Tailoring properties of natural deep eutectic solvents with water to facilitate their applications

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ABSTRACT

Previously it was demonstrated that natural deep eutectic solvents (NADES) are promising green solvents for the extraction of natural products. However, despite their potential, an obvious disadvantage of NADES is the high viscosity. Here we explored the dilution effect on the structures and physicochemical properties of NADES and their improvements of applications using quercetin and carthamin. The results of FT-IR and ^{1}H NMR experiments demonstrated that there are intensive H-bonding interactions between the two components of NADES and dilution with water caused the interactions weaken gradually and even disappeared completely at around 50% (v/v) water addition. A small amount of water could reduce the viscosity of NADES to the range of water and increase the conductivity by up to 100 times for some NADES. This study provides the basis for modulating NADES in a controllable way for their applications in food processing, enzyme reactions, pharmaceuticals and cosmetics.

1. Introduction

A new type of green solvents, natural deep eutectic solvents (NADES) have been proposed by our group to extend the range of ionic liquids (ILs) and deep eutectic solvents (DES) and to explore their applications in life science fields (Choi et al., 2011; Dai, Spronsen, Witkamp, Verpoorte, & Choi, 2013a; Dai, Verpoorte, & Choi, 2014). Natural deep eutectic solvents are composed mostly of natural primary metabolites 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., 2013a). A series of NADES possessing excellent properties as solvents has been developed. These include negligible volatility, liquid state even far below 0 °C, a broad range of polarity, high solubilization power strength for a wide range of compounds, especially poorly water-soluble compounds, high extraction ability and high stabilization ability for some natural products (Choi et al., 2011; Dai et al., 2014; Mamajanov, Engelhart, Bean, & Hud, 2010). From an environmental and economic perspective, NADES also offer many striking advantages including biodegradability, sustainability, low costs and simple preparation. All these properties make them of interest for applications in health-related areas such as pharmaceuticals, foods, and cosmetics. They have already been used to dissolve DNA (Choi et al., 2011; Dai et al., 2013a; Mamajanov et al., 2010), as media for enzyme reactions (Choi et al., 2011; Durand et al., 2013), and biotransformations (Gutiérrez, Ferrer, Yuste, Rojo, & del Monte, 2010; Zhao, Baker, & Holmes, 2011) and extraction of phenolics (Dai, Witkamp, Verpoorte, & Choi, 2013b; Gu et al., 2014; Paiva et al., 2014), processing biomass (Francisco, van den Bruinhorst, & Kroon, 2012; Xia, Baker, Li, Ravula, & Zhao, 2014) and stabilization of natural pigment (Dai et al., 2014).

These solvents, however, share some of the limitations observed in conventional synthetic ILs and DES. Their high viscosity (typically 200–500 mm^2 s^{-1} at 40 °C) (Dai et al., 2013a) is the most obvious issue, which leads to some practical problems, including time-consuming solvent transfer operations and slow mass transfer in dissolutions or extractions. In the case of ILs, this is generally partly overcome by applying external physical forces, such as microwave and stirring at high temperature, thus accelerating the dissolving process (Abe, Fukaya, & Ohno, 2010; Swatloski, Spear, Holbrey, & Rogers, 2002). Another way to get around this problem is diluting the IL with water, which has proved to be an efficient way to decrease their viscosity and to modulate functional solvents for enzyme reaction (Kohno & Ohno, 2012). This could also be useful to design appropriate NADES for specific applications, another of the important issues to be solved.

There is an aspect of the effect of water on NADES that adds additional interest to the understanding of the mechanisms involved in the interaction between water and 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. NADES might be involved in the biosynthesis, storage and transport of various poorly water-soluble compounds (Choi et al., 2011; Dai et al., 2013a). From a 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 in order to further show their applications. Two explorations on adjusting the behavior of protein in DES (urea-choline chloride) have been done as applications (Esquembre et al., 2013; Nardecchia et al., 2012). Thus, we investigated NADES and water mixtures in terms of supermolecular structures, physicochemical properties, and solubilizing capacity for two poorly water-soluble compounds to demonstrate the role of water addition in developing tailor-made NADES for specific applications. This study provides the basis for a deeper understanding of the chemical structures and physical properties of diluted NADES that can modulate NADES in a controllable way for health-related applications.

2. Materials and methods

2.1. Chemicals, material and reagents

All the chemicals for the preparation of NADES and quercetin were purchased from Sigma (St. Louis, MO, USA). Water was of deionized water. Deuterium oxide was obtained from CortecNet (Voisins-Le-Bretonneux, France.) Carthamin (purity >95%) and quercetin (purity >98%) were previously isolated in our laboratory.

2.2. Solvent preparation

Natural deep eutectic solvents including proline-malic acid (PM) and 1,2-propanediol–choline chloride–water (molar ratio, 1:1, PC), were prepared by heating with stirring at 70 °C till a clear liquid was formed. Other NADES including malic acid-choline chloride-water (molar ratio, 1:1:2, MCH), proline–malic acid–water (molar ratio, 1:1:3; PMH), 1,2-propanediol–choline chloride–water (molar ratio, 1:1:1, PCH), lactic acid–glucose–water (molar ratio, 5:1:3, LGH); sucrose–choline chloride–water (molar ratio, 1:4:4, SuCH) and other NADES listed in Table 1 were prepared according to our previously reported 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 (Choi et al., 2011; Dai et al., 2013a). 1,2-Propanediol–choline chloride–water (molar ratio, 1:1:1, PCH)

Table 1
The conductivity (mS cm^{-1}) of NADES at room temperature (27 °C).

Compositions (molar ratio)(abbreviation)	Conductivity
Fructose:glucose:sucrose:water (1:1:1:11) (FGSH)	0.0012
Lactic acid:glucose:water (5:1:3) (LGH)	0.114
Malic acid:β-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
Xylitol:choline chloride:water (1:2:3) (XoCH)	13.55
1,2-Propanediol:choline chloride:water (1:1:1) (PCH)	12.09
Glycerol:choline chloride:water (2:1:1) (GlyCH)	13.78
Water	0.00208
Methanol	0.00424

were diluted with different percentage (v) of deuterium oxide one day before the ^{1}H NMR measurement. For physical properties tests, natural deep eutectic solvents were diluted with deionized water.

2.3. FT-IR and ^{1}H NMR analysis

The ^{1}H NMR spectra of the diluted PCH with deuterium oxide were recorded at 40 °C on a 500 MHz Bruker DMX-500 spectrometer (Bruker, Karlsruhe, Germany). A total of 32,768 data points were recorded covering a spectral window of 9615 Hz; 128 scans of a standard one-pulse sequence with 90° flip angle for excitation and presaturation during 2.0 s relaxation delay. An exponential window function with a line-broadening factor of 0.3 Hz was applied prior to Fourier transformation. The resulting spectra were manually phased and baseline corrected. FT-IR spectra over the range from 4000 to 300 cm^{-1} were collected at room temperature (25 °C) using a Bruker FT-IR spectrometer.

2.4. Physicochemical properties tests

Viscosity test was performed using a viscometer 16983, type U-tube reverse (P.M. Tamson B.V. Zoetermeer, The Netherlands) in a thermostatic bath TVB 445 (Labovisco B.V., The Netherlands) 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). Density test was performed using a density meter (DMA 5000) at 40 °C. Water activity test was performed in a Labmaster water activity equipment (Novasina, Switzerland) at 40 °C. Polarity testing was done with Nile red (NR) as a solvatochromatic probe to estimate polarity of NADES in the form of molar transition energy (E_{NR}). The dye was dissolved in NADES in the concentration of 10^{-5}–10^{-4} M. The absorption spectra of dyes (λ_{max}) was determined with a UV/Vis spectrophotometer and calculated in the following formula to obtain E_{NR}: E_{NR} (kcal mol^{-1}) = $hcNA/\lambda_{max}$ = 28,591/λ_{max} (Ogihara, Aoyama, & Ohno, 2004).

2.5. Solubility tests

Solubility tests were carried out by saturating NADES with an excess of the tested compound in a bottle with a cap and stirring at 40 °C for 2 h. The resulted liquids containing un-dissolved solid compounds were centrifuged and the supernatant was transferred to a 1.5 mL-microtube, diluted with water, and vortexed till a homogeneous solution. The diluted solutions were analyzed by UV spectrometer at wavelength of 370 nm for quercetin and 517 nm for carthamin. All the solubility tests were performed in triplicate.

3. Results and discussion

3.1. The supermolecular structure of NADES

The supermolecular structure of NADES were investigated with ^{1}H–^{1}H-nuclear overhauser enhancements (NOESY) spectroscopy (Dai et al., 2013a), and also further elucidated with FT-IR (Fig. 1) to explore the type of interactions, the atoms involved in the interactions and even the possible ratios of the two components. The FT-IR spectra of solid malic acid, proline and their eutectic mixture malic acid, proline (molar ratio, 1:1) were recorded at room temperature. Compared with single compounds, an upward shift of the stretching vibration band ν_{CO} in malic acid from 1683 to 1717 cm^{-1} and a downward shift of ν_{OH} to ca. 2450 and 1920 cm^{-1} were observed (Fig. 1a), indicative of the appearance
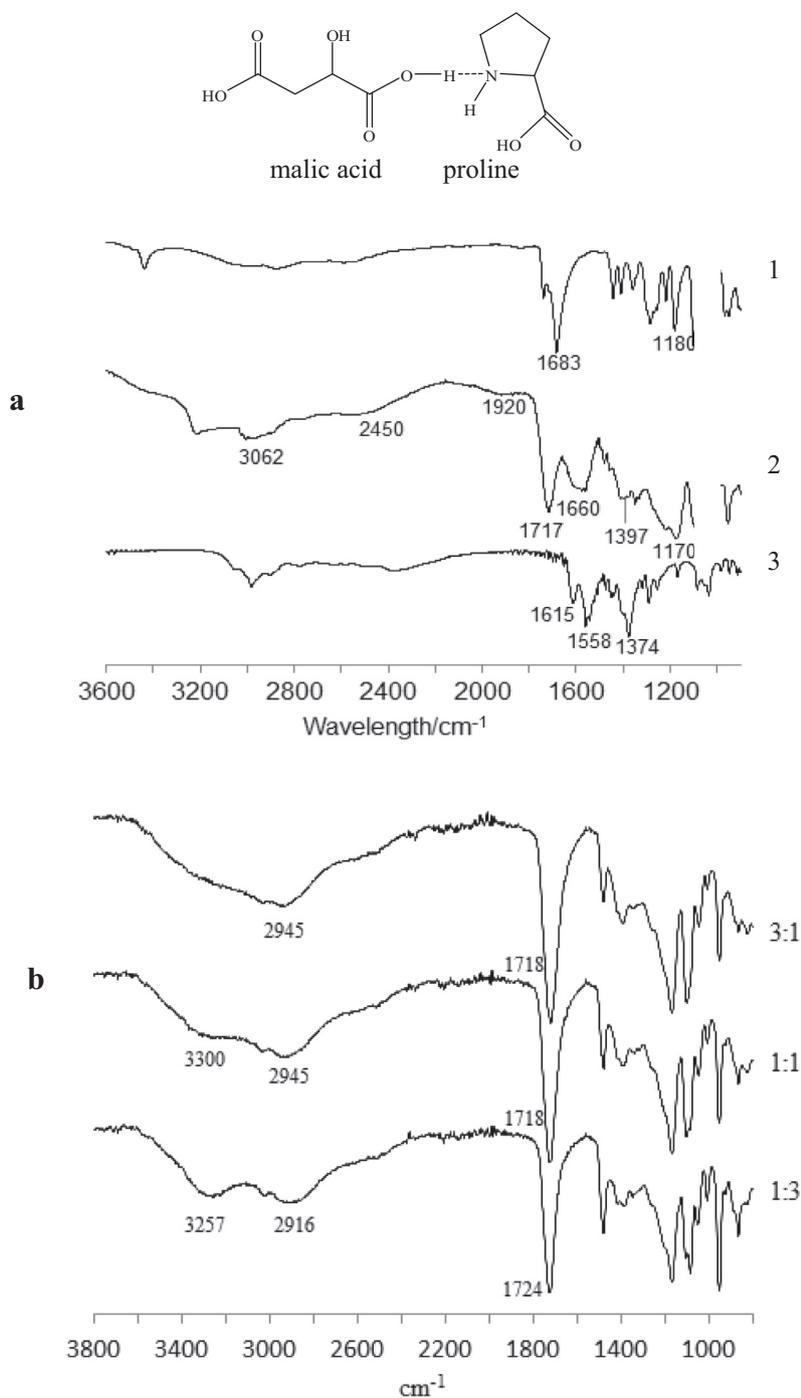
Fig. 1. FT-IR of natural deep eutectic solvents made of malic acid and proline (a) (1: malic acid solid; 2: malic acid:proline (molar ratio, 1:1; PM); and 3: proline at room temperature) and (b) eutectic mixture of malic acid:proline with different molar ratio (3:1; 1:1; 1:3).

of a hydrogen bond (O=C–O–H···N) involving malic acid and proline in PM (Barańska, Kuduk-Jaworska, Szostak, & Romaniewska, 2003; Xu et al., 2005). In PM, the deformation vibration band of δ_{CH2} in malic acid increased in intensity and shifted from 1180 to 1170 cm^{-1}, and in proline the δ_{COO-H} shifted from 1374 to 1397 cm^{-1}, and the δ_{NH} shifted from 1558 to 1660 cm^{-1}. This suggests that both malic acid and proline have different conformations in the PM to form hydrogen bond (Mary, Ushakumari, Harikumar, Varghese, & Panicker, 2009).

Furthermore, the IR spectra of different molar ratios (1:3; 1:1; 3:1) in PM showed that the band of $\nu_{C=O}$ at 1708 cm^{-1} shifts up to 1716 cm^{-1} and the broad band of ν_{OH} in carboxyl group shifts from 2972 to 2980 cm^{-1}, indicating that more H-bonds between proline and malic acid are formed when the ratio of proline–malic acid increased from 1:3 to 1:1 (Fig. 1b). When the amount of proline–malic acid increased to 3:1, there is no shift of the ν_{CO} and ν_{OH} in carboxyl group. This phenomenon suggests that 1:1 is a proper ratio for malic acid and proline to form a eutectic mixture. Similar hydrogen bonds were also detected in the 1,2-propanediol-choline chloride eutectic mixture (PC) (Fig. S-1), including H-bonds between hydroxyl groups in 1,2-propanediol and chloride, and a hydrogen bond with –CH$_2$–OH in choline chloride. All structure

information confirms and extends our reported 2D NMR results of PMH and PCH (Dai et al., 2013a). Clearly, there is an extensive hydrogen-bonding network between the components of NADES; thus, NADES are supermolecules with hydrogen bond network.

3.2. The supermolecular structure of NADES after water dilution

To explore the supermolecular structure of NADES after dilution with water, the eutectic mixture 1,2-propanediol–choline chloride–water (1:1:1) (PCH) was diluted with deuterium oxide and the diluted PCH was investigated with NMR (Fig. S-2). A continuous downfield shift of all signals from the two components except the peaks of methyl and methylene groups vicinal to the nitrogen atom was observed, and the relative number of detected protons on the hydroxyl groups (relative to the protons on the methyl group) decreased during dilution (Table S-1). These observations provide evidence of the progressive rupture of the hydrogen bonds in PCH during dilution, a similar phenomenon was also observed in urea-choline chloride mixture by Gutiérrez, Ferrer, Mateo, and del Monte (2009). The dilution from 25% to 50%, caused all the signals of the hydroxyl groups to disappear, but no shift of the water signal was observed with a further dilution to 75% D_2O, indicating the complete rupture of the hydrogen bonds between 1,2-propanediol and choline chloride with 50% D_2O. These results show that the supermolecular complex structures of PCH are preserved while the content of water is below 50% water, but further dilution produces a solution of the free forms of the individual components in water. These gradual changes in the structure of NADES during dilution may affect their physicochemical properties and also their applications.

3.3. The physicochemical properties of NADES after water dilution

3.3.1. Viscosity

The viscosity of PCH diluted with different percentage of water was measured at room temperature. The viscosity decreased significantly as the water content increased following a function $y = 0.88x^{-1.524}$ ($r^2 = 0.99$) (y: viscosity; x: water weight percentage in PCH) (Fig. 2), similar as the case of glucose-choline chloride-water (GCH) (Dai et al., 2013a). The result shows that the viscosity of NADES is greatly affected by the water content. The high viscosity of NADES is often attributed to the presence of extensive hydrogen-bonding interactions between the components. Dilution with water leads to a large decrease in the viscosity of NADES as a result of the gradually weakened hydrogen-bonding interactions between the components, as revealed in Fig. S-2. After adding 25% water to glucose–choline–water (GCH) and PCH, their viscosity decreased from 397 to 7.2 mm^2 s^{-1} (Dai et al., 2013a), and from 33 to 6.1 mm^2 s^{-1}, respectively, which is near the range of water (0.7 mm^2 s^{-1}). What's more, NADES still possess the supermolecular characteristics with less than 50% water dilution as mentioned above. Thus, dilution with water not only can effectively reduce the viscosity of NADES but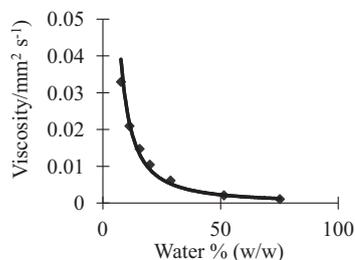 also keep their structure, which may facilitate their different applications. These results reveal the role of water as a diluting agent with respect to its considerable efficiency in adjusting the viscosity of NADES.

3.3.2. Conductivity

The conductivity of 16 typical NADES and five NADES diluted with water was investigated at room temperature. Polyalcohol–choline had the highest conductivity (GlyCH, 13.78 mS cm^{-1}) while sugar–sugar (FGSH, 0.001 mS cm^{-1}) had the lowest value, similar to water (0.002 mS cm^{-1}) and methanol (0.004 mS cm^{-1}) (Table 1). The conductivity of NADES decreases in the following sequence: base-polyalcohol > base-organic acid ≈ base-sugar > organic acid-amino acid (non-polar) > organic acid-sugar > sugar-sugar. NADES with high and low conductivity (PCH, GCH, sucrose-choline chloride-water (SuCH), LGH and PMH) were selected to investigate how the conductivity is affected by water. The conductivity of five NADES diluted with different percentage of water showed that conductivity firstly increased with the increasing water content, and then decreased after reaching a peak value of around 10–100 times higher than that of pure NADES (Fig. 3). GCH, PCH, sucrose–choline chloride–water (SuCH) and PMH had the highest conductivity with 60% of water dilution and LGH reached the highest value with 80% water dilution. Therefore, the conductivity of NADES thus increases as a result of the decreased viscosity by dilution with certain amount of water and the conductivity of NADES can be tailored by changing the water content.

Our results suggest that the conductivity of NADES is correlated with their composition and viscosity. Comparing NADES with one same component, G̲CH (6.81 mS cm^{-1}) was observed to have a much higher conductivity than L̲GH (0.11 mS cm^{-1}), and M̲CH (5.91 mS cm^{-1}) was much more conductive than PM̲H (1.06 mS cm^{-1}), which may be due to the ionic form of choline chloride and partial ionization of lactic acid (pka 3.86) and proline (pka 1.99). Thus, it may be concluded that NADES with choline chloride have a high conductivity while NADES with organic acid

Fig. 2. The relationship curve between the viscosity and water percentage of 1,2-propanediol–choline chloride (1:1; PC).
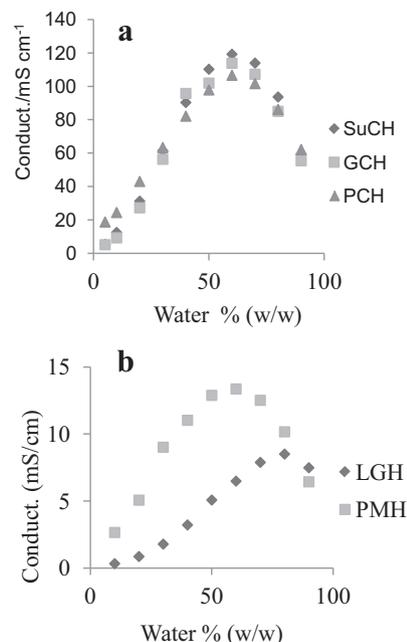

Fig. 3. The relationship curves between the conductivity and water percentage of glucose–choline chloride (molar ratio, 1:2.5; GCH), sucrose–choline chloride (1:4; SuCH), 1,2-propanediol–choline chloride (1:1; PCH), lactic acid:glucose (5:1; LG) and proline:malic acid (1:1; PMH) at room temperature.

or amino acid have low values. The second important factor is viscosity. NADES made of a polyalcohol and a base such as PCH are more conductive than those made of a sugar and a base such as SuCH, which might be correlated to the fact that PCH has much lower viscosity (33 mm^2 s^{-1}) than SuCH (581 mm^2 s^{-1}) (Dai et al., 2013a). Most highly viscous NADES, especially NADES containing a sugar or amino acid (300–600 mm^2 s^{-1}), have a low conductivity (lower than 10) (Dai et al., 2013a).
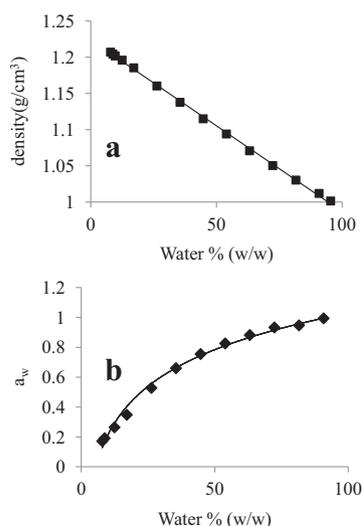
3.3.3. Density, water activity, and polarity

Other physical properties (e.g. density, water activity and polarity) are also affected by the water content. The density, water activity and polarity of some typical NADES diluted with different percentage of water were detected. Results showed that the density of GCH decreased linearly with the increasing water content following a function $y = -0.0024x + 1.224$, $r^2 = 0.99$ (y: density; x: water weight percentage in PCH) (Fig. 4a). A similar linear relationship was also observed in SuCH ($y = -0.0025x + 1.2418$, $r = 0.99$) and PCH ($y = -0.0009x + 1.0873$, $r = 0.99$). With these formulas, the density of diluted NADES can be calculated with known amount of water dilution.

The water activity of NADES increased gradually with their increasing water content following a function $y = 0.354$ $\ln(x) - 0.6008$ for GCH (y: water activity; x: water weight percentage in GCH) (Fig. 4b). Interestingly, above a 50% dilution with water, there was a linear relationship ($y = 0.004x + 0.6016$, $r^2 = 0.99$) between the water content and water activity. This change in water activity might be correlated with the above-described effect of a 50% water dilution on the supermolecular structure of NADES. It implies that the change in the physical properties of NADES may reflect the change in chemical structure during dilution. In addition, NADES covers a wide range of polarity, from more polar than water to a similar polarity to methanol. Water dilution has a great effect on the polarity of NADES resulting in a polarity similar to water itself (Dai et al., 2013a). All this results shows that also the physical properties of NADES can be tailored in a controlled way by adding water.

3.4. Application of water-diluted NADES in natural products research
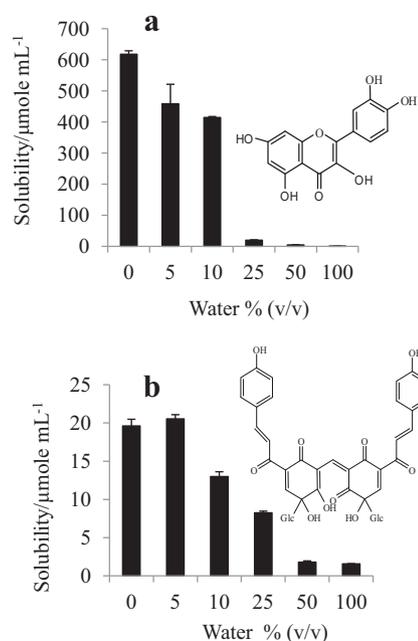
The possibility of adjusting NADES properties quantitatively by adding water allows them to be adapted to different applications such as enzyme reaction (Choi et al., 2011). Another important application of NADES as the third solvent in organs is their high solubilizing capacity for natural products. The solubility of two poorly water-soluble compounds quercetin and carthamin was investigated in PCH with different water contents. The results show that solubility of quercetin was decreased with 5% water in PCH, keeping similar solubility with 10% (v/v) water but decreasing drastically upon dilution with 25% water (v/v) water (Fig. 5). Notably, the case is different for carthamin (a natural dye and antioxidant) (Takahashi et al., 1982; Watanabe, Hasegawa, Yamamoto, Nagai, & Terabe, 1997), which was most soluble in PCH with 5% water, probably due to the medium polarity of carthamin. PCH has the lowest polarity of all our tested NADES (Dai et al., 2013a), and the dilution leads to an increase of its polarity and consequently to a value similar to the polarity of water (Dai et al., 2013a). So the solubility of solutes in diluted PCH will change a lot with water dilution. Most interestingly, 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 a 50% water dilution on the structure of PCH. The decrease of solubility might be correlated with the disappearance of hydrogen bond network with 50% water dilution.

Our results imply that the changed polarity of diluted NADES have a big effect on the solubilization capacity of NADES. This solubility is also correlated with the polarity of solutes, and nonpolar compounds have the highest solubility in pure NADES while a 5–10 v% of water dilution allows the highest solubility of medium polar compounds, which is consist with our former study (Dai et al., 2013a). Furthermore, for different NADES, the optimal water content may be also correlated with the viscosity of NADES. For example, carthamin has the highest solubility in PCH with 5% water and in GCH with 10% water although GCH is more polar than PCH, and this is correlated with the higher viscosity of GCH (Dai et al., 2013a). This points to an extensive hydrogen bonding structure in GCH which results in a high viscosity where there is no space for dissolving various solutes and more water is needed to

Fig. 4. The relationship curves between (a) the density (g cm^{-3}) and water percentage of 1,2-propanediol–choline chloride (1:1) and (b) between the water activity and water percentage of glucose–choline (2:5).

Fig. 5. The solubility of quercetin (a) and carthamin (b) in PCH diluted with a different percentage of water. The data is expressed in mean ± SD ($n = 3$). $^*p < 0.1$; $^{**}p < 0.01$.

loosen the bonded structure and dissolve them. So the physico-chemical characteristics of NADES with different water contents are useful to predict the solubilization capacity of NADES.

4. Conclusion

NADES are supermolecules with hydrogen-bonding interactions between the components. The interactions will be weakened with water dilution and even disappear when the water content is above 50% (v/v). The physicochemical properties can be tailored in a controllable way when diluted with water, as is the case of their viscosity that can be decreased almost to that of water with the addition of 25% water (v/v). The effect of water content on the structure and characteristics of NADES provide a basis to develop tailor-made NADES for various applications, such as the dissolution of compounds, revealing their great potential for diverse uses in health related areas.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.03.123.

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