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1 **Solubilization of *Eucalyptus citriodora* essential oil and citronellal in deep**
2 **eutectic solvents:water:cyclodextrins mixtures**

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13
14 **Abstract**

15 Essential oils (EOs) offer interesting biological properties that can be altered by their high
16 volatility and low solubility. This study examined, for the first time, the effect of the
17 combination of different choline chloride (ChCl) based-deep eutectic solvents (DESs), water
18 and cyclodextrins (CDs) on the retention and solubilization of *Eucalyptus citriodora* EO and
19 its major component, citronellal. The density, viscosity and polarity of the newly prepared
20 DES:water:CD mixtures were measured. The influence of water content, DES type and
21 composition as well as CD type and concentration on the retention capacity were investigated
22 using static headspace-gas chromatography. Results showed that the mixture composed of
23 choline chloride:urea (ChCl:U) (1:2 molar ratio) DES:water (70:30 wt%) in the presence of
24 CD presented the highest retention capacity. Finally, the formation constant (K_f) values of
25 CD/citronellal inclusion complexes and the vapor-liquid partition coefficients of citronellal
26 were determined in ChCl:U DES:water 70:30 wt%.

27
28 **Keywords:** citronellal, cyclodextrins, deep eutectic solvents, essential oil, solubility,
29 volatility.
30

31 **1. Introduction**

32 Essential oils (EOs) are natural multicomponent mixtures that have been widely used due to
33 their various antibiotic, antioxidant, anti-quorum sensing, anti-inflammatory as well as
34 digestive fluids stimulating activities. *Eucalyptus* EO ranks as the world's first traded oil and
35 *Eucalyptus citriodora* EO is the major *Eucalyptus* oil with respect to the trade volume [1].
36 However, incorporating EOs in different formulations remains a challenge due to their low
37 aqueous solubility, low stability and high volatility [2].

38 A recent strategy for enhancing EO solubility is the use of deep eutectic solvents (DESs) [3].
39 DESs are generally defined as mixtures of two or more pure compounds, at specific
40 composition, that are able to associate mainly *via* hydrogen bonds [4]. This interaction causes
41 a great depression in the melting point of the mixture compared to its constituents and to their
42 ideal mixture [5]. Till present, most DESs use choline chloride (ChCl) as hydrogen bond
43 acceptor, due to its low toxicity. ChCl is widely used as a supplement in animal feeding and
44 its use is not expected to substantially increase the concentration in the environment. These
45 solvents have been consequently introduced to replace water and toxic organic solvents for
46 the solvation of different active agents such as curcumin [6], *trans*-anethole as well as star
47 anise and fennel EOs [3].

48 Another approach to overcome such problems is the use of cyclodextrins (CDs),
49 oligosaccharides obtained from the enzymatic degradation of starch. The most common
50 native CDs are α -CD, β -CD, and γ -CD, made up of six, seven or eight (α -1,4)-linked D-
51 glucopyranose units, respectively [7]. CDs possess a hydrophilic surface allowing them to be
52 soluble in water. Also, they have a hydrophobic cavity making them able to form inclusion
53 complexes with lipophilic guests through non-covalent interactions [8]. This usually results in
54 an improvement of the physico-chemical properties of guests, mainly their aqueous
55 solubility. CDs contain several hydroxyl groups that are used for selective functionalization
56 in order to obtain CDs derivatives with enhanced solubility and encapsulation capability, like
57 the hydroxypropylated- β -CD (HP- β -CD) [2]. Many studies focused on using native CDs as
58 well as their derivatives to improve the retention and solubilization of EOs and their
59 components [9–11]. As example, it was shown that native and modified CDs can
60 significantly reduce the volatility of EOs by efficiently binding their individual constituents
61 [12]. It was also proved that different CDs can greatly increase the EOs aqueous solubility
62 [13].

63 Interestingly, it was shown that poorly-soluble native β -CD can be solubilized in choline
64 chloride:urea (ChCl:U) DES up to 1 g.mL^{-1} compared to its solubility in water of 0.018 g.mL^{-1}
65 [14]. Also, it was recently demonstrated that CDs retain their encapsulation capacity in
66 DESs [15]. Thus, the combination of DESs and CDs could give rise to formulations with
67 higher potency of solubilization taking advantage of the encapsulation in CDs and the
68 solvation in DESs. The aim of the present study is to develop new mixtures using CDs and
69 ChCl based-DESs to retain and solubilize *E. citriodora* EO and its major component,
70 citronellal. The density, viscosity and polarity of the new prepared mixtures were determined.
71 Then, the retention and the solubilization potential of different DESs, CDs and the combined
72 DES:water:CD mixtures were studied using static headspace-gas chromatography (SH-GC).
73 Additionally, the influence of water content, DES type, DES composition and CD content on
74 the volatility of the EO were assessed. Finally, the formation constant (K_f) of CD/citronellal
75 inclusion complexes and the vapor-liquid partition coefficients of citronellal were
76 determined.
77

78 2. Experimental section

79 2.1. Materials

80 Choline chloride (ChCl, 98%) was purchased from Sigma-Aldrich, China. Urea (U, 99%) and
81 lactic acid were purchased from Sigma-Aldrich, USA. Levulinic acid was purchased from
82 Sigma-Aldrich, France. Citronellal was provided by Merck group, Germany. Propane-1,3-
83 diol, L(+)-tartaric acid, glycerol, D(-)-sorbitol, D(-)-fructose and D(+)-glucose anhydrous
84 were provided by VWR Chemicals, Belgium. Xylitol and (±)-butane-1,3-diol were provided
85 by Alfa Aesar, Germany. Nile red was provided by Acros, Belgium. β-CD was provided by
86 Wacker-Chemie, Lyon, France. Hydroxypropyl-β-CD (HP-β-CD, MS=0.65) was provided by
87 Roquette Frères, Lestrem, France. *E. citriodora* EO was provided by Herbes et Traditions,
88 Comines, France. The main constituents (> 1%) of the EO are: citronellal (74.77%),
89 citronnellol (4.84%), isopulegol (4.30%), neoisopulegol (2.17%), citronnellate of
90 citronnellyle isomers (2.07%), citronnellyle acetate (1.26%), α-pinene (1.32%), β-pinene
91 (1.04%) and ε-cadinene (1.22%) (data provided by Herbes et Traditions). ChCl was dried at
92 60 °C for at least 2 weeks before use. All other compounds were used as received. Distilled
93 water was used throughout this work.

94 2.2. Preparation of deep eutectic solvents

95 DESs were prepared using the heating method by mixing ChCl as the hydrogen bond
96 acceptor (HBA) with different hydrogen bond donors (HBDs), selected to cover different
97 chemical classes (amines, carboxylic acids, alcohols and sugars); this could help in a better
98 understanding of the EO solubilization in different DESs types. Each ChCl:HBD DES was
99 prepared based on literature data. The mixtures were kept under stirring until the formation of
100 a clear and homogenous liquid (**Table 1**). The water content of all the prepared DESs was
101 determined using the Karl Fisher titration method (Mettler Toledo DL31).

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111 **Table 1-** Composition of the studied deep eutectic solvents.

HBD in ChCl-based DES	Abbreviation	Molar ratio	Heating temperature (°C)	Water content (%)
Urea	ChCl:U	1:2	60	0.05
Levulinic acid	ChCl:Lev	1:2	RT	0.31
Tartaric acid	ChCl:Tar	1:1	80	0.16
Lactic acid	ChCl:Lac	1:2	RT	8.07
Glycerol	ChCl:Gly	1:2	RT	0.20
Xylitol	ChCl:Xyl	1:2	80	0.07
Sorbitol	ChCl:Sor	1:1	80	0.36
Butane-1,3-diol	ChCl:But	1:2	40	0.08
Propane-1,3-diol	ChCl:Pro	1:1	80	0.21
Glucose	ChCl:Glu	2:1	80	0.26
Fructose	ChCl:Fru	2:1	80	2.15

112 ChCl: Choline chloride; DES: Deep Eutectic Solvent; HBA: Hydrogen bond acceptor; HBD:
113 Hydrogen bond donor; RT: Room temperature

114 2.3. Density measurements

115 Density (ρ) measurements of the studied mixtures were carried out using a U-shaped
116 vibrating tube densimeter (Anton Paar, model SVM 3001) operating in a static mode at
117 atmospheric pressure, at 30 or 60 °C. The factory calibration was used and verified before
118 and after each measurement with air. The densimeter performs an analysis with an estimated
119 uncertainty in density and temperature of $\pm 0.0001 \text{ g.cm}^{-3}$ and $\pm 0.005 \text{ °C}$, respectively. For
120 ChCl:U DES:water mixtures at low DES wt%, density values were fitted using a linear
121 equation (1):

$$122 \quad \rho = b + (a \times w) \quad (1)$$

123 With w representing the wt% of ChCl:U DES, a and b representing the fitting parameters of
124 the equation (listed in **Table S1**).

125 2.4. Viscosity measurements

126 The viscosity (η) of the studied mixtures was determined using a Stabinger viscometer
127 (Anton Paar, model SVM 3001) at atmospheric pressure, at 30 or 60 °C. The temperature was
128 controlled to within 0.005 °C. The overall uncertainty on the viscosity was estimated to be
129 2%. For ChCl:U DES:water mixtures at low DES wt%, results were fitted to an exponential
130 equation (2), with a Chi-square up to 0.09.

131
$$\eta = \left(A_1 \times e^{\frac{w}{t_1}}\right) + \left(A_2 \times e^{\frac{w}{t_2}}\right) + y_0 \quad (2)$$

132 With w representing the wt% of ChCl:U DES, y_0 , A_1 , t_1 , A_2 and t_2 representing the fitting
133 parameters of the equation (listed in **Table S2**).

134 **2.5. Polarity measurements**

135 Polarity measurements were carried out for various ChCl:U DES:water mixtures and for the
136 different ChCl-based DES:water mixtures (70:30 wt%). A stock solution of Nile Red (NR)
137 was prepared in ethanol with a concentration of 10^{-4} mol.L⁻¹. A volume of 30 μ L of NR
138 solution was added to 1470 μ L of mixture placed in vials as described previously [16]. Then,
139 solutions were stirred at 20 °C for a few minutes, to ensure the solubilization of NR. UV-Vis
140 spectra were recorded with a PerkinElmer Lambda 2S spectrophotometer, between 400 and
141 700 nm. The wavelength, at which the maximum visible-light absorption of NR occurred
142 (λ_{max}), was determined for each mixture, and the NR polar parameter E_{NR} (in kcal.mol⁻¹) was
143 calculated using equation (3).

144
$$E_{NR} = \frac{28591.44}{\lambda_{max} (nm)} \quad (3)$$

145 **2.6. Solubility studies**

146 The solubilization ability of the different DESs, CDs and the combined DES:water:CD
147 mixtures towards *E. citriodora* EO and citronellal was evaluated using SH-GC.

148 **2.6.1. Preparation of different mixtures**

149 **2.6.1.1. Influence of water content**

150 ChCl:U DES:water mixtures were prepared in various water wt% ranging from 0 to 100 wt%
151 in the absence or presence of 10 wt% HP- β -CD (previously weighted in vials). The final HP-
152 β -CD concentration increased from 73 to 87 mM along with DES wt%.

153 **2.6.1.2. Influence of DES composition**

154 *Screening of different DESs*

155 DES:water (70:30 wt%) mixtures were prepared using different ChCl-based DESs in the
156 absence and presence of 10 wt% HP- β -CD. The studied DESs are listed in **Table 1**.

157 *Influence of the mass fraction of DES components*

158 30 wt% of water and 70 wt% of ChCl:U DES components were mixed using different ChCl
159 and urea (U) mass fractions (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ChCl/U) in the
160 absence and presence of 10 wt% HP- β -CD.

161 **2.6.1.3. Chromatographic conditions**

162 In all experiments, prepared mixtures (3 mL) were placed in 20 mL vials. Then, appropriate
163 amounts of an ethanolic solution of EO or citronellal were added. In each experiment, EO
164 and citronellal concentrations were optimized in order to have satisfying chromatographic
165 signal/noise ratio, thus allowing an accurate detection of the influence of each studied
166 parameter. Vials were subsequently sealed using silicone septa and aluminium foil and
167 thermostated at 30 °C (or 60 °C when varying the mass fractions of DES components) for 24
168 hours under stirring to reach the equilibrium between liquid and gas phases. Then, 1 mL of
169 the headspace was withdrawn from the vial and injected in the chromatographic system for
170 analysis *via* a heated transfer line (250 °C). Measurements were carried out with a Varian
171 Genesis headspace sampler coupled with a Perkin Elmer Autosystem XL gas
172 chromatography (GC) equipped with a DB624 and a flame ionization detector column using
173 nitrogen as carrier gas. The GC column temperature conditions were set as mentioned before
174 [9].

175 Results for EO were expressed as:

$$176 \quad \text{Normalized area (\%)} = \frac{\sum A_m}{\sum A_0} \times 100 \quad (4)$$

177 where $\sum A_m$ and $\sum A_0$ stand for the sum of peak areas of the EO components in the presence of
178 the studied mixture and the corresponding reference, respectively.

179 Results for citronellal were expressed as:

$$180 \quad \text{Normalized area (\%)} = \frac{A_m}{A_0} \times 100 \quad (5)$$

181 where A_m and A_0 stand for the peak area of citronellal in the presence of the studied mixture
182 and the corresponding reference, respectively.

183 **2.6.2. Determination of formation constants**

184 The formation constant (K_f) of the complexes were determined using a SH-GC titration
185 method developed in our laboratory for volatile organic compounds and using an algorithmic

186 calculation [17,18]. Increasing concentrations of CDs (β -CD or HP- β -CD) ranging from 0 to
187 10 wt% of CDs were prepared in ChCl:U DES:water 70:30 wt% mixtures. Then, 40 μ L of the
188 ethanol solutions of EO (0.02 g.mL⁻¹) or citronellal (0.015 g.mL⁻¹) were then added to all
189 mixtures. Vials, equilibrated at 30 °C, were then subjected to SH-GC analysis. The
190 chromatographic conditions were set as in 2.6.1.3.

191

192 **2.6.3. Determination of vapor-liquid partition coefficients of citronellal**

193 The vapor-liquid partition coefficient (K) is the ratio of the concentration of the volatile
194 compound in the vapor phase to its concentration in the liquid phase, when the equilibrium is
195 reached, expressed as [19]:

$$196 \quad K = \frac{C_G}{C_L} \quad (6)$$

197 Where C_G and C_L are the concentrations of the volatile compound in the gas phase and the
198 liquid phase at equilibrium, respectively.

199 K of citronellal was determined in water and in ChCl:U DES:water 70:30 wt% mixture
200 prepared in the absence or the presence of 10 wt% HP- β -CD or 2 wt% β -CD at 30 °C using
201 the phase ratio variation (PRV) method in water (10 μ L of citronellal at 0.005 g.mL⁻¹ were
202 added to different volumes of water in vials) and the vapor phase calibration (VPC) method
203 in the other mixtures (different volumes of citronellal at 0.1 g.mL⁻¹ were added to 3 mL of
204 mixtures in vials) as reported earlier [20].

205

206

207

208 **3. Results**

209 This study was performed to evaluate the solubilization capacities of solutions combining
210 DESs, CDs and water. Different formulations were prepared by varying water content, DESs
211 type and composition as well as CDs content. All formulations were then subjected to
212 physico-chemical characterization. Solubility studies were carried out using SH-GC. When a
213 decrease in the peak areas of the EO or citronellal is observed, this corresponds to a decrease
214 in their volatility, meaning an increase in their solubility in the studied mixture.

215 **3.1. Characterization of the prepared mixtures**

216 **3.1.1. Density measurements**

217 Different wt% of water were added to ChCl:U DES in the absence and presence of 10 wt%
218 HP- β -CD. These solutions were characterized first by measuring their densities. The density
219 of ChCl:U DES decreased linearly when increasing the amount of water in the media in the
220 absence and the presence of CDs (**Figure S1**). Results are coherent with those found in
221 literature with other DESs (ChCl:glucose, ChCl:sucrose, and ChCl:propane-1,2-diol) [21].

222 The influence of ChCl mass fraction variation in ChCl:U DES:water 70:30 wt% was also
223 studied. Results proved that decreasing the ChCl mass fraction in the mixture, meaning
224 increasing the U mass fraction, led to the increase in the density of the mixture (**Table S3**).

225 Further, preparing different types of DESs can give rise to solvents with different properties
226 including their densities [22]. The obtained results (**Table S4**) confirmed that the density of
227 the different types of DES:water 70:30 wt% depended on the nature of the HBD of the DESs,
228 in agreement with literature [23].

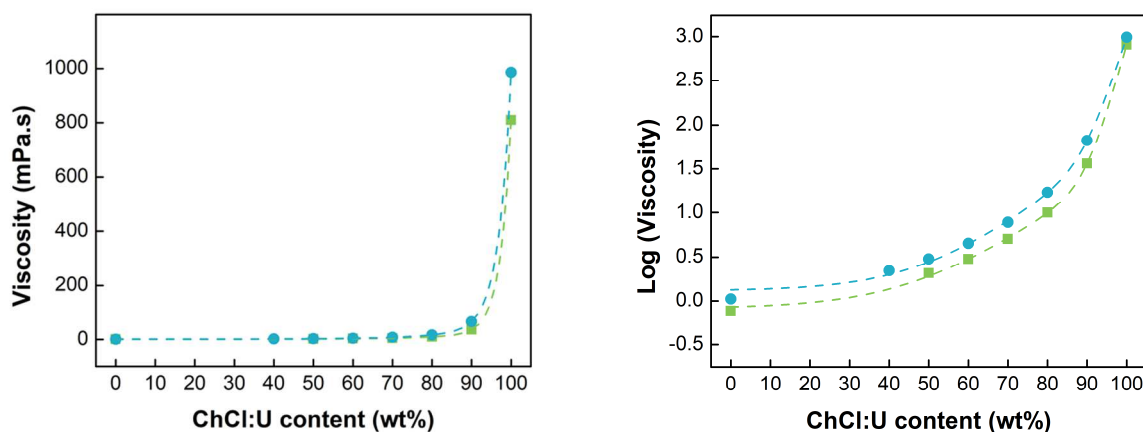
229 For all studied formulations, the presence of 10 wt% of HP- β -CD led to an increase in the
230 density of the mixture compared to CD-free formulations (**Figure S1**). Additionally, the
231 density increased when increasing the wt% of CDs (either HP- β -CD or β -CD) (**Table S5**).
232 Hence, the increase in the density in the presence of CDs can be attributed to the large
233 hydrodynamic volume of CDs.

234

235 **3.1.2. Viscosity measurements**

236 The viscosity (η) of all formulations was then measured. A dramatic decrease in the viscosity
237 was observed upon the addition of a small percentage of water to ChCl:U DES either in the
238 absence or the presence of CDs (**Figure 1**), which is in agreement with the literature [24].

239 This proved the interest of using water to obtain solutions with appropriate viscosity for
240 different applications. In addition, the viscosity of the solutions was higher in the presence of
241 10 wt% HP- β -CD than in its absence. This could be due to the presence of additional
242 hydrogen bonds in the presence of CDs leading to a higher viscosity of the mixture [25].
243 The viscosity of the different ChCl-based DES:water 70:30 wt% mixtures (**Table S4**) was
244 affected by the HBD type of DES, which was always higher in the presence of CDs.



246 **Figure 1:** Viscosity variation as function of the ChCl:U DES content (wt%) in water \blacksquare in the
247 absence and \bullet the presence of 10 wt% of HP- β -CD at 30 °C. The dashed curves are the fitted
248 curves obtained from the experimental values.

249

250 3.1.3. Polarity measurements

251 For a better understanding of the mechanism of solubilization, polarity measurements were
252 conducted for the prepared formulations starting by the different ChCl:U DES:water
253 mixtures. Because of the aggregation phenomena of the Nile red (NR) probe in water, the
254 polarity of only 100, 90, 80, 70, 60, 50 and 40 wt% ChCl:U DES in water mixtures was
255 measured. λ_{\max} and E_{NR} values are depicted in **Table S6**. Polarity values showed that adding
256 water to the media led to the increase in the polarity of the mixture as indicated by the red-
257 shift of the λ_{\max} , in agreement with the literature [26].

258 In addition, polarity measurements of the different synthesized ChCl-based DES:water 70:30
259 wt% mixtures were done (**Table S7**). Even if weak polarity differences between the different
260 mixtures were observed, some trends could be extracted. The least polar mixture was with
261 ChCl:Butane-1,3-diol, while the most polar one was with ChCl:Tartaric acid. This can be
262 explained by the fact that butane-1,3-diol possess a long hydrocarbon chain which explains

263 its lower polarity. Additionally, adding alcoholic and acidic functional groups increase the
264 polarity of the solvent [27]. For example, ChCl:Sorbitol DES:water 70:30 wt% was more
265 polar than ChCl:Xylitol DES:water 70:30 wt%. This could be explained by the presence of an
266 additional alcohol function in sorbitol as compared to xylitol.

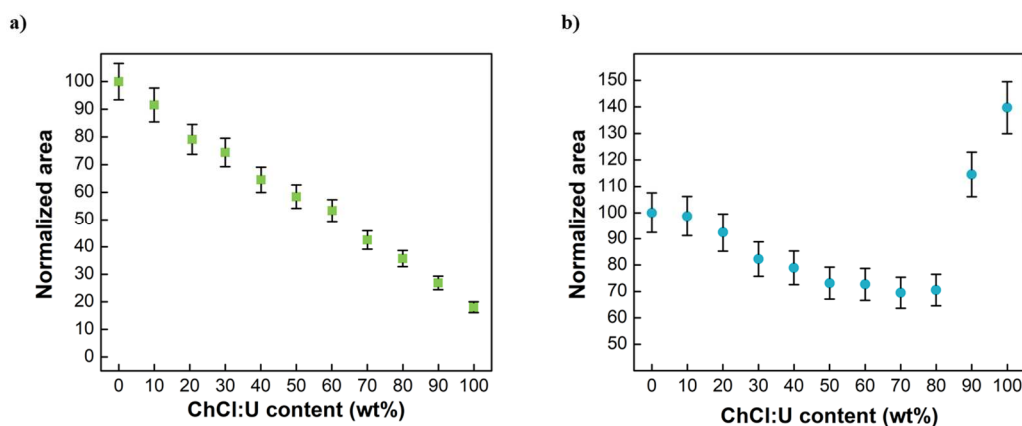
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268 3.2. Solubilization studies

269 3.2.1. Influence of water content

270 Firstly, the influence of water addition on ChCl:U DES on the volatility of the *E. citriodora*
271 EO was studied. **Figure S2**, shows the overlapping chromatograms obtained in water and
272 pure ChCl:U DES. The peaks corresponding to the volatile compounds found in the EO are
273 clearly visible in water but they almost disappeared in the pure DES in accordance with
274 literature [3]. This was obviously observed for the major compound of the EO, citronellal that
275 was eluted at 21 min approximately. This observation led us to optimize the EO and
276 citronellal concentration in each experiment independently in order to obtain satisfying
277 signal/noise ratio allowing accurate evaluation of the influence of each studied parameter.

278 **Figure 2** depicts the evolution of the total area of the EO when increasing the DES wt% in
279 the mixture in the absence and the presence of 10 wt% HP- β -CD.

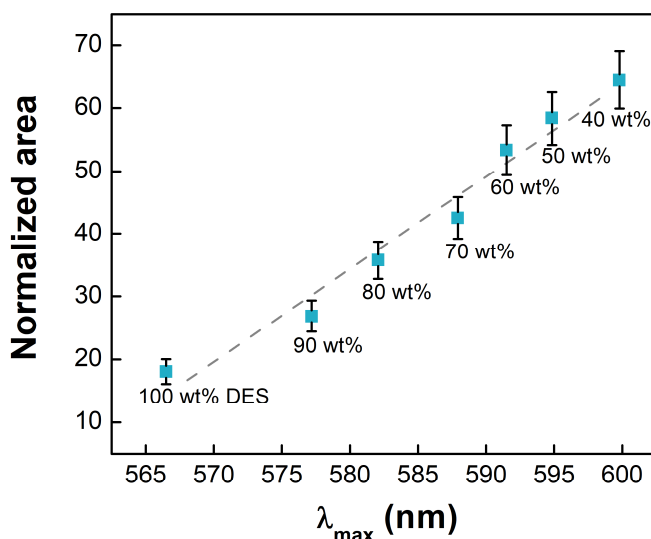


280

281 **Figure 2:** Plots of the EO normalized area, obtained from SH-GC analysis, as a function of
282 the ChCl:U DES content (wt%), a) in the absence of CD where the reference mixture was
283 water (EO concentration = 8.7×10^{-5} g.mL⁻¹) and b) in the presence of 10 wt% of HP- β -CD
284 where the reference mixture was the water containing 10 wt% HP- β -CD, at 30 °C (EO
285 concentration = 1.34×10^{-3} g.mL⁻¹).

286 In the absence of HP- β -CD (Figure 2a), a linear decrease in the total area was observed when
287 increasing the wt% of DES, which indicated a decrease in the volatility of EO due to its
288 increased solubilization. A 5-fold decrease in the volatility was observed in the presence of

289 100 wt% DES as compared to water. The same tendency was observed in literature, where
 290 the increase of water content led to a relatively linear decrease in the solubility of four
 291 phytochemicals in DESs [26]. A correlation between the polarity of ChCl:U DES:water
 292 mixtures and the total areas was observed (**Figure 3**). As can be seen, decreasing DES
 293 content, meaning adding water to the mixture, led concomitantly to the increase in the
 294 polarity of the mixture and to the increase in the EO volatility. This indicated that increasing
 295 the polarity of the mixture led to a decrease in the EO solubility in accordance with the
 296 literature [26]. Therefore, it can be confirmed that the polarity constituted a fundamental
 297 solubilization mechanism herein.



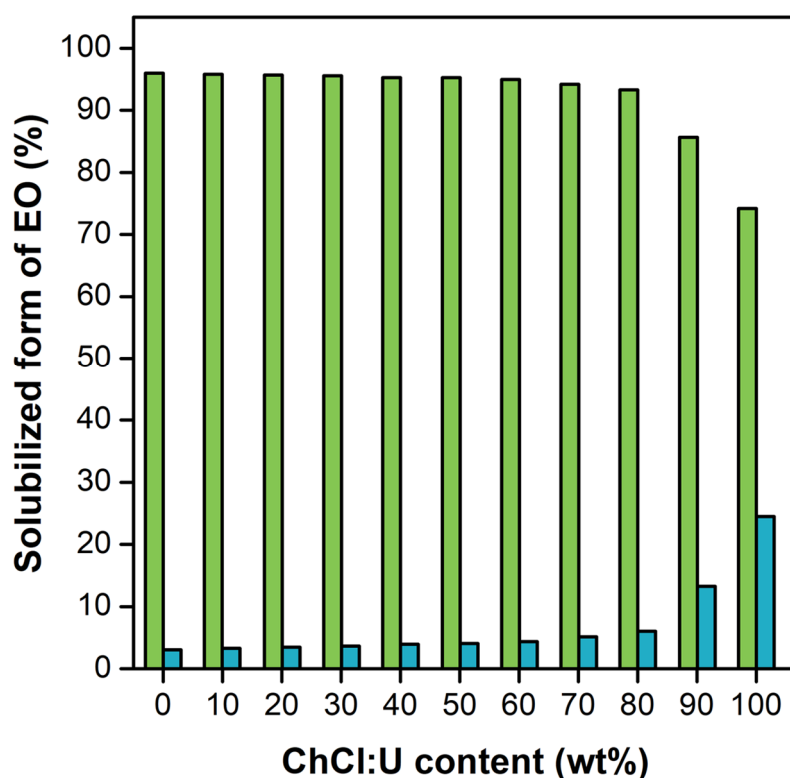
298
 299 **Figure 3:** Correlation between the EO normalized area, using water as reference, obtained
 300 from SH-GC analysis, and the maximum wavelength (λ_{max}) of Nile red probe in the
 301 corresponding ChCl:U DES content (wt%). These data follow the normality test, and possess
 302 a correlation coefficient of 0.9857 with p -value <0.01.
 303

304 As the addition of HP- β -CD to DES:water mixtures resulted in a dramatic decrease in the
 305 total chromatographic peak area of the EO (a 33-fold decrease in the EO total peak area was
 306 observed when adding HP- β -CD to water), a higher EO concentration was used in order to
 307 obtain significant chromatographic peak areas.

308 In the presence of 10 wt% HP- β -CD (Figure 2b), a relatively linear decrease in the EO total
 309 area was observed with increasing the wt% of ChCl:U DES in water from 0 to 70 wt%.
 310 Surprisingly, an increase in the EO volatility was observed above DES:water 80:20 wt%.

311 The apparent HP- β -CD/EO complex formation constant (K_f) values in all DES:water
312 mixtures were determined using the rapid method described earlier [13]. The apparent K_f
313 were calculated using the approximate concentration of citronellal based on its percentage in
314 the EO. Results showed that the K_f values decreased monotonically from 470 to 37 M^{-1} when
315 increasing the ChCl:U DES content in water (**Table S8**).

316 Based on these apparent K_f values, the amounts of free and complexed EO present in each
317 ChCl:U DES:water mixture at equilibrium were determined. Figure 4 shows the percentage
318 of the EO solubilized in each form.



319

320 **Figure 4:** Percentages of the amount of each solubilized form of EO at equilibrium in all
321 ChCl:U DES:water mixtures. EO complexed with HP- β -CD (green histograms) and
322 free EO (blue histograms).

323

324 As we can see, up to ChCl:U DES:water 80:20 wt% more than 93% of the solubilized EO
325 was present in the complexed form. These high complexed amounts observed despite the
326 reduction in the apparent K_f value (Table S8) could be contributed to the high amount of CD

327 present in the medium and the decrease in the polarity of the medium with the DES addition
328 was still not enough to strongly destabilize the inclusion complex.

329

330 Over DES:water 80:20 wt%, a concomitant decrease in the amount of complexed EO form
331 and increase in the amount of free EO form were observed. This could be attributed to the
332 large fall in the polarity of the medium detected when using these high percentages of DES
333 (Figure 3). This led to a significant shift in the equilibrium towards the dissociation of the
334 inclusion complex and thus a significant increase in the amount of free EO in good agreement
335 with the very strong decrease in the apparent K_f values (Table S8). This, consequently, led to
336 the increase in the amount of volatile EO in the gaseous phase (Figure 2b).

337 Altogether, these findings indicated the existence of an optimal DES:water medium observed
338 experimentally in Figure 2b. This results from the fact that the solubilization of EO is
339 governed by two different mechanisms, which follow opposite trends with regards to the
340 solvent composition. Solubilization can occur either in the bulk (solvent) in the free form or
341 as in the form of inclusion complex in the CD cavity. The polarity of the medium affects
342 these two mechanisms in opposite ways. Indeed, decreasing the polarity of the medium
343 increases the contribution of the first mechanism and lessens the latter.

344 Results also proved that, in the presence of more than 20 wt% water, CD could maintain
345 strong encapsulation ability in good agreement with literature. The beneficial effect of the
346 presence of a small proportion of water was confirmed in a recent NMR study on the
347 complexation of piroxicam in β -CD (10 wt%) solubilized in ChCl:U DES. Authors concluded
348 that, upon addition of 25 wt% of water to DES, water molecules tend to interact firstly with
349 the hydroxyl groups of the CDs through hydrogen bonding. This consequently increased the
350 molecular rotational motion of the CDs and allowed it experiencing an aqueous-like
351 environment capable of maintaining its encapsulation capacity [28]. This suggested that the
352 contribution of CDs required a minimum presence of water in order to fully benefit from the
353 inclusion driven by hydrophobic forces.

354 It has also to be underlined that the presence of water improves the convenience of
355 preparation and use of solvents, since water effectively reduces the high viscosity generally
356 observed for DESs, while also improving the solubilization efficiency when using CDs.

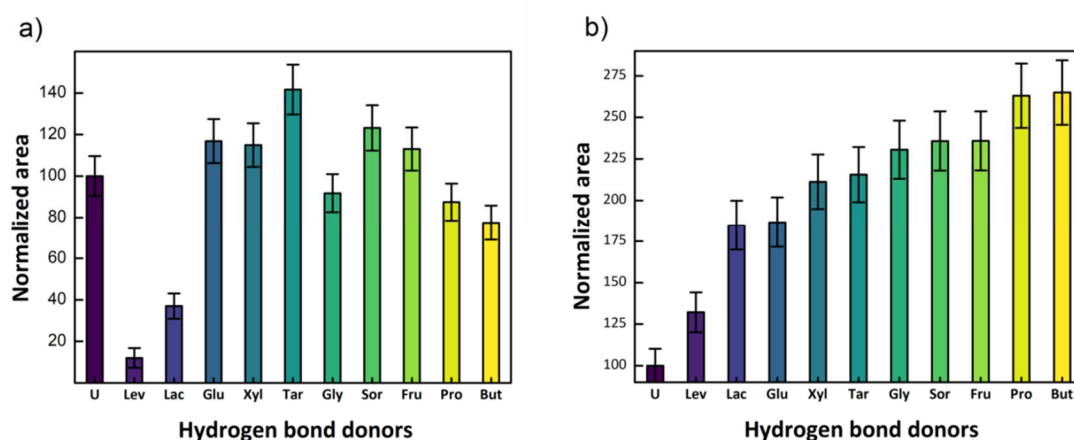
357 As the optimal decrease in the EO volatility, in the presence of HP- β -CD, was detected when
358 the wt% of DES was in the range of 60 to 80 wt% (**Figure 2b**), the ChCl:U DES:water 70:30
359 wt% was used in the following investigations.

360

361 3.2.2. Influence of DES composition

362 3.2.2.1. Screening of different DESs

363 The solubilization capacity of different ChCl-based DES:water 70:30 wt%, in the absence
364 and presence of 10 wt% HP- β -CD, was studied. Results are illustrated in **Figure 5**.



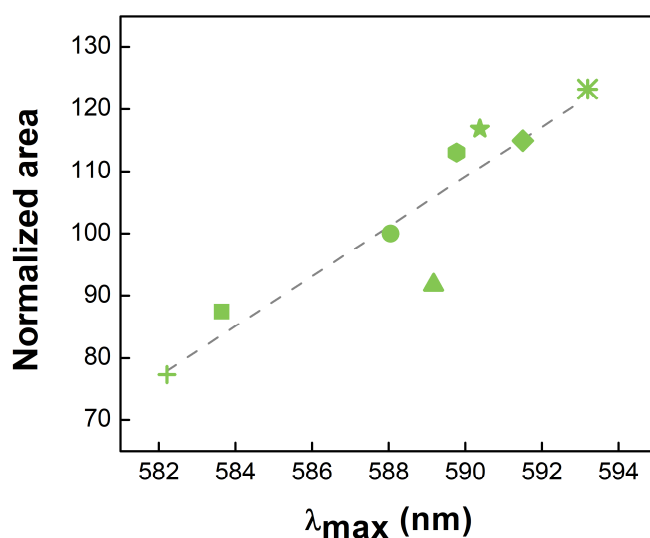
365

366 **Figure 5:** EO normalized area, obtained from SH-GC, for different ChCl-based DES:water
367 70:30 wt% mixtures a) in the absence of CDs where the reference mixture was ChCl:U
368 DES:water 70:30 wt% (EO concentration = 6.7×10^{-5} g.mL $^{-1}$), and b) in the presence of 10
369 wt% HP- β -CD where the reference mixture was ChCl:U DES:water 70:30 wt% containing 10
370 wt% HP- β -CD at 30 °C (EO concentration = 1.07×10^{-3} g.mL $^{-1}$).

371

372 From **Figure 5a**, it can be shown that, in the absence of CDs, the lowest EO volatilities were
373 obtained with ChCl:Levulinic acid and ChCl:Lactic acid DESs. Nevertheless, their acidic
374 nature can therefore limit their applications.

375 When excluding acid-basic effect and thus the three acid-based DESs, a better understanding
376 of the influence of the polarity on the volatility results can be obtained. A correlation was
377 observed between the polarity of the DES:water 70:30 wt% mixtures and the obtained EO
378 chromatographic normalized area for each mixture (**Figure 6**). It can be seen, that when
379 increasing the polarity (a red-shift of the λ_{max}), a higher volatility of the EO was observed,
380 which indicated a decrease in the solubility.



381

382 **Figure 6:** Correlation between the EO normalized area, obtained from SH-GC analysis, and
 383 the maximum wavelength (λ_{\max}) of Nile red probe in the different DES:water 70:30 wt%
 384 mixtures. The reference solvent was the ChCl:U DES:water 70:30 wt%. These data follow
 385 the normality test, and possess a correlation coefficient of 0.8521 with p -value <0.01.

386 + ChCl:But, ■ ChCl:Pro, ▲ ChCl:Gly, ● ChCl:U, ● ChCl:Fru, ★ ChCl:Glu, ◆ ChCl:Xyl,
 387 * ChCl:Sor

388

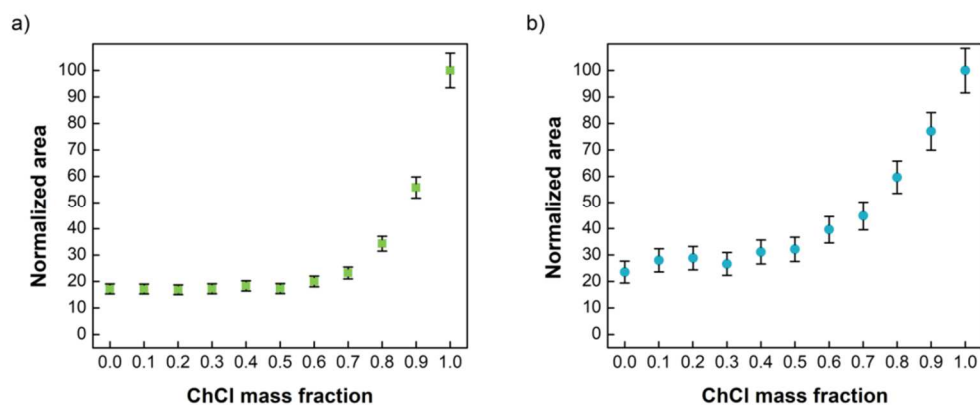
389 **Figure 5b** shows the reduction in EO volatility in the different ChCl-based DES in the
 390 presence of 10 wt% HP- β -CD. Among all, ChCl:U reduced the most the EO volatility and
 391 therefore possessed the best solubilizing ability. This suggests that this system affected the
 392 less the complexation phenomenon comparing to the other DESs. Thus, this optimal mixture
 393 ChCl:U DES:water 70:30 wt% was used in the following investigations.

394

395 3.2.2.2. Influence of the mass fraction of DES components

396 The influence of the ChCl mass fraction in the ChCl:U DES on the volatility of EO in
 397 DES:water 70:30 wt% mixtures was studied in the absence and in the presence of 10 wt%
 398 HP- β -CD. **Figure 7** shows the evolution of the EO volatility as a function of the ChCl mass
 399 fraction in the DES.

400



401

402 **Figure 7:** Plots of the EO normalized area, obtained from SH-GC, as a function of the ChCl
 403 mass fraction in ChCl:U DES:water 70:30 wt% a) in the absence of CDs where the reference
 404 mixture was water containing 1 ChCl mass fraction (EO concentration = 2.7×10^{-5} g.mL⁻¹) and
 405 b) in the presence of 10 wt% of HP-β-CD where the reference mixture was water containing
 406 1 ChCl mass fraction and 10 wt% HP-β-CD at 60 °C (EO concentration = 1.33×10^{-4} g.mL⁻¹).

407

408 It should be noted that the ChCl mass fraction equal to 0.5 roughly corresponds to the
 409 eutectic point 1:2 molar ratio [4]. From **Figure 7**, it can be noticed that increasing the ChCl
 410 mass fraction from 0 to 0.7 (from ChCl:U 0:1 to 1:1 molar ratio, respectively) led to
 411 insignificant volatility variation. Then, above 0.7 ChCl mass fraction, a gradual increase in
 412 the EO volatility was observed. The EO volatility was found to be significantly lower in the
 413 absence of ChCl than in the absence of U. The same tendency was observed in the presence
 414 of 10 wt% HP-β-CD, even if a slightly higher slope was obtained between 0.0- 0.7 ChCl
 415 mass fraction range. This confirms the importance of U for ensuring the best reduction in EO
 416 volatility. Still, the presence of ChCl was also required and was of great importance because
 417 it allows solubilizing U in the solvating mixture and it was therefore necessary to ensure the
 418 liquid state at room temperature. This highlighted the importance of the synergetic effect of
 419 the two DES components for both the solubilization of substances and the improvement of
 420 each other effect in good agreement with literature [29,30]. In fact, U (70 wt%) was not
 421 soluble when associated to 30 wt% of water at room temperature (which led us to carry out
 422 these experiments at 60 °C). To the contrary, a liquid mixture was obtained for 70 wt%
 423 ChCl:U DES with 30 wt% of water. Thus, an effective volatility reduction was achieved in
 424 ChCl:U DES:water 70:30 wt%.

425 As the eutectic point is close to optimal efficiency both in the absence and presence of CDs,
 426 such composition was kept in the following experiments.

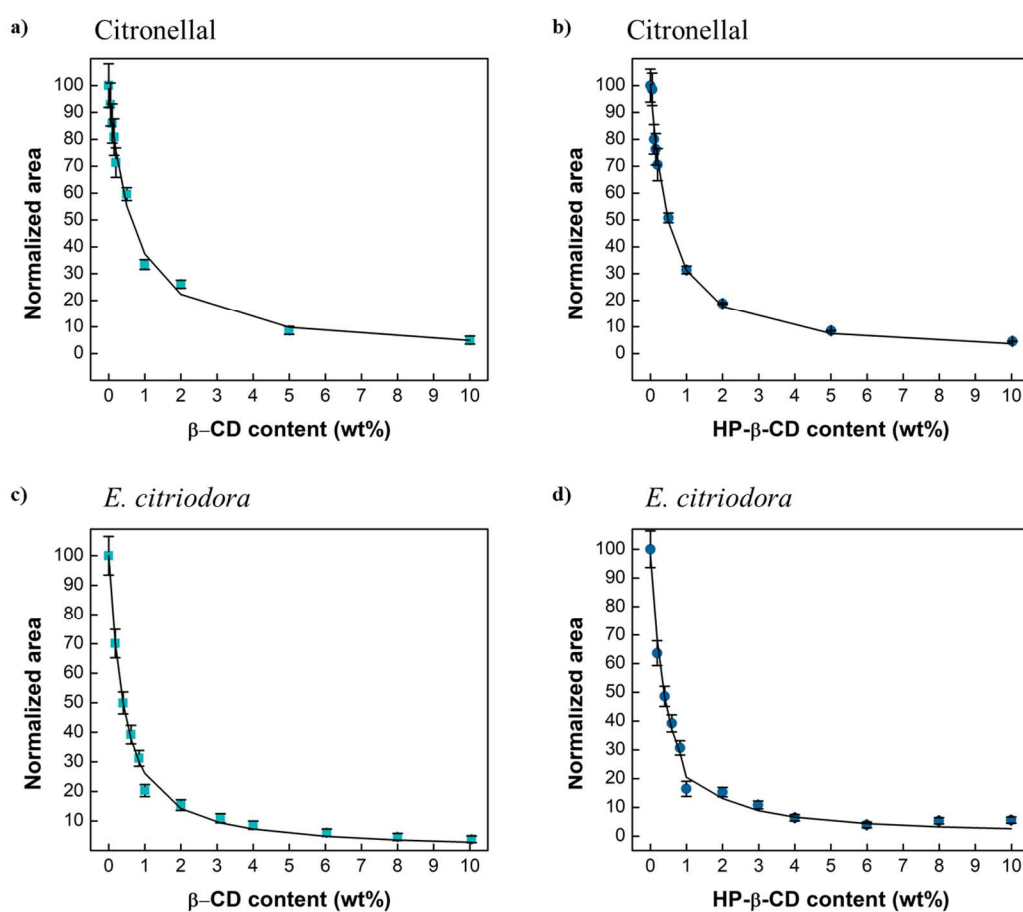
427

428

429 3.2.3. Determination of formation constants

430 Different concentrations of native β -CD and HP- β -CD were added to ChCl:U (1:2)
431 DES:water 70:30 wt%. The evolution of volatility of citronellal and *E. citriodora* EO in the
432 different mixtures are illustrated in **Figure 8**.

433



434

435 **Figure 8:** Complexation curves of a) β -CD/citronellal, b) HP- β -CD/citronellal, c) β -CD/*E.*
436 *citriodora* and d) HP- β -CD/*E. citriodora* in ChCl:U (1:2) DES:water 70:30 wt% at 30 °C. In
437 all these plots, the reference mixture was ChCl:U DES:water 70:30 wt% in the absence of
438 CDs (Citronellal and EO = 1.98×10^{-4} and 2.63×10^{-4} g.mL⁻¹, respectively).

439 An exponential decrease in the volatility of citronellal and EO was observed when increasing
440 the wt% of both CDs. 2 wt% of CDs was sufficient to have an almost maximum retention
441 capacity of the EO. Such an isothermal curve is typical of a complexation phenomenon
442 [31,32]. This variation is in agreement with a 1:1 inclusion complex between citronellal and
443 CDs dissolved in ChCl:U (1:2) DES:water 70:30 wt%. Formation constants (K_f) values for β -

444 CD/citronellal and HP- β -CD/citronellal inclusion complexes were calculated using an
445 algorithmic treatment developed in our laboratory [17] and were equal to $177 \pm 15 \text{ M}^{-1}$ and
446 $303 \pm 28 \text{ M}^{-1}$, respectively. The obtained K_f in DES:water 70:30 wt% were lower than those
447 obtained in water; 1523 M^{-1} and 815 M^{-1} for β -CD/citronellal and HP- β -CD/citronellal,
448 respectively, at $25 \text{ }^\circ\text{C}$ [12]. The same tendency was observed in a previous article that
449 showed the decrease of K_f in the pure ChCl:U DES compared to water [15]. This confirms
450 the early stated hypothesis that the solubilization of guests in DESs lowers their affinity for
451 the hydrophobic cavity of CDs, therefore lowers their encapsulation efficiency.

452 Then, the affinity of CDs towards the whole EO was evaluated. Given that the EO is a
453 mixture of different volatile components, one way to calculate an apparent molar
454 concentration of the EO is to use the percentage of citronellal in the EO (74.77%) and its
455 molar concentration in the vials. Thus, the apparent molar concentration of the EO was
456 calculated by dividing the concentration of citronellal by 0.7477. Apparent K_f of the EO were
457 then determined and were equal to $320 \pm 18 \text{ M}^{-1}$ and $433 \pm 33 \text{ M}^{-1}$ with β -CD and HP- β -CD,
458 respectively. The apparent K_f values obtained for HP- β -CD/EO *via* the rapid (**Table S8**) and
459 titration methods were coherent.

460 **3.2.4. Determination of vapor-liquid partition coefficients of citronellal**

461 As citronellal constitutes the major component of the studied EO, its vapor-liquid partition
462 coefficient (K) in water, ChCl:U DES:water 70:30 wt%, ChCl:U DES:water 70:30 wt% with
463 2 wt% β -CD and ChCl:U DES:water 70:30 wt% with 10 wt% HP- β -CD was determined at 30
464 $^\circ\text{C}$ *via* the PRV and VPC methods, respectively. The corresponding K values were of
465 $3.59 \times 10^{-2} \pm 0.18 \times 10^{-2}$, $1.05 \times 10^{-2} \pm 0.07 \times 10^{-2}$, $3.93 \times 10^{-4} \pm 0.43 \times 10^{-4}$ and $2.54 \times 10^{-4} \pm 0.08 \times 10^{-4}$,
466 respectively. The lower the K value, the higher is the retention or solubilization capacity of
467 the mixture.

468 K value in ChCl:U DES:water 70:30 wt% was approximately 3-times lower than in water,
469 which was similarly obtained in another study [20]. This proved the higher solubilization of
470 citronellal in the mixture. Besides, significantly lower apparent K values were obtained upon
471 the addition of CDs to DES:water mixture, as a consequence of the higher retention of
472 citronellal because of its encapsulation in the CDs. K values were 27 and 41-times lower in
473 the presence of 2 wt% of β -CD and 10 wt% of HP- β -CD, respectively, compared to the
474 DES:water mixture in the absence of CDs.

475

476 **4. Conclusion**

477 With the aim of improving the solubility of *E. citriodora* EO and its major constituent,
478 citronellal, new DES:water:CD mixtures were prepared and characterized by measuring their
479 densities, viscosities and polarities. Solubility studies were conducted by SH-GC and showed
480 that the presence of a minimal amount of water was highly favorable when combining DESs
481 and CDs in the mixture providing the highest solubilization ability compared to the use of
482 each system separately. ChCl:U DES:water 70:30 wt% was shown to be the most efficient
483 mixture for the solubilization of the EO among different ChCl-based DES:water mixtures,
484 when CDs was present. Also, the eutectic point of ChCl:U 1:2 molar ratio was considered as
485 one of the optimal ratios for solubilization purposes. The complexation of the EO and
486 citronellal with β -CD and HP- β -CD was proved in ChCl:U (1:2) DES:water 70:30 wt%. Even
487 if the K_f values of citronellal were lower in DES:water than in water, the contribution of CDs
488 in DESs mixtures was proved to be highly favorable. Lastly, the vapor-liquid partition
489 coefficients of citronellal were determined in ChCl:U (1:2) DES:water 70:30 wt% in the
490 absence and the presence of CDs and were significantly lower compared to water. All these
491 results proved that DES:water:CD mixtures could be efficiently used to solubilize volatile
492 guests. Therefore, these new formulations should be taken into consideration for further
493 research implications in different domains.

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501

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610

I. Optimization

II. Solubility studies

III. Optimal mixture

DES:water:CD mixtures

Presence of
CDs

Volatility ↓
33x in water
4x in DES

Water
content

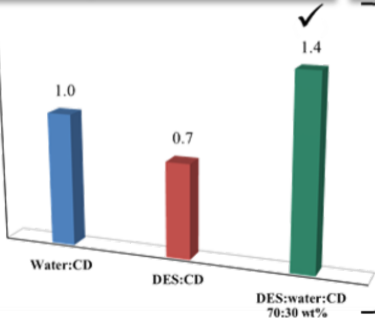
ChCl:U
molar ratio

✓ Eutectic
molar ratio

HBD type

✓ ChCl:U

E. citriodora volatility reduction
normalized to water:CD



ChCl:U
DES:water
70:30 wt%
+CDs

Optimal
mixture