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## Natural deep eutectic solvents and their application in natural product research and development

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## Chapter 4

### The effect of water content on the characteristics of Natural Deep Eutectic Solvents

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#### Abstract

Recently, binary systems made of ionic liquids and co-solvents have been explored in order to expand the applications of ionic liquids. Mixing ionic liquids with organic solvents or water is an efficient and controllable way to design functional ionic liquids through adjusting the physicochemical properties. In this study, binary systems of natural deep eutectic solvents (NADES) and water, the most abundant liquid on earth, were studied in terms of their structures, physicochemical properties, and solubilization capacity. FT-IR spectra demonstrated H-bonding interactions between the two components of NADES. NMR spectra showed that dilution of the NADES causes a gradual weakening of the H-bonding interactions between the two components and that disappears completely at around 50% (v/v) water dilution. A small amount of water (e.g. 5% in weight) can reduce the viscosity of NADES to the range of water. Conductivity of NADES can be increased by up to 100 times with water dilution for some NADES. Water activity and density of NADES have a quantitative relationship with the water content in NADES. The solubility of quercetin decreases significantly in PCH with water dilution, while the solubility of carthamin reaches the maximal value with 5% water (v/v) in PCH. This study provides the basis for modulating NADES for applications in pharmaceuticals, cosmetics, enzyme reactions, and food processing.

**Key words:** Natural deep eutectic solvents; water content; Hydrogen bond interactions; physicochemical properties; solubility.

## 1. Introduction

A new type of green solvents, natural deep eutectic solvents (NADES) have been proposed by our group (Choi *et al.*, 2011; Dai *et al.*, 2013) to extend the range of ionic liquids (ILs) and deep eutectic solvents (DES) and explore their applications in health related fields. Ionic liquids are a class of organic salts with a low melting point (Welton, 1999). Deep eutectic solvents are mixtures of compounds that have a much lower melting point than any of its individual components (Abbott *et al.*, 2004). Natural deep eutectic solvents are composed of natural primary metabolites from organisms such as sugars, sugar alcohols, organic acids, amino acids, and amines, and additionally often contain water in certain molar ratios. They are characterized by extensive intermolecular interactions (Choi *et al.*, 2011; Dai *et al.*, 2013). They can be approximately classified into five main groups: ionic liquids formed by an acid and a base, sugar-based NADES with only neutral compounds, sugar-based NADES with bases, sugar-based NADES with acids and sugar-based NADES with amino acids. Natural deep eutectic solvents possess excellent properties as solvents such as negligible volatility except for the water contained, liquid state even below 0 °C, a broad range of polarity and high solubilization strength for a wide range of compounds, including especially for poorly water-soluble compounds (Dai *et al.*, 2013). As solvents, from an environment and economic perspective, NADES also offer many striking advantages including biodegradability, sustainability, low costs, simple preparation, and low toxicity. All these properties make them of interest for applications in health related areas such as food, pharmaceuticals and cosmetics. They have already been used to dissolve DNA (Mamajanov *et al.*, 2010; Dai *et al.*, 2013), as media for enzyme reactions (Zhao *et al.*, 2011; Choi *et al.*, 2011), and biotransformations (Gutiérrez *et al.*, 2010).

These solvents, however, share some of the limitations observed in synthetic ILs and DES. Their high viscosity (typically 200-500 mm<sup>2</sup>/s at 40 °C) (Dai *et al.*, 2013) is the most obvious issue, which leads to some practical problems. These include time consuming solvent transfer operations and slow mass transfer in dissolutions and extractions. In ILs, this is generally overcome by applying external physical forces, such as microwave (Swatloski *et al.*, 2002) and stirring at high temperature (Abe *et al.*, 2010), thus accelerating the dissolving process. Another way to get around this problem is diluting the ILs with water, and the viscosity of diluted NADES or synthetic ILs can be decreased even down to the range of water (Jacquemin *et al.*, 2006; Dai *et al.*, 2013). Another important issue to be solved is to design appropriate NADES for certain applications. Mixing ILs with organic solvents or water is an efficient way to engineer functional solvents (Kohn and Ohno, 2012). Therefore, dilution with water might be a solution to solve these practical problems and realize or extend the applications of NADES in health related areas.

There is an even more interesting aspect to understand the effect of water on NADES. Water is the most abundant liquid on earth and it plays an important role in biological systems. NADES could play a major role as a third type of solvent in cells and living organisms, besides water and lipids (Choi *et al.*, 2011; Dai *et al.*, 2013). From biological viewpoint, given the coexistence of the two liquids (NADES and water) in organisms it is of great importance to learn more about how water affects NADES.

Different aspects of the effect of water or other organic solvents on ILs have been studied, including structures, physical properties and applications aspects by different groups. In general, the structures and corresponding physicochemical properties of ILs will change with dilution. With the addition of water, the interaction of the two components of ILs is weakened and new hydrogen bonds are formed between the ions and water, as discovered by IR and NMR spectroscopy (Zhang *et al.*, 2010; Mele *et al.*, 2003; Hayes *et al.*, 2012). Certain amounts of water in ILs decrease their viscosity and density (Seddon *et al.*, 2000; Jacquemin *et al.*, 2006), accelerate the diffusion (Schröder *et al.*, 2000), and change the polarity (Fletcher and Pandey, 2003) and conductivity (Widegren *et al.*, 2005) of ILs. ILs diluted with a large amount of water or ethanol or methanol have been used in the extraction of diverse natural products (Du *et al.*, 2007; Cao *et al.*, 2009; Bica *et al.*, 2011; Usuki *et al.*, 2011) and enzyme reactions (Barahona *et al.*, 2006). The extraction yield of certain compounds can be increased by 60-90% through adjusting its water content and the optimal concentrations of ILs for extraction are in the range of 0.5-2 M (Cao *et al.*, 2009; Du *et al.*, 2007; 2009; Dai *et al.*, chapter 1). However, the applications of synthetic ILs in the health-related areas are limited because of the high toxicity of some ingredients, their irritation properties, and high costs of synthesis of the components (Docherty *et al.*, 2005; Quijano *et al.*, 2011). Therefore, in view of their potential applications and the fact that their intermolecular interactions might differ from those in ILs and DES, it is necessary to investigate their behavior when diluted with water.

Thus we explored NADES and water mixtures in terms of supermolecular structures, physicochemical properties, and solubilizing capacity for non-water soluble compounds (quercetin) and a medium polar compound (carthamin) to demonstrate the role of water dilution in developing tailor-made NADES for specific applications. This study provides the basis for deeper understanding the chemical structures and physical properties, and lays the basis for design an adaptable NADES for specific applications in health-related areas.

## 2. Material and methods

### 2.1 Chemicals and material

Water was deionized water. Malic acid, lactic acid, proline, glucose, sucrose, 1,2-propanediol, and choline chloride were purchased from Sigma (St. Louis,

MO, USA). Deuterium oxide was obtained from CortecNet (Voisins-Le-Bretonneux, France.) Carthamin and quercetin were previously isolated in our laboratory.

## 2.2 Solvent preparation

NADES including malic acid-choline chloride (MC); proline-malic acid (PM); and glucose-choline chloride (GC) were prepared by heating with stirring at 70 °C until a clear liquid was formed. Other NADES including malic acid-choline chloride-water (molar ratio, 1:1:2; MCH), proline-malic acid-water (1:1:3; PMH), glucose-choline chloride-water (2:5:5; GHC), 1,2-propanediol-choline chloride-water (1:1:1, PCH), lactic acid-glucose-water (5:1:3, LGH); sucrose-choline chloride-water (1:4:4, SuCH) and other NADES listed in table 1 were prepared with the following method: the mixture of two components with certain amount of water was placed in a bottle with a cap and heated in a water bath at 50 °C with agitation till a clear liquid was formed (Dai *et al.*, 2013). 1,2-propanediol-choline chloride-water (1:1:1, PCH) were diluted with different percentage (v/v) of deuterium oxide one day before the <sup>1</sup>H NMR measurement.

## 2.3 Solubility test

Solubility tests were carried out by saturating NADES with an excess of the tested compound at 40 °C, as reported in our former study (Dai *et al.*, 2013). The resulting solution was analyzed with HPLC-UV at a wavelength of 370 nm for quercetin and 517 nm for carthamin. The solubility test was repeated three times.

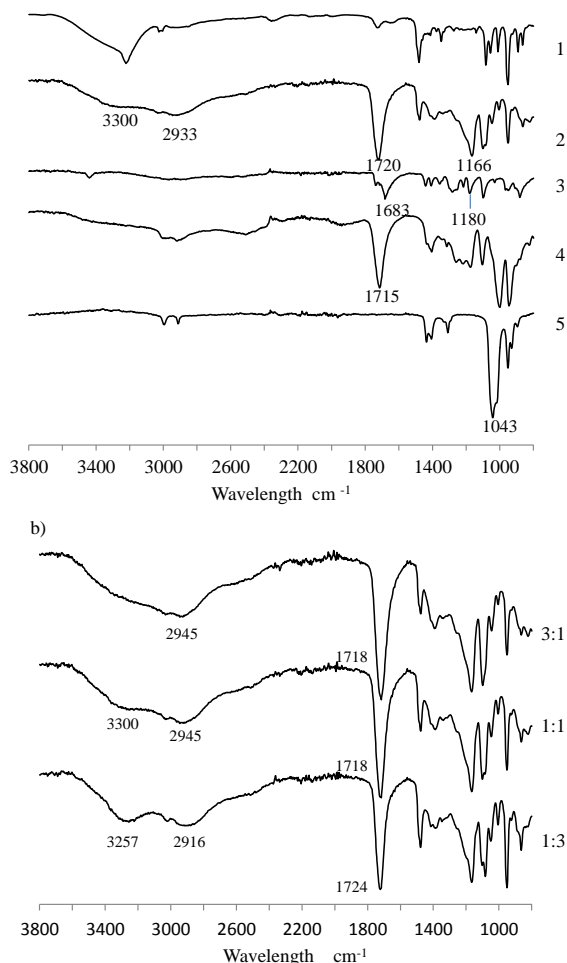
## 2.4 Apparatus and analysis

FT-IR spectra over the range from 4000 to 300 cm<sup>-1</sup> were collected at room temperature (25 °C) using a Bruker FT-IR spectrometer. The <sup>1</sup>H NMR spectra of samples were recorded at 40 °C on a 500 MHz Bruker DMX-500 spectrometer (Bruker, Karlsruhe, Germany) with the parameters previously reported (Dai *et al.*, 2013). Water activity test was performed in a Labmaster water activity equipment (Novasina, Switzerland) at 40 °C. Density tests were performed using a density meter (DMA 5000) at 40 °C. Conductivity test was recorded at ambient temperature (27 °C) on a 756 KF coulometer (Metrohm) equipped with 728 Stirrer and 756 KF Coulometer keyboard). Polarity testing was done with Nile red (NR) as a solvatochromatic probe. The λ<sub>max</sub> was determined with a UV/Vis spectrophotometer and calculated in the following formula to obtain E<sub>NR</sub> (Ogihara, *et al.*, 2004):

$$E_{NR} \text{ (kcal/mol)} = hc N_A / \lambda_{\max} = 28591 / \lambda_{\max}.$$

## 3. Results and discussion

### 3.1 The structure of NADES with and without water



**Fig. 1.** FT-IR **a)** of **1)** choline chloride, **2)** malic acid-choline chloride (1:1, molar ratio; MC), **3)** malic acid solid, **4)** malic acid in DMSO and **5)** DMSO and **b)** malic acid-choline chloride with increasing amount of choline chloride (the molar ratio of malic acid to choline chloride 3:1, 1:1; 1:3) at room temperature.

**3.1.1 The structure of NADES.** Previous studies using NMR spectroscopy showed that there are cross interactions, and particularly hydrogen bonds, between the two components of NADES (Abbott *et al.*, 2004, Dai *et al.*, 2013). The structure of NADES was further investigated using IR to explore the type of interactions, the atoms involved in the interactions and even the possible ratios of the two components. Various categories of changes may occur on the establishment of an hydrogen bond, which can be characterized by the following structural and spectroscopic features (Reichardt, 2003): 1) The distance between the neighboring atoms involved in the hydrogen bonds are

considerably smaller than the sum of their van der Waals radii, resulting in correlation signals in nuclear Overhauser effect spectroscopy (NOESY) or Heteronuclear Overhauser Effect Spectroscopy (HOESY) spectra; 2) The electron density at H-atoms involved in H-bonds is reduced, resulting in downfield shifts of their  $^1\text{H}$  NMR signals; 3) The bond length and corresponding vibrations of H-bonds are changed, which can be detected in IR. The vibrations involve the H-bond donor, such as the bending bond  $\delta_{\text{C-O-H}}$  of a C-O-H group. Vibration changes also occur in the vicinity of the H-bonds, such as the stretching bond  $\nu_{\text{C-OH}}$ , as well as in the H-bond acceptor, such as the  $\nu_{\text{C=O}}$  in carboxyl or carbonyl groups (Maréchal, 2007).

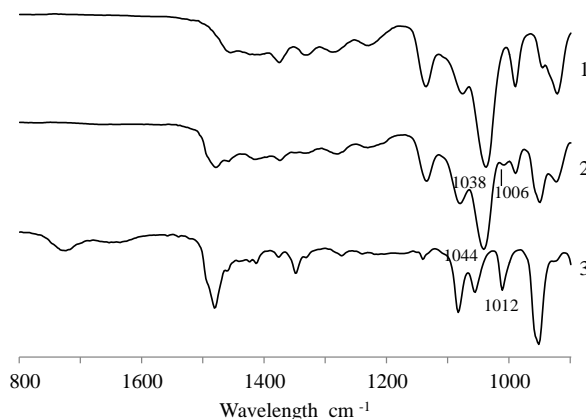
The IR spectrum of malic acid-choline chloride (Fig. 1a) shows broad moderate bands centered around 3300 and 2933  $\text{cm}^{-1}$ , the stretching vibration of hydrogen-bonded hydroxyl group  $\nu_{\text{O-H}}$  in malic acid and choline chloride and carboxyl group in malic acid, which means the formation of hydrogen bonds between malic acid and choline chloride. The strong band at 1683  $\text{cm}^{-1}$  is ascribed to the stretching vibration of carboxylic acid  $\nu_{\text{C=O}}$  dimers. The band of  $\nu_{\text{C=O}}$  is shifted upwards to 1720  $\text{cm}^{-1}$  in malic acid-choline chloride, which is attributed to new H-bonds formed in the malic acid-choline chloride complex with malic acid as H-bond donor. Concerning the strength of the H-bond,  $-\text{COOH}$  is more likely to form an H-bond with  $\text{Cl}^-$  than with the O in choline chloride (Barańska *et al.*, 2003). So, a hydrogen bond ( $-\text{COOH} \cdots \text{Cl}^-$ ) is formed between malic acid and choline chloride. On forming the malic acid-choline chloride complex, the deformation vibration absorption band of  $\delta_{\text{CH}_2}$  increased in intensity and is shifted from 1180  $\text{cm}^{-1}$  down to 1166  $\text{cm}^{-1}$ , suggesting that malic acid has a different configuration in the malic acid-choline chloride eutectic mixture than in solid state (Barańska *et al.*, 2003).

In addition, the type and position of hydrogen bonds were confirmed in the following two experiments. In malic acid DMSO solution, the strong band of the carboxyl group in malic acid  $\nu_{\text{C=O}}$  shifted from 1683  $\text{cm}^{-1}$  upwards to 1715  $\text{cm}^{-1}$  in a malic DMSO solution (Fig. 1a), which is attributed to new H-bonds formation in the malic acid-DMSO complex with malic acid as H-bond donor ( $\text{S=O} \cdots \text{HO-C=O}$ ). It is similar to the malic acid-choline chloride complex. The hydrogen bonds were confirmed by the downwards shift of the stretching vibration band  $\nu_{\text{S=O}}$  from 1042  $\text{cm}^{-1}$  to 1000  $\text{cm}^{-1}$ . The IR spectra of malic acid-choline chloride mixtures with different ratios (Fig. 1b) confirm that there are at least two kinds of H-bonds between malic acid and choline chloride. The formation of an H-bond affects two characteristic absorptions of the carboxylic acid, namely the stretching vibrations  $\nu_{\text{C=O}}$  around 1720  $\text{cm}^{-1}$  and  $\nu_{\text{H-O}}$  from  $\text{COO-H}$  around 3000  $\text{cm}^{-1}$ . With the increasing amount of choline chloride, the upward shift of  $\nu_{\text{C=O}}$  from 1718  $\text{cm}^{-1}$  to 1724  $\text{cm}^{-1}$  and downward shift of  $\nu_{\text{H-O}}$  from 2945  $\text{cm}^{-1}$  to 2916  $\text{cm}^{-1}$  indicates more H-bonds (e.g.  $-\text{COOH} \cdots \text{Cl}^-$ ) are formed between malic acid and choline chloride. The characteristic vibration band of  $\nu_{\text{O-H}}$  around 3300  $\text{cm}^{-1}$  increased in intensity and also shifted downward



to  $3257\text{ cm}^{-1}$  with the increasing amount of choline chloride, suggesting that hydroxyl groups in the mixture also form H-bonds.

Similar hydrogen bonds are also detected in the 1,2-propanediol-choline chloride mixture. The IR spectrum of 1,2-propanediol and choline chloride also indicates the existence of H-bonds ( $\text{CH-OH}\cdots\text{Cl}^-$ ) between 1,2-propanediol and choline chloride (Fig. 2). On forming a eutectic mixture, the  $\nu_{\text{C-OH}}$  of 1,2-propanediol shifted from  $1038\text{ cm}^{-1}$  to  $1044\text{ cm}^{-1}$  indicating the H-bond formation between the hydroxyl groups of 1,2-propanediol and choline chloride with 1,2-propanediol as H-bonding donor. Concerning the strength of the H-bonding, C-OH is more likely to form H-bonding ( $\text{CO-H}\cdots\text{Cl}^-$ ) with  $\text{Cl}^-$  than O in choline chloride. In addition, the formation of a H-bond with  $\text{CH}_2\text{-OH}$  in choline chloride as H-bond donor was revealed by the shift of the vibration band of  $\nu_{\text{C-O}}$  in  $\text{CH}_2\text{-OH}$  of choline chloride from  $1012\text{ cm}^{-1}$  in the solid to  $1006\text{ cm}^{-1}$  in the 1,2-propanediol-choline chloride mixture. All this structural information is in agreement with our reported 2D NMR results (Dai *et al.*, 2013). Clearly, there is an extensive hydrogen-bonding network between the components of NADES; thus NADES are supermolecules.

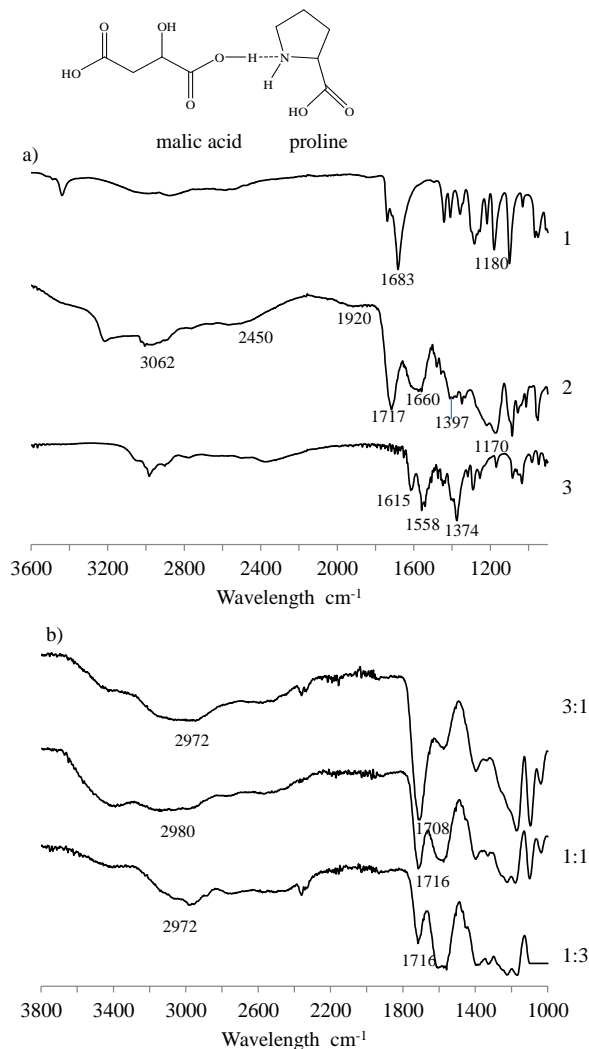


**Fig. 2.** FT-IR of **1**) choline chloride, **2**) 1,2propanediol:choline chloride (molar ratio 1:1) and **3**) choline chloride.

The NOESY spectrum of PMH showed strong interactions between malic acid and proline (Dai *et al.*, 2013). Infrared spectra of the proline-malic mixture, solid malic acid, and solid choline chloride (Fig. 3a) may provide more detailed information on the supermolecular structure of PMH. Proline exists in the ionic form in solid state, as indicated by the stretching bond  $\nu_{\text{C=O}}$  at  $1615\text{ cm}^{-1}$ . A strong band at  $1683\text{ cm}^{-1}$  indicates that malic acid exists in the dimeric form in solid state. In the malic acid-proline mixture, new H-bonds form between the two components, as shown by the broad band at  $3062\text{ cm}^{-1}$  of  $\nu_{\text{OH}}$  in  $\text{COOH}$  and the upward shift of the  $\nu_{\text{C=O}}$  to  $1717\text{ cm}^{-1}$  in malic acid. The stretching bands of hydroxyl groups shifted downwards to ca.  $2450\text{ cm}^{-1}$  and  $1920\text{ cm}^{-1}$  which is

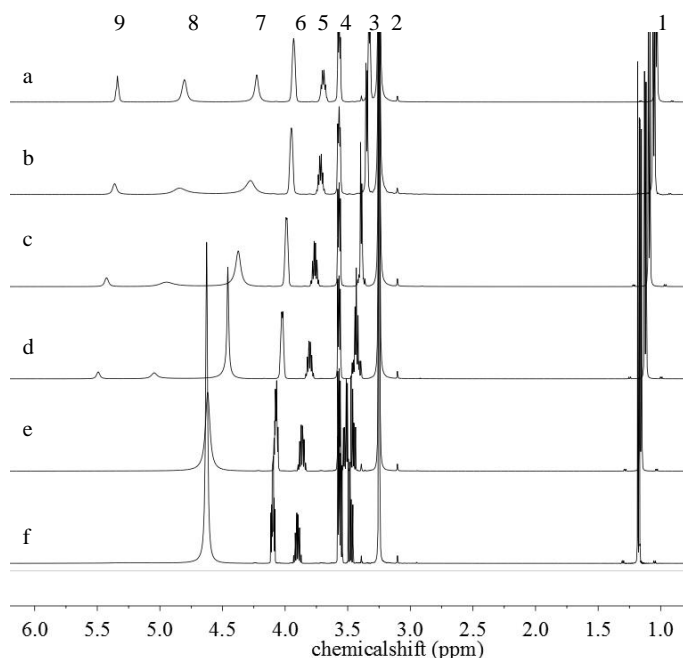
indicative of the appearance of a H-bond interaction ( $\text{O}=\text{C}-\text{O}-\text{H}\cdots\text{N}$ ) between malic acid and proline in the liquid crystal (Barańska *et al.*, 2003; Xu *et al.*, 2005). On forming the malic acid–proline complex, in malic acid the deformation vibration absorption band of  $\delta_{\text{CH}_2}$  increased in intensity and shifted from  $1180\text{ cm}^{-1}$  down to  $1170\text{ cm}^{-1}$ , and in proline the strong in-plane bending vibration of the carboxyl group  $\delta_{\text{OH}}$  shifted from  $1374\text{ cm}^{-1}$  to  $1397\text{ cm}^{-1}$  and the strong broad band of amine  $\delta_{\text{NH}}$  shifted from  $1557\text{ cm}^{-1}$  to  $1660\text{ cm}^{-1}$ , suggesting that malic acid and proline have a different configuration in the malic acid–proline eutectic mixture than in their solid state (Barańska *et al.*, 2003; Sheena Mary *et al.*, 2009). The IR spectra of malic acid–proline mixed in different molar ratios (1:3; 1:1; 3:1) (Fig. 3b) show that the band of  $\nu_{\text{C}=\text{O}}$  at  $1708\text{ cm}^{-1}$  shifts up to  $1716\text{ cm}^{-1}$  and  $\nu_{\text{OH}}$  in carboxyl group shifts from  $2972\text{ cm}^{-1}$  to  $2980\text{ cm}^{-1}$ , indicating that more H-bonds between proline and malic acid are formed when the ratio of proline–malic acid increases from 1:3 to 1:1. When the amount of proline–malic acid increases to 3:1, there is no shift of the  $\nu_{\text{CO}}$  and  $\nu_{\text{OH}}$  in carboxyl group. This phenomenon suggests that 1:1 is a proper ratio for malic acid and proline to form a eutectic mixture.

**3.1.2 The structure of NADES with water dilution.** To further explore the supermolecular structure of NADES after dilution with water, the NADES made mixture of 1,2- propanediol and choline chloride (PCH) was diluted with 0% to 75% (v/v) deuterium oxide and investigated with NMR as an example. FT-IR and the NOESY spectra confirm the existence of hydrogen bonds between the hydroxyl groups of 1,2-propanediol and chloride ion of choline chloride and between all the hydroxyl groups from molecules of 1,2-propanediol, choline and water (Dai *et al.*, 2013). The  $^1\text{H}$  NMR spectra of 1,2-propanediol-choline chloride with increasing amount of  $\text{D}_2\text{O}$  (Fig. 4, Table 1) show a continuous downfield shift of all signals from the two components except the peaks of methyl and methylene groups vicinal to the nitrogen atom, suggesting hydration of the two organic components of the mixture PCH. Also, the relative number of detected protons on the hydroxyl groups (relative to the protons on the methyl group) decreased during dilution (Table 1). The decreasing number of detected hydroxyl groups implies the rupture of the hydrogen bond between 1,2-propanediol and choline chloride. When the dilution increased from 25% to 50%, all the signals of the hydroxyl groups disappeared and there was no further shift of the water signal with a further dilution to 75%  $\text{D}_2\text{O}$ . This indicates the complete rupture of the hydrogen bond between 1,2-propanediol and choline chloride with 50%  $\text{D}_2\text{O}$ . The above results show that the supermolecular complex interactions of PCH are perserved with less than 50% water dilution, but further dilution produce a solution of the free forms of the individual components in water (Gutiérrez *et al.*, 2009). (The further downfield shift of peaks from all methylene or methyne connected to hydroxyl group is due to the effect of concentration as the diluted increased from 50% to 75% (Kim *et al.*, 2006)).



**Fig. 3.** FT-IR **a)** of **1)** malic acid, **2)** malic acid-proline (1:1, molar ratio; PM) and **3)** proline solid at room temperature and **b)** malic acid:proline (PM) with different molar ratio (3:1; 1:1; 1:3).

It was reported that with the addition of water, the interaction of the two components of ILs is weakened (López-Pastor *et al.*, 2006; Kohno and Ohno, 2012). With increasing water concentration, association of water molecules with the anions is observed. With further dilution, the association with water molecules increases and ends with hydrated ions. In agreement with ILs, dilution of NADES with a large amount of water will lead to the hydration of each component, resulting in a solution of two free components.



**Fig. 4.**  $^1\text{H}$  NMR recorded at 40 °C of 1,2-propanediol-choline chloride- $\text{H}_2\text{O}$  (1:1:1) diluted using deuterium oxide from 0% (**a**), 5% (**b**), 15% (**c**), 25% (**d**), 50% (**e**), 75% (**f**)  $\text{D}_2\text{O}$  (v/v) with details of downfield shifts of HDO (peak 7), choline (peaks 2, 4, 6), 1,2 propanediol (peaks 1, 3, 5) and hydroxyl groups from 1,2-propanediol (peaks 8) and choline (peaks 9).

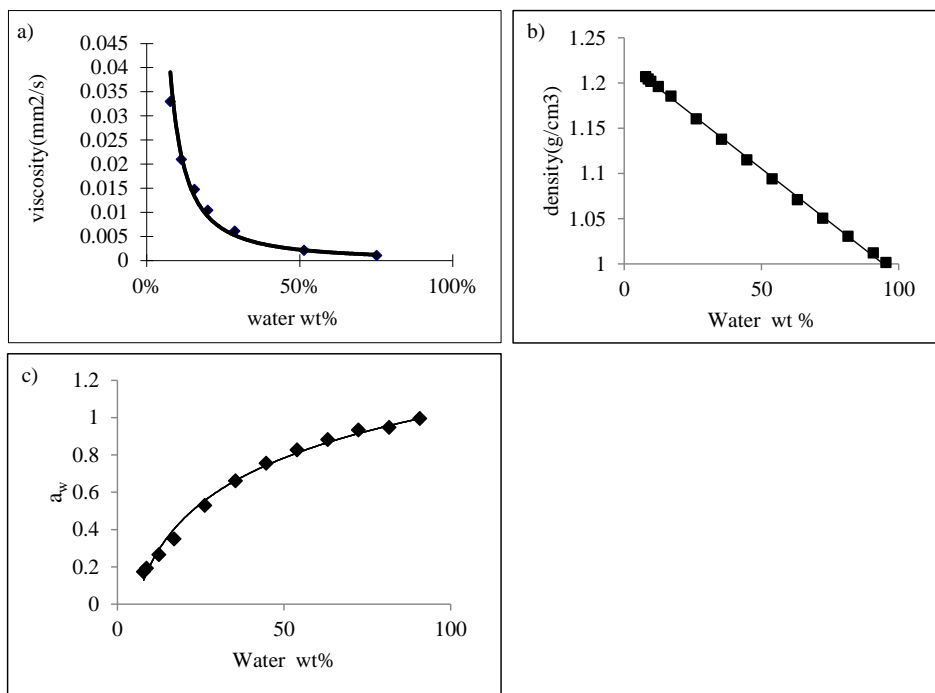
**Table 1.** Chemical shift and some integrated areas of peaks in  $^1\text{H}$  NMR spectroscopy of 1,2-propanediol- choline chloride- $\text{H}_2\text{O}$  diluted with different percentage of deuterium oxide at 40 °C.

PCH% (V)	1,2-propanediol $\text{HOCH}_2(\text{OH})\text{CH}-\text{CH}_3$				choline chloride $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}$				HDO
	$\text{CH}_3$	$\text{HOCH}_2$	$\frac{(\text{OH})}{\text{CH}}$	-OH	$\text{NCH}_3$	$\text{NCH}_2$	$\frac{\text{CH}_2}{\text{OH}}$	$\text{CH}_2\text{OH}$	HDO
	6	4	5	8	3	2	1	9	7
100%	1,04 <sup>a</sup>	3,69	3,33	4.8(1.24H <sup>b</sup> )	3,25	3,57	3,93	5,34(0,6H)	4,23(1,29H)
95%	1,05	3,72	3,35	4.84(0.98H)	3,25	3,57	3,95	5,36(0,47H)	4,28(1,56H)
85%	1,09	3,76	3,39	4.94(0.77H)	3,25	3,57	3,99	5,43(0,38H)	4,37(2,55H)
75%	1,12	3,8	3,43	5.04(0.55H)	3,25	3,57	4,02	5,49(0,27H)	4,46(2,55H)
50%	1,16	3,87	3,49		3,25	3,57	4,07		4,62(6,16H)
25%	1,18	3,90	3,52		3,25	3,56	4,1		4,63(11,64H)

<sup>a</sup> the chemical shift of the proton underlined; <sup>b</sup> the relative integrated area of the proton in hydroxyl groups (relative to the protons on the methyl group).

### 3.2 Properties of NADES diluted with water

**3.2.1 Viscosity.** The viscosity of NADES is greatly affected by the water content. For example, the viscosity of GCH decreases to 1/10 of the original value ( $397.4 \text{ mm}^2/\text{s}$ ) with the addition of 10% water (Dai *et al.*, 2013). Even for NADES with the lowest viscosity, PCH, the viscosity decreases to 1/15 of the original value ( $33 \text{ mm}^2/\text{s}$ ) when diluted with the same volume of water following a function  $y=0.0008x^{-1.524}$  ( $r=0.99$ ) (Fig. 5a). The high viscosity of NADES is often attributed to the presence of extensive hydrogen bonding network between the components (Zhang *et al.*, 2012), which is in agreement with our IR results mentioned above. In fact one may consider the NADES to be liquid crystals. Through dilution with water, the interactions between the components of NADES become weaker, as mentioned before. The change in structure leads to a large decrease in viscosity of NADES. After adding 25% water to GCH and PCH, their viscosity decreased to  $7.2 \text{ mm}^2/\text{s}$  and  $6.10 \text{ mm}^2/\text{s}$ , respectively, which is in the range of the viscosity of water ( $0.66 \text{ mm}^2/\text{s}$ ), whereas they still possess the supermolecular characteristic.



**Fig. 5.** The relationship curves a) between the viscosity and water percentage in weight (w), b) between the density ( $\text{g}/\text{cm}^3$ ) and water percentage and c) between water activity and water percentage in glucose-choline (1:2.5).

**3.2.2 Conductivity.** The conductivity of 16 typical NADES and four kinds of diluted NADES with water was investigated at room temperature. Big differences in conductivity were observed for the NADES, as shown in Table 2.

Polyalcohol-choline has the highest conductivity value (GlyCH, 13.75 mS/cm) while sugar-sugar (FGSH, 0.001 mS/cm) has the lowest, being similar to water (0.002 mS/cm) and methanol (0.004 mS/cm). In this perspective, the NADES made of sugars can be considered as neutral NADES. The conductivity of NADES decreased in the following sequence: base-polyalcohol > base-organic acid  $\approx$  base-sugar > organic acid-non-polar amino acid > organic acid-sugar > sugar-sugar.

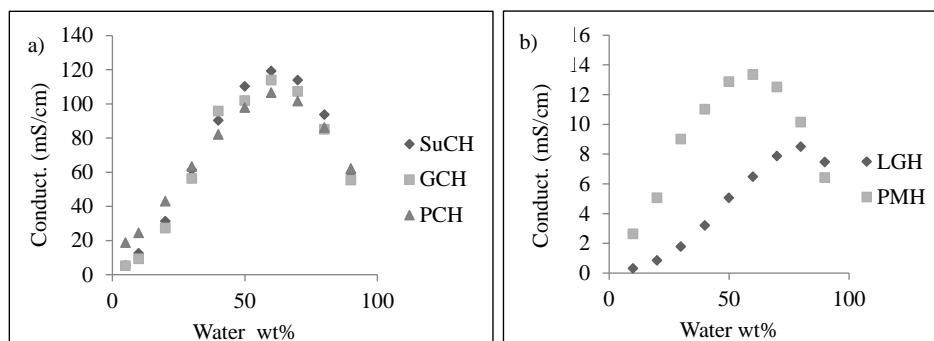
**Table 2.** The conductivity (mS/cm) of natural deep eutectic solvents at room temperature with water and methanol as reference (at 27 °C).

compositions	NADES	conductivity
fructose:glucose:sucrose: water (1:1:1:11)	FGSH	0.0012
lactic acid:glucose: water (5:1:3)	LGH	0.114
malic acid: $\beta$ -alanine:water (1:1:3)	MAH	0.551
proline:malic acid: water (1:1:3)	PMH	1.06
fructose: choline chloride: water (2:5:5)	FCH	3.1
xylose: choline chloride: water (1:2:2)	XCH	3.15
sucrose: choline chloride: water (1:4:4)	SuCH	3.39
malic acid: choline chloride: water(1:1:2)	MCH	5.91
lactic acid: choline chloride: water (1:1)	LC	6.76
sorbitol: choline chloride: water (2:5:6)	SoCH	6.77
glucose: choline chloride: water (2:5:5)	GCH	6.81
1,2-propanediol: choline chloride: water (1:1:1)	PCH	12.09
xylitol:choline chloride: water (1:2:3)	XoCH	13.55
glycerol:choline chloride:water(2:1:1)	GlyCH	13.78
Water	water	0.00208
Methanol	methanol	0.00424

NADES with high and low conductivity values are selected to detect the effect of water content on the conductivity of NADES. The conductivity is first increased with increasing water content, and then decreased after reaching a peak value of around 10-100 times higher than that of pure NADES (Fig. 6). SuCH, GCH, PCH have the highest conductivity with 60% added water and LGH has the highest one with 80% added water. A similar phenomenon was also reported with ILs diluted with different percentage of water, in which the conductivity of ILs increased more than 10 times (Liu *et al.*, 2008). So, the conductivity of NADES can be tailored through changing the water content.

These results show that the conductivity of NADES is correlated with their composition and viscosity, similar as for ILs (Every *et al.*, 2004; Widegren *et*

*al.*, 2005; Chiappe and Pieraccini *et al.*, 2005). Comparing NADES with one same component, GCH ( $6.81 \text{ mS cm}^{-1}$ ) has much higher conductivity than LGH ( $0.11 \text{ mS cm}^{-1}$ ), and MCH ( $5.91 \text{ mS cm}^{-1}$ ) higher than PMH ( $1.06 \text{ mS cm}^{-1}$ ), which may be due to the ionic form of choline chloride and partial ionization of lactic acid ( $pK_a$  3.86) and proline ( $pK_a$  1.99). Thus, it may be concluded that NADES with choline chloride have a high conductivity while NADES with organic acid have low values. The second important factor is viscosity. The second important affecting factor for the conductivity of NADES is their viscosity. NADES made of polyalcohol-base such as GlyCH are highly conductive, and this might be correlated to their low viscosity ( $51 \text{ mm}^2/\text{s}$ ), around 1/10 of that of sugar-choline (Dai *et al.*, 2013). Most NADES with a low conductivity (lower than 10) are highly viscous, especially NADES containing a sugar or amino acid ( $300\text{--}600 \text{ mm}^2/\text{s}$ ). Through dilution with water, the interactions between the components become weaker leading to a decrease of the viscosity of the NADES and as a result the conductivity increases by 10–100 times. No relationship between the conductivity and polarity of NADES is observed.



**Fig. 6.** Conductivity data of diluted NADES at ambient temperature (27 °C) of **a)** sucrose: choline chloride: water (1:4:4, SuCH), glucose: choline chloride: water (2:5:5, GCH), 1,2-propanediol-choline chloride (1:1:1, PCH), and of **b)** lactic acid-glucose (5:1:3, LGH).

**3.2.3 Polarity, density and water activity.** Other physical properties, such as polarity, density and water activity, are also affected by the water content, like in ILs (Seddon *et al.*, 2000). Previous experiments showed that the water content has a great effect on the polarity of NADES (Dai *et al.*, 2013). In those experiment LGH with the highest (44.81 kcal/mol) and PCH with lowest polarity (50.07 kcal/mol) were selected to test the effect of water content on the polarity of NADES (Table 3, chapter 2). Notably, the polarity value of LGH remained the same when the water content increased from 0 to 25%, but increased considerably with 50% water dilution and finally reached a similar polarity value as water (48.2 kcal/mol) at 75% dilution. Conversely, the polarity

value of PCH decreased with the addition of water because the polarity value of PCH is bigger than water (Dai *et al.*, 2013). With 10% percent, there is no obvious change of the polarity of PCH, but it decreased with the addition of 15-50% water and finally stabilizes at 75% dilution. So water dilution results eventually in a polarity similar to water. The density of GCH decreased linearly with the addition of water which can be expressed in a function  $y = -0.0024x + 1.224$ ,  $r = 0.99$ , as shown in Fig. 5b. A similar linear relationship was also observed in SuCH ( $y = -0.0025x + 1.2418$ ,  $r = 0.99$ ) and PCH ( $y = -0.0009x + 1.0873$ ,  $r = 0.99$ ), which is consistent with the findings for synthetic ILs (Seddon *et al.*, 2000). The last property investigated in diluted NADES is the water activity. The water activity of NADES increased gradually with the addition of water, which is quantitatively expressed in a function  $y = 0.354\ln(x) - 0.6008$  for GCH (Fig. 5c). Above a 50% dilution of GCH with water, there is a linear relationship ( $y = 0.004x + 0.6016$ ,  $r = 0.99$ ) between the water content and water activity, which may be correlated with above mentioned effect of water content on the supermolecular structure of NADES.

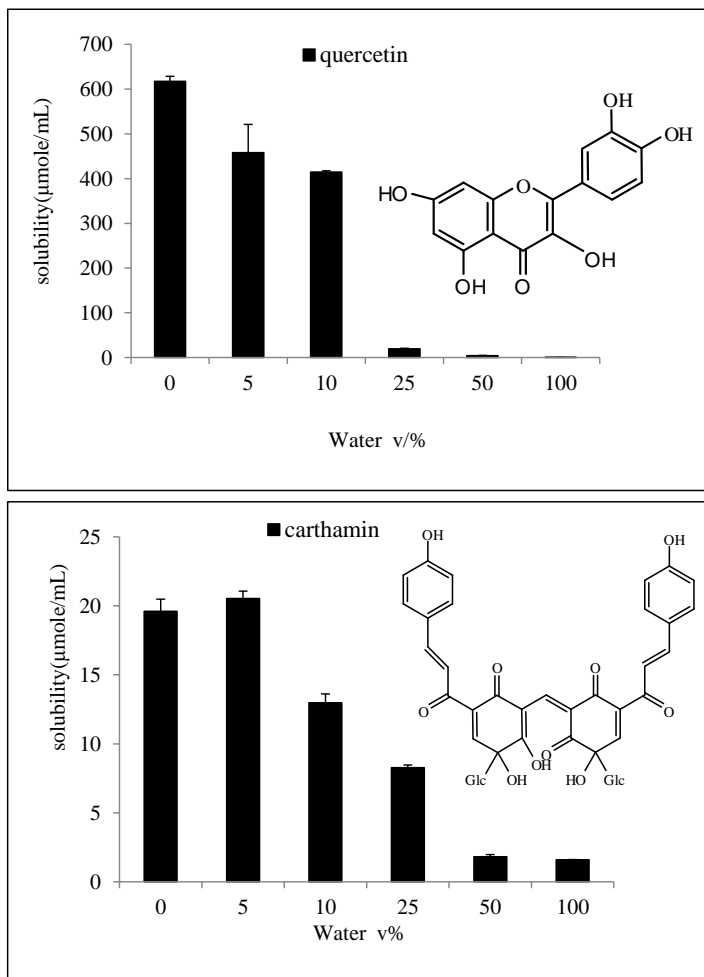
### 3.3 Applications of NADES diluted with water

The possibility of adjusting NADES properties quantitatively by adding water allows them to be adapted to different applications. An important application of NADES is their high solubilizing capacity. The solubility of quercetin (a widely spread flavonoid in plants) and carthamin was investigated in PCH with different water contents. The solubility of quercetin in PCH is 30,000 times higher than in water. However, its solubility changes with an increasing water content in PCH, decreasing in PCH with 5 v% water, keeping similar value with 10% water but decreasing drastically upon dilution with 25% water (Fig. 7). PCH is the NADES with the lowest polarity (50.0 kcal/mol, similar as methanol) of all our tested NADES (Dai *et al.*, 2013). The dilution of PCH leads to an increase of its polarity and consequently to a decrease in the solubility of nonpolar compounds. Notably, the case is different for carthamin (a natural dye and antioxidant), which is most soluble in PCH with 5% water, probably due to its medium polarity. Most interestingly, both the solubility of both quercetin and carthamin decrease strongly when the water content increases from 25% to 50% in PCH, reaching nearly the same values as in water. These phenomena are coherent with the above-mentioned effect of water dilution on the structure of PCH.

The physicochemical characteristics of NADES with different water contents are useful to predict the solubilization capacity of NADES. Our previous study showed that quercetin has the highest solubility in pure GCH, rutin with 5% (v/v) water in GCH and carthamin with 10% (v/v) water in GCH (Dai *et al.*, 2013). Firstly, this is due to the different polarity of the compounds and more water dilution is needed for more polar compounds to reach the highest solubility in NADES. The conclusion can be drawn that nonpolar compounds have the highest solubility in pure NADES and 5-10% water



dilution can increase the solubility of medium polar compounds in NADES. Secondly, the optimal water content is correlated with the viscosity of NADES instead of polarity due to the supermolecular structure of NADES. For example, carthamin has the highest solubility in GCH with 10% (v/v) water and in PCH with 5% (v/v) water. The viscosity of GCH (397 mm<sup>2</sup>/s) is much higher than PCH (33 mm<sup>2</sup>/s). The polarity of GCH is more close to water while PCH is more close to methanol (Dai *et al.*, 2013). However, to reach the highest solubility, more water is needed in GCH than in PCH and so this correlates with the high viscosity of GCH. This points to a strong liquid crystal like structure in GCH with high viscosity where there is no space for dissolving various solutes and more water is needed to loosen the H-bonded structure to dissolve solutes.



**Fig. 7.** The solubility of quercetin and carthamin in 1,2-propanediol-choline chloride-water (1:1:1, molar ratio) diluted with a different percentage of water. The data is expressed in mean  $\pm$  SD ( $n=3$ ).

The second important application of diluted NADES is for enzymatic reactions. For example, the water activity of ILs is an important factor affecting the microenvironment of enzymes and thus their activity. Enzyme activity is often low at both high and low water activity, thus requiring optimization of the conditions. We observed that laccase was inactive in malic acid-choline chloride-water (MCH) but was activated after dilution with 50% water. Similar phenomena were reported, for lipase with a maximum of the initial reaction rate at  $a_w=0.6$  in [bmim][PF<sub>6</sub>] (Barahona *et al.*, 2006) and for horseradish peroxidase being activated in the presence of a small amount of water (4.53%, v/v) in [Bmim][BF<sub>4</sub>] (Wang *et al.*, 2007). This aspect is of interest in connection with the functioning of enzymes in cells, apparently reaction rates can be changed by altering the water environment of the enzyme. It may also explain the germination of seeds and the survival of resurrection plants, which store enzymes in NADES and the enzymes can be activated after absorbing water.

## 4. Conclusion

NADES are supermolecules with hydrogen-bonding interactions between the components, which will be weakened upon water dilution and even disappears when the water content is around 50 v%. The physicochemical properties can be tailored in a controllable way when diluted with water. With increasing water content, viscosity decreases considerably down to the level of water at around 25% water level; Conductivity of NADES covers a broad range and increases with water dilution reaching a peak value with 60-80% water of around 10-100 times that of pure NADES, which is correlated with high viscosity of NADES; Density decreases with water dilution in a linear way; water dilution results in a polarity value similar to water; water activity increases with water dilution.

The changed properties of diluted NADES provide a basis to develop tailor-made for various applications. The water content in NADES significantly affects the solubility of compounds in NADES and enzymatic reactions, revealing their enormous potential for diverse uses in health related areas.

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