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

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### ► To cite this version:

Lamia Nakhle, Miriana Kfoury, Isabelle Mallard, H el ene Greige-Gerges, David Landy. Solubilization of *Eucalyptus citriodora* essential oil and citronellal in deep eutectic solvents:water:cyclodextrins mixtures. *Journal of Molecular Liquids*, 2022, 359, pp.119371. 10.1016/j.molliq.2022.119371 . hal-04320446

HAL Id: hal-04320446

<https://hal.science/hal-04320446>

Submitted on 22 Jul 2024

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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  $\alpha$ -CD,  $\beta$ -CD, and  $\gamma$ -CD, made up of six, seven or eight ( $\alpha$ -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- $\beta$ -CD (HP- $\beta$ -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  $\beta$ -CD can be solubilized in choline  
64 chloride:urea (ChCl:U) DES up to  $1 \text{ g.mL}^{-1}$  compared to its solubility in water of  $0.018 \text{ g.mL}^{-1}$   
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 ( $\rho$ ) 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 ( $\eta$ ) 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<sup>-1</sup>. A volume of 30  $\mu$ L of NR  
138 solution was added to 1470  $\mu$ 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 ( $\lambda_{max}$ ), was determined for each mixture, and the NR polar parameter  $E_{NR}$  (in kcal.mol<sup>-1</sup>) 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- $\beta$ -CD (previously weighted in vials). The final HP-  
152  $\beta$ -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- $\beta$ -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- $\beta$ -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 ( $\beta$ -CD or HP- $\beta$ -CD) ranging from 0 to  
187 10 wt% of CDs were prepared in ChCl:U DES:water 70:30 wt% mixtures. Then, 40  $\mu$ L of the  
188 ethanol solutions of EO (0.02 g.mL<sup>-1</sup>) or citronellal (0.015 g.mL<sup>-1</sup>) 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- $\beta$ -CD or 2 wt%  $\beta$ -CD at 30 °C using  
201 the phase ratio variation (PRV) method in water (10  $\mu$ L of citronellal at 0.005 g.mL<sup>-1</sup> 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<sup>-1</sup> 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- $\beta$ -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].

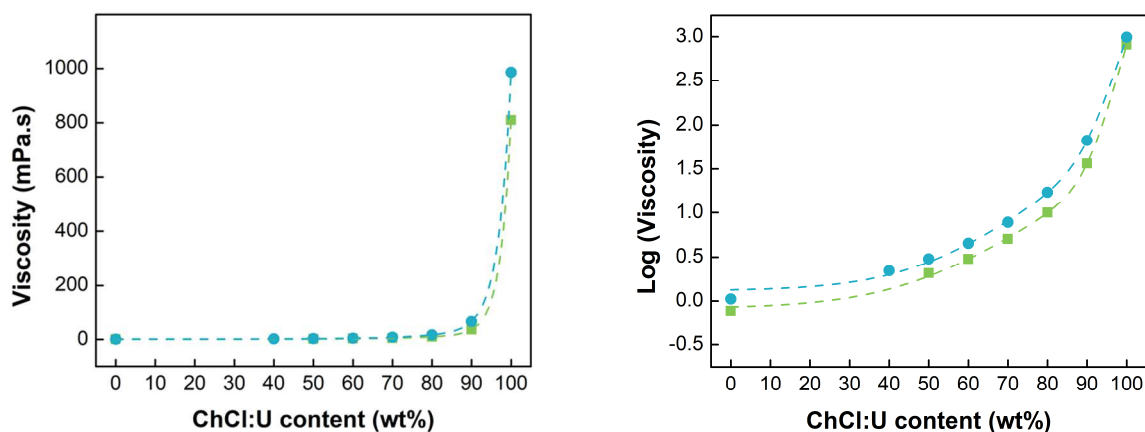
229 For all studied formulations, the presence of 10 wt% of HP- $\beta$ -CD led to an increase in the  
230 density of the mixture compared to CD-free formulations (**Figure S1**). Additionally, the  
231 density increased when increasing the wt% of CDs (either HP- $\beta$ -CD or  $\beta$ -CD) (**Table 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 ( $\eta$ ) 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- $\beta$ -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- $\beta$ -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.  $\lambda_{\max}$  and  $E_{\text{NR}}$  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  $\lambda_{\max}$ , 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.

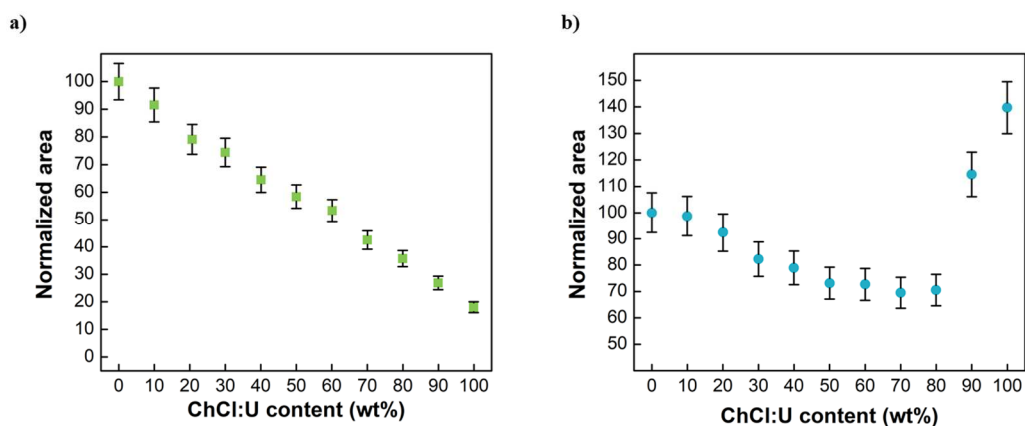
267

### 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- $\beta$ -CD.

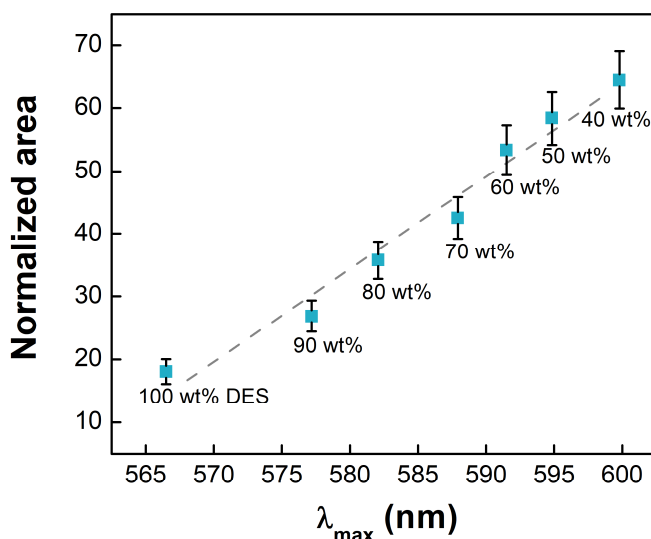


280

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 =  $8.7 \times 10^{-5}$  g.mL $^{-1}$ ) and b) in the presence of 10 wt% of HP- $\beta$ -CD  
284 where the reference mixture was the water containing 10 wt% HP- $\beta$ -CD, at 30 °C (EO  
285 concentration =  $1.34 \times 10^{-3}$  g.mL $^{-1}$ ).

286 In the absence of HP- $\beta$ -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.



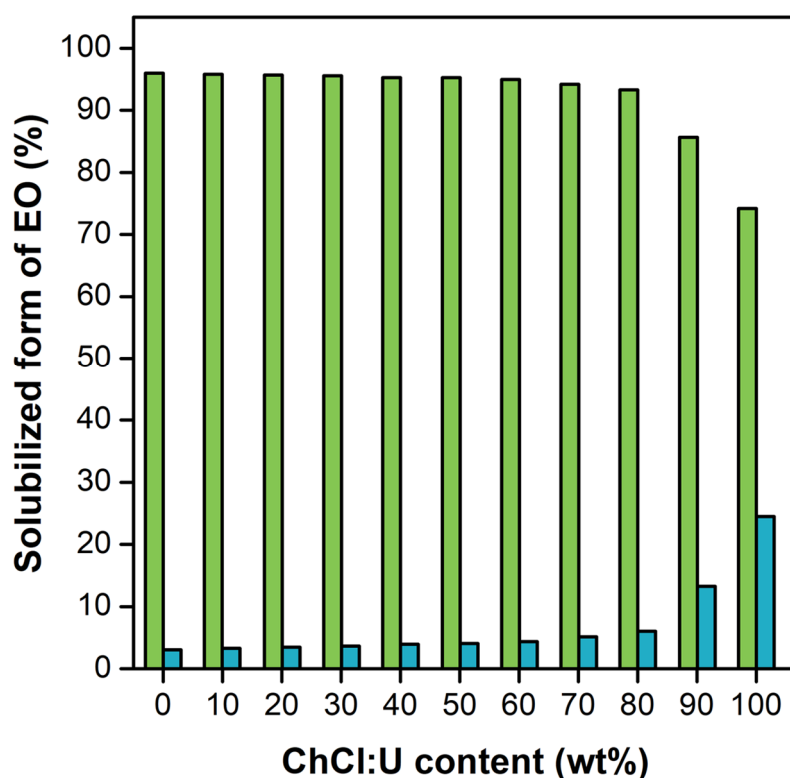
298  
 299 **Figure 3:** Correlation between the EO normalized area, using water as reference, obtained  
 300 from SH-GC analysis, and the maximum wavelength ( $\lambda_{max}$ ) of Nile red probe in the  
 301 corresponding ChCl:U DES content (wt%). These data follow the normality test, and possess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

304 As the addition of HP- $\beta$ -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- $\beta$ -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- $\beta$ -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- $\beta$ -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- $\beta$ -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  $\beta$ -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.

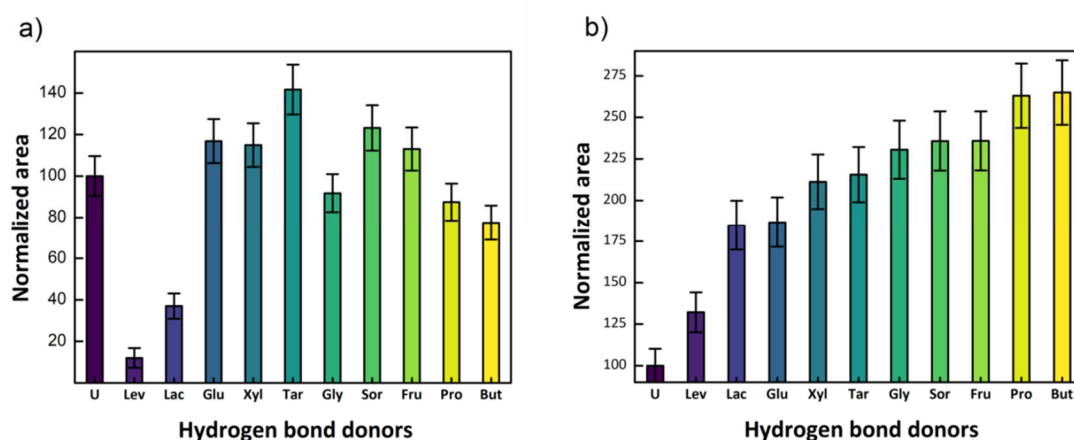
357 As the optimal decrease in the EO volatility, in the presence of HP- $\beta$ -CD, was detected when  
358 the wt% of DES was in the range of 60 to 80 wt% (**Figure 2b**), the ChCl:U DES:water 70:30  
359 wt% was used in the following investigations.

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- $\beta$ -CD, was studied. Results are illustrated in **Figure 5**.



365

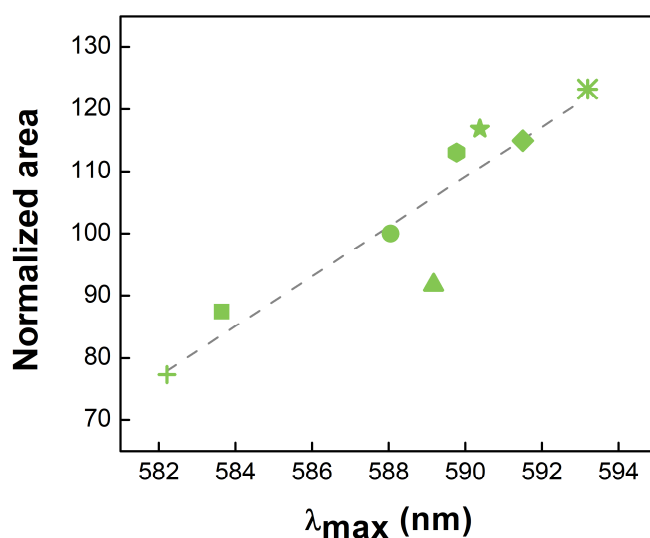
366 **Figure 5:** EO normalized area, obtained from SH-GC, for different ChCl-based DES:water  
367 70:30 wt% mixtures a) in the absence of CDs where the reference mixture was ChCl:U  
368 DES:water 70:30 wt% (EO concentration =  $6.7 \times 10^{-5}$  g.mL<sup>-1</sup>), and b) in the presence of 10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

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  $\lambda_{\max}$ ), 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 ( $\lambda_{\max}$ ) 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

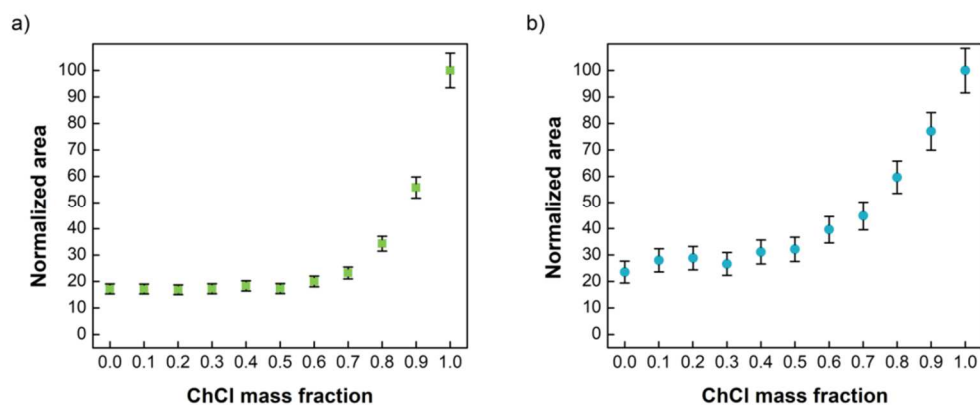
389 **Figure 5b** shows the reduction in EO volatility in the different ChCl-based DES in the  
 390 presence of 10 wt% HP- $\beta$ -CD. Among all, ChCl:U reduced the most the EO volatility and  
 391 therefore possessed the best solubilizing ability. This suggests that this system affected the  
 392 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- $\beta$ -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 =  $2.7 \times 10^{-5}$  g.mL<sup>-1</sup>) and  
 405 b) in the presence of 10 wt% of HP- $\beta$ -CD where the reference mixture was water containing  
 406 1 ChCl mass fraction and 10 wt% HP- $\beta$ -CD at 60 °C (EO concentration =  $1.33 \times 10^{-4}$  g.mL<sup>-1</sup>).

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- $\beta$ -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.

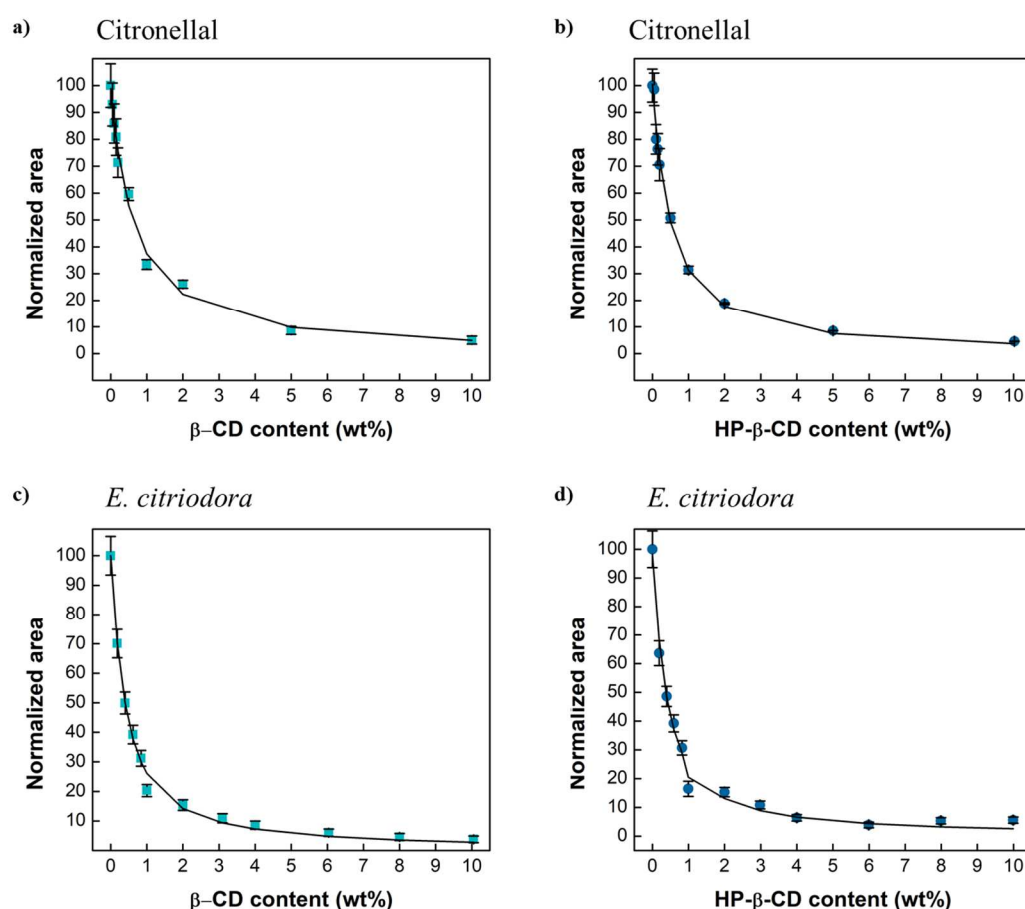
427

428

### 429 3.2.3. Determination of formation constants

430 Different concentrations of native  $\beta$ -CD and HP- $\beta$ -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)  $\beta$ -CD/citronellal, b) HP- $\beta$ -CD/citronellal, c)  $\beta$ -CD/*E.*  
436 *citriodora* and d) HP- $\beta$ -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 =  $1.98 \times 10^{-4}$  and  $2.63 \times 10^{-4}$  g.mL<sup>-1</sup>, 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  $\beta$ -

444 CD/citronellal and HP- $\beta$ -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;  $1523 \text{ M}^{-1}$  and  $815 \text{ M}^{-1}$  for  $\beta$ -CD/citronellal and HP- $\beta$ -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  $\beta$ -CD and HP- $\beta$ -CD,  
458 respectively. The apparent  $K_f$  values obtained for HP- $\beta$ -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%  $\beta$ -CD and ChCl:U DES:water 70:30 wt% with 10 wt% HP- $\beta$ -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  $\beta$ -CD and 10 wt% of HP- $\beta$ -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  $\beta$ -CD and HP- $\beta$ -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.

#### 494 **Acknowledgements**

495 The authors would like to acknowledge the National Council for Scientific Research of  
496 Lebanon (CNRS-L) and Université du Littoral Côte d'Opale (ULCO) for granting a doctoral  
497 fellowship to Lamia Nakhle. This work is a contribution to the CPER (Contrat de Plan Etat-  
498 Région) research project IReNE (Innovation et Recherche en Environnement) and is  
499 supported by the French Ministère de l'Enseignement Supérieur, the region Hauts-de-France  
500 and the European Regional Development Fund.

501

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610



## I. Optimization

DES:water:CD mixtures

Presence of  
CDs

Volatility ↓  
33x in water  
4x in DES

Water  
content

ChCl:U  
molar ratio

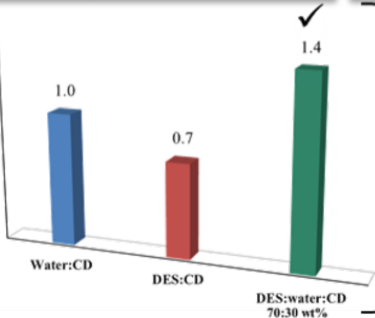
✓ Eutectic  
molar ratio

HBD type

✓ ChCl:U

## II. Solubility studies

*E. citriodora* volatility reduction  
normalized to water:CD



## III. Optimal mixture

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

Optimal  
mixture