

Insights into the Lignin Dissolution Mechanism of Water Content Tailored-choline Chloride (ChCl) Based Green Solvents for Biomass Pretreatment

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(Received 6 July 2022, Accepted 11 September 2022)

This work studies the ternary deep eutectic solvents (DESs) as green solvents to enhance the performance of biomass dissolution and tunability of DESs for varying applications. For this purpose, water is extremely suitable, as it is both a hydrogen bond acceptor and a hydrogen bond donor. There are limited studies on modulating the properties of DES. ChCl/Malic acid 1:1 (CCMA) and ChCl/Lactic acid 1:18 (CCLA) DESs with varying water content were synthesized and characterized. *Fourier transform infrared spectroscopy* (FTIR) demonstrated the formation of hydrogen bonds. The latter was concluded from broadened OH stretching, diminished choline chloride characteristic peak associated with its OH group, and bathochromic shift of the C=O stretching associated with carboxylic acids. The addition of water also caused 2.8% and 3.6% reduction in the density of CCMA and CCLA, respectively, due to the lowered free volume of DESs with 50 wt% of water content. CCLA showed the highest lignin solubility at 6.32 wt%, and further addition of water resulted in weakened structure that negatively affected the dissolution performance. Both CCMA and CCLA with water content are green solvents that hindered self-degradation by esterification reaction, showing great potential for use in biomass pretreatment.

Keywords: Green solvents, Choline chloride, Hydrogen bonding, Lignin dissolution, Stabilizing capacity

INTRODUCTION

The main goal of research on green solvents is to introduce innovative alternatives to conventional solvents that are harmful to the environment. Excessive use of solvents in industries of all sorts is against the environmental sustainability [1]. Furthermore, most of these conventional solvents are caustic, poisonous, volatile, and imperishable causing adverse health impacts. In this regard, recent research works on green solvents attempt to search for the

eco-friendly and customizable solvents as an alternative to conventional solvents. However, some of these state-of-the-art green solvents have high cost and technological constraints; this further highlights the needs for new solvents that are different from those existed conventional. The desired green solvents should have low cost while exhibiting exceptional performance that can challenge the conventional solvents. Today's, the most commonly reported green solvents in literatures include water, supercritical fluids, ionic liquids (ILs), deep eutectic solvents (DESs), and bio-based solvents [1].

DESs have been widely recognized as green solvent; it is

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analogous to ILs developed to be categorized as green solvents [2]. Briefly, DESs describes a new group of tailored solvent which consists of natural and renewable materials that are readily acquirable and abundant in nature, assuring long-term sustainability. The term DESs was used by Abbott *et al.* (2003) for the first time [3], due to its characteristic similar to eutectic systems. Later, advancements in the green solvent research discovered that most of these solvents, in fact, exhibited glass transitions instead of eutectic (melting) points. Therefore, the original term DES was found to be inaccurate for naming the new class of solvents. Hence, the term 'low transition temperature mixtures' (LTTMs) was introduced to describe to a group of solvents that are synthesized by mixing two or more solid constituents which are capable of establishing hydrogen bond interactions and further yields a liquid phase with a melting point that is much lower than that of its respective constituents [4]. However, in literatures the both terms (*i.e.* LTTM and DES) are used interchangeably.

DESs can be prepared using natural starting materials from readily available sources, which are considered nearly inextinguishable, making it an inexpensive alternative to the conventional solvents. The broad liquid range, high tunability, and befitting physicochemical properties are the key properties of DESs that are beneficial for use in solvent-related applications. DESs can be prepared from a wide array of constituents with relatively simple preparation methods. The only requirement is a pair of compatible hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The physicochemical properties of DESs can be easily modulated to obtain the desired properties (by altering the constituents or the composition of DESs). Consequently, DESs can be used in wide range of applications including biomass valorization, which is of great interest due to the abundance of lignocellulosic biomass worldwide.

Following the use of DESs, ChCl has been extensively explored across various studies. The immense interest in ChCl is due to its non-toxic nature making it suitable for use in green chemistry applications [5]. Although active researches have been focused on application of DESs, comprehensive studies for characterization of physicochemical properties of DESs are still lacking [6]. In most of the existing works, the effects of water are often not addressed despite the fact that most DESs are known to be

hygroscopic in nature, which implies that the absorption of moisture from the ambient air in real life applications is difficult to avoid [4]. Water molecules possess the ability to form hydrogen bonds across a wide range of solutes and the high polarity of water enables it to act as both the HBA and HBD [7]. Consequently, water may compete with existing constituents of the DESs for hydrogen bond formations and promote the disintegration of the DESs [7-9]. Thus, proper characterization of water content tailored-DESs is necessary in order to address the ambiguity in the building principles, incomprehensive knowledge on the mechanisms of formation, as well as the intermolecular interactions within the solvent in biomass pretreatment. This research aims to screen the compatibility of lactic acid and malic acid as potential HBD with choline chloride as HBA for the formation of DESs. The effect of water on the physicochemical properties of resultant DESs in lignin solubilization mechanism is also investigated

EXPERIMENTAL

Materials

Analytical reagent (AR) grade choline chloride 99%, DL-Malic acid 99+ % and DL-Lactic acid 90% for synthesis of DESs were obtained from ACROS Organics. Biochemical reagent (BR) grade Kraft lignin, general reagent (GR) grade soluble starch and cellulose microcrystalline powder for biopolymers solubility test were purchased from Nanjing Dulai Biotechnology and Sigma-Aldrich.

Methods

Synthesis of DESs and compatibility screening. The starting materials were weighed and mixed together in a glass beaker. The mixture was heated at 70 °C and stirred with magnetic stirrer until the formation of homogeneous and colorless DESs. Two types of DESs were synthesized namely ChCl/Malic acid 1:1 (CCMA) and ChCl/Lactic acid 1:18 (CCLA). Different water content ranging from 10 wt% to 50 wt% were then gradually added to both CCMA and CCLA. The DESs were cooled to room temperature and visually screened for its compatibility by checking whether the DESs remain as stable, homogenous, and colorless solution.

Fourier transform infrared spectroscopy (FTIR) analysis. All samples were analyzed by using Attenuated Total Reflectance (ATR) sampling method over the spectral range from 4000 cm^{-1} to 400 cm^{-1} with 32 scans per spectrum at a resolution of 4 cm^{-1} . The FTIR-ATR spectra of the starting materials and DESs of different water content were compared and analyzed to identify the conformational changes in functional groups and evidence of hydrogen bond formation between the constituents of the DESs.

Measurement of density. Analytical approach (*i.e.* gravimetric method) was used for the measurement of density of the DESs under influence of different water content. An adjustable micropipette was used to transfer DESs of known volume into a glass beaker weighed on the A&D Apollo GX-2002A Advanced Analytical Balance.

Biopolymers solubility test. The biopolymers tested in this project involves Kraft lignin, starch, and cellulose as the representative constituents to imitate those in lignocellulosic biomass that typically consist of lignin, cellulose, and hemicelluloses [10]. The cloud point method was employed to determine the solubility of the biopolymers, following the procedures proposed by Francisco *et al.* (2012) [11]. 2 g of DESs were transferred into glass vials and heated in a water bath at a specific pretreatment temperature followed by gradual addition of small amount of biopolymer samples (around 0.2 to 1 mg) while continuously stirred until attaining maximum dissolution [12]. The pretreatment temperature was set at 100 °C for ChCl/Malic acid DESs and 60 °C for ChCl/Lactic acid DESs due to the higher viscosity in ChCl/Malic acid DESs [4]. The biopolymer solubility was registered if the solutes did not dissolve into the solvents after kept for 24 h [12]. The biopolymers solubility was calculated by using the formula as shown in Eq. (1).

$$\text{Biopolymer solubility} = m_{\text{solute}}/m_{\text{solvent}} \times 100\% \quad (1)$$

Here, m_{solute} is the maximum mass of biopolymers dissolved, and m_{solvent} is the fixed mass of DESs used for the test.

RESULTS AND DISCUSSION

Compatibility of DESs

Both malic acid and lactic acid as HBDs were proven to

be compatible with ChCl as the HBA. All the DESs appeared as stable, clear, and homogenous liquids at room conditions. ChCl/Malic acid DESs displayed a slight yellowish tone while ChCl/Lactic acid DESs appeared as a completely clear and colourless solution as shown in Fig. 1a. In addition, Fig. 1b and Fig. 1c show that the water content (10 wt% to 50 wt%) bound perfectly with ChCl/Malic acid and ChCl/Lactic acid DESs, respectively; this was due to the interference of coordination sphere of the ions and water, enhancing the entropic state and interactions of the resultant solvents [4].

Supermolecule Structure of DESs

The strong and wide peak in the 3650 cm^{-1} to 3200 cm^{-1} range which centered around 3221 cm^{-1} (shown in Fig. 2 and Fig. 3) is assigned to the hydroxyl peak associated with OH-Cl of ChCl [7].

The ChCl hydroxyl peak at 3221 cm^{-1} diminished in the CCMA (Fig. 2) and CCLA (Fig. 3) spectra, indicating a significant dissociation of the original OH-Cl bonds in ChCl to form hydrogen bond with the hydroxyl group of the carboxylic acids. The absorbance associated with the ChCl hydroxyl stretching vibration within the range of 3700 cm^{-1} to 3200 cm^{-1} significantly broadened and intensified, indicating the extensive hydrogen bond formation between ChCl and the carboxylic acids, indicating the successful formation of DESs by both HBDs. The broad band within the absorption region from 3200 cm^{-1} to 2500 cm^{-1} is assigned to the OH stretching frequency associated with carboxylic acids. Both CCMA and CCLA exhibited lower absorption of OH stretching associated with carboxylic acid, indicating the significantly lower amount of free carboxylic acid in the synthesized CCMA and CCLA compared to their starting materials. The sharp strong peak within the range of 1725 cm^{-1} to 1680 cm^{-1} is attributed to the C=O stretching of carboxylic acids which demonstrated a bathochromic shift (red shift) from 1680 cm^{-1} to 1720 cm^{-1} in CCMA and from 1718 cm^{-1} to 1723 cm^{-1} in CCLA. Similar redshift was observed in malic acid-based DESs which can be taken as the evidence for a hydrogen bond formation, specifically associated with the O=C-O-H...N bond interaction between ChCl and malic acid as shown in Fig. 2 [13].

The spectra of the DESs after water addition showed some changes mainly due to the alternative availability of

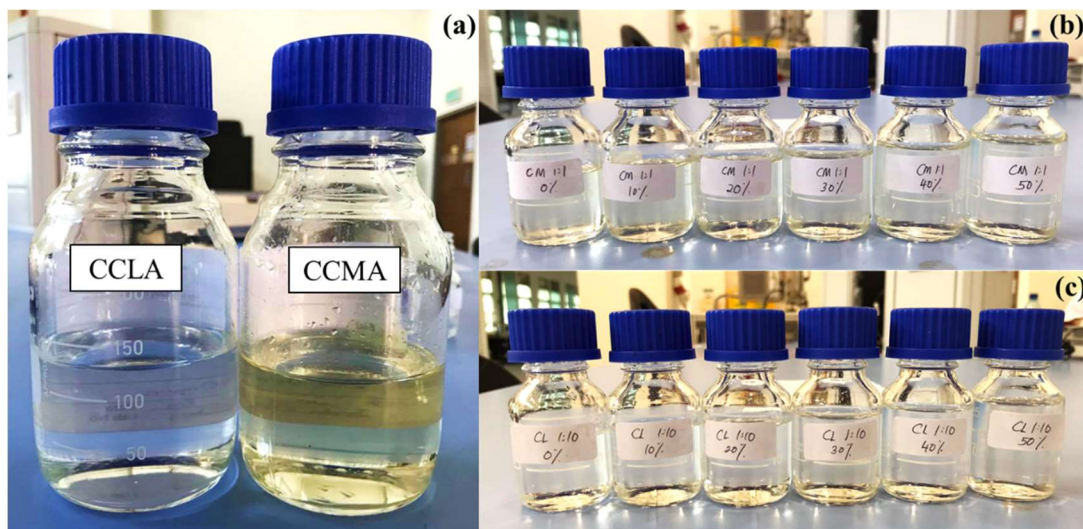


Fig. 1. Physical appearance of (a) DESs without water; (b) ChCl/Malic acid 1:1 with different water content; and (c) ChCl/Lactic acid 1:18 with different water content (0-50 wt%).

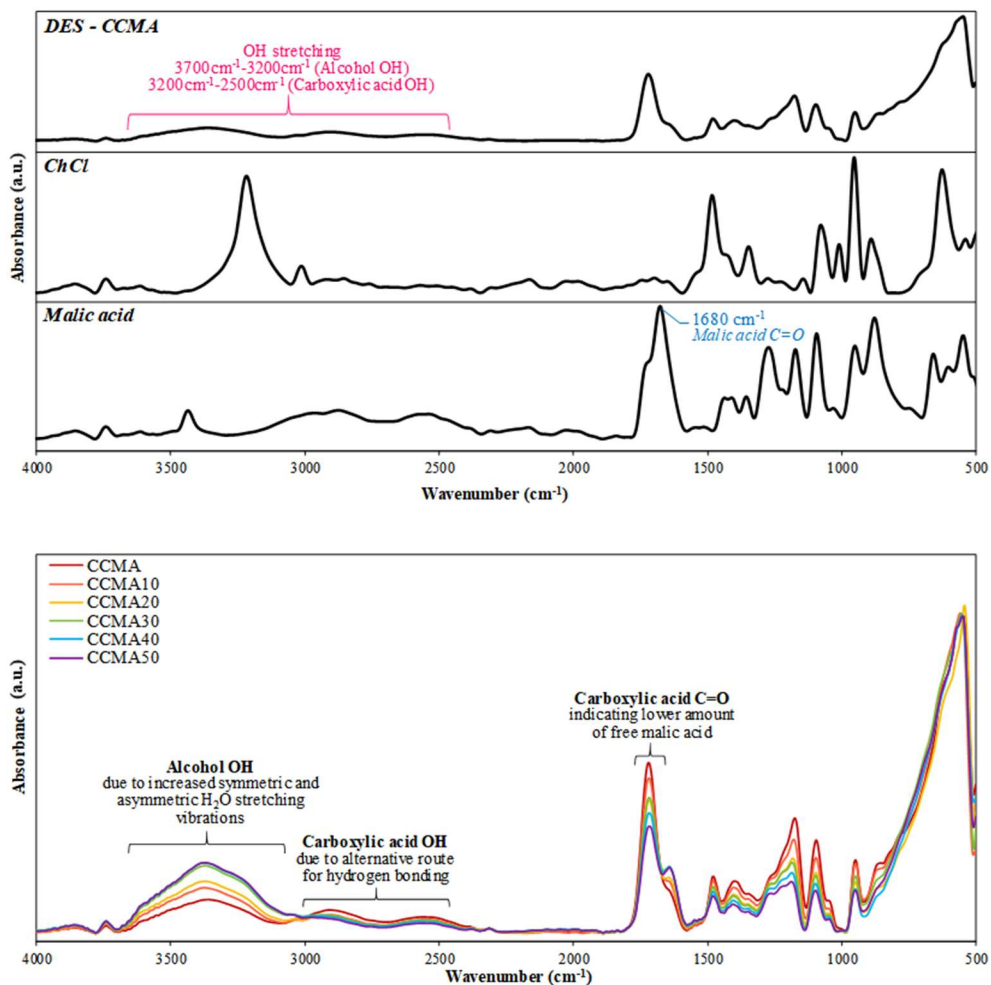


Fig. 2. FTIR Spectra for ChCl/Malic acid DESs.

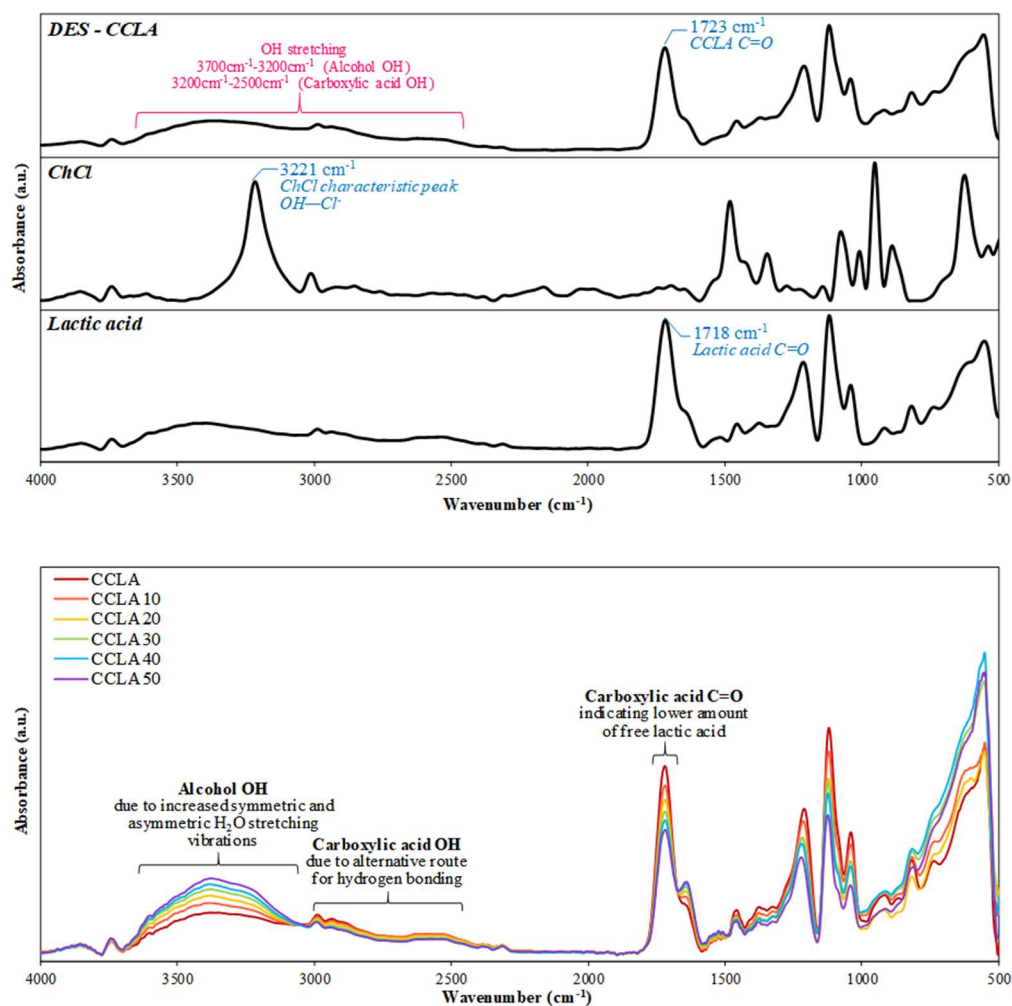


Fig. 3. FTIR Spectra for ChCl/Lactic acid DESs.

hydrogen bonding since water can act as both the HBA and HBD and interact with the other DESs constituents in the ternary system. The observations were applicable for both types of DESs. The hydroxyl (OH) stretching is associated with the alcohol group within the region 3700 cm^{-1} to 3200 cm^{-1} as shown in Fig. 2 and Fig. 3, demonstrating hyperchromic effect (toward higher absorbance) at higher water content due to the increased symmetric and asymmetric H_2O stretching vibrations [12]. The intensity of the carboxylic acid OH stretching detected within the range 3200 cm^{-1} to 2500 cm^{-1} demonstrates the slight hypochromic effect (toward lower absorbance) with the addition of water due to the increased dissociation of the intermolecular OH bond within the carboxylic acid. Apart from this, the C=O

peak centered around 1720 cm^{-1} (ChCl/Malic acid) or 1723 cm^{-1} (ChCl/Lactic acid) is associated with the carboxyl group of the malic acid or lactic acid, showing weaker signal with water addition, which is indicative of reduced free carboxylic acid in the DESs [7]. These changes are attributed to the increased availability of alternative routes for hydrogen bonding interactions between water molecules and the DESs components, which results in the weakening and disintegration of existing hydrogen bond network within the DESs [7,14].

The high retention of the overall DESs supramolecular structure could be observed even after substantial addition of water content up to 50 wt%; the phenomenon was ascribed to the high tolerance of the DESs nanostructure [7]. The ability

of the DESs to retain most of its original nanostructure was further attributed to the solvophonic sequestration of water molecules into the nanostructure, which surrounds the choline cations through short-range Coulombic and hydrogen-bonding interaction [7,14]. The interactions in the ChCl-based DESs were dominant even at high level of water addition up to 50 wt%; This did not cause severe disruption of the overall structure and did not substantially affect the eutectic nature of DESs [15]. However, there is an upper limit for the hydration of DESs such that the “*DES-in-water*” regime dominate after extensive dilution; this is opposed to the “*water-in-DES*” regime, in which an aqueous or diluted DESs solution is formed instead of a ternary DESs, although the supramolecular structure is mainly retained [14].

Density of DESs

Most DESs exhibited higher density than water and the experimental findings showed that the densities of ChCl/Malic acid and ChCl/Lactic acid DESs are in line with the typical range of DES densities from 1.04 g cm^{-3} to 1.63 g cm^{-3} [6]. The measurement of DESs density by using gravimetric method indicated that ChCl/Malic acid DESs are generally denser than ChCl/Lactic acid DESs. The difference in density between ChCl/Malic acid and ChCl/Lactic acid DESs is attributed to the different density of the starting materials (*i.e.* ChCl 1.10 g cm^{-3} , Malic acid 1.61 g cm^{-3} , Lactic acid 1.209 g cm^{-3} , and distilled water 0.9970 g cm^{-3} at $25 \text{ }^{\circ}\text{C}$), as well as the different ratio of ChCl to HBD that was used. Haghbakhsh *et al.* (2019) [16] explained that the variance of DESs densities is due to the difference in the constituents or their concentration in the DESs.

All the DESs showed decreasing densities upon addition of water, due to the fact that water exhibits relatively lower density and viscosity compared to most DESs, which consequently produced a dilution effect lowering the density of the DESs. Higher water content reduced the density of the DESs such that the density was close to that of water at extended dilution [7]. The decreased density with water addition was further attributed to the lowered free volume of the overall DESs due to addition of water [8]. The density of ChCl/Lactic acid DESs with varying water content is presented in Fig. 4.

Density is an important thermophysical property that is crucial in the design, operation and optimization of any

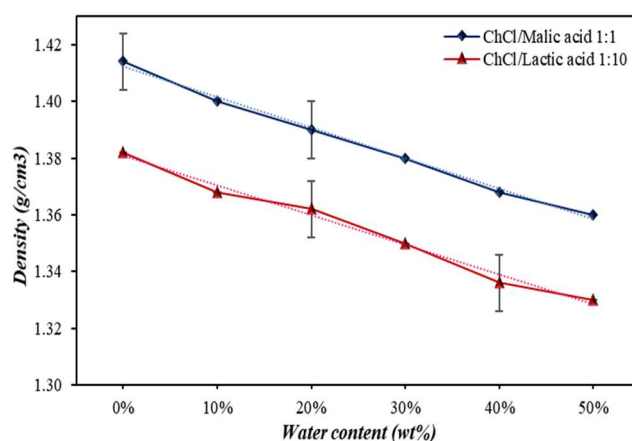


Fig. 4. Density of ChCl/Malic acid and ChCl/Lactic acid DESs with different water content measured by gravimetric method at $30 \text{ }^{\circ}\text{C}$.

application [16]. Solvents of lower density are often considered more economical ones due to the fact that solvents are often purchased in bulk by weight [16]. Thus, the density of DESs makes it practical for applications and possible to be modulated through water addition.

Biopolymers Solubility Capacity of DESs

The solubility of biopolymers in the synthesized DESs is important since they can be taken as benchmarks for the applicability of DESs for biomass pretreatment. The biopolymers solubility test was performed with Kraft lignin, starch, and cellulose to imitate the typical components in lignocellulosic biomass [10]. The mechanism of lignin dissolution by DESs was associated with the hydrogen bonding between the halogen component of ChCl and the hydroxyl groups of lignin that were involved in the cleavage of ether or ester bonds among hemicellulose and lignin [18]. The recalcitrance nature of lignocellulosic biomass is due to the covalent bonding of lignin to hemicellulose containing benzyl ester, benzyl ether, and phenyl glycoside groups, and also with further cross-linking through strong hydrogen-bonding interactions [19]. The strong hydrogen-bonding interactions in the lignin are possibly weakened due to the competing hydrogen-bond formation between the chloride ions of the DES and hydroxyl groups in the lignin-carbohydrate complex, which eventually results in the cleavage of the lignin-carbohydrate linkages [19]. The

solubility of lignin in DESs is dependent on the hydrogen bond between the choline ion and the hydroxyl group of the HBD, where a stronger hydrogen bond typically results in higher lignin dissolution [20]. The biopolymers solubility involving lignin, starch, and cellulose in ChCl/Malic acid and ChCl/Lactic acid DESs are shown in Fig. 5.

In contrast, higher lignin dissolution capacity was observed in ChCl/Lactic acid DESs due to the involvement of lactic acid molecule in the hydrogen bond interaction with the hydroxyl group of lignin, instead of chloride ion as in other typical solvent system [20]. In ChCl/Malic acid DESs, the dissolution of lignin in CCMA showed 2.73 wt% maximum solubility, which was in close agreement with the reported value by Francisco *et al.* (2012) [11]. Addition of water to the CCMA was found to improve lignin solubility and the highest lignin solubility was observed at CCMA40 with 3.67 wt% maximum solubility. ChCl/Lactic acid DESs showed a decreased in lignin solubility with water addition from 6.32 wt% in CCLA to 3.28 wt% in CCLA50. The different effects of water addition on the ChCl/Malic acid and ChCl/Lactic acid were due to the two different mechanisms of lignin solubilization (*i.e.* hydrotropy and co-solvency) [22]. The hydrotropic behavior refers to the phenomenon of increased solubility of water-insoluble or sparingly water-soluble organic compounds in aqueous solutions with the presence of hydrotropes. Meanwhile, the co-solvency behavior is based on the solvation of the solute by a mixed solvent (*i.e.* water + cosolvent). Hydrotropy solubilization is characterized by a sigmoidal solubilization profile and in some cases, showing a maximum point over the explored range while the solvation ability in co-solvency systems are often intermediate to those of pure water and the cosolvent itself [22]. The co-solvency solubilization profile is often linked to a linear or monotonic function of the DES concentration. In this scenario, the ChCl/Malic acid DESs showed hydrotropy behavior involving cooperative intermolecular reactions while the ChCl/Lactic acid DESs showed co-solvency solubilization behavior in water.

Starch often do not dissolve in solvents containing carboxylic acids as their HBD even when subjected to pretreatment at elevated temperature after prolonged duration [23]. CCMA was found to be highly capable of dissolution of starch up to 6.57 wt%, which is in agreement with the value reported in Francisco *et al.* (2012) [11]. Further addition of

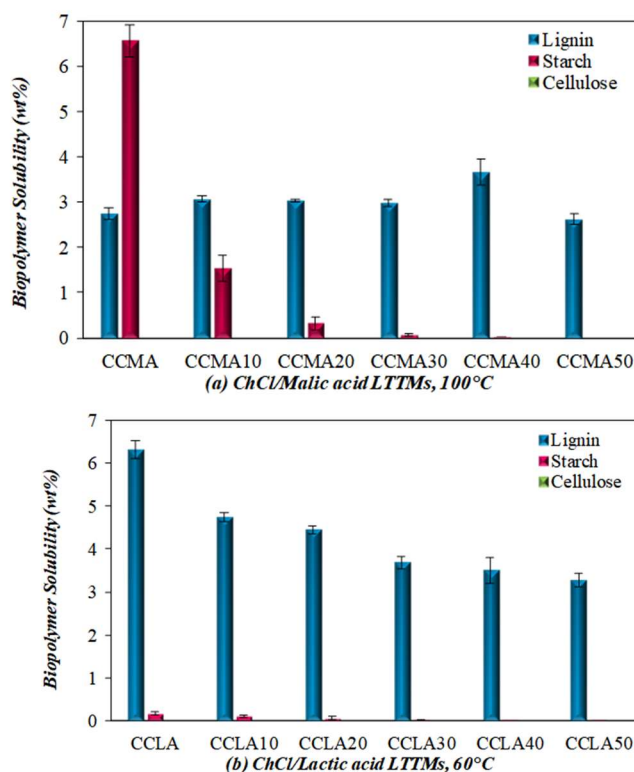


Fig. 5. Biopolymers solubility in (a) ChCl/Malic acid; and (b) ChCl/Lactic acid DESs at the specified pretreatment temperature.

water significantly reduced the starch dissolution capability to a very low solubility and ultimately zero solubility obtained for CCMA40 and CCMA50 samples. Meanwhile, the ChCl/Lactic acid demonstrated extremely low solubility of starch where the CCLA attained only 0.17 wt% starch solubility, which further reduced with addition of water to ultimately zero solubility in CCLA50 samples. The low solubility can be attributed to the preparation of CCLA from aqueous lactic acid which initially carried about 15 wt% of water [24]. The DESs with higher water content showed reduced dissolution capability for starch, mainly due to the insolubility of starch in water.

Cellulose was found to be insoluble in all the DESs which is in good agreement with the findings of Majová *et al.* (2017) [25]. This is attributed to the high tendency of carboxyl group (-COOH) of the HBD toward the hydroxyl group (-OH) of cellulose to form monoesters or cross-linked diesters, which hinders the dissolution of cellulose during acid hydrolysis

[20]. The experimental findings suggested that tailoring physicochemical properties of DESs by the adding water was feasible and could possibly improve biopolymers solubility and selectivity of the DESs, demonstrating hydrotropic solubilisation behavior.

The selectivity for lignin was higher in ChCl/Lactic acid due to the extremely low solubility of starch in ChCl/Lactic acid DESs. Another advantage of ChCl/Lactic acid was the lower pretreatment temperature at 60 °C compared to 100 °C for ChCl/Malic acid (due to the higher viscosity in ChCl/Malic acid), indicating a potential for energy savings in the long run. Nonetheless, ChCl/Lactic acid required an environment with strict control on moisture or humidity since the lignin solubility decreased even by moisture absorbed from the surrounding due to the hygroscopic nature of ChCl based DESs upon high number of hydrophilic groups [26].

Hindering Degradation in DESs

The ChCl/Malic acid and ChCl/Lactic acid DESs were kept at room conditions over a period of 30 days and all the DESs exhibited degraded performance in terms of lignin dissolution as shown in Fig. 6.

This phenomenon was due to the esterification reaction in DESs, occurring as an undesired side reaction over an extended period of time even at room conditions, and the degradation would be further accelerated at elevated values [27]. The addition of water was shown to prevent or slow down the degradation of DESs, due to the displacement of the reaction equilibrium by adding water [28]. In ChCl/Malic acid DESs, the addition of water resulted in lower degradation in CCMA50 (4.45%) and CCMA40 (10%) as compared to CCMA (44.2%). However, the addition of water to ChCl/Lactic acid DESs did not show the same trend as in ChCl/Malic acid DESs since the degradation were around 36.8% to 47.2% regardless of water content due to the high water content in the starting material. Hence, further addition of water could not further slowdown the esterification beyond the optimum state.

CONCLUSIONS

In conclusion, addition of water was proven to cause changes in the composition of DESs, which further altered their physicochemical properties. The effect of water on

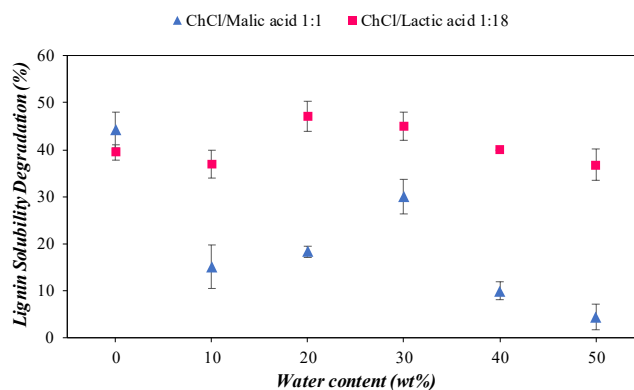


Fig. 6. Performance drop in lignin dissolution by using ChCl/Malic acid and ChCl/Lactic acid DESs with different water content after leaving for 30 days at room conditions.

DESs was found beneficial by a proper adjustment of water content. The addition of water resulted in decreasing density due to the lowered free volume of the overall DESs. FTIR spectra demonstrated the hydrogen bond formation between the HBA and HBD, which confirmed the role of water in forming ternary DESs instead of diluted DESs. The presence of an upper limit for water addition into DESs was also discovered, as finite capacity of hydrogen bonding restricted the further tuning of DESs composition by water addition. Biopolymers solubility test validated the effectiveness of ChCl/Malic acid and ChCl/Lactic acid DESs in lignin dissolution, hence indicating their high potential to be applied in biomass pretreatment. Addition of water to CCMA was found to improve lignin solubility up to 3.67 wt% over 2.73 wt%. The self-degradation of ChCl and carboxylic acids DESs due to esterification reaction was also prevented with the addition of water, which further enhanced their stabilizing capacity. CCMA with 50 wt% water addition was found to be able to suppress the degradation of CCMA DES to 10 times lower compare to its pure variant. The research findings from this study can contribute to the development of green solvents, particularly those involving DESs for overcoming the Achilles' heel of biomass-to-energy transformation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding

supports from Small Grant Scheme UNIMAS, F02/SGS/1992/2020, with finance ID of 05-FA020700-0613-0017.

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