_liquids/flammable_liquids.html, Accessed July 15, 2015.

144. Brennecke, J. F.; Maginn, E. J., Ionic liquids: innovative fluids for chemical processing. *American Institute of Chemical Engineers. AIChE Journal* **2001,** *47* (11), 2384.

145. Abbott, A. P.; Davies, D. L.; Capper, G.; Rasheed, R. K.; Tambyrajah, V. Ionic liquids and their use as solvents. WO2002026701A2, 2002.

146. Nardecchia, S.; Gutierrez, M. C.; Ferrer, M. L.; Alonso, M.; Lopez, I. M.; Rodriguez-Cabello, J. C.; del Monte, F., Phase behavior of elastin-like synthetic recombinamers in deep eutectic solvents. *Biomacromolecules* **2012,** *13* (7), 2029-36.
147. Liu, Y.-T.; Chen, Y.-A.; Xing, Y.-J., Synthesis and characterization of novel ternary deep eutectic solvents. *Chinese Chemical Letters* **2014,** *25* (1), 104-106.
148. Sze, L. L.; Pandey, S.; Ravula, S.; Pandey, S.; Zhao, H.; Baker, G. A.; Baker, S. N., Ternary Deep Eutectic Solvents Tasked for Carbon Dioxide Capture. *ACS Sustainable Chemistry & Engineering* **2014,** *2* (9), 2117-2123.
149. Lunkenheimer, K.; Wantke, K.-D., Determination of the surface tension of surfactant solutions applying the method of Lecomte du Noüy (ring tensiometer). *Colloid and Polymer Science* **1981,** *259* (3), 354-366.
150. Finizio, A.; Vighi, M.; Sandroni, D., Determination of n-octanol/water partition coefficient (Kow) of pesticide critical review and comparison of methods. *Chemosphere* **1997,** *34* (1), 131-161.
151. https://hamptonresearch.com/product_detail.aspx?cid=4&sid=71&pid=139, Accessed July 15, 2015.
152. Mjalli, F. S.; Ahmed, O. U., Characteristics and intermolecular interaction of eutectic binary mixtures: Reline and Glyceline. *Korean J. Chem. Eng.* **2016,** *33* (1), 337-343.
153. Mjalli, F. S.; Ahmed, O. U., Physical properties and intermolecular interaction of eutectic solvents binary mixtures: reline and ethaline. *Asia-Pac. J. Chem. Eng.* **2016**, 549–557.

154. Visser, A. E.; Swatloski, R. P.; Rogers, R. D., pH-dependent partitioning in room temperature ionic liquids provides a link to traditional solvent extraction behavior. *Green Chemistry* **2000,** *2* (1), 1-4.

155. Huddleston, J. G.; Willauer, H. D.; Swatloski, R. P.; Visser, A. E.; Rogers, R. D., Room temperature ionic liquids as novel media for 'clean'liquid–liquid extraction. *Chemical Communications* **1998,** (16), 1765-1766.

156. http://www.inchem.org/documents/sids/sids/67481.pdf, Accessed July 15, 2015.

157. Al-Badr, A. A.; El-Obeid, H. A., Acetylcholine Chloride: Physical Profile. *Profiles of Drug Substances, Excipients and Related Methodology* **2005,** *31*, 1-19.

158. http://pubchem.ncbi.nlm.nih.gov/compound/urea#section=Top, Accessed July 15, 2015.

159. http://pubchem.ncbi.nlm.nih.gov/compound/glycerol#section=Solubility, Accessed July 15, 2015.

160. Abbott, A. P.; Capper, G.; Gray, S., Design of improved deep eutectic solvents using hole theory. *Chemphyschem* **2006,** *7* (4), 803-6.

161. Leron, R. B.; Soriano, A. N.; Li, M.-H., Densities and refractive indices of the deep eutectic solvents (choline chloride+ethylene glycol or glycerol) and their aqueous mixtures at the temperature ranging from 298.15 to 333.15K. *Journal of the Taiwan Institute of Chemical Engineers* **2012,** *43* (4), 551-557.

162. Mjalli, F. S., Mass connectivity index-based density prediction of deep eutectic solvents. *Fluid Phase Equilib.* **2016,** *409*, 312-317.

163. Mjalli, F. S.; Vakili-Nezhaad, G.; Shahbaz, K.; AlNashef, I. M., Application of the Eötvos and Guggenheim empirical rules for predicting the density and surface tension of ionic liquids analogues. *Thermochimica Acta* **2014,** *575*, 40-44.

164. Shah, D.; Mjalli, F. S., Effect of water on the thermo-physical properties of Reline: An experimental and molecular simulation based approach. *Phys Chem Chem Phys* **2014,** *16* (43), 23900-7.

165. Abbott, A. P.; Harris, R. C.; Ryder, K. S., Application of hole theory to define ionic liquids by their transport properties. *The Journal of Physical Chemistry B* **2007,** *111* (18), 4910-4913.

166. Hsieh, Y.-P.; Leron, R. B.; Soriano, A. N.; Caparanga, A. R.; Li, M.-H., Diffusivity, density and viscosity of aqueous solutions of choline chloride/ethylene glycol and choline chloride/malonic acid. *Journal of Chemical Engineering of Japan* **2012,** *45* (12), 939-947.

167. Hong-Zhen, S.; Jing-Mei, Y.; Qing-Shan, L.; Chang-Ping, L., Properties of Four Deep Eutectic Solvents: Density, Electrical Conductivity, Dynamic Viscosity and Refractive Index. *ACTA PHYSICO-CHIMICA SINICA* **2015,** *31* (8), 1468-1473.

168. Abbott, A. P.; Barron, J. C.; Ryder, K. S.; Wilson, D., Eutectic-Based Ionic Liquids with Metal-Containing Anions and Cations. *Chemistry–A European Journal* **2007,** *13* (22), 6495-6501.

169. Shahbaz, K.; Baroutian, S.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M., Densities of ammonium and phosphonium based deep eutectic solvents:

Prediction using artificial intelligence and group contribution techniques. *Thermochimica Acta* **2012,** *527*, 59-66.

170. Tshibangu, P. N.; Ndwandwe, S. N.; Dikio, E. D., Density, viscosity and conductivity study of 1-butyl-3-methylimidazolium bromide. *Int. J. Electrochem. Sci* **2011,** *6* (6), 2.

171. Fredlake, C. P.; Crosthwaite, J. M.; Hert, D. G.; Aki, S. N.; Brennecke, J. F., Thermophysical properties of imidazolium-based ionic liquids. *Journal of Chemical & Engineering Data* **2004,** *49* (4), 954-964.

172. Tariq, M.; Forte, P.; Gomes, M. C.; Lopes, J. C.; Rebelo, L., Densities and refractive indices of imidazolium-and phosphonium-based ionic liquids: Effect of temperature, alkyl chain length, and anion. *The Journal of Chemical Thermodynamics* **2009,** *41* (6), 790-798.

173. Jacquemin, J.; Husson, P.; Padua, A. A.; Majer, V., Density and viscosity of several pure and water-saturated ionic liquids. *Green Chemistry* **2006,** *8* (2), 172-180.

174. Riddick, J. A.; Bunger, W. B.; Sakano, T. K., Organic solvents: physical properties and methods of purification. **1986**.

175. Hoyt, L., New table of the refractive index of pure glycerol at 20 C. *Industrial & Engineering Chemistry* **1934,** *26* (3), 329-332.

176. Siongco, K. R.; Leron, R. B.; Li, M.-H., Densities, refractive indices, and viscosities of N,N-diethylethanol ammonium chloride–glycerol or –ethylene glycol deep eutectic solvents and their aqueous solutions. *The Journal of Chemical Thermodynamics* **2013,** *65*, 65-72.

177. Thormählen, I.; Straub, J.; Grigull, U., Refractive index of water and its dependence on wavelength, temperature, and density. *Journal of physical and chemical reference data* **1985,** *14* (4), 933-945.

178. Speight, J. G., *Lange's handbook of chemistry*. Vol. 1.

179. Iglesias-Otero, M. A.; Troncoso, J.; Carballo, E.; Romaní, L., Density and refractive index in mixtures of ionic liquids and organic solvents: Correlations and predictions. *The Journal of Chemical Thermodynamics* **2008,** *40* (6), 949-956.

180. Wagle, D. V.; Zhao, H.; Baker, G. A., Deep eutectic solvents: sustainable media for nanoscale and functional materials. *Acc Chem Res* **2014,** *47* (8), 2299-308.

181. Shahbaz, K.; Mjalli, F.; Hashim, M.; AlNashef, I., Prediction of the surface tension of deep eutectic solvents. *Fluid Phase Equilibria* **2012,** *319*, 48-54.

182. http://www.surface-tension.de/, Accessed July 15, 2015.

183. Law, G.; Watson, P. R., Surface tension measurements of N-alkylimidazolium ionic liquids. *Langmuir* **2001,** *17* (20), 6138-6141.

184. Beek, B.; Böhling, S.; Bruckmann, U.; Franke, C.; Jöhncke, U.; Studinger, G., The assessment of bioaccumulation. In *Bioaccumulation–New Aspects and Developments*, Springer: 2000; pp 235-276.

185. http://www.inchem.org/documents/sids/sids/57136.pdf, Accessed July 15, 2015.

186. http://www.inchem.org/documents/sids/sids/56815.pdf, Acessed July 15, 2015.

187. Sangster, J., Octanol-water partition coefficients of simple organic compounds. *Journal of Physical and Chemical Reference Data* **1989,** *18* (3), 1111-1229.

188. Ropel, L.; Belvèze, L. S.; Aki, S. N.; Stadtherr, M. A.; Brennecke, J. F., Octanol–water partition coefficients of imidazolium-based ionic liquids. *Green Chemistry* **2005,** *7* (2), 83-90.

189. Likhachev, E., Dependence of water viscosity on temperature and pressure. *Technical Physics* **2003,** *48* (4), 514-515.

190. Speight, J. G., *Lange's handbook of chemistry*. McGraw-Hill New York: 2005; Vol. 1.

191. Maugeri, Z.; Domínguez de María, P., Novel choline-chloride-based deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols. *RSC Adv.* **2012,** *2* (2), 421-425.

192. Craveiro, R.; Aroso, I.; Flammia, V.; Carvalho, T.; Viciosa, M. T.; Dionisio, M.; Barreiros, S.; Reis, R. L.; Duarte, A. R. C.; Paiva, A., Properties and thermal behavior of natural deep eutectic solvents. *J. Mol. Liq.* **2016,** *215*, 534-540.

193. Ren, H.; Chen, C.; Wang, Q.; Zhao, D.; Guo, S., *The Properties of Choline Chloride-based Deep Eutectic Solvents and their Performance in the Dissolution of Cellulose*. 2016; Vol. 11.

194. Shahbaz, K.; Mjalli, F. S.; Hashim, M.; Al-Nashef, I. M., Using deep eutectic solvents for the removal of glycerol from palm oil-based biodiesel. *Journal of Applied Sciences* **2010,** *10* (24), 3349-3354.

195. Wang, S.; Peng, X.; Zhong, L.; Jing, S.; Cao, X.; Lu, F.; Sun, R., Choline chloride/urea as an effective plasticizer for production of cellulose films. *Carbohydrate polymers* **2015,** *117*, 133-139.

196. Ngo, H. L.; LeCompte, K.; Hargens, L.; McEwen, A. B., Thermal properties of imidazolium ionic liquids. *Thermochim. Acta* **2000,** *357-358*, 97-102.

197. Clark, J. H.; Macquarrie, D. J., *Handbook of green chemistry and technology*. John Wiley & Sons: 2008.

198. Katritzky, A. R.; Fara, D. C.; Yang, H.; Tämm, K.; Tamm, T.; Karelson, M., Quantitative measures of solvent polarity. *Chemical reviews* **2004,** *104* (1), 175-198.

199. Giernoth, R., Solvents and Solvent Effects in Organic Chemistry 4th Ed by Christian Reichardt and Thomas Welton. *Angew. Chem., Int. Ed.* **2011,** *50* (48), 11289.

200. Reichardt, C., Solvatochromic dyes as solvent polarity indicators. *Chemical Reviews* **1994,** *94* (8), 2319-2358.

201. Ab Rani, M. A.; Brant, A.; Crowhurst, L.; Dolan, A.; Lui, M.; Hassan, N. H.; Hallett, J. P.; Hunt, P. A.; Niedermeyer, H.; Perez-Arlandis, J. M.; Schrems, M.; Welton, T.; Wilding, R., Understanding the polarity of ionic liquids. *Phys Chem Chem Phys* **2011,** *13* (37), 16831-40.

202. Kamlet, M. J.; Taft, R., The solvatochromic comparison method. I. The. beta.-scale of solvent hydrogen-bond acceptor (HBA) basicities. *Journal of the American chemical Society* **1976,** *98* (2), 377-383.

203. Taft, R.; Kamlet, M. J., The solvatochromic comparison method. 2. The. alpha.-scale of solvent hydrogen-bond donor (HBD) acidities. *Journal of the American Chemical Society* **1976,** *98* (10), 2886-2894.

204. Kamlet, M. J.; Abboud, J. L.; Taft, R., The solvatochromic comparison method. 6. The. pi.* scale of solvent polarities. *Journal of the American Chemical Society* **1977,** *99* (18), 6027-6038.

205. Shukla, S. K.; Khupse, N. D.; Kumar, A., Do anions influence the polarity of protic ionic liquids? *Phys Chem Chem Phys* **2012,** *14* (8), 2754-61.

206. Barbosa, J., Solvatochromism by P. Suppan and N. Ghoneim. *TrAC, Trends Anal. Chem.* **1999,** *18* (3), 235, X.

207. Furniss, B. S., *Vogel's textbook of practical organic chemistry*. Pearson Education India: 1989.

208. Reichardt, C.; Harbusch-Görnert, E., Über Pyridinium-N-phenolat-Betaine und ihre Verwendung zur Charakterisierung der Polarität von Lösungsmitteln, X. Erweiterung, Korrektur und Neudefinition der ET-Lösungsmittelpolaritätsskala mit Hilfe eines lipophilen penta-tert-butyl-substituierten Pyridinium-N-phenolat-Betainfarbstoffes. *Liebigs Annalen der Chemie* **1983,** *1983* (5), 721-743.

209. Reichardt, C., Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes. *Green Chemistry* **2005,** *7* (5), 339-351.

210. Gu, Y.; Jérôme, F., Glycerol as a sustainable solvent for green chemistry. *Green Chemistry* **2010,** *12* (7), 1127-1138.

211. Imperato, G. New organic solvents based on carbohydrates. Ph.D Dissertation, University of Regensburg, Regensburg, 2006.

212. Diaz-Alvarez, A. E.; Francos, J.; Lastra-Barreira, B.; Crochet, P.; Cadierno, V., Glycerol and derived solvents: new sustainable reaction media for organic synthesis. *Chem Commun (Camb)* **2011,** *47* (22), 6208-27.

213. Pandey, A.; Rai, R.; Pal, M.; Pandey, S., How polar are choline chloride-based deep eutectic solvents? *Phys Chem Chem Phys* **2014,** *16* (4), 1559-68.

214. Buhvestov, U.; Rived, F.; Ràfols, C.; Bosch, E.; Rosés, M., Solute–solvent and solvent–solvent interactions in binary solvent mixtures. Part 7. Comparison of the enhancement of the water structure in alcohol–water mixtures measured by solvatochromic indicators. *Journal of physical organic chemistry* **1998,** *11* (3), 185-192.

215. Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R., Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters,. pi.*,. alpha., and. beta., and some methods for simplifying the generalized solvatochromic equation. *The Journal of Organic Chemistry* **1983,** *48* (17), 2877-2887.

216. Reichardt, C.; Welton, T., *Solvents and solvent effects in organic chemistry*. John Wiley & Sons: 2011.

217. Laurence, C.; Nicolet, P.; Dalati, M. T.; Abboud, J.-L. M.; Notario, R., The Empirical Treatment of Solvent-Solute Interactions: 15 Years of. pi.*. *The Journal of Physical Chemistry* **1994,** *98* (23), 5807-5816.

218. Khupse, N. D.; Kumar, A., Delineating solute− solvent interactions in binary mixtures of ionic liquids in molecular solvents and preferential solvation approach. *The Journal of Physical Chemistry B* **2010,** *115* (4), 711-718.

219. Kamlet, M. J.; Taft, R. W., Linear solvation energy relationships. Part 1. Solvent polarity–polarizability effects on infrared spectra. *Journal of the Chemical Society, Perkin Transactions 2* **1979,** (3), 337-341.

220. Jessop, P. G.; Jessop, D. A.; Fu, D.; Phan, L., Solvatochromic parameters for solvents of interest in green chemistry. *Green Chemistry* **2012,** *14* (5), 1245.

221. Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T., Solvent–solute interactions in ionic liquids. *Physical Chemistry Chemical Physics* **2003,** *5* (13), 2790-2794.

222. Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T., Solvent?solute interactions in ionic liquidsElectronic supplementary information (ESI) available: synthesis of ionic liquids. See http://www.rsc.org/suppdata/cp/b3/b303095d. *Physical Chemistry Chemical Physics* **2003,** *5* (13), 2790.

223. Ohno, H.; Fukaya, Y., Task specific ionic liquids for cellulose technology. *Chemistry Letters* **2009,** *38* (1), 2-7.

224. Wu, Y.; Sasaki, T.; Kazushi, K.; Seo, T.; Sakurai, K., Interactions between spiropyrans and room-temperature ionic liquids: photochromism and solvatochromism. *The Journal of Physical Chemistry B* **2008,** *112* (25), 7530-7536.

225. Yoshida, Y.; Baba, O.; Saito, G., Ionic liquids based on dicyanamide anion: influence of structural variations in cationic structures on ionic conductivity. *The Journal of Physical Chemistry B* **2007,** *111* (18), 4742-4749.

226. Tokuda, H.; Tsuzuki, S.; Susan, M. A. B. H.; Hayamizu, K.; Watanabe, M., How ionic are room-temperature ionic liquids? An indicator of the physicochemical properties. *The Journal of Physical Chemistry B* **2006,** *110* (39), 19593-19600.

227. Zhang, S.; Qi, X.; Ma, X.; Lu, L.; Deng, Y., Hydroxyl ionic liquids: the differentiating effect of hydroxyl on polarity due to ionic hydrogen bonds between hydroxyl and anions. *The Journal of Physical Chemistry B* **2010,** *114* (11), 3912-3920.

228. Chiappe, C.; Pieraccini, D., Determination of ionic liquids solvent properties using an unusual probe: The electron donor-acceptor complex between 4, 4'-bis (dimethylamino)-benzophenone and tetracyanoethene. *The Journal of Physical Chemistry A* **2006,** *110* (14), 4937-4941.

229. Jeličić, A.; García, N.; Löhmannsröben, H.-G.; Beuermann, S., Prediction of the ionic liquid influence on propagation rate coefficients in methyl methacrylate radical polymerizations based on Kamlet− Taft solvatochromic parameters. *Macromolecules* **2009,** *42* (22), 8801-8808.

230. Mellein, B. R.; Aki, S. N.; Ladewski, R. L.; Brennecke, J. F., Solvatochromic studies of ionic liquid/organic mixtures. *The Journal of Physical Chemistry B* **2007,** *111* (1), 131-138.

231. Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D., Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chemistry* **2001,** *3* (4), 156-164.

232. Muldoon, M. J.; Gordon, C. M.; Dunkin, I. R., Investigations of solvent–solute interactions in room temperature ionic liquids using solvatochromic dyes. *Journal of the Chemical Society, Perkin Transactions 2* **2001,** (4), 433-435.

233. Lee, J.-M.; Ruckes, S.; Prausnitz, J. M., Solvent polarities and kamlet-taft parameters for ionic liquids containing a pyridinium cation. *The Journal of Physical Chemistry B* **2008,** *112* (5), 1473-1476.

234. Coleman, S.; Byrne, R.; Minkovska, S.; Diamond, D., Investigating nanostructuring within imidazolium ionic liquids: a thermodynamic study using photochromic molecular probes. *The Journal of Physical Chemistry B* **2009,** *113* (47), 15589-15596.

235. Fukaya, Y.; Hayashi, K.; Wada, M.; Ohno, H., Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. *Green Chem.* **2008,** *10* (1), 44-46.

236. Snyder, L., Changing reversed-phase high performance liquid chromatography selectivity Which variables should be tried first? *Journal of Chromatography B: Biomedical Sciences and Applications* **1997,** *689* (1), 105-115.

237. Snyder, L., Classification of the solvent properties of common liquids. *Journal of Chromatography A* **1974,** *92* (2), 223-230.

238. Snyder, L.; Carr, P.; Rutan, S., Solvatochromically based solvent-selectivity triangle. *Journal of Chromatography A* **1993,** *656* (1-2), 537-547.

239. Marcus, Y., *Introduction to liquid state chemistry*. Wiley: 1977.

240. Lin, C.-M.; Leron, R. B.; Caparanga, A. R.; Li, M.-H., Henry’s constant of carbon dioxide-aqueous deep eutectic solvent (choline chloride/ethylene glycol,

choline chloride/glycerol, choline chloride/malonic acid) systems. *The Journal of Chemical Thermodynamics* **2014,** *68*, 216-220.

241. Sato, B. M.; de Oliveira, C. G.; Martins, C. T.; El Seoud, O. A., Thermo-solvatochromism in binary mixtures of water and ionic liquids: on the relative importance of solvophobic interactions. *Physical Chemistry Chemical Physics* **2010,** *12* (8), 1764-1771.

242. Baker, S. N.; Baker, G. A.; Bright, F. V., Temperature-dependent microscopic solvent properties of 'dry'and 'wet'1-butyl-3-methylimidazolium hexafluorophosphate: correlation with ET (30) and Kamlet–Taft polarity scales. *Green Chemistry* **2002,** *4* (2), 165-169.

243. Trivedi, S.; Malek, N. I.; Behera, K.; Pandey, S., Temperature-dependent solvatochromic probe behavior within ionic liquids and (ionic liquid+ water) mixtures. *The Journal of Physical Chemistry B* **2010,** *114* (24), 8118-8125.

244. Cammarata, L.; Kazarian, S.; Salter, P.; Welton, T., Molecular states of water in room temperature ionic liquids. *Physical Chemistry Chemical Physics* **2001,** *3* (23), 5192-5200.

245. Sarkar, A.; Pandey, S., Solvatochromic absorbance probe behavior and preferential solvation in aqueous 1-butyl-3-methylimidazolium tetrafluoroborate. *Journal of Chemical & Engineering Data* **2006,** *51* (6), 2051-2055.

246. Sarkar, A.; Ali, M.; Baker, G. A.; Tetin, S. Y.; Ruan, Q.; Pandey, S., Multiprobe spectroscopic investigation of molecular-level behavior within aqueous 1-butyl-3-methylimidazolium tetrafluoroborate. *The Journal of Physical Chemistry B* **2009,** *113* (10), 3088-3098.

247. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustainable Chemistry & Engineering* **2014,** *2* (5), 1063-1071.

248. Hayyan, M.; Hashim, M. A.; Hayyan, A.; Al-Saadi, M. A.; AlNashef, I. M.; Mirghani, M. E.; Saheed, O. K., Are deep eutectic solvents benign or toxic? *Chemosphere* **2013,** *90* (7), 2193-5.

249. Jhong, H.-R.; Wong, D. S.-H.; Wan, C.-C.; Wang, Y.-Y.; Wei, T.-C., A novel deep eutectic solvent-based ionic liquid used as electrolyte for dye-sensitized solar cells. *Electrochemistry Communications* **2009,** *11* (1), 209-211.

250. Hayyan, M.; Hashim, M. A.; Al-Saadi, M. A.; Hayyan, A.; AlNashef, I. M.; Mirghani, M. E., Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. *Chemosphere* **2013,** *93* (2), 455-9.

251. Radosevic, K.; Bubalo, M. C.; Srcek, V. G.; Grgas, D.; Dragicevic, T. L.; Redovnikovic, I. R., Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. *Ecotoxicol Environ Saf* **2015,** *112*, 46-53.

252. de Morais, P.; Gonçalves, F.; Coutinho, J. A. P.; Ventura, S. P. M., Ecotoxicity of Cholinium-Based Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering* **2015**.

253. Wen, Q.; Chen, J. X.; Tang, Y. L.; Wang, J.; Yang, Z., Assessing the toxicity and biodegradability of deep eutectic solvents. *Chemosphere* **2015,** *132*, 63-69.

254. Florin, I.; Rutberg, L.; Curvall, M.; Enzell, C. R., Screening of tabacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* **1980,** *15* (3), 219-232.

255. Lewinski, N.; Colvin, V.; Drezek, R., Cytotoxicity of nanoparticles. *small* **2008,** *4* (1), 26-49.

256. Abe, K.; Matsuki, N., Measurement of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction activity and lactate dehydrogenase release using MTT. *Neuroscience research* **2000,** *38* (4), 325-329.

257. Docherty, K. M.; Hebbeler, S. Z.; Kulpa Jr, C. F., An assessment of ionic liquid mutagenicity using the Ames Test. *Green Chemistry* **2006,** *8* (6), 560-567.

258. Juneidi, I.; Hayyan, M.; Mohd. Ali, O., Toxicity profile of choline chloride-based deep eutectic solvents for fungi and Cyprinus carpio fish. *Environ. Sci. Pollut. Res.* **2016**, Ahead of Print.

259. Modica-Napolitano, J. S.; Aprille, J. R., Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. *Advanced drug delivery reviews* **2001,** *49* (1), 63-70.

260. Kandala, H. Synthesis, Properties, and Utilization of 1-Ethyl-3-methylimidazolium Alkylbenzene Sulfonate Ionic Liquids. Ph.D Dissertation, South Dakota State University, Brookings, 2016.

261. Weyermann, J.; Lochmann, D.; Zimmer, A., A practical note on the use of cytotoxicity assays. *Int. J. Pharm.* **2005,** *288* (2), 369-376.

262. Dewulf, J.; Van Langenhove, H., *Renewables-based technology: sustainability assessment*. John Wiley & Sons: 2006.

263. Klass, D. L., *Biomass for renewable energy, fuels, and chemicals*. Academic press: 1998.

264. Hendriks, A.; Zeeman, G., Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource technology* **2009,** *100* (1), 10-18.

265. Anwar, Z.; Gulfraz, M.; Irshad, M., Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. *Journal of radiation research and applied sciences* **2014,** *7* (2), 163-173.

266. Alvira, P.; Tomás-Pejó, E.; Ballesteros, M.; Negro, M., Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource technology* **2010,** *101* (13), 4851-4861.

267. Balan, V., Current challenges in commercially producing biofuels from lignocellulosic biomass. *ISRN biotechnology* **2014,** *2014*.

268. Tan, S. S. Y.; MacFarlane, D. R., Ionic liquids in biomass processing. In *Ionic liquids*, Springer: 2010; pp 311-339.

269. Mora-Pale, M.; Meli, L.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S., Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnology and bioengineering* **2011,** *108* (6), 1229-1245.

270. Procentese, A.; Johnson, E.; Orr, V.; Garruto Campanile, A.; Wood, J. A.; Marzocchella, A.; Rehmann, L., Deep eutectic solvent pretreatment and subsequent saccharification of corncob. *Bioresour Technol* **2015,** *192*, 31-6.

271. Bhatia, L.; Johri, S.; Ahmad, R., An economic and ecological perspective of ethanol production from renewable agro waste: a review. *Amb Express* **2012,** *2* (1), 1.

272. Pham, T. P. T.; Cho, C.-W.; Yun, Y.-S., Environmental fate and toxicity of ionic liquids: a review. *Water research* **2010,** *44* (2), 352-372.

273. Francisco, M.; van den Bruinhorst, A.; Kroon, M. C., New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing. *Green Chemistry* **2012,** *14* (8), 2153-2157.

274. Škulcová, A.; Kamenská, L.; Kalman, F.; Ház, A.; Jablonský, M.; Čížová, K.; Šurina, I. In *Deep Eutectic Solvents as Medium for Pretreatment of Biomass*, Key Engineering Materials, Trans Tech Publ: 2016; pp 17-24.

275. Loong, Y. C.; Yusup, S.; Uemura, Y., Delignification of Empty Fruit Bunch (EFB) Using Low Transition Temperature Mixtures (LTTMs): A Review. *Applied Mechanics and Materials* **2014,** *625*, 741-744.

276. Kroon, M. C.; Casal, M. F.; Van den Bruinhorst, A. Pretreatment of lignocellulosic biomass and recovery of substituents using natural deep eutectic solvents/compound mixtures with low transition temperatures. WO2013153203A1, 2013.

277. Xia, S.; Baker, G. A.; Li, H.; Ravula, S.; Zhao, H., Aqueous Ionic Liquids and Deep Eutectic Solvents for Cellulosic Biomass Pretreatment and Saccharification. *RSC Adv* **2014,** *4* (21), 10586-10596.

278. Gedye, K.; Gonzalez-Hernandez, J.; Ban, Y.; Ge, X.; Thimmapuram, J.; Sun, F.; Wright, C.; Ali, S.; Boe, A.; Owens, V., Investigation of the transcriptome of prairie cord grass, a new cellulosic biomass crop. *The Plant Genome* **2010,** *3* (2), 69-80.

279. Gonzalez-Hernandez, J.; Sarath, G.; Stein, J.; Owens, V.; Gedye, K.; Boe, A., A multiple species approach to biomass production from native herbaceous perennial feedstocks. *In Vitro Cellular & Developmental Biology-Plant* **2009,** *45* (3), 267-281.

280. Di Marino, D.; Stöckmann, D.; Kriescher, S.; Stiefel, S.; Wessling, M., Electrochemical depolymerisation of lignin in a deep eutectic solvent. *Green Chemistry* **2016**.

281. Lian, H.; Hong, S.; Carranza, A.; Mota-Morales, J. D.; Pojman, J. A., Processing of lignin in urea–zinc chloride deep-eutectic solvent and its use as a filler in a phenol-formaldehyde resin. *RSC Adv.* **2015,** *5* (36), 28778-28785.

282. Troter, D. Z.; Todorović, Z. B.; Đokić-Stojanović, D. R.; Stamenković, O. S.; Veljković, V. B., Application of ionic liquids and deep eutectic solvents in biodiesel production: A review. *Renewable and Sustainable Energy Reviews* **2016,** *61*, 473-500.

283. Kumar, A. K.; Parikh, B. S.; Pravakar, M., Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. *Environmental Science and Pollution Research* **2015**, 1-11.

284. Dominguez de Maria, P., Recent trends in (ligno)cellulose dissolution using neoteric solvents: switchable, distillable and bio-based ionic liquids. *J. Chem. Technol. Biotechnol.* **2014,** *89* (1), 11-18.

285. Yang, G. H.; Wu, T. Y.; Loow, Y.-L.; Ang, L. Y., Application of deep eutectic solvents in biomass valorization.

286. Vasco, C. A.; Ma, R.; Quintero, M.; Guo, M.; Geleynse, S.; Ramasamy, K. K.; Wolcott, M.; Zhang, X., Unique Low-molecular-weight Lignin with High Purity Extracted from Wood by Deep Eutectic Solvents (DES): A Source of Lignin for Valorization. *Green Chemistry* **2016**.

287. Irvine, G., The glass transitions of lignin and hemicellulose and their measurement by differential thermal analysis. *Tappi Journal* **1984,** *67* (5), 118-121.

288. Achinivu, E. C.; Howard, R. M.; Li, G.; Gracz, H.; Henderson, W. A., Lignin extraction from biomass with protic ionic liquids. *Green Chemistry* **2014,** *16* (3), 1114-1119.

289. Tan, S. S.; MacFarlane, D. R.; Upfal, J.; Edye, L. A.; Doherty, W. O.; Patti, A. F.; Pringle, J. M.; Scott, J. L., Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chemistry* **2009,** *11* (3), 339-345.

290. Bathula, N. V. Comprehensive Structural and Thermal Characterization of Selected Grass Lignins. Ph.D Dissertation, Brookings, 2013.

291. Tadesse, H.; Luque, R., Advances on biomass pretreatment using ionic liquids: an overview. *Energy & Environmental Science* **2011,** *4* (10), 3913-3929.

292. Brandt, A.; Gräsvik, J.; Hallett, J. P.; Welton, T., Deconstruction of lignocellulosic biomass with ionic liquids. *Green Chemistry* **2013,** *15* (3), 550-583.

9 APPENDIX

Table 9.1. pH, density, refractive indices, surface tension, octanol-water partition coefficient, and viscosity of DESs at room temperature.

DES	pH	Density (g mL^{-1})	RI	Surface tension (dynes cm^{-1})	K_{OW}	Viscosity (Pa.s)
DES01	9.44	9.41	1.5069	57.10	0.0050	0.261
DES02	7.76	7.73	1.4867	59.70	0.0186	0.254
DES03	7.28	7.25	1.5017	68.15	0.0241	3.827
DES04	5.94	5.91	1.4824	69.83	0.0481	0.412
DES05	8.52	8.49	1.4963	59.70	0.0142	0.319
DES06	9.22	9.19	1.5012	57.60	0.0096	0.408
DES07	7.82	7.79	1.4910	59.00	0.0209	0.294
DES08	6.59	6.56	1.4918	64.60	0.0321	0.604
DES09	6.91	6.88	1.4956	59.60	0.0209	1.380
DES10	6.34	6.31	1.4874	62.40	0.0376	0.389
DES11	6.80	6.77	1.5029	74.70	0.0185	1.400
DES12	7.23	7.2	1.5046	72.10	0.0123	1.370
DES13	6.42	6.39	1.5036	78.10	0.0204	3.360
DES14	5.55	5.52	1.4838	73.60	0.0313	0.371
DES15	6.36	6.33	1.8430	71.00	0.0213	0.276
DES16	5.26	5.23	1.4838	77.90	0.0389	0.306