

**Advances in the Development of Novel Green Liquids:
Thymol/Water, Thymol/Urea and Thymol/Phenylacetic Acid
as Innovative Hydrophobic Natural Deep Eutectic Solvents**

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ABSTRACT

Deep Eutectic Solvents (DESs) are increasing their relevance thanks to their ecologically favourable properties, their interesting structural features and their catalytic properties.

In this paper we present three novel hydrophobic DESs mixtures prepared by mixing thymol with water, with urea and with phenylacetic acid. These natural DESs represent important advances in the development of green DESs liquids: Thymol/Water mixture is the first binary, water-based DES with hydrophobic properties; in Thymol/Urea DES, urea and its chelating properties were put in a water-separable phase; finally, Thymol/Phenylacetic acid DES is a mixture of two hydrogen-bond donor molecules showing hydrophobic and slightly acidic properties. The hydrogen bond properties within the different mixtures were investigated via DFT, while *ab initio* molecular dynamics results showed a substantial interaction between thymol and water in the Thymol/Water DES. Moreover, the investigated DESs show excellent extraction properties of heavy metal salts and phenols from aqueous phases.

KEYWORDS

Deep Eutectic Solvents; Sustainable Chemistry; DFT; Molecular Dynamics; Liquid-Liquid Extractions.

INTRODUCTION

Deep Eutectic Solvents (DESs) are a novel class of organic liquids with increasing relevance thanks to their green characteristics, structural features and catalytic properties. (<https://doi.org/10.1002/anie.200900134>. <https://doi.org/10.1016/j.cogsc.2019.09.004>. DOI: 10.1039/C5RA05746A. 10.1016/j.molliq.2017.05.084. <http://dx.doi.org/10.1002/ejoc.201501197>). Developed as a subclass of Ionic Liquids (ILs), DESs share with them many favourable properties such as low or absent vapor pressure, low flammability, easy recycle and recovery. However, DESs represent an advancement to ILs thanks to their much lower ecological impact and their lower toxicity. DESs formed from natural compounds (NADESs, Natural Deep Eutectic Solvents) additionally profit from their biocompatibility (<https://doi.org/10.1016/j.crci.2018.04.002>. <https://doi.org/10.1021/sc500096j>. <https://doi.org/10.1021/acs.jnatprod.7b00945>).

DESs are formed via weak interactions, mainly hydrogen bonds (H-bonds), between two species: a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) molecule (<https://doi.org/10.1002/anie.202110520>. DOI: 10.1039/C7CP01286A). H-bonds forming between HBD-HBA species as well as HBD-HBD and HBA-HBA(DOI: 10.1039/C7CP01286A. <https://doi.org/10.1002/aic.17382>) lead to the liquid nature of DESs at specific HBD/HBA molar ratios, as a regular crystal lattice formation is suppressed. The molecules used for the design of DESs belong to different classes of HBDs, e.g. polyols, alcohols, carboxylic acids, or sugars, while HBAs are usually choline chloride, betaine, generic halide ammonium or phosphonium salts (<https://doi.org/10.1021/cr300162p>. <https://doi.org/10.1016/j.fluid.2017.01.022>. 10.1039/C4RA10628H.).

One of the outstanding aspects contributing to the green properties of DESs is their synthesis routes, which do neither require the use of any solvent or catalyst nor need additional purification procedure (<https://doi.org/10.1021/acs.jpcclett.9b01980>. <https://doi.org/10.1016/j.seppur.2020.118015>. <https://doi.org/10.1002/slct.201800713>). DESs are in fact prepared by mixing and heating two (or more) pure substances, often solids. This is of utmost importance as no solvent is needed to obtain these liquid systems, which maximizes the greenness of the DES synthetic procedures.

Moreover, DESs can possess catalytic properties whenever used as substitutes of organic volatile solvents in chemical transformations(10.1039/C6RA04721A. <https://doi.org/10.1515/gps-2019-0003>. DOI: 10.1039/C9GC03465J). DESs with acidic (both of Brønsted and of Lewis features),

reducing, and also basic properties are reported in literature (<https://doi.org/10.1016/j.molliq.2018.07.060>. <https://doi.org/10.1016/j.biortech.2018.05.016>. <https://doi.org/10.1016/j.cclet.2019.09.055>. DOI: 10.1039/C5RA05746A. 10.1002/ejoc.201801884). Chiral DESs (CDEs) further may be employed as organocatalysts in asymmetric synthesis (10.1002/ejoc.202100385. 10.1016/j.molliq.2020.113573. 10.1016/j.molliq.2018.04.096). The structural features of the DES seem to have an important role in determining their properties. Some DES can act as basic catalysts even if they are formed with acidic molecules. This is due to the formation an extended network of H-bonds in which the acidic proton of the reactant could participate in (<https://doi.org/10.1002/ejoc.202100698>). This behavior, even if these studies are in their infancy, seems to be similar to the ones observed with protic solvents.

Water participates in a peculiar way within the H-bond network of DESs. In fact, water molecules can create water-rich microdomains retaining the HBD-HBA interactions of the DESs, or dissolve the H-bond network at concentrations higher than 50-60% w/w (<https://doi.org/10.1039/C8CS00325D>. <https://doi.org/10.1016/j.foodchem.2015.03.123>. <https://doi.org/10.1016/j.molliq.2019.111301>). The role of the water in DESs still is poorly explored and deserves stronger attention in particular with focus on hydrophobic DESs, which are mixtures based on hydrogen bonds that however are water-separable (<https://doi.org/10.1002/cssc.201900147>. <https://doi.org/10.1021/acssuschemeng.8b03520>. <https://doi.org/10.1021/acssuschemeng.0c00559>. <https://doi.org/10.1063/5.0052200>). In these liquid mixtures, water can be absorbed in small amounts (<10% w/w), while it separates from the mixture at increasing concentration (doi.org/10.1021/acs.jpcb.1c08170). Previous works emphasize that the hydrophobicity/hydrophilicity of the HBD determines the hydrophobicity of the resultant liquid (10.1016/j.molliq.2019.02.107. DOI: 10.1039/c5gc01451d).

In this work, we report the realization of novel thymol-based hydrophobic DESs: Thymol/Phenylacetic Acid, Thymol/Urea and Thymol/Water DESs, see Figure 1. All the three mixtures are Natural Deep Eutectic Solvents. Thymol, already being largely used in DESs chemistry (<https://doi.org/10.1016/j.chroma.2019.01.022>. <https://doi.org/10.1016/j.chroma.2018.07.070>. <https://doi.org/10.1007/s10953-018-0793-1>), is derived from thyme plant and is used in many pharmacological applications because of its activities as antibacterial, antifungal, antioxidant, free radical scavenging, anti-inflammatory, analgesic, antispasmodic, antiseptic and antitumor agent (<https://doi.org/10.1016/j.foodchem.2016.04.111>. <https://doi.org/10.1016/j.arabjc.2020.11.009>.

<https://doi.org/10.3389/fphar.2017.00380>); urea is widely used as fertilizer and is also produced by humans and animals in urea cycle for nitrogen excretion (<https://doi.org/10.1007/BF01048783>); phenylacetic acid is a natural organic acid that is present in many matrices such as honey (<https://doi.org/10.1099/mic.0.2006/002444-0>. <https://doi.org/10.1021/jf3004477>); water is undoubtedly the most abundant and important molecule in nature for all the living beings.

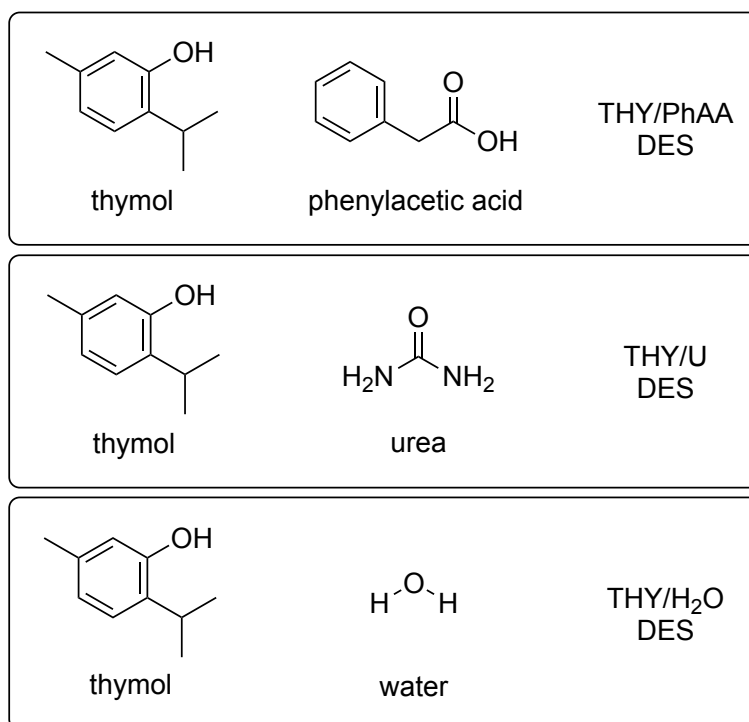


Figure 1: Structures of the molecules used for the development of the three novel hydrophobic thymol-based Deep Eutectic Solvents: Thymol/Phenylacetic acid (THY/PhAA); Thymol/Urea (THY/U); Thymol/Water (THY/H₂O).

We provide an extensive discussion on the preparation, the solid-liquid phase diagrams and the extraction capabilities of these novel liquids, supported by theoretical calculations on the structural features of the DES via DFT as well as *ab initio* molecular dynamics. We believe the novel structural features and the excellent extracting properties showed by these three novel DESs mixtures could inspire further studies on novel water-based and urea-based DESs. Moreover, the possibility of preparation and extraction directly *in situ* of the Thymol/Water mixture, made by adding solid thymol to a water solution and then heating, could lead to the use of this extraction methodology in different matrices.

RESULTS AND DISCUSSION

The first step of this work was a screening on possible hydrophobic DESs mixtures. A wide set of molecules were tested in binary mixtures at molar ratios spanning from 1/5 to 5/1 (with increases by integers of molar ratios) by mixing and heating them with the aim of finding novel water-separable liquid systems. The molecules were chosen based on their H-bonding, or more generally weak interaction, capability. In order to find hydrophobic liquids, a set of low-water-soluble molecules were tested, mainly carboxylic acids and a natural-source phenol: dodecanoic acid, decanoic acid, octanoic acid, sebacic acid, terephthalic acid, anisic acid, phenylacetic acid, benzoic acid *p*-dodecyloxybenzoic acid, *p*-cetyloxybenzoic acid and thymol. Hydrophilic molecules were also used in combination with the hydrophobic ones because if their role is as HBAs in the DESs the resulting liquid is water-separable (<https://doi.org/10.1016/j.molliq.2019.02.107>. DOI: 10.1039/c5gc01451d). Therefore, also trimethylglycine, choline chloride, glycolic acid, sorbic acid, (RS)-lactic acid, oxalic acid, (RS)-mandelic acid and urea were tested in combination with the hydrophobic molecules. The water solubility of all the tested compounds and their melting points are reported in Supporting Information section.

From this screening, we obtained seven novel mixtures which were liquid at room temperature: Thymol/Urea (THY/U, 5/1 molar ratio); Thymol/Phenylacetic Acid (THY/PhAA, 3/1 molar ratio); Thymol/Benzoic Acid (THY/BENZ, 4/1 molar ratio); Octanoic Acid/Benzoic Acid (OCT/BENZ, 4/1 molar ratio); Octanoic Acid/Phenylacetic Acid (OCT/PhAA, 3/1 molar ratio); Decanoic Acid/Phenylacetic Acid (DEC/PhAA, 4/1 molar ratio); Octanoic Acid/Urea (OCT/U, 4/1 molar ratio). To understand whether these liquids were simple mixtures showing a eutectic point or if they represent DESs, we investigate the solid-liquid phase diagrams of all liquids. The results were compared with the theoretical curves obtained from Equation S3, Supporting Information. In this way we can detect an eventual shift from the ideal curves and therefore determine the liquids identities (<https://doi.org/10.1002/cssc.202001331>. <https://doi.org/10.1007/s10953-018-0793-1>). The activity coefficients γ were also determined for all the mixtures at the different molar ratios (see Figure S2, Supporting Information for the diagrams). OCT/PhAA, OCT/U and DEC/PhAA liquids result as simple mixtures as the experimental melting curves strongly correlated with the theoretical curves of the pure substances, thus indicating a negligible interaction between the species. The melting point curve as well as γ of OCT/BENZ shows a moderate deviation from the pure substances;

therefore, also this liquid cannot be considered as a DES. THY/BENZ shows a moderate shift in the melting point both in terms of temperature and of molar ratio; also, the activity coefficients deviate only slightly from the values of 1. Therefore, THY/BENZ cannot undoubtedly be considered as a DES, but may be characterized only as a eutectic liquid.

THY/PhAA (3/1 molar ratio) and THY/U (5/1 molar ratio) mixtures are the only ones within the large set that can fulfill the requirements of DESs. Both systems show relevant shifts in the eutectic points, in terms of temperature and of molar ratio, and deviate from ideality in the activity coefficients, as illustrated in Figure 2A and B respectively. The molecules employed commonly were reported to represent the HBDs in previous DESs (<http://dx.doi.org/10.1016/j.molliq.2017.05.084>, <https://doi.org/10.1016/j.chroma.2019.01.022>, DOI: 10.1039/B210714G). Moreover, both liquids showed separation from added water. In the case of THY/U, the hydrophobicity of the liquid, considering the high solubility in water of urea, suggested the role of thymol as HBD in the mixture according to literature (<https://doi.org/10.1016/j.molliq.2019.02.107>, DOI: 10.1039/c5gc01451d) (see further on in DFT experiments section). However, the HBD-HBA role of the molecules is not according to their pKa values, but it must be considered that the pKa values are measured in water solution rather than as pure compounds in mixture.

As the role of molecules as HBDs or HBAs depends on their counterpart within the mixture, we mixed thymol with H₂O to try to obtain a DES, considering that water is undoubtedly the most capable molecule for H-bond formation. Surprisingly, by mixing and heating thymol in 5/1 and 4/1 molar ratios with water, a stable, uncolored, and water-separable liquid was obtained (THY/H₂O). The solid-liquid phase diagram and the activity coefficient profile of this mixture is reported in Figure 2C.

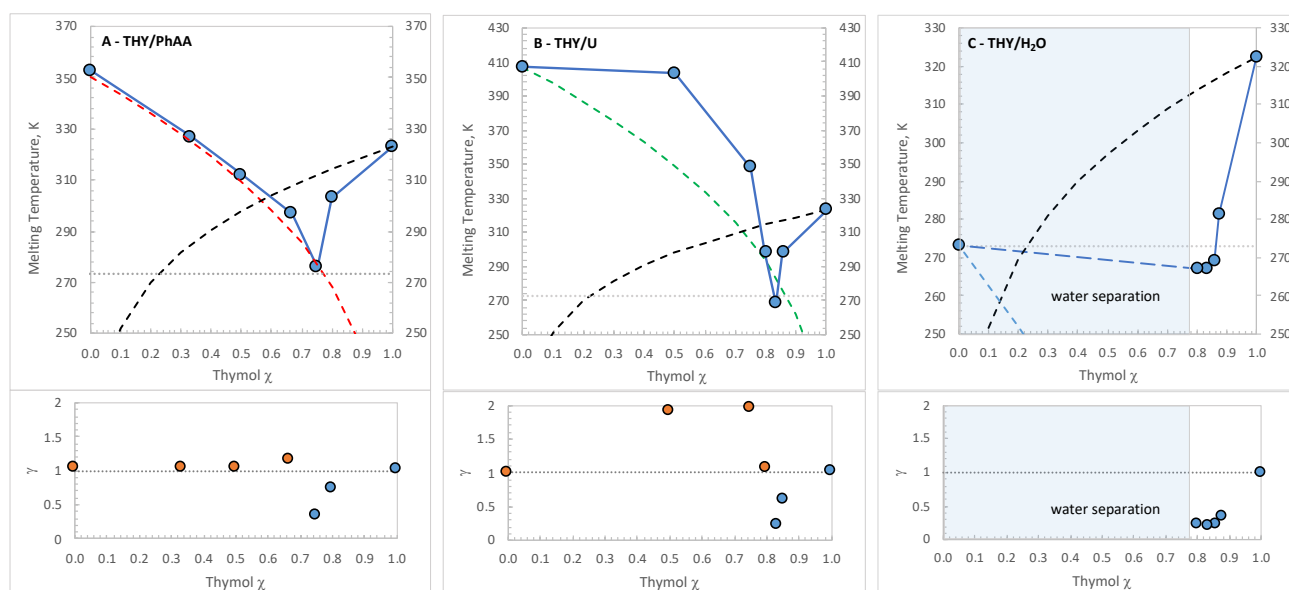


Figure 2: Solid-liquid phase diagrams of the three novel thymol-based hydrophobic DESs. Panel A – THY/PhAA (Thymol/Phenylacetic acid) up: experimental melting points (blue dots), Thymol melting point theoretical curve (black dashed line), Phenylacetic Acid melting point theoretical curve (red dashed line); down: activity coefficients profile. Panel B – THY/U (Thymol/urea) up: experimental melting points (blue dots), Thymol melting point theoretical curve (black dashed line), Urea melting point theoretical curve (green dashed line); down: activity coefficients profile. Panel C – THY/H₂O (Thymol/Water) up: experimental melting points (blue dots), Thymol melting point theoretical curve (black dashed line), Water melting point theoretical curve (light blue dashed line); down: activity coefficients profile.

Because of the natural source of the molecules forming them, the three DESs can be considered as NADESs (Natural Deep Eutectic Solvents). This tremendously increases their importance as green solvents. There are several interesting peculiarities in these novel thymol-based liquids. As mentioned before, THY/PhAA is a mixture of molecules both commonly reported as HBDs. The same observation holds for the THY/U, which further provides the possibility of having urea, and its chelating properties, in a hydrophobic/water separable phase. THY/H₂O represent a novel, peculiar and unique case of a binary, and especially hydrophobic DES liquid formed with water as one of the two components. The liquid-solid phase diagram of THY/H₂O, Figure 2c, showed a highly peculiar behavior as water separates from the liquid mixture itself when we increase the amount of water beyond the 4/1 molar ratio (THY/H₂O). The existence of a liquid mixture is therefore limited to small amounts of added water, from $\chi = 0.875$ until 0.8 of thymol. The 5/1 and the 4/1 points, detected as minimums in the melting points of the mixtures at different molar ratios, both showed melting temperatures of the mixtures of -6°C, being only slightly lower than the one of water (0°C). However, THY/H₂O shows strong deviations from the theoretical one in terms of molar ratio ($\chi_{\text{THY}} = 0.15$) and in the activity coefficients which are much smaller than 1. Also, substantial differences between the melting temperature curve of thymol and the THY/H₂O is

visible. Interestingly, even though thymol enters the mixture with high molar fraction, its melting point is strongly decreased from 49°C to -6°C upon adding 1/5 of moles of water, together with a large shift from the ideal thymol melting curve. For these reasons, THY/H₂O can be considered as a DES. It is impossible to determine if the 5/1 or 4/1 molar ratios are the eutectic point of this mixture as water separates from the DESs at increased molar ratios (doi.org/10.5286/ISIS.E.RB2010042, doi.org/10.1021/acs.jpcc.1c08170, doi.org/10.1021/acs.cgd.1c01477). According to literature, DESs can be defined as HBD-HBA, HBD-HBD and HBA-HBA adducts. The hydrophobicity of the 4/1 THY/H₂O mixture suggests that clusters of water-water adducts, which may formed at larger water concentration, only weakly interact with the THY/H₂O adducts. However, there are many DESs reported that showed their unique properties even if the molar ratio of the eutectic point is not determined because they showed melting temperatures lower than -70°C at different molar ratios, therefore undetectable precisely (<https://doi.org/10.1016/j.molliq.2019.111301>, <https://doi.org/10.1016/j.molliq.2021.116676>, <https://doi.org/10.1016/j.tca.2021.179055>); this could be considered a similar case.

The preparation of these novel THY-based DESs (see Experimental section in Supplementary Information) required higher temperatures (about 120°C) compared to the other common DESs reported in literature, but with lower heating times of <20 mins for about 20 mL sample and <30 s for about 1 mL sample with a heating gun. As thymol easily sublimates and water evaporates at these temperatures, a reduction in the headspace of the sealed container and heavy shaking was necessary to obtain stable homogeneous liquids. To properly prepare THY/H₂O DES, a larger amount of water can also be added (also about 10/1 molar ratio over thymol). After the formation of the liquid in the upper phase, the water phase can be separated via centrifugation of the mixture resulting in a stable DES. With this procedure of preparation, we obtained a molar ratio of 4.8/1 THY/H₂O measured via Karl Fisher titration and NMR measures in different samples batches, see Experimental in Supporting Information. The melting point of the 4.8/1 adduct is retained at -6°C. An additional relevant aspect of THY/H₂O mixture is the capability of an *in-situ* preparation in water by simply adding the solid thymol with subsequent heating and shaking of the sealed container with a heat gun for few seconds. This leads to the formation of the DES liquid directly above the solution in the upper phase. Note that this procedure was applied also in the extraction experiments which are discussed later on in this work.

In Table 1, we summarize the molar ratios of the novel thymol-based DESs, their melting points, their densities, the molar volumes and the water amount after mixing with a water phase

(50/50 w/w) and then centrifugation. All the densities are lower than the one of water. The molar volumes show values similar to the ones reported in literature for other DESs (<http://dx.doi.org/10.1016/j.cjche.2016.05.047>); THY/PhAA mixture showed the highest molar volume, indicating a weaker intimacy of the HBD-HBA couple (DOI: 10.1002/apj.1978.). The water amount of the starting DESs is not reported as it can change substantially in different experimental conditions (i.e. different water amounts in the reagents). We thus reported the maximum water content in the DESs phase in Table 1; over these values water separates in a lower phase that persists in all the percentages of added water (until >95% w/w).

Table 1: Molar ratios, melting points, densities, molar volumes, and water content after separation from water phases for the thymol-based DES mixtures.

	Acronym	Molar Ratio (THY/HBA)	Melting point (°C)	Density (g/ml)	Molar Volume (cm ³ /mol)	Maximum water content (% w/w)
Thymol / Phenylacetic acid	THY/PhAA	3/1	3	0.9771	150.14	5.50
Thymol / Urea	THY/U	5/1	-5	0.9794	138.04	2.35
Thymol / Water	THY/H ₂ O	4.8/1	-6	0.9400	136.36	2.45

Density functional theory (DFT) calculations were performed in order to study the intermolecular interactions occurring between the molecule pairs within the thymol DESs. We consider the hydrogen bonding within all potential HBD-HBA configurations to clarify the role of HBD or HBA in the DESs mixtures (see section S2.1, Supporting Information, for computational details). The interaction between the HBA and the HBD in the DESs is quantified by the relative formation energy $E_{rel} = E(\text{HBD} \rightarrow \text{HBA}) - E(\text{HBA}) - E(\text{HBD})$, with the energy of the interacting HBD-HBA complex, $E(\text{HBD} \rightarrow \text{HBA})$, and the energy of the isolated HBA and HBD molecules, $E(\text{HBA})$ and $E(\text{HBD})$, respectively. In THY/U and THY/H₂O, THY acts as HBD with the HBAs being urea and water with E_{rel} of -6.6 kcal/mol and -5.29 kcal/mol, respectively (Table 2 and Figure 3). In THY/PhAA, lowest E_{rel} between THY and PhAA of -4.79 kcal/mol is obtained. Considering also the structures (Figure 3) THY and PhAA both act as HBD and as HBA at the same time. The strong PhAA- \rightarrow PhAA interaction with E_{rel} of -10.95 kcal/mol, further suggests that the DES may contain PhAA rich domains, which can explain the similarity in the melting curve of PhAA and THY/PhAA DES, Figure 2A.

Table 2: Relative formation energy E_{rel} for thymol-based DES THY/PhAA, THY/U, and THY/H₂O. The notation A→B represents configurations with species A as hydrogen bond donor and species B as hydrogen bond acceptor. *In PhAA→THY, PhAA and THY both act as HBD and HBA at the same time.

Molecule A	Molecule B	A→A (kcal/mol)	B→B (kcal/mol)	A→B (kcal/mol)	B→A (kcal/mol)
Thymol	Phenylacetic acid	-3.56	-10.95	-3.88	-4.79 (*)
Thymol	Urea	-3.56	-7.64	-6.69	-2.85
Thymol	Water	-3.56	-4.27	-5.29	-2.95

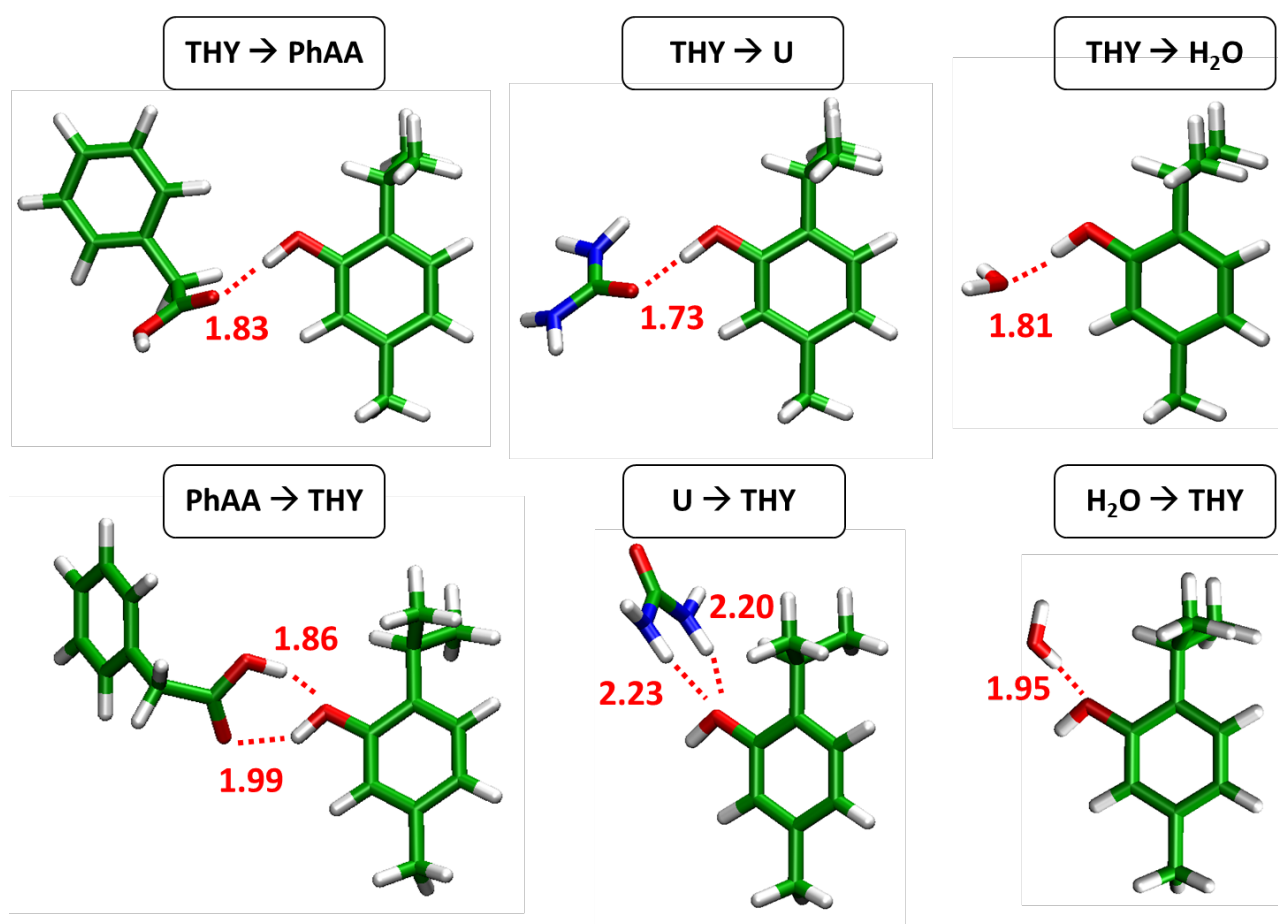


Figure 3: Visualization of the hydrogen bonds within the thymol-based DES THY/PhAA, THY/U, THY/H₂O. The hydrogen bonds are indicated by dashed lines and the bond length is given in units of Angstroms for each hydrogen bond.

One of the most studied and applied properties of novel hydrophobic green liquids is their use for the decontamination of water phases from various pollutants or for the extraction/preconcentration of valuable and relevant compounds from different matrices

(<https://doi.org/10.1016/j.foodchem.2017.09.141>. <https://doi.org/10.1016/j.cej.2020.127648>. <https://doi.org/10.1016/j.chroma.2019.460695>). For these reasons, we investigated the capabilities of the three novel hydrophobic DESs for the extraction of model pollutants and phenolic compound from water solutions via a simple mixing and mild centrifugation procedure (see Supporting Information for the experimental details). THY/PhAA, THY/U and THY/H₂O DESs were tested by mixing them in equal weight with water solutions containing various contaminants: transition heavy metals salts Co(II), Ni(II) and Cu(II) (as chlorides and perchlorates in order to analyze different softness of the anions; all • 6H₂O, except CuCl₂• 2H₂O); 2-nitrophenol and 2,4-dinitrophenol; malachite green and methylene blue, representing two dyes formed by aromatic cations; and umbelliferone. All the structures are reported in Figure 4.

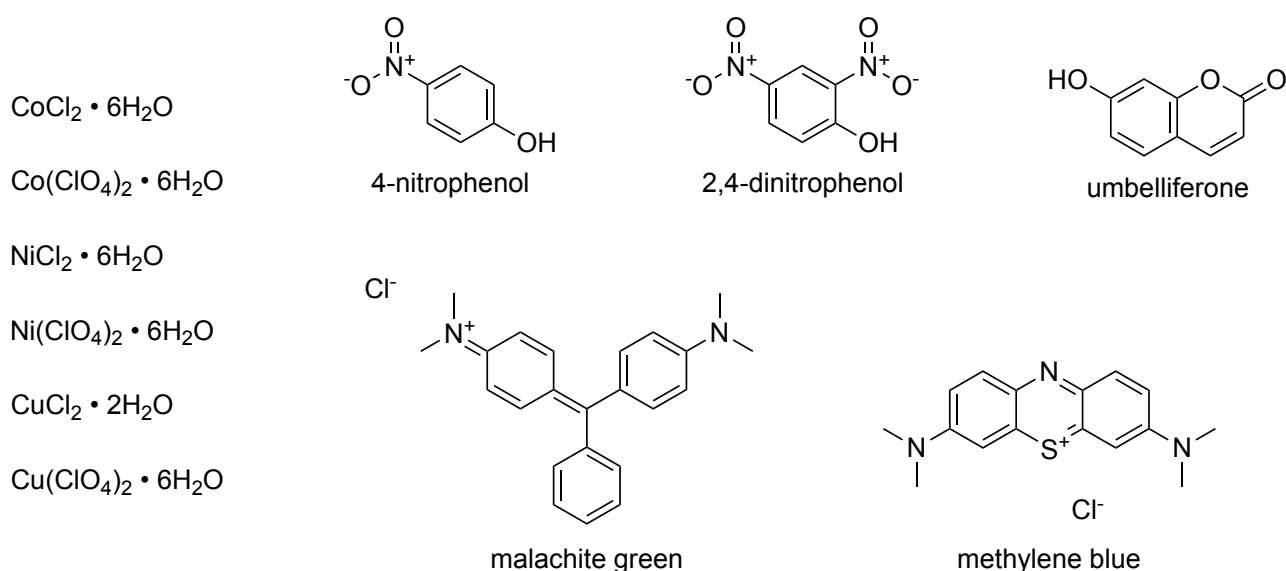


Figure 4: Structures of the heavy metal salts and of the molecules tested for the extractions from water solutions with novel hydrophobic DESs.

Transition heavy metal salts are known for their toxic activity at high concentrations and for their bioaccumulation (<https://doi.org/10.1007/s10311-010-0297-8>. [https://doi.org/10.1016/S0269-7491\(00\)00179-2](https://doi.org/10.1016/S0269-7491(00)00179-2). [https://doi.org/10.1016/S0140-6736\(11\)61516-X](https://doi.org/10.1016/S0140-6736(11)61516-X)). Nitrophenols, as well as the heavy metal salts, are water pollutants listed as the priority water pollutants of EPA (US Environmental Protection Agency)(<https://doi.org/10.1016/j.envpol.2018.05.089>. [https://doi.org/10.1016/S0021-9673\(01\)87845-6](https://doi.org/10.1016/S0021-9673(01)87845-6)). Dyes are largely used in many industrial applications and their difficult removal and biodegradation, as well as their toxicity, is a matter in

various chemical applications (10.6088/ijes.2012030133002.
<https://doi.org/10.1016/j.aquatox.2003.09.008>. [https://doi.org/10.1016/S0022-3468\(03\)00278-1](https://doi.org/10.1016/S0022-3468(03)00278-1)).
Umbelliferone is an antioxidant phenol offering great pharmacological activity and it can be
extracted from many different matrices (<https://doi.org/10.1016/j.bfopcu.2017.05.001>.
<https://doi.org/10.1556/jpc.26.2013.5.6>). The extraction efficiencies E% of the three liquids,
evaluated via UV-Vis analyses of the water phases, are reported in Figure 5. With Ni(II) and Cu(II)
salts the UV-Vis analyses were performed by adding DDC (Sodium diethyldithiocarbamate) in order
to shift the absorbance to a spectral region with no possible overlapping (DOI
<https://doi.org/10.1039/AN9952000255>). In Supporting Information section all the spectra before
and after the extraction are reported.

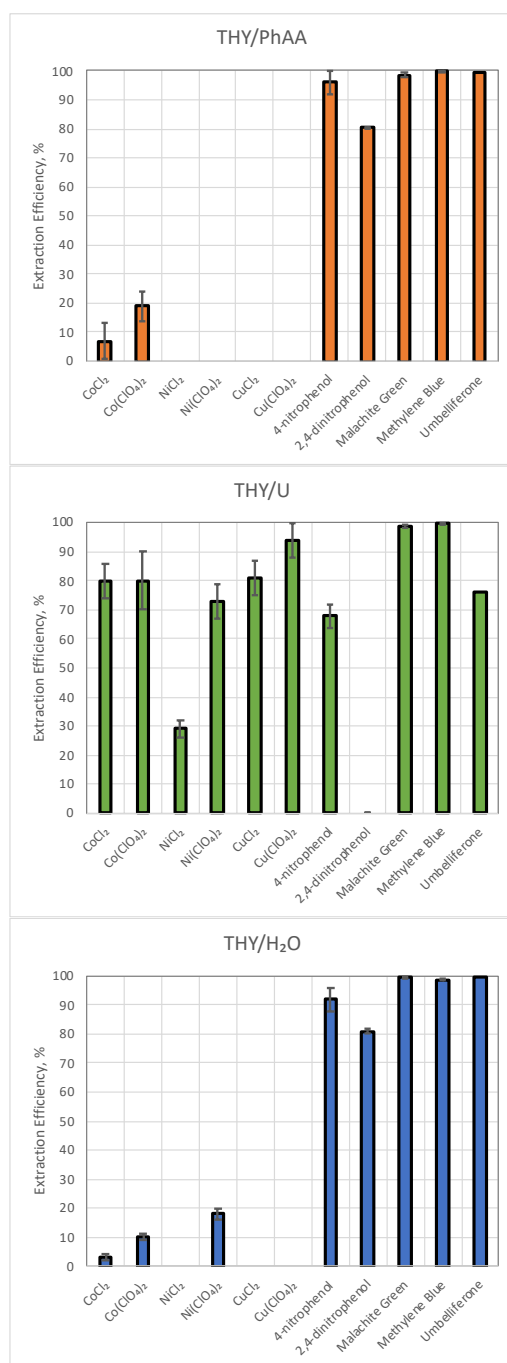


Figure 5: Extraction efficacies (E%) of the hydrophobic DESs (up: Thymol/Phenylacetic acid, THY/PhAA; middle: Thymol/Urea, THY/U; down: Thymol/water, THY/H₂O) of polluting compounds and the umbelliferone in water solutions: from left to right CoCl₂; Co(ClO₄)₂; NiCl₂; Ni(ClO₄)₂; CuCl₂; Cu(ClO₄)₂; 4-nitrophenol; 2,4-dinitrophenol; Malachite Green; Methylene Blue; Umbelliferone. With Ni(II) and Cu(II) salts the UV-Vis analyses were performed by adding DDC (Sodium diethyldithiocarbamate) in order to shift the absorbance to a spectral region with no possible overlapping. See Supporting Information for the concentrations. The measures were made in triplicate and the error bars reported are the standard deviations of the three samples.

THY/PhAA mixture was not effective in the extraction of heavy metal ions; Co(II) salts are the only ones that can be extracted but with very little efficacies of 7% and 19% for chloride and

perchlorate anions, respectively. However, it shows excellent E% for the two phenolic compounds, for umbelliferone and for the two dyes. The E% spans between high values of 95% to 99%, and only 2,4-dinitrophenol solution showed a slightly lower E% value of 81%.

The THY/U DES is the only liquid of the investigated set that shows extraction capabilities of the heavy metal ions, with excellent values spanning from 75% to 80%. The only two exceptions are NiCl_2 that shows a slight lower capability of 29%, and $\text{Cu}(\text{ClO}_4)_2$ that is the highest in the heavy metal salts set (E%=95%). There is no observed correlation with the softness of the anion passing from chloride to perchlorate as the values are similar in all the set except for the two exceptions. Urea is a well-known and well-studied molecule being capable to chelate cations (<https://doi.org/10.1039/B511809C>), which is retained also in the resulting Urea-based DES. This confirms that the DES properties depend on the properties of its constituents (DOI: 10.1039/C9GC03465J). The most relevant aspect of this liquid is given by the presence of urea in a hydrophobic, water-separable and excellently extracting liquid for these heavy metal ions. The capabilities of extraction of the phenols, the dyes and umbelliferone are excellent (65% to 99%) like the other DESs, with the peculiar case that no extraction at all is observed for 2,4-dinitrophenol.

THY/ H_2O DES can only extract few elements of the heavy metal ions set with very low E% (CoCl_2 , $\text{Co}(\text{ClO}_4)_2$ and $\text{Ni}(\text{ClO}_4)_2$) of 3% to 18%. Excellent extraction was observed for the phenols, the dyes and umbelliferone, with values spanning from 92% to 99%. Once again, 2,4-dinitrophenol represented an exception as its E% value is about 81%. The behavior of this liquid is practically identical to the one of the THY/PhAA hydrophobic DES, with the exception of $\text{Ni}(\text{ClO}_4)_2$ which shows a reduced E%. This strengthens again our identification of this liquid as a DES.

The capability of extraction of phenols and dyes was carried out by all the three novel hydrophobic DESs, but THY/U mixture showed a further advantage in terms of capability of extraction of heavy metal salts. 2,4-dinitrophenol showed the lowest E% values within the set of phenols and dyes for all three DESs. The low extraction efficiency seems to correlate with the pKa value of the HBAs in the DESs, as the slight acidic phenylacetic acid-based one is the most efficacious DES in the set. The pKa value of phenylacetic acid is, in fact, comparable to the one of 2,4-dinitrophenol ($\text{pK}_{\text{aPhAA}} = 4.31$; $\text{pK}_{\text{a2,4-dinitrophenol}} = 4.09$). The other two liquids, which have neutral molecules as HBAs (water and urea), show worse extraction properties. Therefore, we performed a set of extraction experiments of the phenolate of this phenol, using 2,4-dinitrophenolate sodium salt, with the three DESs liquids. THY/PhAA showed an almost ideal extraction with E% = 99%, while THY/ H_2O and THY/U give a reduced E% of 32% and even lower E% = 8%, respectively. Moreover,

when the water solution of the phenolic salt is mixed with THY/PhAA DES, a change of its color from yellow to colorless can be observed. This could be attributed to the protonation of the phenolate thanks to the higher acidity of Phenylacetic acid compared with the other counterparts in the liquids. Recent studies (DOI: 10.1039/d0cp01209b) demonstrated that phenolate introduces a local ordering of hydrogen-bonds between water molecules expanding beyond the first solvation shell of the phenolate oxygen atom, which is substantially reduces in case of phenol. DFT simulations show lowest protonation energies of the 2,4-dinitrophenol for PhAA, see Table S3, Supporting Information; furthermore, the water-2,4-dinitrophenol hydrogen-bond is the weakest with respect to the other components of the thymol-based DESs, see Table S4, Supporting Information. Thus, we may rationalize our experimental observation by the protonation of the 2,4-dinitrophenol by PhAA, leading to a strong reduction of the water-phenol interaction and promoting the extraction of 2,4-dinitrophenol. Again, the properties of a DES liquid are dependent on the properties of its components and this data promotes THY/PhAA DES as a novel hydrophobic and slightly acidic liquid. These experiments could lead to further future studies on the suppressed extraction of this organic aromatic anion, as on the contrary the two organic aromatic cationic dyes were efficaciously extracted in all the DESs.

As water is a component of THY/H₂O DES, the novel hydrophobic liquid can be prepared directly *in situ* by adding solid thymol to a water solution, then heating and shaking the sample. With this approach we repeated the extraction procedures by adding solid thymol to the polluted water solutions. 0.93 grams of thymol were added for each mL of solution (considering the density of the liquid and the decrease in volume of the water phase as the thymol subtracts it to form the DES); then the samples were heated with a heat gun, mixed for few seconds and then centrifuged. The results are reported in Figure 6 in comparison with the ones obtained by mixing the pre-formed DES to the water solutions.

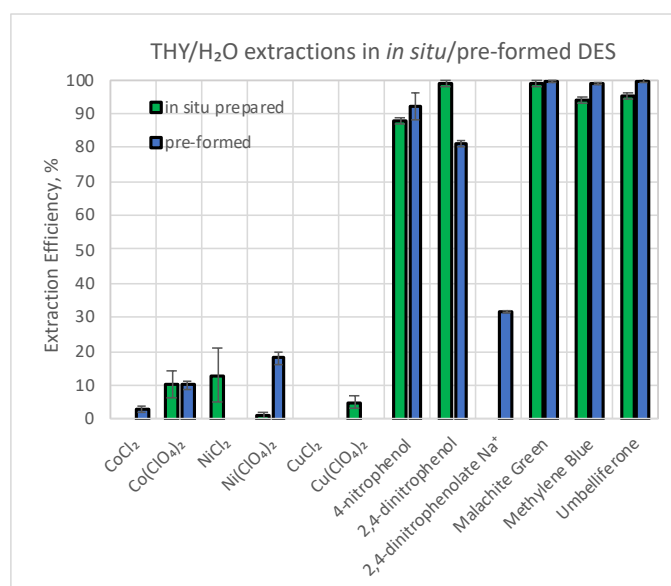


Figure 6: Comparison of Extraction Efficiency (E%) of THY/H₂O DES prepared *in situ* via heating the sample after adding solid thymol (green columns), and pre-preparing the DES (blue columns). The measures were repeated in triplicates and the reported error bars as standard deviations of the three samples.

As it emerges from Figure 6, the *in situ* preparation and the pre-preparation of the DES liquid show little differences in the E% values, with the exception of the 2,4-dinitrophenolate Na⁺. The E% *in situ* prepared DES is suppressed while substantial extraction can be seen for the pre-formed DES. The reported results demonstrates that our presented produce is a simple and highly efficient method for the decontamination of a water phase. *In situ* preparation of hydrophobic binary DESs has been reported previously, but in contrast to existing results we could demonstrate the effective decontamination of water by only adding thymol to the water solutions without the need for a pre-formed hydrophobic DESs (<https://doi.org/10.1016/j.chroma.2019.460730>. <https://doi.org/10.1016/j.foodchem.2019.125870>).

The interesting properties of the water-based, but hydrophobic THY/H₂O DES raises the need for a detailed understanding of the chemical interaction between thymol and water molecules when being mixed. We performed *ab initio* molecular dynamics simulations (see section S2.2, Supporting Information, for computational details) on the THY/H₂O DES to understand the chemical interaction and the type of H-bonds forming in solution. We setup a model system of THY/H₂O using a 5:1 molar ratio (40:8 molecules) of thymol to water molecules, see Figure 7a. We adopt a cubic cell (a=b=c=with a volume of 22.14 Å³) to reproduce the experimental DES density reported in Table 1. The initial configuration is constructed of local clusters made of 5 thymol molecules and 1 water molecule which are connected by a H-bond network, see Figure S11, Supporting Information. We

run 20 ps to equilibrate the system and then sample additional 25 ps to gain a sufficient statistical description of the H-bond properties, see Figure S12, Supporting Information.

During the equilibration phase, the system mixes by the spontaneous rearrangement of molecules, which results in a substantial decrease of THY→THY H-bonds (Figure S12, Supporting Information) and homogeneously dispersed water molecules. In the equilibrated system, we observe a large amount of hydrogen bonds, i.e. 0.68 H-bonds per mole of water, between water molecules as HBA and thymol as HBD, see Figure 7b. The contribution of H₂O→THY H-bonds per mole of water is substantially less with a value of 0.26. The mixing results in only small contributions of THY→THY H-bonds (0.17 per mole of thymol) and H₂O→H₂O H-bonds (0.01 per mole of water). The radial distribution function, RDF, between the thymol H atoms, H_{THY}, and water O atoms, O_W, shows a strong peak centered at 1.74 Å, representing the THY→H₂O H-bonds, and a less pronounced second peak at 3.3-3.4 Å. The H₂O→THY H-bonds results in a much weaker peak in the H_W/O_{THY} RDF at 1.87 Å. The second peak at 3.26 Å can be attributed THY→H₂O H-bonds. The comparably negligible O_{THY}/O_{THY} peak and the lack of any RDF peaks in the O_W/O_W distribution, Figure 7b, further underlines the well-mixed nature of the THY/H₂O system. Note that the RDFs show a lack of long-range correlation, underlining the liquid nature of the THY/H₂O DES, Figure S13, Supporting Information. Even though the H₂O→H₂O hydrogen bond energies in gas phase are comparable to the ones of THY→H₂O hydrogen bonds (see Table 2), the excess of thymol suppressed the formation of water clusters in our AIMD simulations. In excess of water, we may expect that water hydrogen bond networks may form, while the competing THY→H₂O bonding can bond some water molecules. The steric hindrance of thymol and simultaneously the low density of THY hydrogen bond donors, due to the large size of thymol, potentially limits the density of water molecules which mix with thymol, explaining the experimental observations.

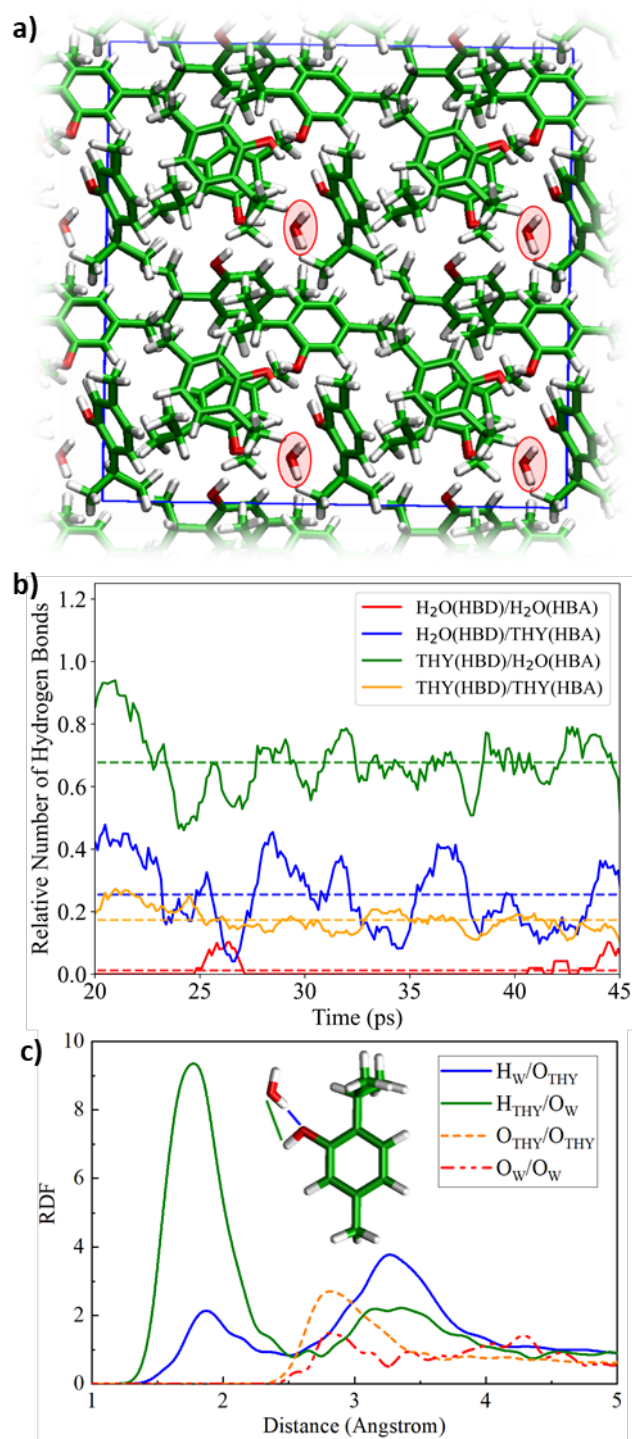


Figure 7: (a) Structural model of the THY/H₂O mixture, water molecules are explicitly highlighted. (b) Time evolution of the number of the intermolecular hydrogen bonds between thymol (THY) and water molecules. The number of hydrogen bonds is normalized on the number of water molecules for H₂O/H₂O, H₂O/THY, and THY/H₂O; for THY/THY, we normalize the number of hydrogen bonds on the number of thymol molecules. (c) Radial distribution function, RDF, between the water H atom, H_w, and the thymol O atom, O_{THY}, (blue), and between the thymol H atom of the hydroxyl group, H_{THY}, and the water O atom, O_w, (green), schematically shown by the inset. Dashed lines give the RDF between O_{THY} atoms (orange) and between O_w atoms (red).

CONCLUSIONS

In this study, we reported novel hydrophobic natural deep eutectic solvents based on thymol, in particular using Thymol/Water, Thymol/Urea and Thymol/Phenylacetic Acid, representing a step-ahead in the development of green solvents. For the first time to our knowledge, water was used as a component of a binary DES mixed with thymol showing surprise properties in terms of water separability/hydrophobicity. The structural properties of the different solvents, with a strong focus on the thymol/water DES, were characterized by first-principles DFT and *ab initio* molecular dynamics simulations. The AIMD simulations predict a high miscibility of water with thymol, dominated by hydrogen bonding between thymol as HBD and water as HBA. In Thymol/Urea DES, the urea and its chelating properties were put in a water separable/hydrophobic liquid. Thymol/Phenylacetic acid is a mixture of two commonly known HBD components. We demonstrate excellent extraction capabilities especially of heavy metal salts thanks to the Urea in THY/U mixture, which promotes these solvents as for many different applications. We strongly believe that our results open novel approaches for the development of novel green liquids. Especially the use of water within a binary mixture represents, in fact, a great step ahead in the realization of novel water-based DESs and green solvents.

SUPPORTING INFORMATION

Advances in the Development of Novel Green Liquids: Thymol/Water, Thymol/Urea and Thymol/Phenylacetic Acid as Innovative Hydrophobic Natural Deep Eutectic Solvents

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S1 EXPERIMENTAL SECTION

S1.1 REAGENTS AND METHODS

S1.1.2 Reagents

All the reagents were purchased from Merck, Sigma-Aldrich, Acros, Alfa-Aesar and Carlo Erba suppliers, all the purities are >99%. Dodecanoic acid, decanoic acid, octanoic acid, sebacic acid, terephthalic acid, anisic acid, phenylacetic acid, benzoic acid, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$; 2-nitrophenol, 2,4-dinitrophenol, 2,4-dinitrophenolate Na^+ , malachite green, methylene blue, umbelliferone were used without any purification procedure. Betaine, choline chloride, glycolic acid, sorbic acid, (RS)-lactic acid, oxalic acid, (RS)-mandelic acid and urea were dried under vacuum under P_2O_5 prior using them. *p*-dodecyloxybenzoic acid, *p*-cetyloxybenzoic acid were synthesised following procedures reported elsewhere (<https://doi.org/10.1002/ejoc.201100720>). Thymol was purchased from Merck and it was purified via sublimation, otherwise the resulting systems were unstable and gave slightly higher melting temperatures. The water used was bidistilled purity grade. The water solubility of the tested compounds and their melting points are reported in Table S1. (Yalkowsky, Samuel H., Yan He, and Parijat Jain. Handbook of aqueous solubility data. CRC press, 2016 - <https://doi.org/10.1002/ejoc.201100720>):

Table S1: Maximum water solubility and melting points of the tested molecules.

Compound	Maximum water solubility, g/L	Melting Point, °C
Thymol	0.90 (25°C)	49
Dodecanoic acid	0.055 (20°C)	44
Decanoic acid	0.15 (20°C)	31
Octanoic acid	0.68 (20°C)	16
Sebacic acid	1.00 (20°C)	131
Terephthalic acid	0.015 (20°C)	>400
Anisic acid	0.20 (20°C)	184
Phenylacetic acid	16.6 (20°C)	77
<i>p</i> -dodecyloxybenzoic acid	1.094 (25°C)	95
<i>p</i> -cetyloxybenzoic acid	0.36 (25°C)	96
Benzoic acid	3.44 (25°C)	121
(RS)-Mandelic acid	140 (25°C)	120

Sorbic acid	1.60 (20°C)	135
(RS)-Lactic acid	>500	17
Oxalic acid	86 (25°C)	190
Betaine	>500	310
Choline Chloride	>500	302
Glycolic acid	>500	63
Urea	>500	133

S1.1.3 DESs Preparation and Characterization

The components were weighed at proper molar ratios in a flask fitted with a stopper. The mixtures were magnetically stirred and heated at temperatures spanning from 40° to 100°C until homogeneous liquids were formed in times spanning from 30 mins to 2 hours. Thymol/Phenylacetic Acid and Thymol/Urea mixtures were prepared at higher temperatures (120°C) with shorter times (<20 mins for about 20 mL sample, <30 s for about 1 mL sample with a heating gun) in screw-capped septum vials with low headspace; the samples were shaken vigorously in order not to subtract the thymol from the mixture that can sublime in these conditions. Thymol/Water mixtures was prepared via the same method of the other two liquids or via adding larger amounts of water (about 10/1 molar ratio over thymol) then centrifugation of the sample (Beckman Coulter Allegra 64 R, 5000 RPM, 15 min, 25 °C) to obtain an upper-phase hydrophobic DES.

Water contents of the mixtures were measured via Karl-Fischer titrator Metrohm 684 KF Coulometer. The THY/H₂O DES water content was confirmed also via NMR experiments (Bruker Avance 400 HD, 400 MHz): two samples were prepared with the same CD₃CN solvent batch, one of the DES in CD₃CN, and the other with CD₃CN only by adding the same amounts of deuterio-solvent (550 µl); the water peak integration was considered as water amount by subtracting the absolute integration value of the sample with only the solvent; the samples were performed with the same RG values in the same instrument. The results were identical to the ones obtained via Karl-Fischer titration.

The molar volumes (V_M) of all the DESs mixtures were determined with the formula (S1):

$$V_M = \frac{M_{DES}}{\rho} \quad (S1)$$

Where ρ is the density (in g/cm³); M_{DES} is calculated with formula (2):

$$M_{DES} = \frac{\chi_{HBD} MW_{HBD} + \chi_{HBA} MW_{HBA}}{\chi_{HBD} + \chi_{HBA}} \quad (S2)$$

Where χ are the molar fractions and MW are the molecular weights of the components of the DESs. The densities were determined at 25.0 ± 0.1 °C via weighting samples in volumetric flasks.

S1.1.4 Solid-Liquid Phase Diagrams

All the mixtures were characterized on the basis of the comparison between the experimental and the theoretical solid-liquid phase diagrams. The experimental solid-liquid phase curves were obtained by measuring the melting points of the different samples at the different molar ratios with a thermometer via immersion of the samples in an ice/NaCl mixture or solid CO₂/acetone mixture in a Dewar. The melting temperatures were evaluated in triplicate to avoid any kinetic effect on the melting of the mixtures and they showed a standard deviation <1°C in the triplicates.

The solid-liquid theoretical curves were determined by using the following Formula (S3) that represents the solid-liquid equilibrium curve in a eutectic mixture:

$$\ln(\chi_i \cdot \gamma_i) = \frac{\Delta_m h_i}{R} \cdot \left(\frac{1}{T_{m,i}} - \frac{1}{T} \right) + \frac{\Delta_m C_{p,i}}{R} \cdot \left(\frac{T_{m,i}}{T} - \ln \frac{T_{m,i}}{T} - 1 \right) \quad (S3)$$

where χ_i is the mole fraction of component i, γ_i is its activity coefficient in the liquid phase, $\Delta_m h_i$ and $T_{m,i}$ are its melting enthalpy and temperature, respectively, $\Delta_m C_{p,i}$ is its heat capacity change upon melting, R is the ideal gas constant, and T is the absolute temperature of the system. This equation can be simplified by considering the heat capacity change upon the melting of a substance as negligible, therefore equation (S4) was used:

$$\ln(\chi_i \cdot \gamma_i) = \frac{\Delta_m h_i}{R} \cdot \left(\frac{1}{T_{m,i}} - \frac{1}{T} \right) \quad (S4)$$

The theoretical melting temperatures were determined from the theoretical curves by considering the activity coefficients $\gamma_i = 1$. The eutectic points were determined as the minimum in the experimental curves and they were compared to the theoretical ones.

The experimental γ_i values were determined via equation (5) by using the experimentally observed melting temperatures:

$$\gamma_i = \frac{\exp\left[\frac{\Delta_m h_i}{R} \left(\frac{1}{T_{m,i}} - \frac{1}{T}\right)\right]}{\chi_i} \quad (S5)$$

S1.1.5 Extractions Procedure

The DESs were mixed with equal weight water solutions of the analytes; a typical experiment was carried out by adding 0.5 grams of DES and 0.5 grams of water solution in a 1.5 mL Eppendorf. Then the mixtures were vortexed for 1 min, then centrifuged (5000 RPM, 20°C, 15 minutes, Beckman Coulter Allegra 64 R for larger samples or ALC 4224 Centrifuge for smaller samples in Eppendorf). The water phases were analyzed via UV-Vis absorption spectra (Agilent 8453 diode array UV-Vis spectrophotometer, temperature controlled at 25°C with a Peltier system Agilent 890890A) comparing the spectra with the ones of the starting water solutions at the same dilutions (Formula 6):

$$E_{\%} = \frac{A_0 - A_i}{A_0} \cdot 100 \quad (S6)$$

where A are the absorbances of analyte before (0) and after (i) the extractions. All the experiments were repeated in triplicate and the errors were calculated as standard deviations of the samples. The wavelengths used for the different analytes, their starting concentrations and the dilutions in cuvette are reported in Table S2. For 4-nitrophenol and 2,4-dinitrophenol, in Figure S1 the isosbestic point determinations in ethanol (made with subsequent additions of NaOH $5 \cdot 10^{-4}$ M water solution) are reported.

Table S2: Wavelengths used for the different analytes, their starting concentrations and the dilutions in cuvette.

Compound	Water solution concentration, M	Absorbance Wavelength, nm	Dilution in Cuvette (2 mL Ethanol), μ L
CoCl ₂	4.83 E-5	403	100
Co(ClO ₄) ₂	5.32 E-4	403	100
NiCl ₂	5.00 E-4	390	100 ^b
Ni(ClO ₄) ₂	5.00 E-4	390	100 ^b
CuCl ₂	4.83 E-4	438	50 ^b
Cu(ClO ₄) ₂	6.27 E-4	438	50 ^b
4-nitrophenol	0.0101	342 ^a	25
2,4-dinitrophenol	9.84 E-4	326 ^a	80

2,4-dinitrophenolate Na ⁺	9.73 E-4	326 ^a	80
Malachite Green	1.78 E-4	620	100
Methylene Blue	3.30 E-4	655	50
Umbelliferone	1.01 E-3	326	100

^a = isosbestic points. ^b = DDC (Sodium diethyldithiocarbamate) added to the ethanol solutions (0.01 M).

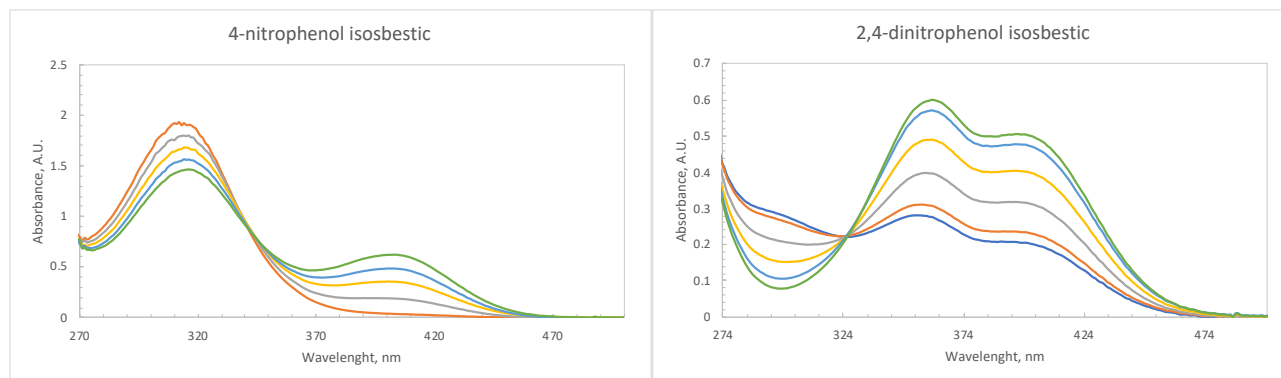


Figure S1: Isosbestic points determination of 4-nitrophenol and 2,4-dinitrophenol in ethanol.

S1.1.6 *In Situ* Extraction Procedure

The *in situ* preparation of Thymol/water DES and the extractions were performed by directly adding the solid thymol to the water solutions of the compounds to be extracted in ratio 0.93 grams of thymol for each mL of solution. Then the samples were heated with a heat gun for times < 30 seconds and heavily shaken to prevent sublimation of the thymol and evaporation of water on the top of the sealed vial. Then the samples were centrifuged and analyzed in the same manners of the other samples reported in “Extractions procedures”.

S1.2 SOLID-LIQUID PHASE DIAGRAMS

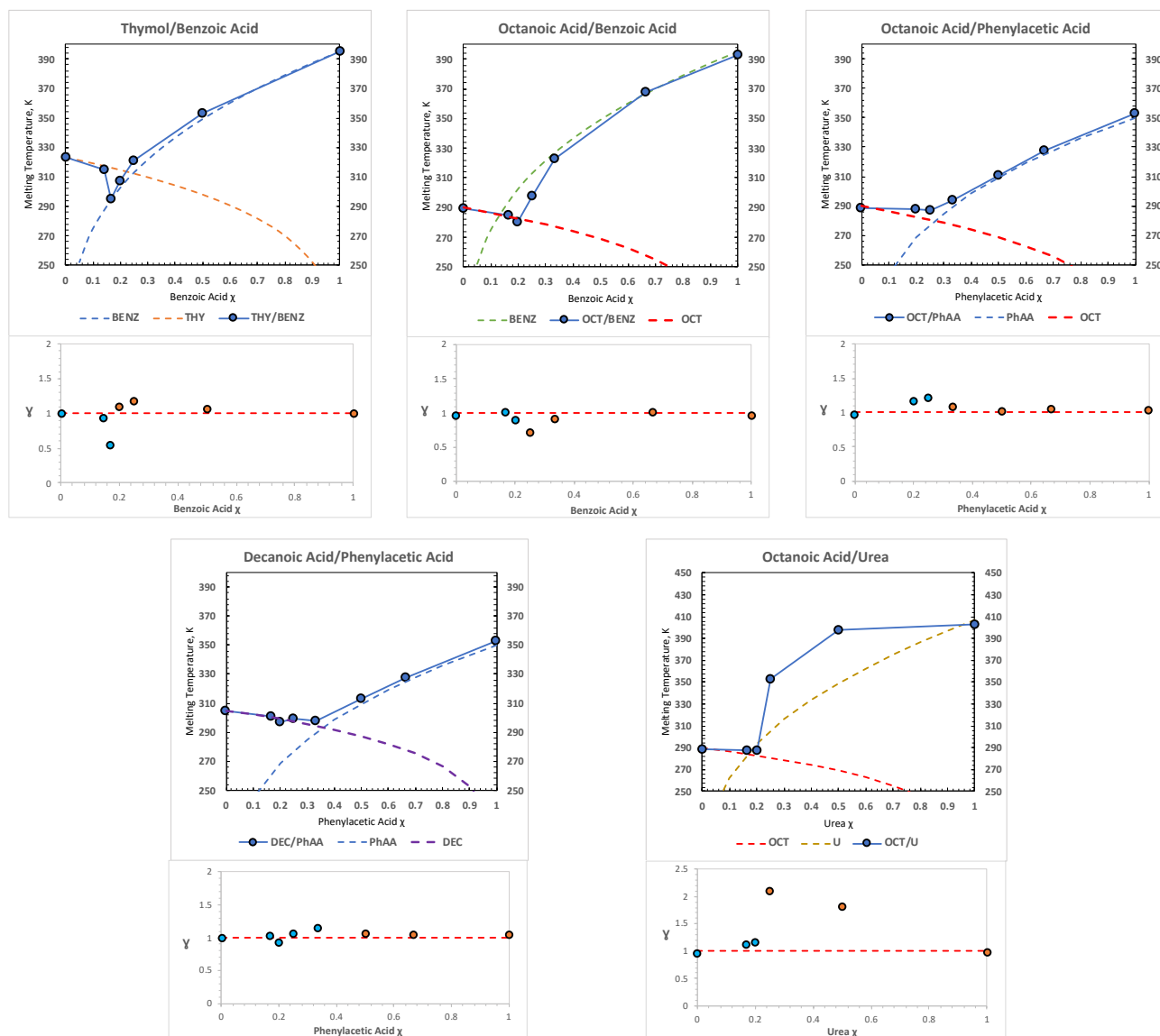


Figure S2: Solid-Liquid Phase diagrams of Thymol/Benzoic Acid, Octanoic Acid/benzoic Acid, Octanoic Acid/Phenylacetic Acid, Decanoic/Phenylacetic Acid, Octanoic Acid/Urea mixtures.

S1.3 UV-VIS SPECTRA OF WATER PHASES BEFORE AND AFTER EXTRACTION PROCEDURES

- Thymol/Phenylacetic acid DES (THY/PhAA)

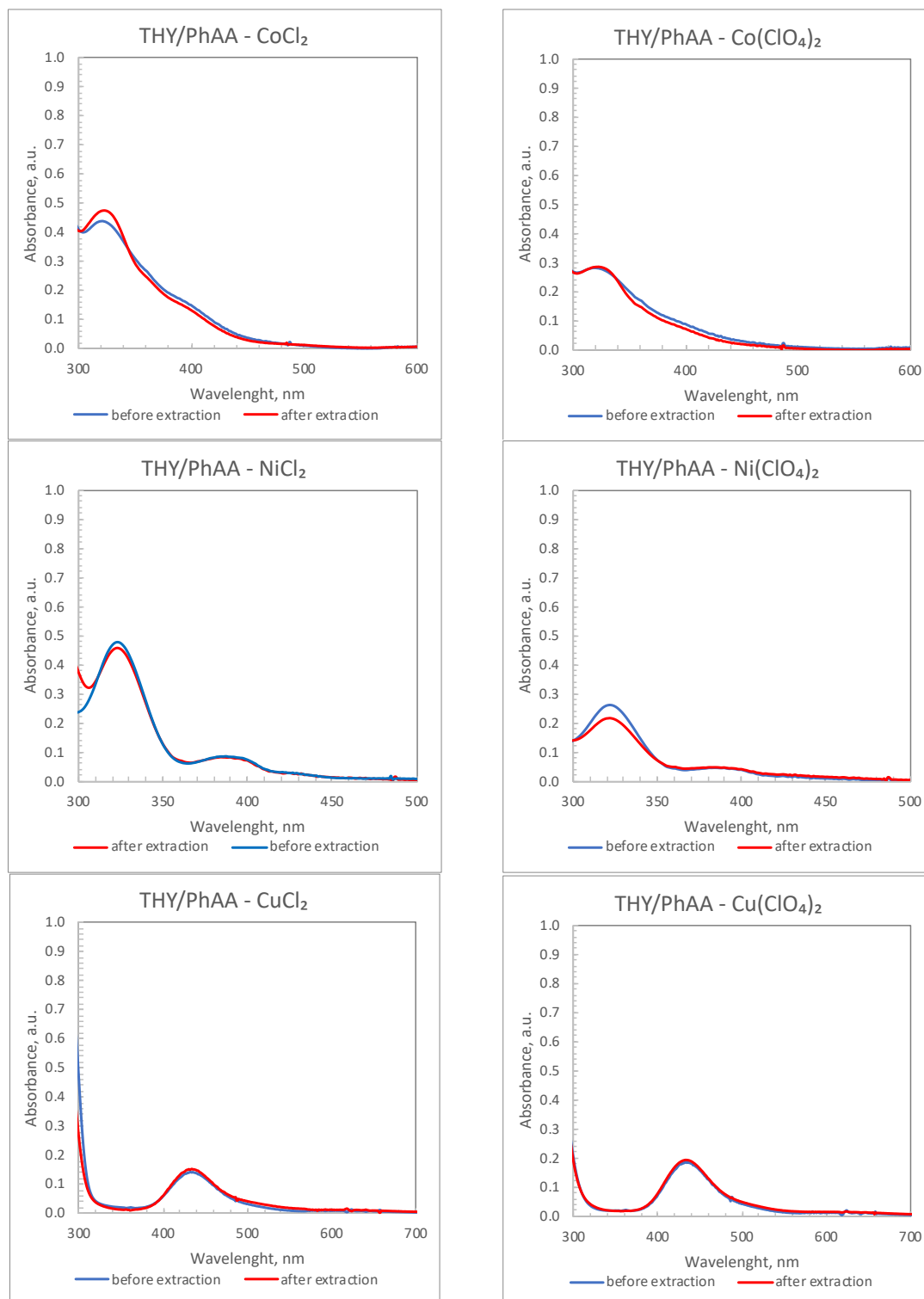


Figure S3: UV-VIS spectra of water phases before and after extraction procedures Thymol/Phenylacetic acid DES (THY/PhAA) – heavy metal salts.

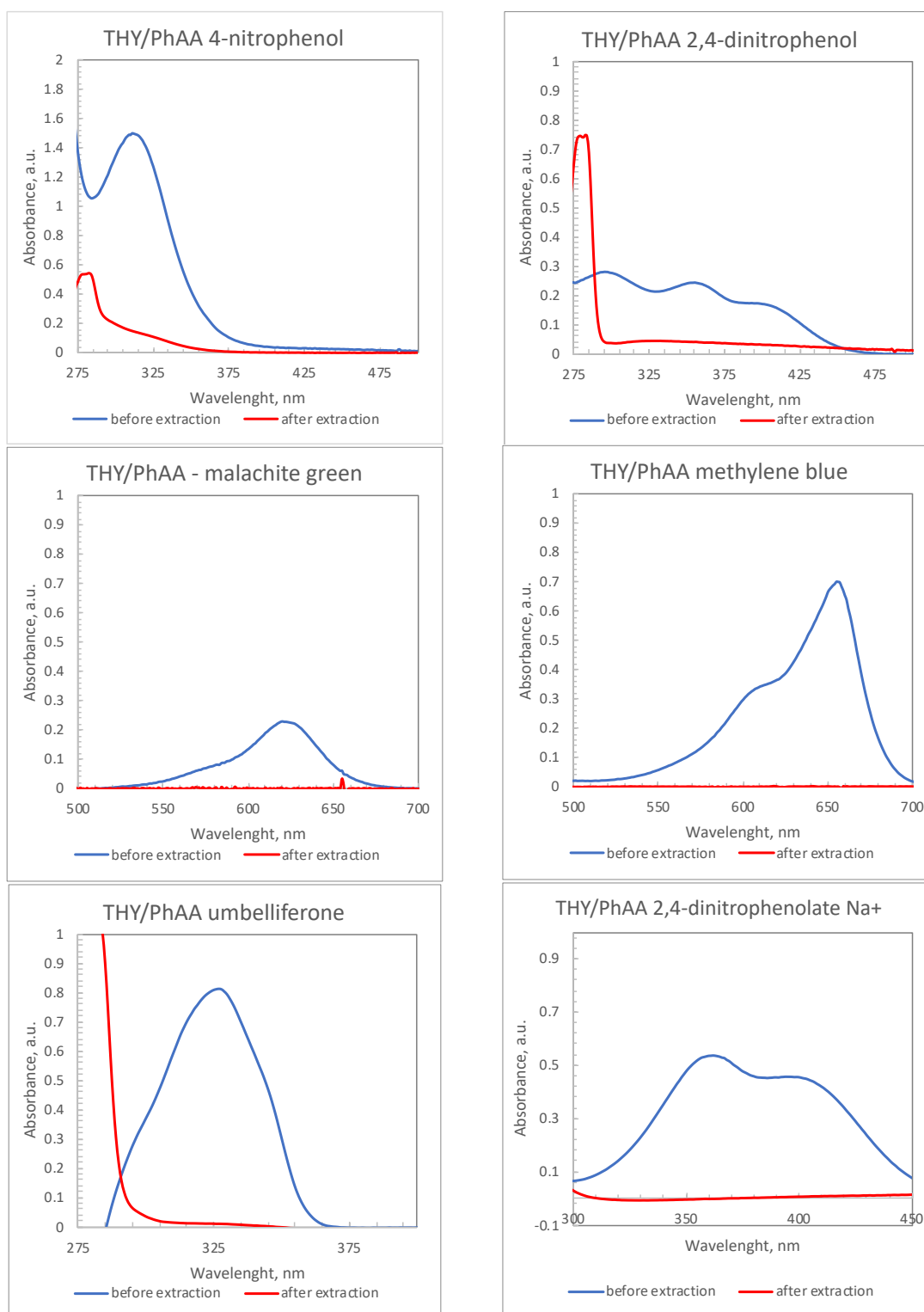


Figure S4: UV-VIS spectra of water phases before and after extraction procedures Thymol/Phenylacetic acid DES (THY/PhAA) – phenols and dyes.

- Thymol/Urea DES (THY/U)

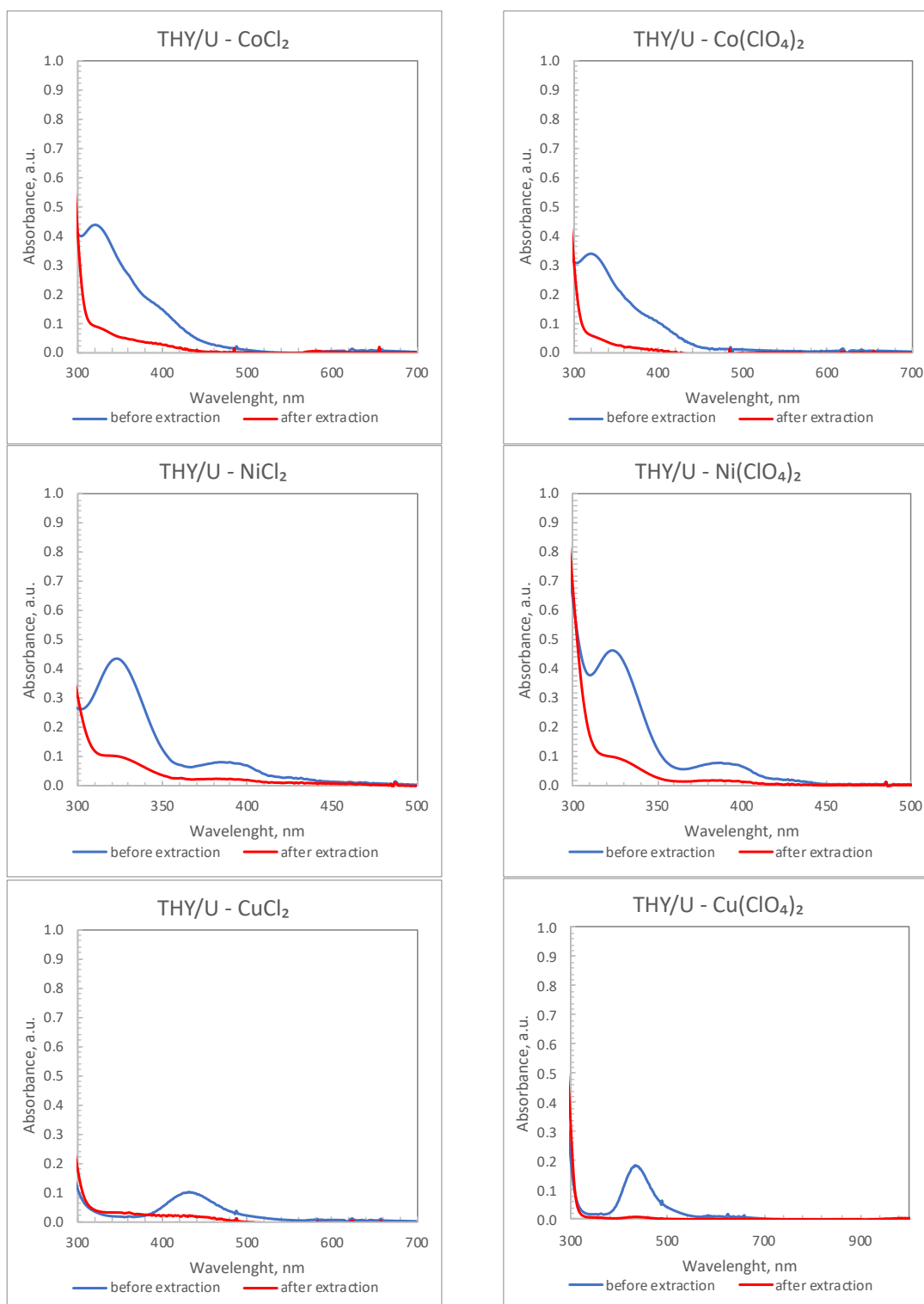


Figure S5: UV-VIS spectra of water phases before and after extraction procedures Thymol/U DES (THY/U) – heavy metal salts.

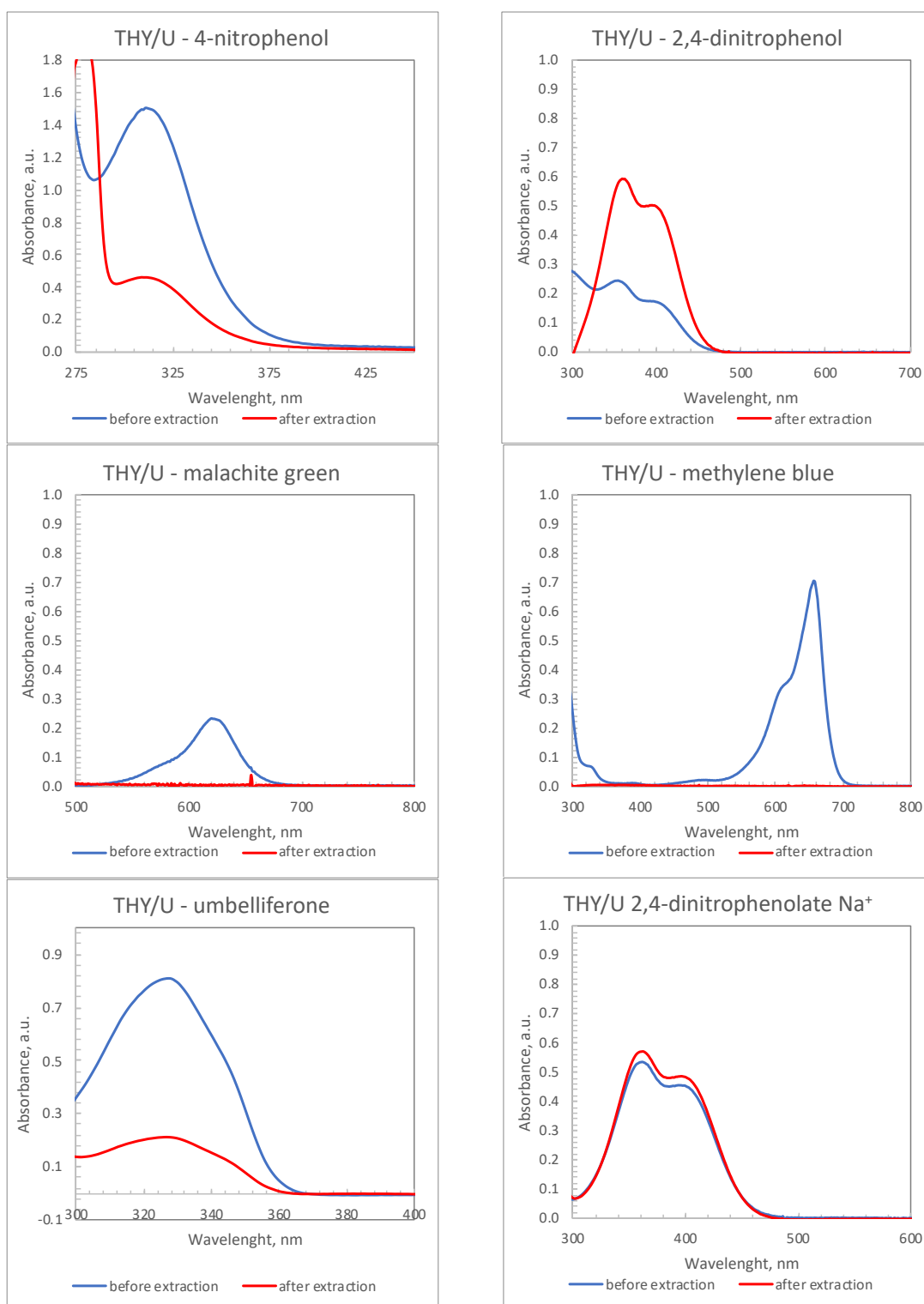


Figure S6: UV-VIS spectra of water phases before and after extraction procedures Thymol/U DES (THY/U) – phenols and dyes.

- Thymol/Water DES (THY/H₂O)

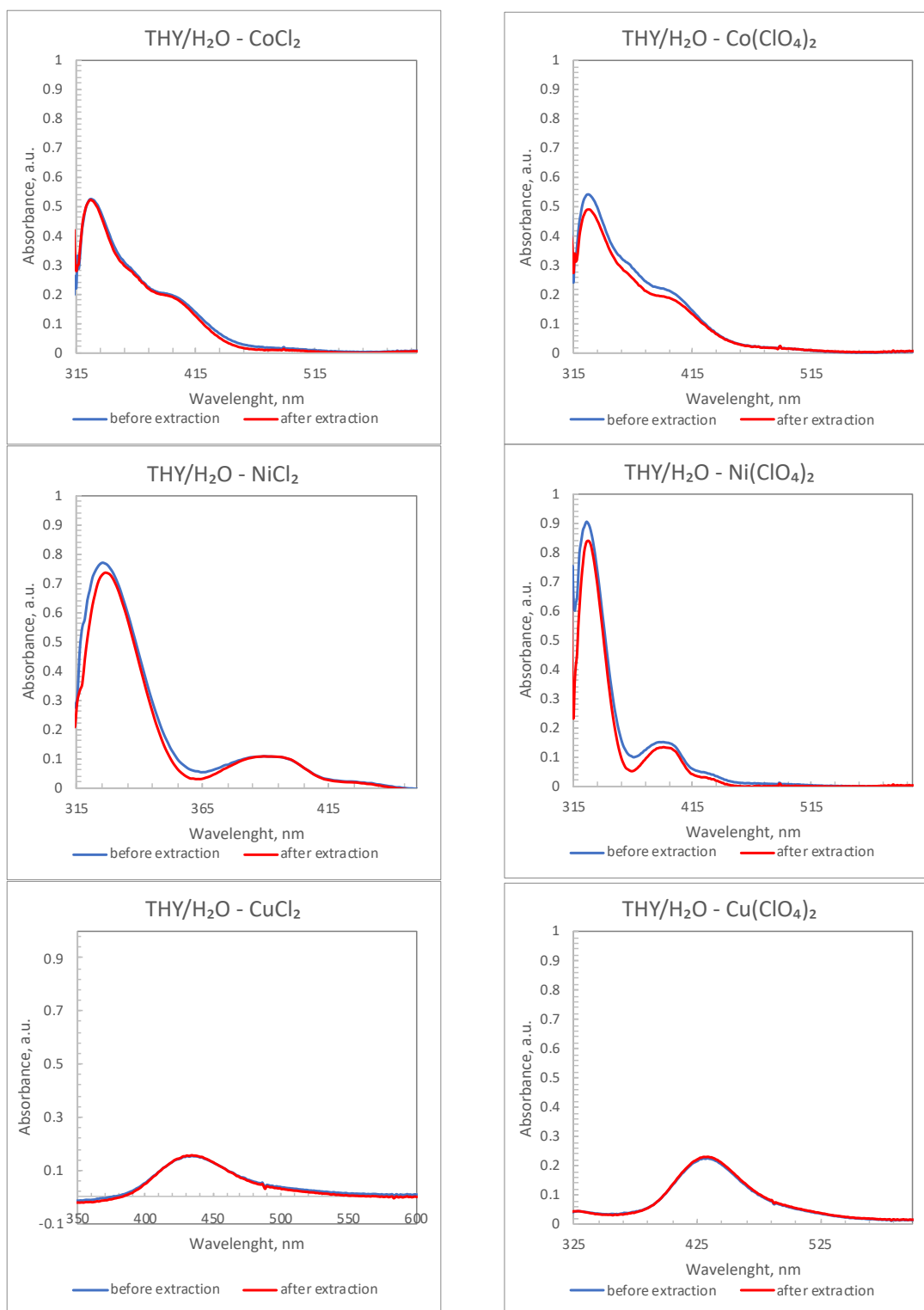


Figure S7: UV-VIS spectra of water phases before and after extraction procedures Thymol/H₂O DES (THY/U) – heavy metal salts.

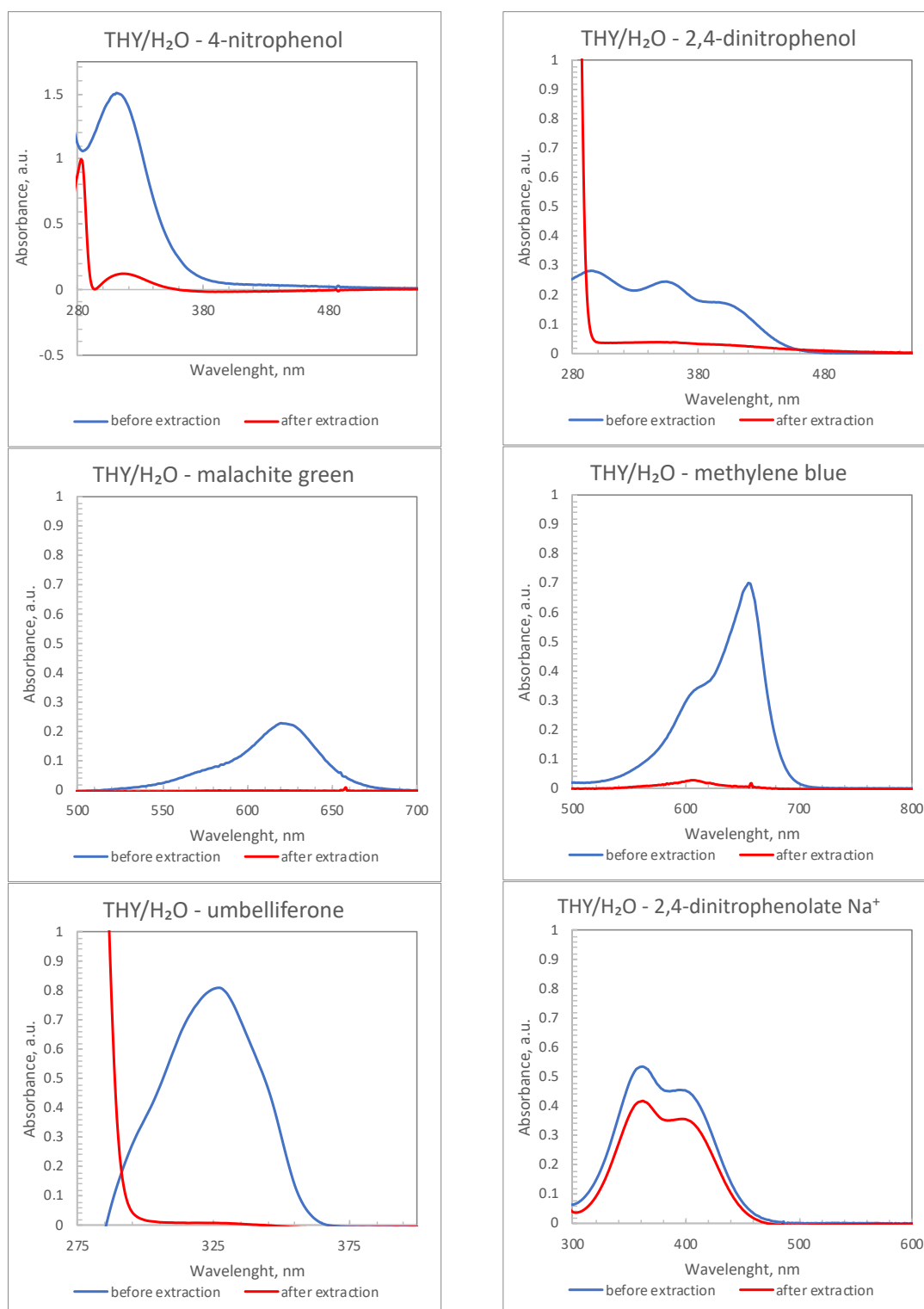


Figure S8: UV-VIS spectra of water phases before and after extraction procedures Thymol/H₂O DES (THY/U) – phenols and dyes.

- Thymol/Water DES (THY/H₂O) prepared *in situ*

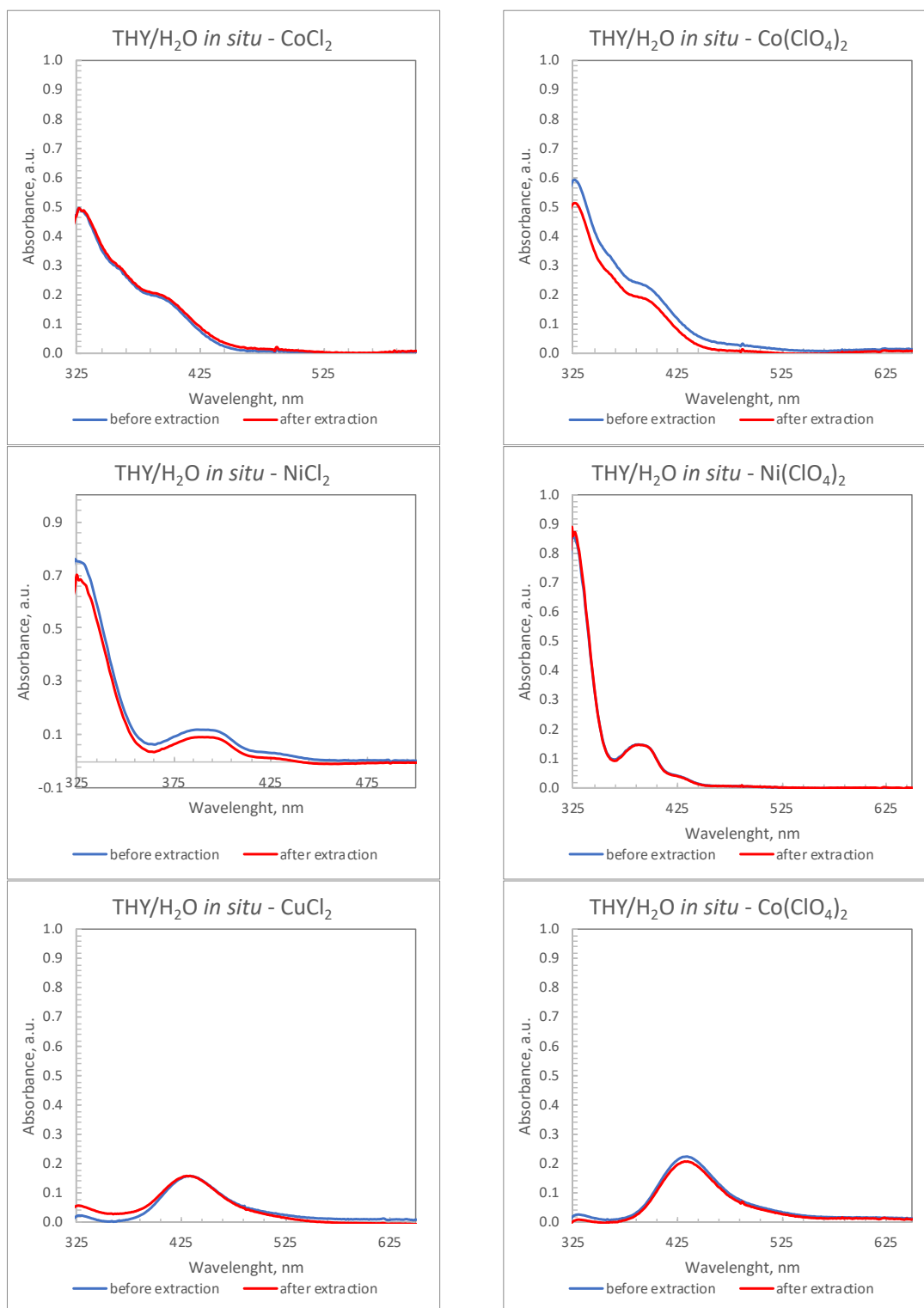


Figure S9: UV-VIS spectra of water phases before and after extraction procedures Thymol/H₂O DES (THY/U) – heavy metal salts *in situ*.

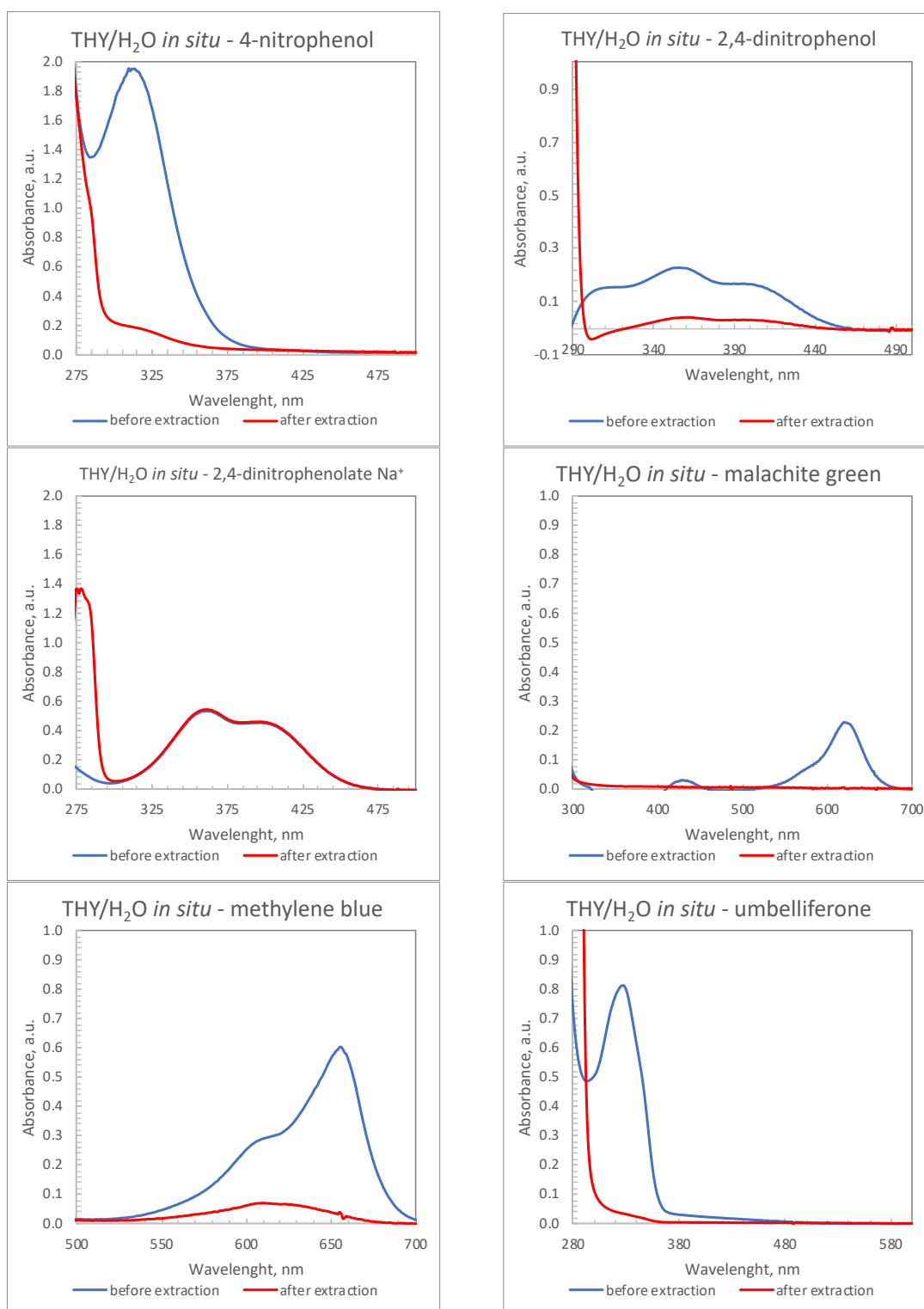


Figure S10: UV-VIS spectra of water phases before and after extraction procedures Thymol/H₂O DES (THY/U) – phenols and dyes *in situ*.

S2. COMPUTATIONAL STUDIES

S2.1 Density Functional Theory Calculations

Density functional theory (DFT) calculations of molecular and electronic structure calculations were performed using the Gaussian 09 [Frisch, M.J. et al. Gaussian 09, Revision D. 01; Gaussian, Inc.: Wallingford, CT, 2016.] software package. For all atoms, we employed the 6-311++G** basis set and used the B3LYP exchange-correlation functional [cite: 10.1063/1.464913; 10.1103/PhysRevB.37.785; 10.1021/j100096a001]. Solvent effects were employed with the CPCM polarizable conduction calculation method [cite: 10.1021/jp9716997; 10.1002/jcc.10189], using acetone as solvent for all considered material systems.

S2.2 Car–Parrinello Molecular Dynamics Simulations

Car–Parrinello molecular dynamics [10.1103/PhysRevLett.55.2471] (CPMD) simulations have been carried out with the Quantum Espresso package [10.1103/PhysRevLett.55.2471] along with the GGA-PBE [10.1103/PhysRevLett.77.3865] functional. For all calculations, electron–ion interactions were described by scalar relativistic ultrasoft pseudopotentials with electrons from O, N and C 2s, 2p; H 1s explicitly included in the calculations. Plane-wave basis set cutoffs for the smooth part of the wave functions and the augmented density were 25 and 200 Ry, respectively. CPMD simulations have been performed with an integration time step of 10 au, for a total simulation time of ca. 45 ps. We run 20 ps to equilibrate the system and then sample additional 25 ps to gain a sufficient statistical description of the H-bond properties, see Figure S12. The fictitious mass used for the electronic degrees of freedom is 1000 au, and we set the atomic masses to an identical value of 6 amu to enhance the dynamical sampling. Initial randomization of the atomic positions has been used to reach a temperature of 380 ± 30 K.

S2.3 Additional Data

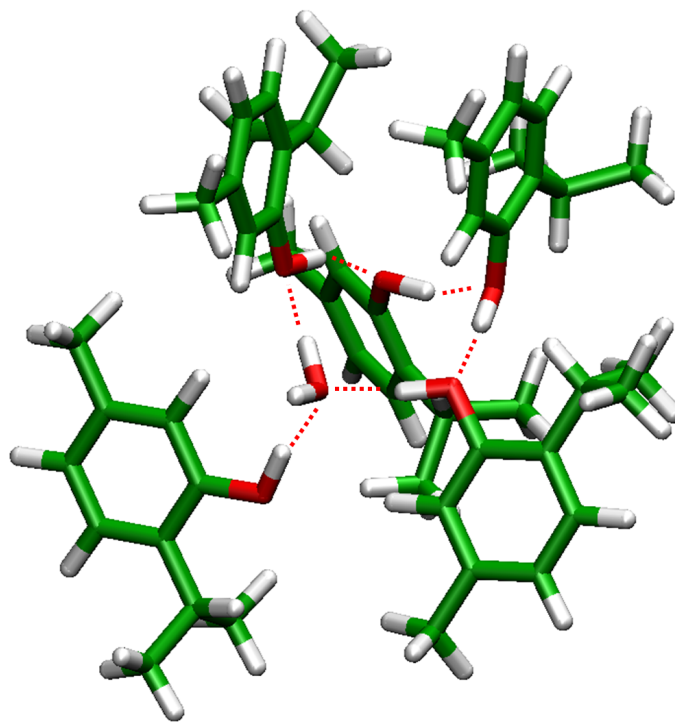


Figure S11: DFT-optimized cluster made of 5 thymol molecules and 1 water molecule. Hydrogen bonds are highlighted by dotted lines.

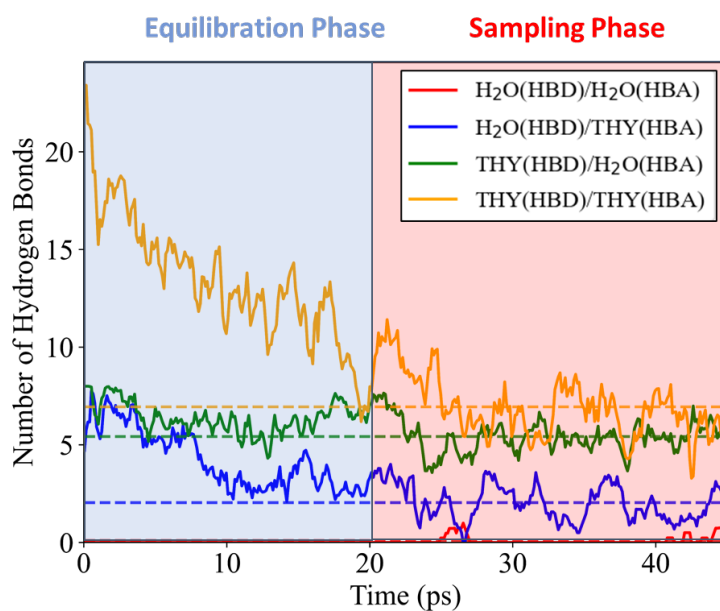


Figure S12: Time evolution of the number of hydrogen bonds between the different species, H₂O and THY. The blue colored region highlights the equilibration phase, the red one the sampling phase which is used for the statistical analysis in the main text.

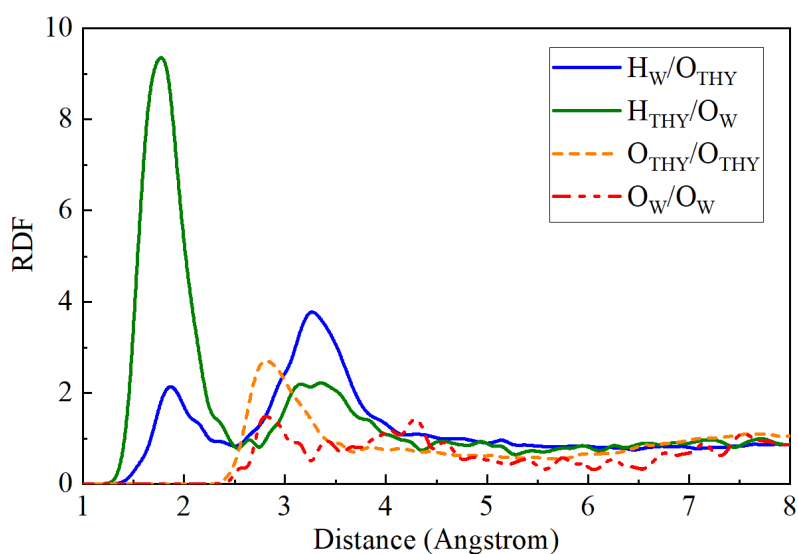


Figure S13: Long-range radial distribution function, RDF, between the hydrogen and oxygen water atoms (index W) and the ones of thymol (index THY). The peaks at short distances of the H/O RDFs represent the hydrogen bonds. The lack of further peaks beyond 4 Angstrom demonstrates the liquid nature of the system as there is lack of long-range correlation.

Table S3: Protonation energy E_p of the 2,4-dinitrophenolate to 2,4-dinitrophenol by the different molecules of the thymol-based DES THY/PhAA, THY/U, and THY/H₂O. E_p is calculated by $E_p = E(2,4\text{-dinitrophenol}) + E(A^-) - E(2,4\text{-dinitrophenolate}) - E(A \cdot H)$.

Molecule	E_p (kcal/mol)
Water	41.98
Urea	35.97
Thymol	25.83
Phenylacetic acid	13.89

Table S4: Hydrogen bond formation energy E_{rel} of the thymol-based DES molecules, given as molecule A, as hydrogen bond donor and the 2,4-dinitrophenol as hydrogen bond acceptor.

Molecule	E_{rel} (kcal/mol)
Water	-2.80
Urea	-4.04
Thymol	-3.81
Phenylacetic acid	-8.55