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ROLE OF THE HYDROGEN BOND DONOR COMPONENT FOR A PROPER DEVELOPMENT OF NOVEL HYDROPHOBIC DEEP EUTECTIC SOLVENTS

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ABSTRACT

Novel solvents with green properties are relevant in order to reduce the environmental impact of chemical applications. In this field, Deep Eutectic Solvents (DESs), mixtures of a hydrogen bond donor (HBD) molecule and a hydrogen bond acceptor (HBA) one at the proper molar ratio, are promising liquids owing to their low toxicity, high eco-compatibility and high ease and "greenness" of preparation. In this paper, we present the preparation of novel hydrophobic deep eutectic solvents and the studies of their properties: density, eutectic profiles, ranges of water separation, contamination of the separated phases, extraction capabilities of phenol model polluting molecules, capabilities of extraction at acidic and basic conditions. From these studies on a set of DESs with properly-chosen components, interesting results emerged about the role of their components. Their capabilities were dependent on the nature of the HBD molecule, and in particular on its hydrophobicity. Even the DESs with highly water-soluble HBA showed to be easily separable from water and really efficacious as extracting agents when prepared with hydrophobic HBDs. The results of the extractions of pollutants in acid and basic conditions showed the capability of water separation and extraction efficiency of these mixtures even with water at pH = 2 and pH = 9; therefore the phenols could interact with these liquids without involvement of any acid/base-type of interactions.

KEYWORDS

Deep Eutectic Solvents; Hydrogen Bond Donor; Green Liquids; Liquid-Liquid Extractions; Phenols; Hydrophobic.

1. INTRODUCTION

Novel organic liquids that potentially possess green advantages over the commonly used organic solvents are representing an advance in the green chemistry framework[1,2]. This is thanks to their advantageous green properties, such as low flammability, low or absent vapour pressure, high recycle and reuse capability. The most commonly used and known liquids so far are represented by Ionic Liquids (ILs): molten salts which are liquid at temperatures below 100 °C due to bulky, asymmetric cations and weakly coordinating anions that destabilize the crystal lattice[3,4]. Even if the applications of ILs are spread with advantageous properties in many relevant topics (such as organic synthesis, extraction media, biocatalysys, separation, etc.[5–8]), recent studies are revealing their disadvantageous characteristics in terms of their toxicity[9]. ILs, in fact, are active towards all levels of life and many ecosystems are vulnerable to their contamination[10,11]. Moreover, the synthesis of ILs involves the use of organic solvents, therefore sometimes overriding their green advantages[12].

In recent years Deep Eutectic Solvents (DESs) are rapidly emerging as a side-class of ILs with the same advantageous characteristics but with better properties in terms of their "greenness"[13,14]. DESs could be divided into four classes depending on the molecules involved, but they can simply be defined as mixtures of a hydrogen bond donor molecule (HBD) and a hydrogen acceptor (HBA) molecule at the proper molar ratio[15,16]. The network of hydrogen bonds occurring between the molecules (most of the times solids) leads to a strong decrease in the melting points of the mixtures, often over 100°C, then to a formation of stable liquids at temperatures generally lower than 70-80°C, or even at room temperature (RTDESs: Room Temperature Deep Eutectic Solvents)[17]. The synthesis of DESs is performed by simply heating and mixing the two components at temperatures between 70°C to 100°C for times spanning from minutes to hours; a liquid is then obtained with a yield of 100% and with an atom economy of 100%. This represents the first "green" advantage of the DESs over ILs. The second advantage is represented by their low or absent toxicity, as it is emerging from recent papers[18–20]. A peculiar and highly relevant class of DESs is represented by NADESs (Natural Deep Eutectic Solvents): mixtures of molecules of natural source, therefore with high bioavailability and biocompatibility[21,22]. Besides the green advantages, DESs share with ILs many practical advantages: in synthetic chemistry, "out of the hood" procedures can be performed thanks to the absent volatility of these mixtures (with a strong economic advantage in industrial applications)[23,24]. Moreover, no anhydrification of the media is required using DESs in commonly anhydrous-condition reactions: Pd-catalysed

C-H bond activation[25], and Grignard and organo-lithium reactions[26] are recent relevant examples of this topic.

A further advantage in the use of DESs is their "active" role: they can act as acid catalysts preventing the use of harmful acids in many chemical reactions, or the components of the mixtures could be reactants of a chemical process[27,28]. Recently gold nanoparticles were obtained without the use of any reducing agents thanks to the action of the NADESs (Oxalic acid/Betaine)[29]. Chiral DESs (made with chiral components) revealed to be structured liquids that can permit to determine an enantiomeric excess in a probe chemical reaction, with values that are the same observed in literature in the same process[30].

A relevant field in which DESs are finding fruitful applications, is their use as extraction and separation agents; their high solubilization capabilities permit to use them as extracting agents of relevant substances from biological matrixes[31]. DESs have been currently widely studied as extraction agents of phenols and phenolic compounds from different matrices as these molecules revealed a great affinity with them[32]. Therefore, preconcentration/separation analytical steps can be successfully performed in these novel green and sustainable mixtures[33,34]. In recent literature, novel hydrophobic Deep Eutectic Solvents are emerging as highly promising water-insoluble media, with applications as extracting and removal agents from aqueous samples of transition metal ions, of polycyclic aromatic hydrocarbons, of pesticides, of phytocannabinoids and so on.[35–38]

Although the most common used DESs in literature are mixtures of choline chloride with urea, ethylene glycol and glycerol[39], the differences and the different properties and capabilities that can be obtained by changing the HBD and/or the HBA components of the mixtures promote a fine tuning of the DESs for specific applications[40]. This pushes forward for a better comprehension of the DESs components role and its effect on the liquid properties.

In this work, we present the preparation of novel hydrophobic Deep Eutectic Solvents, the characterization of their properties (eutectic profiles, density, water separation, contamination of the separated phases) and their extraction capabilities from aqueous media of polluting nitro-substituted phenols and a dye, even at acid and basic pH. The HBD and the HBA molecules were properly chosen in order to obtain structure/activity relationships, revealing that the HBD has a prominent role for an effective separation and for the extraction capabilities from water. Moreover, some of these mixtures are NADESs, thanks to the natural source of the components, increasing the "greenness" of these liquids. The environmental favourable properties of these DESs and their cost-effective preparation promote them as a promising tool for water liquid-liquid extraction processes.

2. EXPERIMENTAL

2.1 Reagents and Materials

Glycolic acid (GLY), phenylacetic acid (PhAA), thymol (THY), trimethylglycine (TMG), 4-nitrophenol (PNP), 2,4-dinitrophenol (DNP), Phenol Red (PhRed), 2-phenoxypropanoic acid, methyl 4-hydroxybenzoate, 2-(3-methoxyphenyl)acetic acid, (1S)-(+)-camphorsulfonic acid, 3,4,5-trihydroxybenzoic acid and L-menthol were purchased from Sigma-Aldrich/Merck (purities >99%). The solids were dried with P_2O_5 under vacuum overnight before use. N,N-dimethyl-N,N-didodecylammonium chloride (DDDACl) was synthesised using a procedure previously published[41]: N,N-dimethyldodecyl amine was synthesised from 1-dodecylamine, formic acid and formaldehyde via Leuckart-Wallach reaction; DDDACl was obtained by refluxing N,N-dimethyldodecyl amine with 1-dodecylchloride in acetonitrile. M.p. = 129-131°C. ^{1}H NMR (400 MHz, MeOD) δ 3.33-3.28 (m, 4H), 3.07 (s, 6H), 1.80–1.74 (m, 4H), 1.41–1.31 (m, 36H), 0.93-0.89 (t, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.9, 51.7, 32.1, 29.8-26.4, 23.0-22.9, 14.3. (Spectra reported in Supporting Information section, Figures SI-1 and SI-2).

2.2 DESs preparation

The HBA and the HBD solid components were weighed at proper molar ratios in a flask fitted with a stopper. The solid mixtures were magnetically stirred and heated at temperatures spanning from 40° to 100°C until homogeneous liquids were formed.

2.3 Eutectic profiles

In a 10 mL round-bottomed flask equipped with a thermometer, weighed amounts of HBD and HBA solid components were introduced and gently heated and mixed until homogenous liquids were formed. The freezing points of the DESs were determined by cooling the mixtures in an ice bath (or an acetone/liquid nitrogen mixture for the mixtures with very low melting temperatures) and determining the freezing temperature with the thermometer. The eutectic profiles were determined by repeating the procedure at different HBA/HBD molar ratios. All the experiments were repeated in triplicate and the errors were calculated as standard deviations of the samples.

2.4 Density

The density of the DESs were determined via weighing 1mL of liquid in a flask at 25.0 °C using an analytical balance: the flasks were held in a thermostated bath for 1 hour then the volume

was adjusted ~~by~~ using a Pasteur pipette. The experiments were repeated in triplicate and the errors were calculated as standard deviations of the samples.

2.5 Water content

The water content of all the DESs was evaluated via Karl-Fischer titration with Metrohm 684 KF Coulometer. The measures were performed when the values of the samples observed were stable and reproducible (in times spanning from minutes to hours depending on the sample analysed)[42].

2.6 Water separation

DESs were weighed in centrifuge tubes and specific amounts of bidistilled H_2O were added to the liquids. The mixtures were centrifuged (Beckman Coulter Allegra 64 R, 6500 RPM, 30 min, 25°C) whenever they remained opalescent after stirring and mixing at room temperature, giving bi-phasic solutions. The samples were then centrifuged after each water addition.

2.7 Phases contamination

After separation of the phases, the water content of the DESs phases were measured via Karl-Fisher titration. The experiments were repeated in triplicate. The aqueous phases were analysed via ^{1}H-NMR measurements (Bruker Avance 400, 400 MHz) using D_2O for the separation and an internal standard (Maleic acid, Sigma ≥99%) for the evaluation of the amounts of DESs components in water: specific amounts of D_2O phases were dissolved in MeOD solutions of the standard (3.30×10^{-3} M). The spectra were acquired with only one scan in order to avoid any error due to possible different relaxation times of the different nuclei. Absolute integral values (TopSpin 3.5 software, Bruker) were used for the quantitative analysis. The experiments were repeated in triplicate and the errors were calculated as standard deviations of the samples.

2.8 Extraction efficiency

The DESs were mixed with water solutions of 4-nitrophenol (1×10^{-2} M), 2,4-dinitrophenol (1×10^{-3} M), Phenol Red (1×10^{-3} M) at 35% w/w (25% w/w for Glycolic acid/ N,N-dimethyl-N,N-didodecylammonium chloride DES). The solutions were stirred for 1 minute at room temperature with a vortex, then centrifuged (Beckman Coulter Allegra 64 R, 6500 RPM, 30 min, 25°C). 25 µL of aqueous phases were dissolved in 2 ml of water (in 2 ml of NaOH 0.01 M for Phenol Red samples) and the UV-Vis spectra (Agilent 8453 UV-VIS spectrophotometer,

temperature controlled at 25.0°C with a Peltier system Agilent 890890A) were registered and compared with the spectra of the starting aqueous solutions at the same dilution. The extraction efficiency percentage ($E_{\%}$) was determined as a ratio of the absorbances using the formula:

$$E_{\%} = \frac{A_o - A_i}{A_0} \cdot 100$$

where A are the absorbances of analyte before (0) and after (i) the extractions.

The wavelengths considered were: 318 nm for 4-nitrophenol (Molar Extinction Coefficient: 9092.2 $M^{-1}cm^{-1}$); 359 nm for 2,4-dinitrophenol (11651 $M^{-1}cm^{-1}$); 559 nm for phenol red at pH=12 (56325 $M^{-1}cm^{-1}$).

The acid and basic solutions experiments were carried out in the same manners but using acidic (0.01 M HCl, pH ≈ 2) or basic ($1x10^{-5}$ M NaOH, pH ≈ 9) solutions of 2,4-dinitrophenol mixing them at 35% w/w with the DESs. 25 µL of aqueous phases were dissolved in 2 ml of neutral water then analysed via UV-VIS with the same procedure described above.

All the experiments were repeated in triplicate and the errors were calculated as standard deviations of the samples.

3. RESULTS AND DISCUSSION

3.1 DESs PREPARATION

A set of differently structured molecules (carboxylic acids, alcohols, phenols) were at first analysed as HBD for their capacity of liquid formation when mixed with the HBA N,N-dimethyl-N,N-didodecylammonium chloride. Phenylacetic acid, 2-phenoxypropanoic acid, methyl 4-hydroxybenzoate, 2-(3-methoxyphenyl)acetic acid, thymol, (1S)-(+)-camphorsulfonic acid, 3,4,5-trihydroxybenzoic acid, glycolic acid and L-menthol gave homogeneous liquids at the temperatures between 25°C and 50°C. The DESs investigated in this work were prepared by combining three of these HBD compounds and two HBA molecules: Thymol (THY), Phenylacetic acid (PhAA) and Glycolic acid (GLY) as HBD and Trimethylglycine (TMG) and N,N-dimethyl-N,N-didodecylammonium chloride (DDDACl) as HBA. The choice of these molecules was made to evaluate the effect of the hydrophobicity of the components of the DESs on the hydrophobicity of the resulting liquids and because the resulting DESs are liquid at room temperature. These molecules have, in fact, different

solubility in water; THY, PhAA and DDDACl have low water solubility ($5.7x10^{-3}$ M, 0.13 M and 0.0125 M maximum water solubility respectively at 25.0°C), while TMG and GLY can be considered as highly soluble (5.22 M and 8.02 M maximum water solubility respectively)[43,44]. Figure 1 shows the structures of these molecules; in red the ones scarcely soluble in water, and in blue the hydrophilic ones.

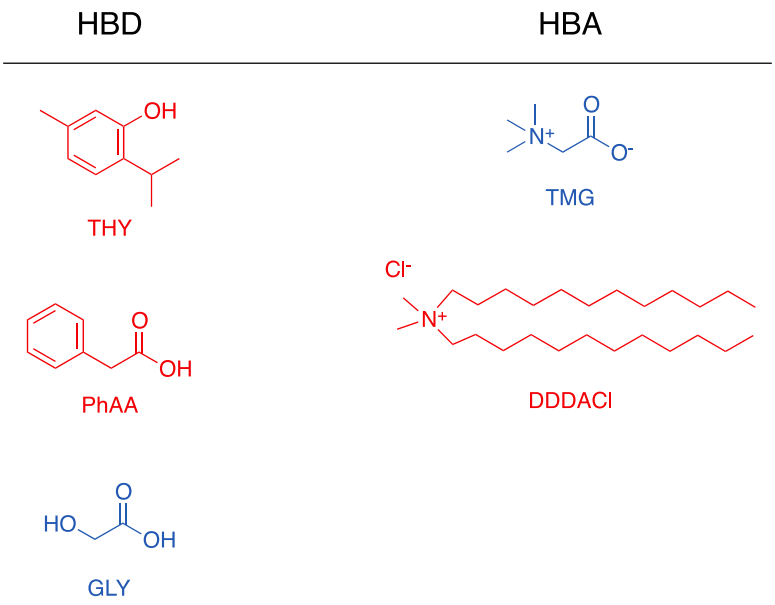

Figure 1: HBD molecules HBA molecules used for hydrophobic DESs preparation: Thymol (THY), Phenylacetic acid (PhAA) and Glycolic acid (GLY) as HBD and Trimethylglycine (TMG) and N,N-dimethyl-N,N-didodecylammonium chloride (DDDACl) as HBA. The molecules with water solubility higher than 1 M are coloured in blue, the ones with water solubility lower than 1 M are coloured in red.

The three HBD and the two HBA molecules were mixed to give six deep eutectic solvents. PhAA/TMG and GLY/TMG DESs were previously synthesized in our laboratories[20], and their hydrophobic properties were analysed in this study in order to obtain a homogeneous set of liquids. The other mixtures (THY/DDDACl, PhAA/DDDACl, GLY/DDDACl and THY/TMG) are novel DESs prepared and characterized in this work. In Table 1 the eutectic ratios, the melting points and the densities of these DESs are reported, in Supporting Information sections all the eutectic profiles are reported (Figure SI-3).

Table 1: DESs used in this work[a].

HBD	HBA	Eutectic ratio (HBD:HBA)	DES freezing point, °C	Density, g/mL (25°C)
THY	DDDACl	2:1	-20 ± 1	0.884 ± 0.022
PhAA	DDDACl	2:1	-7 ± 1	0.941 ± 0.024
GLY	DDDACl	3:1	5 ± 1	1.040 ± 0.024
THY	TMG	3:1	-15 ± 1	0.959 ± 0.026
PhAA	TMG	2:1	-7 ± 1	1.161 ± 0.030
GLY	TMG	2:1	-36 ± 2	1.274 ± 0.031

[a] PhAA/TMG and GLY/TMG mixtures values were previously measured[20].

All of these mixtures are liquid at temperatures lower than 0°C, except for GLY/DDDACl that showed a freezing point slightly above this value (5°C). Three of these liquids (the ones with TMG as HBA) are NADESs and this increases the "greenness" of these mixtures.

3.2 WATER SEPARATION

The capabilities of separation from water were evaluated for all the DESs. Specific amounts of H_2O were added to the liquids, and the mixtures were centrifuged whenever they remained opalescent after stirring and mixing at room temperature. The amounts of water in the pure DESs were measured via Karl-Fisher titration and were added to the values of water added in the mixtures. GLY/DDDACl DES did not show any water separation at any water concentration. Therefore we prepared another DES with the same components but with a different molar ratio: we used a 2:1 HBD:HBA molar ratio even if the eutectic point was at 3:1; in this way the hydrophilic component amount in the DES was decreased. With this molar ratio, the melting point was still below room temperature (21°C) so it was applied in the experiments giving a water separation.

In Figure 2 the phase separations of the liquids are reported, as well as the molar ratios of the DESs, all the measurements were performed at 25°C.

DES		DES position in DES/H_2O separation	Water Content, % w/w									
HBD	HBA		10	20	30	40	50	60	70	80	90	
THY	DDDACl	upper phase										
PhAA	DDDACl	upper phase										
GLY	DDDACl [a]	upper phase										
THY	TMG	upper phase										
PhAA	TMG	lower phase										
GLY	TMG	n.a.										

	Mono-Phasic
	Bi-Phasic
	Precipitation/Gel

Figure 2: Relative position of the DESs phase in water separation: in orange those liquids with densities lower than water and in pink the ones higher than water; phase separation table of the DESs from water (blue: monophasic; green: bi-phasic; yellow: precipitation of solid materials or gel formation). T = 25°C. a = DES prepared at HBD:HBA ratio of 2, density = 0.959 g/mL.

All the DESs solubilized amounts of water at values lower than 10% w/w. This could be due a "structural" role of the water that could participate to HBD-HBA interactions with the components[21,45–47]. THY/DDDACl DES showed a very good separation from water at about 10-15% w/w of water, and the two phases exist in all the % range until over 90% w/w. The same behaviour was observed for the DES with the same HBD molecule but with TMG as HBA (THY/TMG DES), which showed a separation at slightly lower % values (about 10-12% w/w). PhAA/DDDACl DES showed a range of separation spanning between 18-23% w/w until 50-53% w/w; after these values the mixture became "gel-like", possibly due to the organization of amphiphilic DDDACl with the aromatic portion of the acid[48]. PhAA/TMG DES showed also a small range of separation from water after centrifugation, and it spans from 30-31% w/w to 40-42% w/w; at higher amounts of water a precipitate is observed. These data were really interesting as these PhAA-based DESs could be considered as both hydrophobic and hydrophilic as they can be efficaciously separated from water in a certain range of concentrations and they can be water-miscible at other concentrations (from 0% until 20-30% w/w). This is relevant and promising for the use of these DESs in extraction applications as they can be separated both from water and from organic apolar solvents. The PhAA/DDDACl is formed by synthetic HBA molecule (DDDACl), therefore it has a lower "greenness", while TMG is a natural source molecule as well as PhAA, therefore PhAA/TMG

DES is a NADES (Natural Deep Eutectic Solvent). Glycolic acid based DESs showed difficult separations from water: GLY/TMG DES is totally hydrophilic and it was not possible to separate it from water in all the concentration range. GLY/DDDACl showed a small range of separations from water, spanning from 20-22% w/w to 31-35% w/w and it must be considered that this DES was separated from water only when the ratio between HBD and HBA molecules was decreased in terms of the hydrophilic one. This behaviour showed to impact also on the other properties of this DES as it will be shown in this work.

These data showed the role of the components of these DESs in their water separation: the HBD molecule, and in particular its hydrophobicity/hydrophilicity is the driving force that leads to an efficacious separation from water. Also the DESs formed with highly water-soluble HBA (such as TMG) revealed to be separable from water in case of hydrophobic HBD counterpart. The glycolic acid (that is highly water soluble) gave, in fact, water-total soluble DES (GLY/TMG) or a DES (GLY/DDDACl) that resulted separated once used in a HBD:HBA ratio with lower amounts of hydrophilic HBD, and in a small range of concentration. The DESs with PhAA as HBD revealed to be the best in our set in terms of their separation from water, since the separation can be modulated with small changes of water amount; PhAA/TMG is even more relevant because it is a NADESs.

The densities of these liquids are lower than the density of water except PhAA/TMG that has a density higher than water. GLY/DDDACl DES showed a change in its density by changing the molar ratio of HBD to HBA, with a value of 0.959 g/mL with 2:1 HBD:HBA molar ratio, while it was 1.040 g/mL in the case of 3:1 HBD:HBA.

3.3 PHASES CONTAMINATION

The efficacy of the separation of the two DES/water phases was evaluated with two techniques. The water amount in the DES phase after the centrifugation was evaluated by Karl-Fisher titration. The contamination/solubilization of HBD and HBA components in water was evaluated after separation of D_2O phases and then dissolving an amount of them in a MeOD solution of an internal standard (maleic acid); the absolute integral values of the relative peaks of ^{1}H-NMR spectra were used for an estimation of the molecules in water. In these experiments all the DESs/water samples were prepared at 35% w/w of water. This was made for many reasons: at this value all the DESs show an effective separation; it is the lower values of DESs that can be used for a separation; all the measured data can be compared between the different mixtures. GLY/DDDACl DES was used at 25% w/w of water because it

has a small range of water separation and it cannot be separated from water at 35% w/w (Figure 2).

In Table 2 the DESs analyzed, the amount of water percent in the DESs phase (the starting of the pure DESs and after the separation) and the amount (M) of the HBD and HBA components in the water phases after the centrifugation are reported; in the same Table the data of the molar ratio of HBD:HBA in water phases are reported. In Figure 3 the ^{1}H-NMR spectra of the water phases of PhAA/TMG DES is reported (25 µL of D_2O phases in CD_3OD solution); all the other ^{1}H-NMR spectra are reported in Supporting Information (Figures SI-4-5-6-7).

Table 2: Phase contaminations of the DESs/water biphasic systems [a].

DES		H_2O in DESs phase, %		DES components in H_2O, M		
HBD	**HBA**	**Pure DES**	**After separation**	**HBD**	**HBA**	**HBD:HBA**
THY	**DDDACl**	2.2 ± 0.2	7.2 ± 0.2	*traces*	*traces*	*n.a.*
PhAA	**DDDACl**	2.1 ± 0.1	21.4 ± 0.6	$(6.3 \pm 0.3)\times10^{-5}$	*traces*	*n.a.*
GLY	**DDDACl**	3.2 ± 0.1	28.3 ± 0.8	2.8 ± 0.5	1.3 ± 0.3	≈ 2:1
THY	**TMG**	0.4 ± 0.1	4.4 ± 0.1	0.020 ± 0.003	1.8 ± 0.2	≈ 1:90
PhAA	**TMG**	1.6 ± 0.1	23.9 ± 0.4	0.42 ± 0.06	2.4 ± 0.4	≈ 1:5.7
GLY	**TMG**	4.2 ± 0.8	*n.a.*	*n.a.*	*n.a.*	*n.a.*

[a] Water amount 35% w/w except for GLY/DDDACl DES (25% w/w of water); water percent in the DESs phase (pure DES, after the separation) evaluated via Karl-Fisher titration; amount (M) of the HBD and HBA components and their ratio in the water phases after the centrifugation measured via ^{1}H-NMR spectroscopy. All the experiments were repeated in triplicate and the errors were evaluated as standard deviations of the samples.

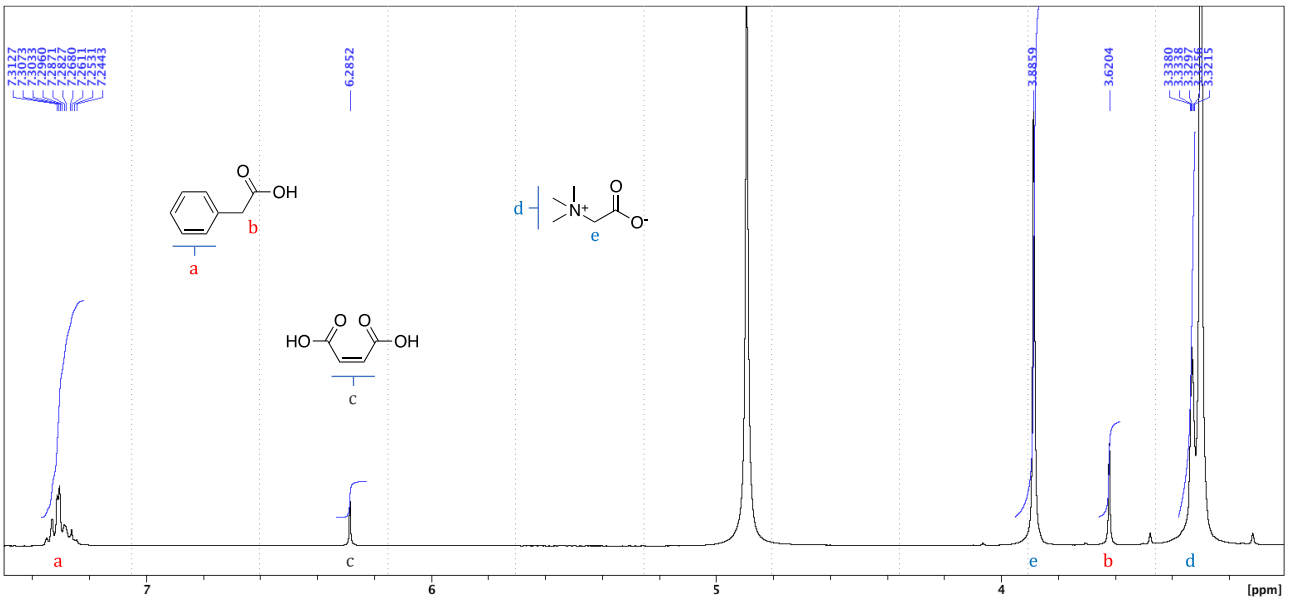

Figure 3: ^{1}H-NMR spectra of the D_2O phases separated from PhAA/TMG DES in CD_3OD. Maleic acid as internal standard.

Almost all the pure DESs have low initial water amounts, under 3.3% w/w; GLY/TMG DES showed the highest value in the set (4.2% w/w), due to its hydrophilicity. After the separation with water by centrifugation, the differently structured DESs absorbed different amounts of water. Even in this case the properties showed a correlation with the hydrophobicity of the HBD portion. THY-based liquids showed an amount of 7.2% and 4.4% w/w of water with the HBA counterparts (DDDACl and TMG respectively). PhAA HBD ones showed higher values (spanning from 21.4% to 23.9% w/w), while the GLY/DDDACl liquid showed the highest value in the set (28.3% w/w); GLY/TMG did not show any water separation; therefore the water amount value after separation could not be measured.

The contamination of the water phases with HBD and HBA molecules showed that the DES with both hydrophobic portions (THY/DDDACl) released only traces of the DES molecules in water ($<1x10^{-7}$ M); in the water contamination, this DES showed the best properties in the set. The same HBD with TMG as HBA showed amounts of HBD in water about 0.02 M and 1.8 M of TMG. The increase of hydrophilicity of the DES led to higher amounts of HBD and HBA molecules in water: PhAA/DDDACl mixture did not show the presence of the HBA in the water phases and very low amounts (about $6x10^{-5}$ M) of the HBD; PhAA/TMG DES showed amounts of HBD about 0.42 M and 2.4 M of the HBA. GLY/DDDACl DES showed the higher amounts of HBD and HBA molecules in the water phase: 2.8 M of HBD and 1.3 M of HBA. These values are interesting if we consider the solubilities in water of the single HBD and HBA components (THY = $5.7x10^{-3}$ M; PhAA = 0.13 M; DDDACl = 0.0125 M; TMG = 5.22 M; GLY = 8.02 M). PhAA/TMG DES released an amount of PhAA in water that is higher than its solubility in water, with a HBD:HBA ratio of about 1:5.7, this could mean that the HBD/HBA components could be present in the water phases also as DES-structured. Even in the case of THY/TMG DES the amount of HBD in water was higher than its maximum solubility, with a HBD:HBA ratio in water of about 1:90; this could be due to the same effect. The most peculiar case in the set is represented by GLY/DDDACl DES that showed an amount of HBA in water phase higher than its solubility and in a molar ratio that is the same of the DES itself, suggesting the solubilization of the molecules as DES-structured. These data, while suggesting the need of further investigations with more specific techniques for a better elucidation, hint at these HBD and HBA molecules being differently structured in the DES and that these interactions lead to DESs with properties (such as the water solubility) that are different from the starting materials as expected.

3.4 EXTRACTION EFFICIENCY

The extraction efficiency from aqueous media of phenols and a dye were evaluated for all the novel hydrophobic deep eutectic solvents. These model liquid-liquid extraction experiments were carried out to determine the efficacy of extraction from water of pollutants of the differently structured DESs with a simple and fast liquid-liquid extraction procedure. Three model-compounds were chosen as analytes: 4-nitrophenol (**PNP**), 2,4-dinitrophenol (**DNP**) and Phenol Red (PhRed). The chosen phenols are reported in the list of priority pollutants of water[49,50]; the -NO_2 substituted ones were chosen in this set because their UV-Vis spectra do not overlap the ones of the DESs components present in water after the separation. Phenol Red is widely used as pH indicator and dye but it has a high impact on the environment because it is found as pollutant in wastewaters due to its difficult degradation[51]. Figure 4 shows their chemical structures.

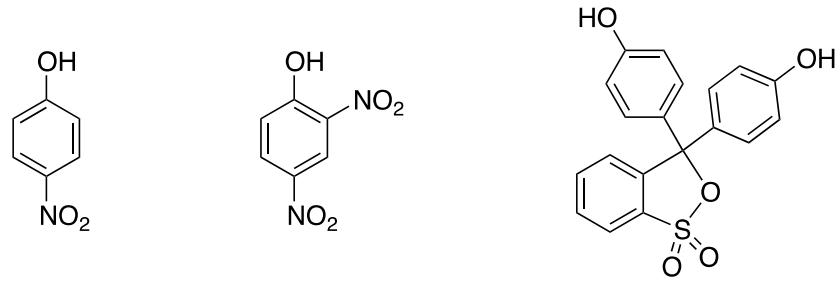

Figure 4: Chemical structures of 4-nitrophenol, 2,4-dinitrophenol and Phenol Red used in this work.

The experiments were carried out with a simple and fast procedure: first the water solutions of the pollutants were mixed for 1 minute with the hydrophobic DESs at room temperature; then the samples were centrifuged for 30 minutes at 6500 RPM at 25°C giving separated phases; finally, the water phases were analyzed via UV-Vis spectra analysis comparing their spectra with the ones before extraction. The mixing time was determined via an experiment (see Supporting Information) that showed that the extraction efficacy did not change significantly by mixing the phases in times spanning from 1 minute to 3 hours. All the experiments were carried out at 35% w/w of water solutions in the DESs: this value was chosen as the minimum amount of DESs that could be separated from water for all the liquids and to compare the results in same conditions for all the mixtures. GLY/DDDACl DES was used at 25% w/w of water solutions because at 35% it is not separable from water.

In Table 3 the results of the extractions of the two phenols and of the Phenol Red are reported for all the DESs; in Figure 5 the same results are reported as histograms, all the UV-Vis spectra are reported in Supporting Information (Figures SI-8-21).

Table 3: Extraction efficiency (%) of hydrophobic Deep Eutectic Solvents[a].

DES		$E_{\%}$		
HBD	**HBA**	**PNP**	**DNP**	**PhRed**
THY	**DDDACl**	98.9 ± 0.5 %	97.3 ± 1.1 %	99.8 ± 0.2 %
PhAA	**DDDACl**	98.9 ± 0.3 %	98.2 ± 1.5 %	99.9 ± 0.1 %
GLY	**DDDACl**	73.9 ± 3.2 %	75.6 ± 24.2 %	n.a.
THY	**TMG**	98.1 ± 0.9 %	89.9 ± 1.1 %	98.1 ± 1.9 %
PhAA	**TMG**	93.9 ± 3.1 %	96.7 ± 1.3 %	89.7 ± 1.2 %
GLY	**TMG**	n.a.	n.a.	n.a.

[a] 4-nitrophenol (PNP, starting solution $1x10^{-2}$ M), 2,4-dinitrophenol (DNP, starting solution $1x10^{-3}$ M) and Phenol Red (PhRed, starting solution $1x10^{-2}$ M). Times of mixing = 1minute; centrifugation at 6500 RPM for 30 minutes at 25°C; 25 µL of aqueous phases were dissolved in 2 ml of water (in 2 ml of NaOH 0.01 M for Phenol Red samples) and the UV-Vis spectra were compared with the spectra of the starting solutions at the same dilution. All experiments were repeated in triplicate and the errors were evaluated as standard deviations of the samples.

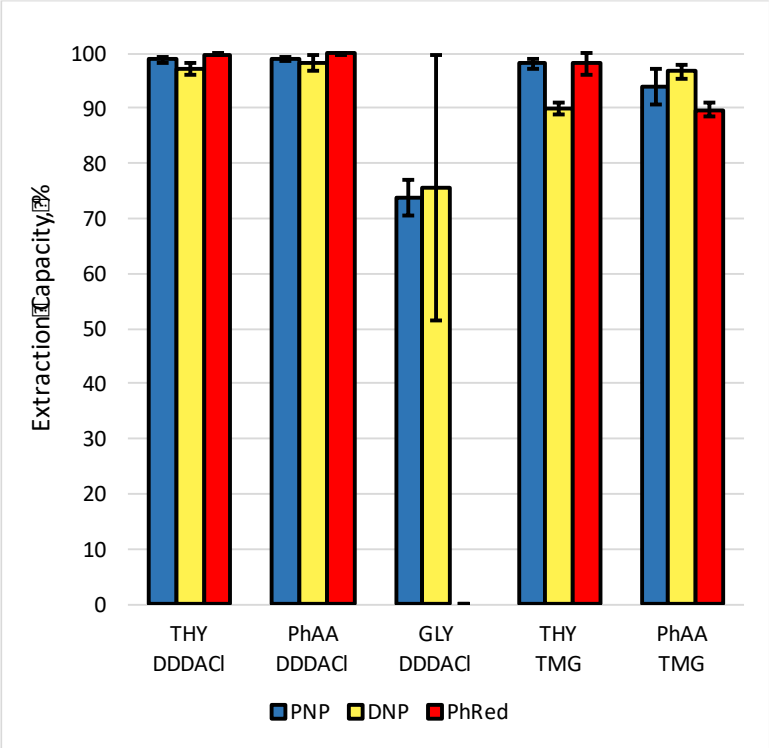

Figure 5: Histogram of extraction efficiencies (%) of hydrophobic Deep Eutectic Solvents of 4-nitrophenol (blue, PNP, starting solution $1x10^{-2}$ M), 2,4-dinitrophenol (yellow, DNP, starting solution $1x10^{-3}$ M) and Phenol Red (red, PhRed, starting solution $1x10^{-2}$ M). All the experiments were repeated in triplicate and the errors were evaluated as standard deviations of the samples.

The DESs showed almost complete extraction of all the phenols from the water phases, with values spanning from 90% to 99% for all the samples for all the phenolic compounds. The only DES that showed lower efficacy (and lower reproducibility) was GLY/DDDACl. This DES did not show any extracting capability of PhRed (because of high samples contamination) and about 75% for the other two (PNP, DNP). This result is similar to that observed in its capacity of separation from water in terms of the contamination of the two phases. More efficacious separation leaded to more efficacious extraction. Again, the HBD compound, and in particular its hydrophilicity, was responsible for the extraction. For all the other DESs, it seemed that there was not a correlation between the nature of the components and their extraction efficacy as they act all about the same $E_{\%}$. GLY/TMG DES was not analyzed as it cannot be separated from water.

These results showed again the great affinity of phenolic compounds with Deep Eutectic Solvents, as it is shown also in literature[32,52,53]. For a deeper understanding of the mechanism of interaction between the DESs and the phenolic compounds, and therefore of the extraction capabilities of this class of molecules, we performed an experiment changing the pH of the water phases. In these manners information about the capability of water separation at different pH of the DESs and, above all, eventual acid-base interactions occurring between the phenols and the DESs HBA/HBD components could be achieved. Two sets of experiments were performed using DNP as analyte dissolving it in acid and basic solutions (pH 2 and 9). The pH values were chosen considering the pK_a of the components of the DESs and of DNP (THY = 10.62; PhAA = 4.31; GLY = 3.83; TMG = 1.84; DNP = 4.09)[54–57] so that at these values all the components result fully protonated/deprotonated.

In Table 4 the $E_{\%}$ of DNP of the DESs at pH = 2 and pH = 9 are reported. The experiments were carried out at 35% w/w of acidic/basic water (25% for GLY/DDDACl). In Figure 6 the same data are reported as histograms; the experiment at pH = 7 is also reported as comparison. All the spectra are reported in Supporting Information section (Figures SI-22-31).

Table 4: Extraction efficiency (%) of 2,4-dinitrophenol of the hydrophobic DESs in basic and in acid water phases[a].

DES		DNP E%, %	
HBD	**HBA**	**pH = 9**	**pH = 2**
THY	**DDDACl**	92.7 ± 1.6 %	96.2 ± 0.6 %
PhAA	**DDDACl**	92.6 ± 0.2 %	90.3 ± 3.8 %
GLY	**DDDACl**	54.6 ± 8.4 %	40.4 ± 15.9 %
THY	**TMG**	91.0 ± 3.7 %	94.9 ± 2.6 %
PhAA	**TMG**	97.1 ± 1.1 %	91.2 ± 5.5 %
GLY	**TMG**	n.a.	n.a.

[a] (DNP, $1x10^{-3}$ M); experiments carried out at 35% w/w of acidic/basic water, 25% w/w for GLY/DDDACl mixture. Basic conditions: pH=9, NaOH $1x10^{-5}$ M; acid conditions: (pH=2, HCl 0.01 M). All the experiments were repeated in triplicate and the errors were evaluated as standard deviations of the samples.

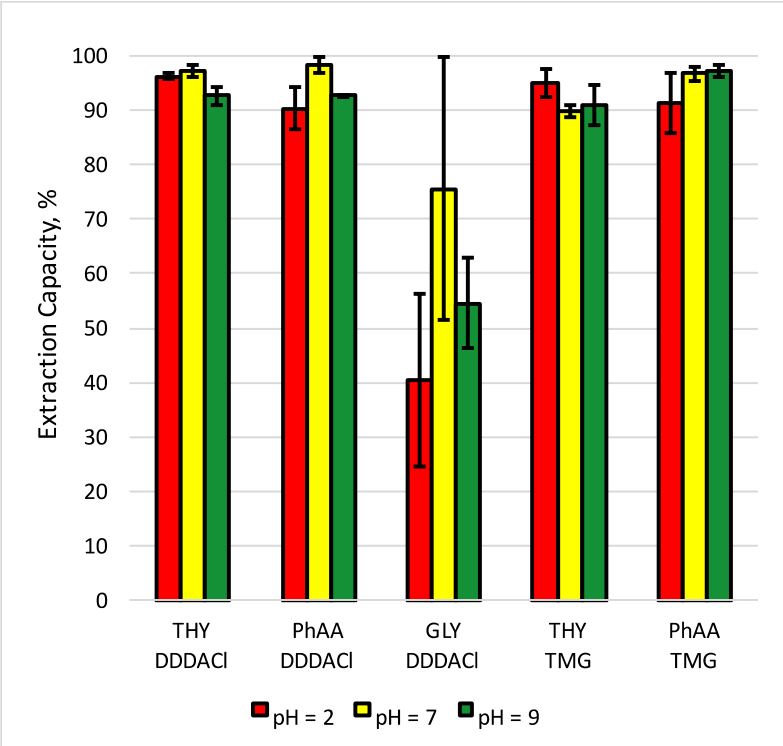

Figure 6: Extraction Efficiency E% (%) of DNP of DESs at pH = 2 (red), at pH = 7 (yellow) and at pH = 9 (green). All the experiments were repeated in triplicate and the errors were evaluated as standard deviations of the samples.

The phase separations occurred both in acidic and basic conditions and the extractions are excellent and spanning from about 90% to 97% E%. These values are slightly below the ones observed in neutral conditions. Even in this case GLY/DDDACl DES showed a lower efficiency (from 40% to 54% E%); also in this case, the stability of the phases could impact on the E%. These experiments revealed two relevant information: the DESs are water phase-separated

and efficacious for the phenols extractions even at acid and basic pH; the phenols could interact with these liquids with interactions that did not involve any acid/base-type of interactions. This was demonstrated also by the UV-Vis analysis of the CH_3CN diluted DES phases of THY/DDDACl mixture (see Supporting Information, Figure SI-32): in this case, the spectra of the analyte extracted both as phenol- and as phenate- were observed.

4. CONCLUSIONS

The Hydrophobic Deep Eutectic Solvents proposed in this work revealed to be excellent tools for the extraction/removal from water phases of polluting phenolic compounds. In this study, the role of the components of the liquids has been revealed, showing the HBD compound (and in particular its hydrophobicity/hydrophilicity) to be fundamental for an efficacious separation from water and for an effective removal/extraction of organic phenol polluting molecules from water. The phase separation and the extraction efficacy of these liquids was not dependent on the pH of the water phases, revealing these liquids to be effectively separated under these conditions and still active in their extraction capabilities. Some of these mixtures, the ones with betaine as HBA, are NADESs (Natural Deep Eutectic Solvents) due to the natural source of their components, promoting the bio-availability of these mixtures. Phenylacetic acid/betaine (PhAA/TMG) mixture revealed to be a promising tool in the extraction topic thanks to its miscibility with water that can be considered modular.

These liquids are promising tools as hydrophobic extraction agents from different matrices and hydrophobic green reaction media.

DECLARATIONS OF INTEREST

None

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GRAPHICAL ABSTRACT