Solubility of Bioactive Compounds from Mediterranean Plants in Natural Deep Eutectic Solvents Estimated by COSMO-RS Software

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Abstract: Natural deep eutectic solvents (NADES) represent a promising group of green solvents employed for the extraction of bioactive compounds from medicinal plants. Due to the wide range of polarities of investigated solutes, and an outstanding number of combinations of NADES components, fine tuning and selection by software for modeling and evaluation of thermodynamic properties of solvents and solutes is a necessary step preceding the extraction from real samples. In this study, four hydrophilic NADES were chosen for the prediction of solubility of 19 bioactive compounds from Mediterranean plants, which was performed by COSMO-RS software. NADES structure optimization was performed by pseudocomponent approach and individual constituent approach. Change of hydrogen bond donor components of NADES did not show a significant influence on the solubility of screened solutes. According to both approaches, the investigated systems represent promising solvents for extraction of hydrophilic and moderately lipophilic flavonoids, terpenoids, and phenylpropanoids, while being unsuitable for extraction of the most lipophilic ($\log k_{ow} \geq 4$) natural products and compounds lacking polar functional groups due to the absence of sites participating in hydrogen bond formation.

Keywords: solubility prediction, NADES, mediterranean herbs, COSMO-RS, choline chloride, eutectic, extraction.

INTRODUCTION

NOWADAYS, a significant accent is being placed on environmentally friendly industrial processes characterized by lower energy consumption, decreased carbon footprint, and application of biodegradable materials.[1] One approach to the „greener" industry is exemplified by the development of substitutes to conventional organic solvents. Deep eutectic solvents (DES) are constituted by combining two or more solid components, forming a eutectic with a substantially decreased melting point in comparison to the individual components.[2] Several classes of DES are known, ranging from salt mixtures to nonionic DES. Recently, NADES (natural deep eutectic solvents) emerged as a special case of DES entirely composed of at least two naturally occurring primary plant metabolites.[3] Some authors consider NADES as a media alternative to water in living cells, involved in the biosynthesis of water insoluble biomolecules, heavy metal sequestration and dissolution of secondary metabolites.[4] Most of the described NADES fall within the Type III of DES, containing a hydrogen bond acceptor (HBA), usually an organic salt containing a bulky cation and a Lewis base, usually a halide anion, and also a hydrogen bond donor (HBD), a neutral organic species. The most common natural HBA used for NADES preparation is choline chloride (ChCl) or betaine, while urea, various polyols, carboxylic acids, and sugars are employed as HBD.[5] In comparison to traditional solvents, NADES have a pronounced chemical and thermal stability, low vapor pressure, low thermal conductivity, lower corrosiveness, and non-flammability.[6] NADES are prepared easily from readily available and cheap starting materials, they are biodegradable, and less hazardous to the environment in comparison to ionic liquids.[7] One of the important aspects of

NADES is a decrease in volumes required for extraction and lower waste generation.[8]

Another useful property of DES and NADES which makes them a subject of recent search for green solvents is possibility of tuning of the physicochemical properties such as polarity and viscosity, which might significantly affect the extraction efficiency.[9] The high viscosity of NADES may greatly diminish the transport phenomena and extraction rate of solutes from solid samples into the liquid phase.[10] In the case of NADES, a criterion of naturally occurring components must be fulfilled in order to guarantee full biocompatibility and suitability for application in the food, cosmetic and pharmaceutical industries. The fine tuning of NADES properties is usually performed by variations in the ratio of individual components, type of HBD and HBA used, water addition, or variation of extraction temperature.[11] It is usually noted that the increase of temperature will result in decreased polarity due to intensive reorientation of the dipoles,[12] which could be useful for extraction of certain lipophilic compounds, but could also result in decreased extraction yield due to thermal decomposition of the solutes.[13] Change of the HBD structure, most notably the alkyl chain length, can influence the viscosity of NADES.[14] Water addition allows for a significant decrease in NADES viscosity, but above certain ratio, it causes a disruption in the initial NADES hydrogen bonds and induces structural changes which can result in decreased extraction capability and rate.[10]

As mentioned, the melting point of ionic NADES is depressed due to the charge delocalization and weaker interaction between cation and anion, resulting from the formation of hydrogen bonds and contribution from van der Waals and electrostatic interactions.[15] The molecular and supramolecular structures of DES and NADES were partially elucidated by experimental techniques. HOESY NMR was applied to a mixture of ChCl/propane-1,2-diol/water (1 : 1 : 1) to identify the protons of the HBD interacting with the methyl and methylene groups in choline chloride.[4] Hydrogen bonds were identified by FT-IR and ^{1}H NMR experiments in the same solvent used for the extraction of quercetin.[10] In a mixture of sucrose and malic acid (1 : 1), hydrogen bonds were investigated by NOESY NMR.[3] Neutron diffraction was used to clarify that, apart from urea acting as the HBD, the hydroxyl group moiety of choline in the system ChCl/urea can also act as a hydrogen bond donor.[16]

NADES show a high solubilizing capacity of various biomolecules and were recently used to extract natural products from various sources. As the most thoroughly evaluated class of NADES, deep eutectics based on choline chloride were used to extract flavonoids from the medicinal plant *Sophora japonica*.[6] NADES made from ChCl/ethane-1,2-diol (1 : 2) showed improved extraction efficiency of phenylpropanoids from nuts and olives when compared to the conventional solvents.[17] Other choline chloride-based NADES were successfully used for extraction of various flavonoids,[18] phenylpropanoids,[17] terpenoids,[19] and coumarin derivatives[20] from herbs, edible oils, and food production waste such as peels, lees, and pomace. In the case of lipophilic solutes such as cannabinoids and vanillin, nonionic NADES based on DL-menthol or ionic DES based on quaternary ammonium halides as HBA represent a better choice of extraction solvent.[21,22]

Due to a vast number of possible combinations between compounds forming NADES and different outcomes of extraction resulting from simple modifications of NADES, *in silico* methods are used to select the most suitable candidates for a specific application before any experimental procedure. COSMO-RS software was developed by Klamt and coworkers as a statistical thermodynamic method for the calculation of molecular descriptors and physicochemical properties based on a quantum chemical approach.[23] COSMO-RS prediction is performed by an initial step in which the observed compound is placed in a dielectric continuum to probe the charge density on the molecular surface and to perform energy minimization. In this way, the σ-profile and σ-potential curves are generated, giving the information on the polarity distribution of molecules. In the second step, the thermodynamic behavior of the compound is calculated and the energy of interacting surface segments is determined. COSMO-RS allows the chemical potential of a screened component to be predicted, as well as the molecular contributions for the affinity towards target compounds, as the generated σ-potential profile is indicative of interactions between solvent and solute.[24] From the chemical potential determined by COSMO-RS, individual properties and thermodynamic behavior of NADES can be predicted. In the domain of natural product solubility prediction, COSMO-RS was recently applied to elucidate the partitioning of vanillin and other natural products in choline chloride and betaine NADES.[25] The solubility and activity of rutin at infinite dilution in NADES were correlated by COSMO-RS.[26] The solubility of sulfonamide antibiotics in the ChCl/polyol mixtures was predicted, with identification of the ChCl/glycerol (1 : 1) mixture as the most suitable solvent, with the solubility almost 43 times higher compared to water.[27] COSMO-RS was used to screen a series of terpenoids and NADES to recover volatile fatty acids from aqueous solutions with lipophilic NADES composed of a medium-chain fatty acid and thymol as the best candidates.[28] The efficiency of the anthocyanins and pectins extraction from *Myrciaria cauliflora* was predicted and the molecular interactions between the target molecules and NADES were studied by COSMO-RS. This study recognized the ChCl/propane-1,2-diol as the best candidate for anthocyanin extraction.[29]

Hydrophobic NADES were tested for removal of chlorophenols from wastewater, with good accordance between experimental data and predictions by COSMO-RS.[30] This Preliminary Communication proposes a "green" and sustainable approach useful for extraction of bioactive compounds from natural sources. The aim was to assess the applicability of natural deep eutectic solvents for extraction of bioactive compounds from Mediterranean herbs. These data represent a root for further prospective studies and possible implementation of these findings in the food and cosmetics industry procedures which are more affordable, environmentally friendly and require reduced energy consumption.

CALCULATION

Target Compounds and Screened NADES

The following solutes were screened: terpenes, terpenoids and related compounds (camphor, carvacrol, carnosol, myrcene, oleanolic acid, oleuropein, thujone, thymol); phenylpropanoids and related compounds (caffeic acid, coumarin, chlorogenic acid, ferulic acid, rosmarinic acid); flavonoids and related compounds (apigenin, disometin, luteolin, quercetin); and others (*p*-cymene, hydroxytyrosol). These compounds often found in the Mediterranean plants extracts have pronounced biological activity and are a continuous subject of our broader research. The screened NADES solvents were: choline chloride/urea (1 : 2); choline chloride/glycerol (1 : 3); choline chloride/propane-1,2-diol (1 : 2) and choline chloride/malonic acid (1 : 1).

COSMOthermX Calculations

COSMOthermX program (version C30 release 13.01) was used to calculate the relative solubility of solutes in NADES in terms of the logarithm of the solubility in mole fractions ($\log_{10}(x_{solub})$). As in the previous reports by our group, the logarithm of the best solubility was set to 0 while the other solvents were shown relative to the best solvent. Additionally, the logarithm was expressed into a probability of solubility (%). The calculation was performed at 25 °C and 30 °C for each solvent. As the structures of the solutes were absent from the COSMOtherm database, their molecular geometries were optimized to the lowest energy minimum using TURBOMOLE GUI (TmoleX). For every tested solute, a single conformer generated by Turbomole was used for solubility screening. Solvents were input into prediction calculations as single conformers. NADES were modeled both by pseudocomponent approach and individual constituent approach. Both approaches have been explored as the current reports indicate a complex, incompletely elucidated nature of NADES structure and their interaction with solutes, which might in turn influence the predicted solubility

values. Initial structures for each compound were input as SMILES and the geometry optimization was performed at Hartree-Fock level and 6-31G* basis set which accounts for polarization effects of the particular complex species. For the pseudocomponent approach, the most probable structures of the NADES were chosen and the length of the intermolecular bonds between HBD and HBA was adjusted to 2 Å. A single point computation was performed for the optimized geometry of each species using density functional theory with Becke-Perdew functional and triple-ζ valence potential basis set. The thermodynamic properties of compounds were calculated by COSMOthermX program by importing generated COSMO files containing optimized conformation using the BP_TZVP_C30_1201 parametrization file implemented in COSMOthermX package. The predicted solubility values for all the solutes are reported in the Tables 1–4.

RESULTS AND DISCUSSION

The aim of this preliminary study was to explore the potential of NADES as environmentally friendly and affordable solvents for concurrent extraction of polar and nonpolar bioactive compounds (Figure S1) from various Mediterranean herbs by *in silico* approach. Up to date, there are only a few reports on the computational modeling of NADES for purpose of extraction of bioactive compounds from natural sources. Solubility prediction was performed by COSMO-RS software under different initial conditions for a group of bioactive components from Mediterranean plants in four selected NADES. Structural optimization of the selected NADES was performed by individual constituent approach and pseudocomponent approach. The referent compound for extractions of bioactive compounds, against which the series of NADES were compared, was *n*-hexane, a commonly used solvent with low polarity, optimal boiling point, and easy procedure of extract recovery.

According to the recent literature, NADES based on choline chloride are suitable for the extraction of moderately hydrophilic natural products. The main rationale for the choice of ChCl and urea-based NADES in this work is the fact that the intermolecular structure of NADES made from these components is the most studied and clarified among all the NADES. In addition, NADES containing two polyols (glycerol and propane-1,2-diol) and one organic acid (malonic acid) were also tested as readily available and described in the literature. The ratio of solvents in the eutectic was chosen on the basis of known NADES with these solvents reported in the previous papers, and due to the stoichiometric ratio of the components which form the eutectic. Water addition was not performed, as this study is focused on pure eutectics solely, and there was a chance that the water addition would inhibit the extraction of the most

Table 1. Relative solubility of screened compounds at 25 °C against four NADES predicted by COSMO-RS with individual constituent approach. Green: high solubility; yellow: sparingly soluble; red: poor solubility. (ChCl – choline chloride).

Solvents / Solutes	ChCl / urea (1 : 2)	ChCl / glycerol (1 : 3)	ChCl / propane-1,2-diol (1 : 2)	ChCl / malonic acid (1 : 1)	*n*-Hexane
Flavonoids					
Apigenin	100.00	100.00	100.00	100.00	0.00
Luteolin	100.00	100.00	100.00	100.00	0.00
Quercetin	100.00	100.00	100.00	100.00	0.00
Diosmetin	100.00	100.00	100.00	100.00	0.00
Terpens, terpenoids, derivatives					
Myrcene	1.15	0.52	2.13	1.76	77.62
Carvacrol	100.00	100.00	100.00	100.00	12.59
Thymol	65.09	13.78	89.40	76.37	19.22
Thujone	3.51	1.86	5.06	3.63	48.98
Camphor	4.73	2.83	6.61	4.61	50.36
Oleuropein	100.00	100.00	100.00	100.00	0.00
Carnosol	94.39	7.50	94.78	62.35	0.39
Oleanolic acid	27.70	1.26	57.56	24.51	1.16
Phenylpropanoids					
Rosmarinic acid	100.00	100.00	100.00	100.00	0.00
Coumarin	24.52	5.97	14.37	18.06	3.30
Ferulic acid	100.00	100.00	100.00	100.00	0.00
Caffeic acid	100.00	100.00	100.00	100.00	0.00
Chlorogenic acid	100.00	100.00	100.00	100.00	0.00
Other					
p-Cymene	1.60	0.65	2.61	2.37	69.79
Hydroxy-tyrosol	100.00	100.00	100.00	100.00	0.00

Table 2. Relative solubility of screened compounds at 30 °C against four NADES predicted by COSMO-RS with individual constituent approach.

Solvents / Solutes	ChCl / urea (1 : 2)	ChCl / glycerol (1 : 3)	ChCl / propane-1,2-diol (1 : 2)	ChCl / malonic acid (1 : 1)	*n*-Hexane
Flavonoids					
Apigenin	100.00	100.00	100.00	100.00	0.00
Luteolin	100.00	100.00	100.00	100.00	0.00
Quercetin	100.00	100.00	100.00	100.00	0.00
Diosmetin	100.00	100.00	100.00	100.00	0.00
Terpens, terpenoids, derivatives					
Myrcene	1.23	0.51	2.17	1.87	78.17
Carvacrol	100.00	100.00	100.00	100.00	13.80
Thymol	77.31	14.73	89.40	90.53	20.66
Thujone	3.79	1.84	5.17	3.84	49.92
Camphor	5.10	2.80	6.76	4.86	51.24
Oleuropein	100.00	100.00	100.00	100.00	0.00
Carnosol	100.00	7.79	100.00	74.75	0.49
Oleanolic acid	36.49	1.32	68.97	31.55	1.49
Phenylpropanoids					
Rosmarinic acid	100.00	100.00	100.00	100.00	0.00
Coumarin	25.65	5.76	14.23	18.66	3.30
Ferulic acid	100.00	100.00	100.00	100.00	0.00
Caffeic acid	100.00	100.00	100.00	100.00	0.00
Chlorogenic acid	100.00	100.00	100.00	100.00	0.00
Other					
p-Cymene	1.71	0.64	2.65	2.51	70.43
Hydroxy-tyrosol	100.00	100.00	100.00	100.00	0.01

lipophilic compounds. The results of the solubility predictions for ChCl/glycerol (1 : 3) calculated by pseudocomponent approach were discarded due to the poor quality and performance of the model (Table 3 and 4).

COSMO-RS calculations were performed by two different approaches. The first, individual constituent approach, is performed by modeling the NADES as a real mixture of defined molar ratios of individual components, which are input separately in the COSMO-RS software, i.e., if a mixture of choline chloride and urea in a ratio 1 : 2 behaves as a deep eutectic at the simulation temperature, then the system is regarded as a mixture of choline cation, chloride anion and two molecules of urea as separate compounds (Figure S2). The second, pseudocomponent approach,

observes NADES as a single molecular entity (Figure S3) with HBD and HBA species joined by the hydrogen bond. It comprises an additional step, computed by TURBOMOLE 17 software and performed before the solubility screening. In this step, energy minimization and a selection of the most stable conformation of NADES candidate is performed. The individual constituent approach was applied as it presents a more physical representation of NADES with their reduced eutectic point, while the pseudocomponent approach was tested as it allows easier handling and parametrization due to a reduced number of species.

This preliminary study was focused on the choice of the NADES systems composed exclusively of choline chloride as a HBA, while the HBD and thus the final composition

of the NADES was chosen by applying additional three criteria: *i*) the NADES system is known and described in the literature *ii*) the eutectic point of the NADES is below or near 25 °C and *iii*) the pH of the HBD and the resulting NADES should not cause the degradation of screened solutes. The initial structures of the choline chloride-HBD clusters are created according to the models reported elsewhere.[31–33] Frequently modeled ChCl/polyol systems were selected due to their lower reported viscosity and lower polarity, similar to methanol,[4] which could make them suitable for the extraction of less polar compounds. Due to the increase in number of hydroxyl groups present, such NADES systems represent potentially good solvents for

polyphenols and other solutes abundant in hydroxyl groups.[34] Although anthocyanins, which show optimal extraction performance with ChCl/acid NADES due to increased solubility in acidic media,[35] were not the target solutes in this study, one example of acid-based NADES (ChCl/malonic acid) was evaluated to assess the influence on the extraction of solutes with ionizable groups.

The main purpose of the NADES screened is to extract nonpolar and polar bioactive compounds from various Mediterranean herbs. Using the individual constituent approach, all four screened solvents exhibited excellent solvating properties (Table 1–4) for four target flavonoids, a majority of phenylpropanoids, and the terpene and

Table 3. Relative solubility of screened compounds at 25 °C against four NADES predicted by COSMO-RS with pseudo-component approach. *Choline chloride/glycerol (1 : 3) model rejected due to poor performance.

Solvents Solutes	ChCl / urea (1 : 2)	ChCl / glycerol (1 : 3)*	ChCl / propane- 1,2-diol (1 : 2)	ChCl / malonic acid (1 : 1)	*n*- Hexane
Flavonoids					
Apigenin	100.00	0.03	100.00	100.00	0.00
Luteolin	100.00	0.22	100.00	100.00	0.00
Quercetin	100.00	0.03	100.00	100.00	0.00
Diosmetin	100.00	0.01	100.00	100.00	0.00
Terpens, terpenoids, derivatives					
Myrcene	0.11	0.01	1.27	1.05	77.62
Carvacrol	100.00	0.05	100.00	100.00	12.59
Thymol	67.48	0.04	36.24	63.82	19.22
Thujone	0.42	0.27	3.74	2.12	48.98
Camphor	0.65	0.87	5.22	2.73	50.36
Oleuropein	100.00	0.00	100.00	100.00	0.00
Carnosol	2.30	0.02	16.48	29.50	0.39
Oleanolic acid	0.05	0.00	2.43	4.45	1.16
Phenylpropanoids					
Rosmarinic acid	100.00	0.00	100.00	100.00	0.00
Coumarin	7.26	4.38	12.89	16.68	3.30
Ferulic acid	100.00	0.56	100.00	100.00	0.00
Caffeic acid	100.00	0.38	100.00	100.00	0.00
Chlorogenic acid	100.00	0.01	100.00	100.00	0.00
Other					
p-Cymene	0.17	0.01	1.62	1.52	69.79
Hydroxy-tyrosol	100.00	0.96	100.00	100.00	0.00

Table 4. Relative solubility of screened compounds at 30 °C against four NADES predicted by COSMO-RS with pseudo-component approach. *Choline chloride/glycerol (1 : 3) model rejected due to poor performance.

Solvents Solutes	ChCl / urea (1 : 2)	ChCl / glycerol (1 : 3)*	ChCl / propane- 1,2-diol (1 : 2)	ChCl / malonic acid (1 : 1)	*n*- Hexane
Flavonoids					
Apigenin	100.00	0.03	100.00	100.00	0.00
Luteolin	100.00	0.27	100.00	100.00	0.00
Quercetin	100.00	0.03	100.00	100.00	0.00
Diosmetin	100.00	0.01	100.00	100.00	0.00
Terpens, terpenoids, derivatives					
Myrcene	0.11	0.01	1.23	1.07	78.17
Carvacrol	100.00	0.05	100.00	100.00	13.80
Thymol	68.23	0.04	39.09	74.12	20.66
Thujone	0.42	0.33	3.64	2.15	49.92
Camphor	0.64	1.12	5.11	2.77	51.24
Oleuropein	100.00	0.00	100.00	100.00	0.00
Carnosol	2.51	0.03	17.32	34.33	0.49
Oleanolic acid	0.06	0.00	2.58	5.41	1.49
Phenylpropanoids					
Rosmarinic acid	100.00	0.00	100.00	100.00	0.00
Coumarin	7.19	5.54	12.46	16.88	3.62
Ferulic acid	100.00	0.65	100.00	100.00	0.00
Caffeic acid	100.00	0.40	100.00	100.00	0.00
Chlorogenic acid	100.00	0.01	100.00	100.00	0.00
Other					
p-Cymene	0.17	0.01	1.57	1.55	70.43
Hydroxy-tyrosol	100.00	1.06	100.00	100.00	0.01

terpenoid compounds with higher polarity ($\log k_{ow} \leq 4$). Choline chloride-based NADES belong to the group of fairly hydrophilic solvents and are less suitable for extraction of the most hydrophobic natural products. NADES are ideal for the extraction of moderately hydrophilic solutes, while tailored hydrophobic DES might show better extraction capability of the most lipophilic solutes studied here.

In all the modeled NADES, a change of HBD did not show significant influence on the predicted solubility, except in the case of two solutes, thymol and carnosol. Comparison of NADES modeled by pseudocomponent and individual constituent approach shows a similar trend in solubility of natural products within tested NADES classes, with a few differences noticed, the major being the poor performance of the ChCl/glycerol (1 : 3) NADES for solvation of all the solutes when the solvent was modeled by pseudocomponent approach. Solutes thymol, carnosol, oleanolic acid, and coumarin showed better extraction properties with NADES modeled by individual constituent approach. Solubility of thujone, camphor, and coumarin, all the compounds characterized by lower lipophilicity ($\log k_{ow}$ 2.40, 1.94 and 1.28, respectively, Figure 1), is predicted to be very low compared to the compounds with similar $\log k_{ow}$ values, and this could be explained by the abundance of nonpolar alkyl- or aryl- fragments in their structure, with only one carbonyl oxygen present in all three molecules, thus significantly decreasing the chance for the formation of hydrogen bonds with NADES. A similar situation is noticed with *p*-cymene and myrcene, even more lipophilic hydrocarbons ($\log k_{ow}$ = 4.09 and 4.42), which have no potential sites for interaction with the solvent components. Conversely, these compounds have a very good predicted solubility in *n*-hexane, which is a nonpolar aliphatic hydrocarbon. A phenolic diterpene carnosol, which has $\log k_{ow}$ values very similar to myrcene, shows good solubility in all the NADES modeled by individual constituent approach except the system ChCl/urea. This could be explained by the presence of two hydroxyl groups and the ester group in carnosol, which are absent in myrcene. Carnosol is practically insoluble in the NADES modeled by pseudocomponent approach, with the possible influence of increased molecule bulkiness. Oleanolic acid, with the highest $\log k_{ow}$ value of all the studied compounds (6.06) shows highly variable solubility which might be the consequence of bulky structure, composed of five fused rings, and the two groups responsible for hydrogen bond formation being very distant from each other, which in turn decreases the chance of interaction between a single NADES cluster with both groups of the solute molecule. Comparison between positional isomers carvacrol and thymol shows a decreased solubility of the latter, explained by the closeness of the hydroxyl group, the only hydrogen bond forming group in the molecule, to the relatively bulky isopropyl group. NADES composed of
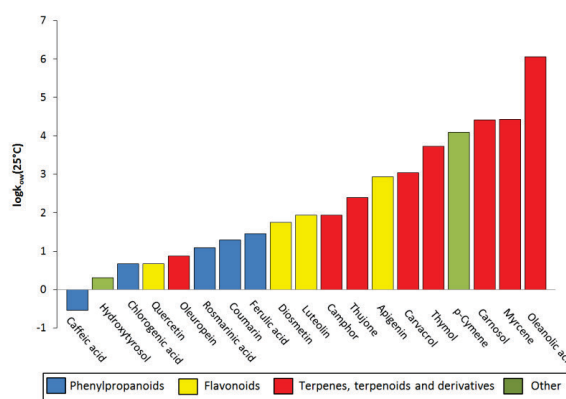

Figure 1. Lipophilicity of solutes expressed by $\log k_{ow}$ values as calculated by COSMO-RS.

ChCl/glycerol (1 : 3) was particularly unsuitable for extraction of this compound. This study is focused on the investigation of the low-energy input methods for extraction of bioactive compounds from Mediterranean herbs. Ideally, these extractions will be carried out at 25 °C in order to avoid heating linked to energy expense, and possibly to avoid degradation of some solutes. The simulations were additionally run at 30 °C solely to check the influence of a slightly higher temperature on the solubility outcome. A minute increase of simulation temperature did not exert a significant influence on the extraction probability, and only a minor increase in the solubility of a few selected compounds was observed. Only in the case of thymol and oleanolic acid did the temperature increase result in up to an 18 % raise in solubility. In other cases, the solubility increase was negligible.

The σ-profile and σ-potential curve of the screened NADES and solutes provides information about molecular polarity distribution by identifying the HBA, HBD and hydrophobic region, and allows interpretation of the possible interactions between a solvent and a solute. All the evaluated NADES show negative values in both donor (σ < −0.008 e Å⁻²) and acceptor (σ > 0.008 e Å⁻²) hydrogen bond regions, which would conversely make them suitable for extraction of amphoteric solutes. The positive regions of the σ-profile of NADES originate from the present oxygen atoms, responsible for the HBA behavior of NADES, while the negative regions correspond to the hydrogen atoms and donating ability of the particular NADES fragment. The NADES containing carboxyl group showed higher values in the HBD region than the polyol-based ones, as the carboxyl group enhances the polarization of the −OH group in the acid HBD, which enhances the dipole strength and leads to stronger hydrogen bond interaction. However, this behavior did not influence the predicted solubility of the majority of solutes screened. In the case of ChCl/polyol NADES, the presence of multiple hydroxylic groups allows for an increased number of possible hydrogen bonds generated

between solvent and solute. The flavonoid and phenylpropanoid solutes show significant groups available for interaction in the HBD and HBA regions, with a very good predicted solubility. By contrast, hydrocarbons *p*-cymene and myrcene showed nonpolar groups in the σ-profile, which would account for a very low predicted affinity of all the screened NADES for nonpolar solutes.

The interaction of individual contributors to NADES at the intermolecular level is not fully elucidated. As choline chloride NADES are the earliest and most studied class of these solvents, their modeling has been reported in several publications. Due to diverse possible conformations, a study by Jeliński required five choline chloride isomers to fully represent its characteristics relevant to the experimental properties of existing NADES, while glycerol, used as the HBD was represented by ten different conformers.[33] Choline chloride-based NADES studies by molecular dynamics indicated the presence of hydrogen bonds between chloride anion and HBD component, and interaction between the hydroxyl group of choline with an acidic HBD.[36] Even within a series of NADES employing the same HBA, the character of hydrogen bonds formed can be radically different if different HBD are used. Stefanovic *et al.* (2017) have shown that NADES ChCl/urea has a mesoscale hydrogen bonding dissimilar to the choline chloride NADES with glycerol or ethylene glycol as HBD.[37] Within a single NADES, different cluster conformations between HBA and HBD are possible. Simulation of hydrogen bond formation possibility between choline chloride and urea has resulted in a series of statistically possible HBD-HBA pairs, but also a high probability of urea-urea interactions, i.e. HBD-HBD pairs, which further complicates the structure of the NADES.[32] This finding makes the comparison of NADES for the selection of the most suitable extraction solvent tedious, and practical experimentation is necessary to confirm the predicted solubility.

Results of the COSMO-RS solubility assessment (Table 1–4) showed a similar trend for both pseudocomponent approach and individual constituent approach. It can be concluded that the tested NADES are suitable for extraction of bioactive compounds from Mediterranean herbs. According to the COSMO-RS prediction results, by evaluating compounds shaded with green (very good solubility), followed by yellow (medium solubility) and red (poor solubility), a general solvation behavior of the screened solvents can be established as follows: ChCl/urea (1 : 2) ≈ ChCl/propane-1,2-diol (1 : 2) ≈ ChCl/malonic acid (1 : 1) > ChCl/glycerol (1:3) > *n*-hexane. It should be noted, however, that the solvent ChCl/glycerol (1 : 3) modeled by pseudocomponent approach was excluded from this solvation behavior trend, as the model was rejected. Results show that choline chloride-based NADES possess a high dissolving capacity of flavonoids and phenylpropanoids

containing several hydroxylic and carboxylic groups, good potential for extraction of terpenes and terpenoids with smaller molecular mass containing at least few polar functional groups, while showing decreased or poor performance for the extraction of hydrocarbons, lacking any polar groups, and also the most lipophilic bulky solutes. A drawback of COSMO-RS application for such systems which could lead to erroneous conclusions is the unknown degree of dissociation of weak acid HBD, thus rough assumptions have to be made prior to validation with experimental solubility data.[26] Predictive model reliability might be further explored by task-specific fine-tuning of the COSMO-RS parameters or by applying electrolyte models to salt based DES such as COSMO-RS-ES[38] or machine learning algorithms using the molecular descriptors calculated by COSMO-RS to build nonlinear predictive models.

CONCLUSIONS

The solubility of bioactive compounds were predicted by COSMO-RS and a solvation behavior in four selected NADES was established. The individual constituent approach for NADES modeling for solubility prediction showed more promising results, which should be confirmed by practical extraction experiments, while the pseudocomponent approach was unstable in the case of one modeled solvent system and will not be followed. Choline chloride-based NADES were previously reported as good flavonoids and phenolics solubilizers, and should be tested for the extraction of these secondary plant products. These alternative green solvents have a higher potential for extraction of bioactive compounds compared to *n*-hexane, and should be used for the extraction of compounds from medicinal plants. Additionally, *n*-hexane showed low potential for extraction of most of the bioactive compounds evaluated. It should be mentioned, however, that the practical results could show significant deviation from the computed predictions, since COSMO-RS approach does not consider macroscopic factors and transfer phenomena governing the extraction. In all the investigated cases, the results need experimental confirmation, especially in the case of NADES where the structures of clusters in the solvent bulk phase are not completely clarified.

Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3863.
PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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