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1           **Cyclodextrin-based low melting mixtures as a solubilizing**  
2           **vehicle: Application to non-steroidal anti-inflammatory drugs**

3

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9

10 **Keywords:** cyclodextrins, deep eutectic solvents, low melting mixtures, non-steroidal anti-  
11 inflammatory drugs, solubility

12

## 13 Abstract

14 Poor water solubility represents a serious issue for drug formulation and delivery. Cyclodextrins  
15 (CDs) and cosolvents are valuable approaches to overcome this issue. Currently, there is a  
16 growing interest in deep eutectic solvents (DESs) with high solubilizing ability and low toxicity.  
17 Low melting mixtures (LMMs) based on CDs have recently emerged, combining the solubilizing  
18 properties of solvents and drug carriers. In this study, we evaluated the solubility of naproxen,  
19 ketoprofen and flurbiprofen in two mixtures based on levulinic acid (Lev). The effect of water on  
20 the solubilizing potential and viscosity of these mixtures was assessed. Also, the stability of the  
21 studied drugs was monitored up to one year. The LMM based on randomly methylated- $\beta$ -  
22 cyclodextrin (RAMEB) showed the highest solubilizing potential (up to 5012-fold the aqueous  
23 solubility) compared to the DES based on choline chloride. Formation of RAMEB/drug inclusion  
24 complex in the LMM was evidenced by nuclear magnetic resonance spectroscopy (NMR). Adding  
25 water (10 wt%) sharply reduced the viscosity of both mixtures while maintaining their high  
26 solubilizing ability. Moreover, the studied drugs were stable up to 90% in both mixtures after a  
27 year at 25°C. Such low-viscous mixtures endowed with high solubilizing and stabilizing properties  
28 have great potential for pharmaceutical applications.

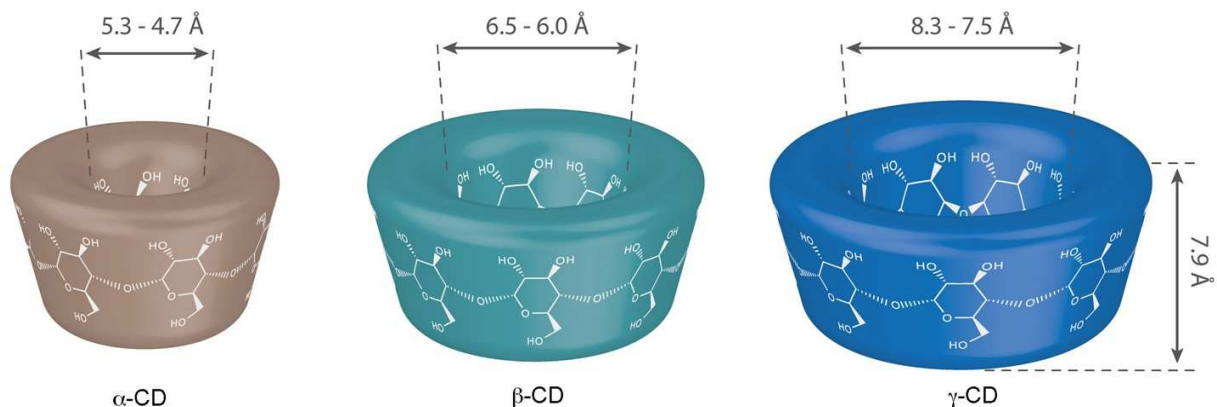
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## 30 1. Introduction

31 Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed and  
32 used medicines [1]. They possess anti-pyretic, analgesic, and anti-inflammatory properties [2,3].

33 The main mechanism of action of NSAIDs is the inhibition of the cyclooxygenase activity and  
34 therefore the synthesis of prostaglandins. NSAIDs are divided into different chemical classes that  
35 have different therapeutic efficacy [4]. Ketoprofen, naproxen and flurbiprofen belong to the  
36 propionic acid family and are known to be highly permeable but barely soluble in water. They are  
37 classified as class II drugs according to the Biopharmaceutics Classification System (BCS) [5]. Their  
38 poor solubility results in insufficient and uneven bioavailability. Therefore, doses higher than  
39 those required for therapeutic effect are administered causing severe adverse effects and  
40 reducing patient compliance [6]. The most prevalent adverse effect of NSAIDs usage is local  
41 irritation of the gastric mucosa [7]. Several approaches have been tested to reduce the  
42 gastrointestinal irritation of NSAIDs, including prodrug formation, addition of neutralizing  
43 excipients and co-prescription of anti-ulcer agents [8]. Cyclodextrins (CDs) have been shown to  
44 form complexes with NSAIDs thus providing protection against gastrointestinal disorders caused  
45 by these drugs [9–11]. Moreover, CDs have been used successfully to increase the solubility of  
46 NSAIDs and several are currently marketed as solid or solution-based CD complex formulations  
47 [12].

48 CDs are cyclic oligosaccharides formed by 6 ( $\alpha$ -CD), 7 ( $\beta$ -CD) or 8 ( $\gamma$ -CD) ( $\alpha$ -1,4)-linked D-  
49 glucopyranose units. They are cone-shaped molecules with a hydrophilic surface and a  
50 hydrophobic cavity (Figure 1).



51

52 Figure 1: Structure of the three native cyclodextrins. (Adapted from de Santana et al. 2020 [13])

53

54 CDs are used as functional pharmaceutical excipients due to their ability to form inclusion  
 55 complexes with lipophilic, poorly water-soluble compounds. The complex formation increases  
 56 the solubility and stability of the encapsulated molecule and masks some of its undesired  
 57 properties such as gastrointestinal and ocular irritation as well as unpleasant smell or taste [14].

58 In order to improve the native CDs' performance, more precisely seeking solubility and binding  
 59 enhancement, a series of methylated, hydroxypropylated and sulfobutylated CDs derivatives  
 60 were developed [15,16].

61 Other strategies have been proposed to improve the dissolution characteristics of NSAIDs such  
 62 as encapsulation in polymer and lipid-based carriers [17], entrapment in dendrimers [18] and the  
 63 use of cosolvents [19,20]. The latter approach is a simple and effective method commonly used  
 64 in the pharmaceutical industry [21], where non-toxic cosolvents are used to enhance the  
 65 solubility of poorly water-soluble compounds.

66 In this context, deep eutectic solvents (DESs), have emerged as promising alternatives to  
 67 conventional solvents [22–25]. DESs consisting of components from natural origin, also called

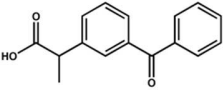
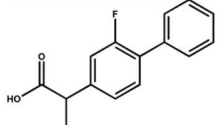
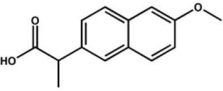
68 natural deep eutectic solvents (NADESs), hold great promise for pharmaceutical applications  
69 because of their biodegradability, low toxicity and ability to dissolve chemically diverse  
70 compounds, including water-insoluble ones [26–30]. DESs are obtained by mixing organic  
71 compounds, generally a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD),  
72 generating eutectic mixtures with melting points that are much lower than those of the individual  
73 components. The exact definition of DESs is still a controversial topic since there is a lack in  
74 addressing the fundamentals and the nature of these solvents [31]. However, the number of  
75 studies focusing on their applications is in continuous growth, what lead to the generation of  
76 numerous new combinations with versatile properties. It is important to note that researchers  
77 are becoming more conscious to the use of the right term for naming their solvents and hence  
78 more general terms, like “low melting mixtures” (LMMs), are being used to designate the new  
79 mixtures with melting points lower than the forming compounds.

80 Recently, El Achkar et al. have introduced new low melting mixtures (LMMs) based on CDs that  
81 are liquid at room temperature. These mixtures showed an interesting solubilizing potential. For  
82 example, *trans*-anethole was up to 1300 times more soluble in the CD-based LMM randomly-  
83 methylated- $\beta$ -CD:Levulinic acid (RAMEB:Lev) than in water [32]. Moreover, it was shown that,  
84 despite being part of the solvent, the CD retained its complexing ability and inclusion complexes  
85 were identified in the intact LMM, also upon water addition [33].

86 The aim of this work was to evaluate the effect of CD on NSAIDs’ solubility by comparing the  
87 solubilizing potential of RAMEB:Lev with that of a conventional DES, choline chloride:levulinic  
88 acid (ChCl:Lev). For this, three poorly water-soluble NSAIDs (ketoprofen, naproxen, and  
89 flurbiprofen) were chosen as a model (Table 1). In addition, the effect of water on the viscosity

90 and solubilization properties of the used mixtures was assessed, and the formation of inclusion  
91 complexes in the LMM and in the (LMM-water) binary systems was highlighted.

92 Table 1: Chemical structures, logP and water solubility values and absorption status of the studied  
93 NSAIDs.

| NSAID               | Structure   | LogP  | Solubility in water at   |                                      | Absorption |
|---------------------|---|-------|--------------------------|--------------------------------------|------------|
|                     |   |       | 25°C (mg/mL)             |                                      |            |
| <b>Ketoprofen</b>   |    | 3.12* | 0.11 [20]                | Rapidly and completely absorbed [34] |            |
| <b>Flurbiprofen</b> |    | 4.16* | 0.03 [35]<br>0.04 [36]   | Rapidly and completely absorbed [37] |            |
| <b>Naproxen</b>     |  | 3.18* | 0.025 [38]<br>0.065 [39] | Rapidly and completely absorbed [40] |            |

94 \*: pubchem.ncbi.nlm.nih.gov

## 95 2. Experimental section

### 96 2.1. Materials

97 Levulinic acid (98%) and choline chloride ( $\geq 98\%$ ) were purchased from Sigma-Aldrich, France.  
98 Randomly methylated- $\beta$ -CD (RAMEB, DS = 12.9) was provided by Roquette Frères (Lestrem,  
99 France). Ketoprofen was purchased from Alfa Aesar, France. Naproxen and flurbiprofen were  
100 provided by LIGAND Pharmaceuticals (San Diego, CA, USA).

101 All reagents were used as received except for choline chloride that was incubated at 60 °C for at  
102 least two weeks before use; to ensure it was dry enough and ready to be used.

103

## 104 2.2. Preparation of the low melting mixtures

105 The low melting mixtures were prepared by mixing at the appropriate proportions the HBA and  
106 the HBD (Table 2). These mixtures were then stirred and heated at 50°C until a clear homogenous  
107 liquid was formed. The resulting mixtures were left to cool at room temperature before being  
108 used. Their water content (wt%) was determined using a coulometric Karl Fischer titrator  
109 (Mettler Toledo C20S) (Table 2).

110 Table 2: Composition and water content of the prepared low melting mixtures.

| <b>Solvent</b>   | <b>HBA</b>               | <b>HBD</b> | <b>HBA:HBD<br/>molar ratio</b> | <b>Water<br/>content (wt%)</b> |
|------------------|--------------------------|------------|--------------------------------|--------------------------------|
| <b>ChCl:Lev</b>  | Choline chloride         | Levulinic  | 1:2                            | 0.84 ± 0.08                    |
| <b>RAMEB:Lev</b> | Randomly methylated β-CD | acid       | 1:27                           | 3.04 ± 0.06                    |

111

## 112 2.3. Viscosity measurements

113 Viscosity ( $\eta$ ) measurements were carried out using a Stabinger SVM 3001 (Anton Paar). The  
114 temperature was controlled to within 0.005°C. The overall uncertainty on the viscosity was  
115 estimated to be 2%. All measurements were performed at atmospheric pressure and at  
116 temperatures ranging between 20 and 40°C.



## 117 2.4. Solubility assessment

118 For each NSAID, an excess amount was added to a vial containing a well-defined amount of the  
119 mixture. The resulting suspension was stirred at 25°C for 24 h to ensure that the solubility  
120 equilibrium was attained. Then, the undissolved remaining excess was removed by filtration  
121 through 0.45 µm glass-fiber syringe filter, and the clear saturated solution was used to determine  
122 the solubility. In this aim, appropriately diluted solutions were prepared in ethanol and assayed  
123 for their concentrations using a UV-Vis spectrophotometer (Perkin Elmer Lambda 2S) at the  
124 corresponding maximum wavelength ( $\lambda_{max}$ ).

125 For assessing the solubility of the studied NSAIDs in the systems with different water content (0-  
126 100 wt%), the mixture-water solutions were prepared prior to the addition of the NSAIDs. The  
127 undissolved excess of the drugs was then filtered, and the samples were used for the solubility  
128 assessment as well as for the diffusion ordered spectroscopy (DOSY) experiments with D<sub>2</sub>O insert.

129

## 130 2.5. Phase solubility study

131 The solubility study was carried out according to the method reported by Higuchi and Connors  
132 [41]. Excess amounts of the NSAIDs were added to aqueous solutions containing increasing  
133 concentrations of RAMEB (0 – 10 mM). The flasks were then sealed and stirred at 25°C for 24 h  
134 to ensure that the equilibrium was reached. Subsequently, the suspensions were filtered by 0.45  
135 µm glass-fiber syringe filters, appropriately diluted in ethanol and analysed for their  
136 concentrations using UV-Vis spectrophotometry. The formation constant ( $K_f$ ) of the complex was  
137 calculated from the slope of the phase-solubility diagram, using equation (1):

138 
$$K_f = \frac{\text{slope}}{S_0(1-\text{slope})} \quad (1)$$

139 where  $S_0$  is the intrinsic solubility of the NSAID in pure water and the slope is the slope of the  
140 phase solubility profile.

141

## 142 2.6. Nuclear magnetic resonance studies

143 All NMR experiments have been recorded on a Bruker Avance III spectrometer operating at 400  
144 MHz for the proton nucleus, equipped with a multinuclear z-gradient BBFO probe head. In all  
145 experiments, the probe temperature was maintained at 30°C and standard 5 mm NMR tubes  
146 with D<sub>2</sub>O insert were used.

147 The <sup>1</sup>H spectra were recorded with the following acquisition parameters: time domain 55 K with  
148 a digital resolution of 0.20 Hz, relaxation delay: 2 s, and 16 scans.

149 2D-ROESY spectrum of RAMEB/NSAID complex in RAMEB:Lev solvent was acquired with a mixing  
150 time of 800 ms during spin-lock, using off resonance pulse program troesyph, and States-TPPI  
151 method with a 2048 K time domain in F2 and 512 experiments in F1 with 142 scans.

152 2D-DOSY spectra have been performed using the bipolar longitudinal eddy current delay (BPPLIED  
153 – Bipolar Pulsed Field Gradient Longitudinal Eddy Delay) pulse sequence. In each DOSY  
154 experiment, a series of 16 spectra with 20 K points were collected, with 16 scans. The pulse  
155 gradients have been incremented in 16 steps from 2 to 98 % of the maximum gradient strength  
156 in a linear ramp. Diffusion times ( $\Delta$ ) and gradient pulse durations ( $\delta$ ) were optimized for each  
157 experiment in order to achieve a 95 % decrease in resonance intensity with respect to the guest

158 molecule; typically,  $\delta$  has been set between 5 and 1.5 ms and  $\Delta$  between 400 and 75 ms. After  
159 Fourier transformation, phase and baseline correction, the diffusion dimension of the 2D-DOSY  
160 spectra was processed by means of MestRenova software (version 11.0.2). The diffusion  
161 constants were calculated by Peak Heights Fit method with 128 points in diffusion dimension.  
162 Among the DOSY measurements, only two were performed in duplicates, and the uncertainty  
163 was estimated to be between 2 and 4%.

164

## 165 2.7. Stability studies

166 The stability of the studied NSAIDs was determined by comparing their solubility in both mixtures  
167 after 3 months and one year. For this purpose, the samples used for solubility assessment were  
168 stored at room temperature and away from light for the desired periods.

169

## 170 3. Results and discussion

### 171 3.1. Solubility assessment

172 The solubility of the three NSAIDs, naproxen, ketoprofen and flurbiprofen, was assessed in  
173 ChCl:Lev (1:2) which is a conventional DES and in RAMEB:Lev (1:27) which is a CD-based LMM  
174 [33]. The results, represented as the mean  $\pm$  SD of at least three independent experiments, are  
175 summarized in Table 3 along with the solubility advantages (ratio of solubility of the NSAID in the  
176 mixtures to its solubility in water).

177 Table 3: Solubility of naproxen, ketoprofen, and flurbiprofen in the solvents under study and  
 178 solubility advantage compared to water at 25°C.

179

| NSAID               | Solubility (mg/mL) |                |               | Solubility advantage |          |
|---------------------|--------------------|----------------|---------------|----------------------|----------|
|                     | Rameb:Lev (1:27)   | ChCl:Lev (1:2) | Water         | Rameb:Lev            | ChCl:Lev |
| <b>Ketoprofen</b>   | 189.65 ± 5.74      | 149.14 ± 4.24  | 0.12 ± 0.01   | 1580                 | 1243     |
| <b>Flurbiprofen</b> | 135.33 ± 4.89      | 115.86 ± 4.3   | 0.027 ± 0.004 | 5012                 | 4291     |
| <b>Naproxen</b>     | 50.05 ± 0.9        | 50.71 ± 1.17   | 0.03 ± 0.001  | 1668                 | 1690     |

180

181 The results show a great solubility enhancement for all studied NSAIDs in both mixtures  
 182 compared to their water solubility (solubility advantages range between 1243 and 5012). Few  
 183 data is available in the literature for the solubility of ketoprofen and naproxen in DESs. Indeed,  
 184 their solubility was determined in ChCl:Lev (1:2) at 37°C as equal to 192.9 ± 3.62 and 31.67 ± 0.40  
 185 mg/mL, respectively [26]. Taking into account the effect of temperature on viscosity and the  
 186 effect of viscosity on experimental errors, we can say that our findings are in accordance. In the  
 187 same study, the solubility of these NSAIDs was determined in a series of other DESs where those  
 188 based on levulinic acid and propylene glycol showed the highest solubilizing potential with  
 189 solubility advantages up to 980 and 3108 in the tetrapropylammonium bromide:propylene glycol  
 190 (1:2) DES for ketoprofen and naproxen, respectively. Naproxen's solubility was also investigated  
 191 in other DESs, and despite the fact that the values of solubility advantage vary from a study to  
 192 another, the order of solubility enhancement is always respected [27,42–44].

193 Moreover, the solubility values determined for ketoprofen, flurbiprofen and naproxen in water  
194 are in good agreement with those found in the literature (Table 1).

195 Additionally, our results show that the solubility of the drugs is either equally enhanced in both  
196 mixtures (naproxen) or more enhanced in RAMEB:Lev (ketoprofen and flurbiprofen) (Table 3).

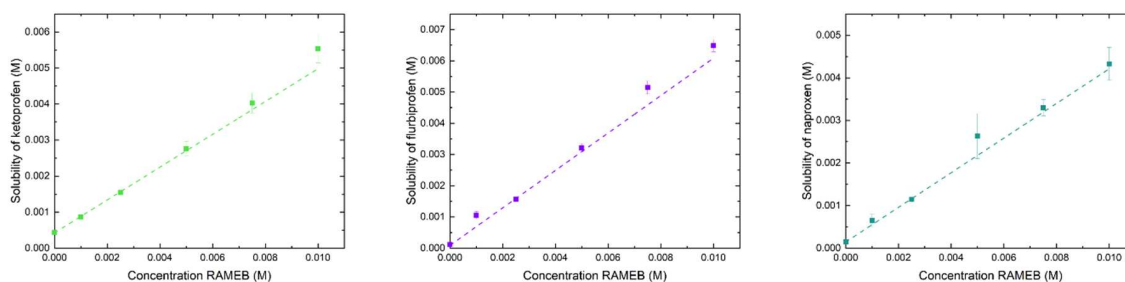
197 This difference in the solubilizing potential taken along with the complexing abilities of  
198 RAMEB:Lev raises the question of whether the solubility may be due, in part, to the formation of  
199 inclusion complexes.

200

## 201 3.2. Evidence of inclusion complex formation

### 202 3.2.1. Phase solubility analysis

203 It is well known that CDs interact with poorly water-soluble compounds and increase their  
204 apparent solubility, through the formation of non-covalent dynamic inclusion complexes. Phase  
205 solubility analysis is a traditional approach to determine the stability constant of the inclusion  
206 complex and the stoichiometry at equilibrium [12,45]. The phase solubility diagram, obtained by  
207 plotting the apparent solubility of the studied NSAIDs against RAMEB concentration, is reported  
208 in Figure 2.



209

210 Figure 2: Phase solubility diagrams of ketoprofen, flurbiprofen and naproxen in RAMEB aqueous  
211 solutions at 25°C (n=3).

212

213 The solubility of ketoprofen, flurbiprofen and naproxen increases linearly as function of RAMEB  
214 concentration ( $R^2 = 0.998$ ,  $R^2 = 0.997$  and  $R^2 = 0.999$  respectively). This linear correlation  
215 indicates the formation of 1:1 inclusion complex ( $A_L$ -type phase solubility diagram) between  
216 RAMEB and each NSAID. The formation constant of the complexes (RAMEB/Ketoprofen) and  
217 (RAMEB/naproxen) were calculated as  $1960 \pm 723 \text{ M}^{-1}$  and  $4710 \pm 927 \text{ M}^{-1}$ , respectively. These  
218 values are in agreement with the values ( $2000 \text{ M}^{-1}$  and  $6900 \text{ M}^{-1}$ ) determined by Mura et al. for  
219 the complex between methylated- $\beta$ -cyclodextrin and ketoprofen (Me- $\beta$ -CD/Ketoprofen), and  
220 naproxen (Me- $\beta$ -CD/naproxen) in the same temperature conditions [46]. The authors also  
221 determined the formation constants of the inclusion complexes of ketoprofen with  $\beta$ -CD ( $810 \text{ M}^{-1}$ ),  
222 hydroxyethyl- $\beta$ -CD ( $780 \text{ M}^{-1}$ ), and hydroxypropyl- $\beta$ -CD ( $970 \text{ M}^{-1}$ ). The formation constants for  
223 the inclusion complexes of naproxen with the same CDs were also determined:  $\beta$ -CD ( $1700 \text{ M}^{-1}$ ),  
224 hydroxyethyl- $\beta$ -CD ( $2200 \text{ M}^{-1}$ ), and hydroxypropyl- $\beta$ -CD ( $2600 \text{ M}^{-1}$ ). The values obtained can be  
225 explained by the fact that the methyl groups of Me- $\beta$ -CD expand the hydrophobic region and  
226 hence increase substrate binding through hydrophobic interactions. Expanding the hydrophobic  
227 region with hydroxyethyl and hydroxypropyl groups was not as advantageous. This may be due  
228 to the bulk effect of the substituents that hinders the interactions between the CD and the NSAID.  
229 Expanding the hydrophobic region with methyl groups was also more advantageous for  
230 flurbiprofen complexation. As evaluated by Cirri et al., the formation constants were as follow:  
231  $\beta$ -CD ( $1700 \text{ M}^{-1}$ ), hydroxyethyl- $\beta$ -CD ( $2775 \text{ M}^{-1}$ ), and Me- $\beta$ -CD ( $11570 \text{ M}^{-1}$ ) [47]. This value is

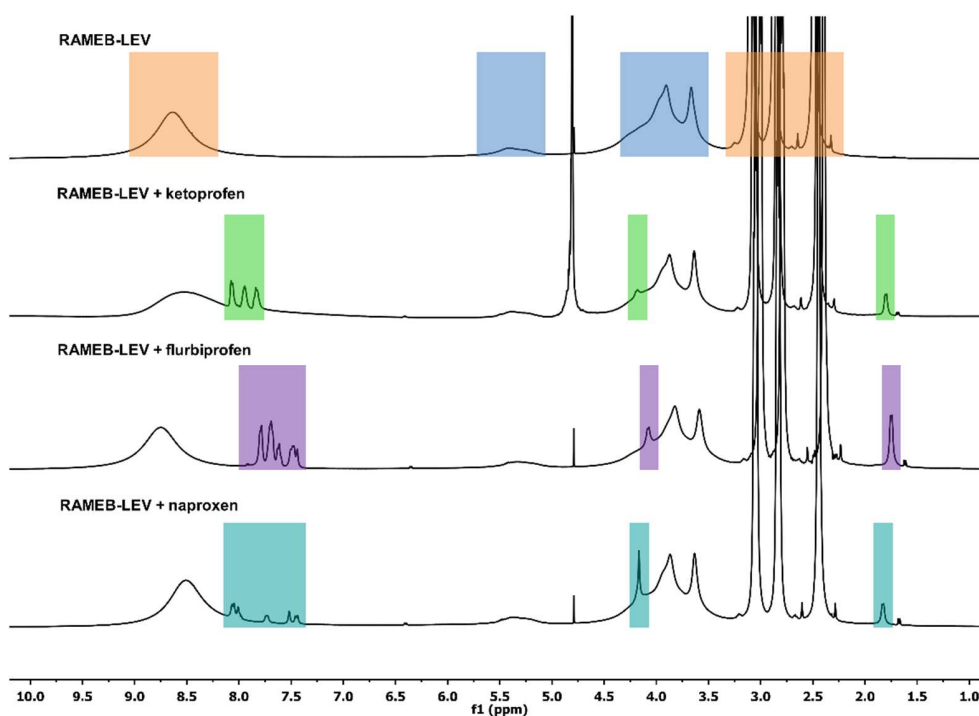
232 consistent with the formation constant found for the complex RAMEB/flurbiprofen in our case  
233 ( $16300 \pm 485 \text{ M}^{-1}$ ).

234

### 235 3.2.2. RAMEB/NSAID inclusion complex in the LMM

236 Knowing that RAMEB can form an inclusion complex with the different studied NSAIDs in aqueous  
237 solution, we were interested in finding evidence of this complexation upon the solubilization of  
238 these NSAIDs in the LMM RAMEB:Lev. The classical approach described earlier for proving the  
239 formation of inclusion complex cannot be used here, since the CD is now a component of the  
240 solvent. Therefore, the 2D ROESY NMR spectroscopy was used. We started by identifying the  
241 peaks of the  $^1\text{H}$  NMR spectrum of ketoprofen, flurbiprofen and naproxen in RAMEB:Lev. Since  
242 the viscosity of the solvent hinders the acquisition of well-defined NMR spectra (Figure 3), peaks  
243 attribution was achieved by analogy to the  $^1\text{H}$ -NMR spectra of these NSAIDs in the following  
244 references [48], [49] and [50].

245



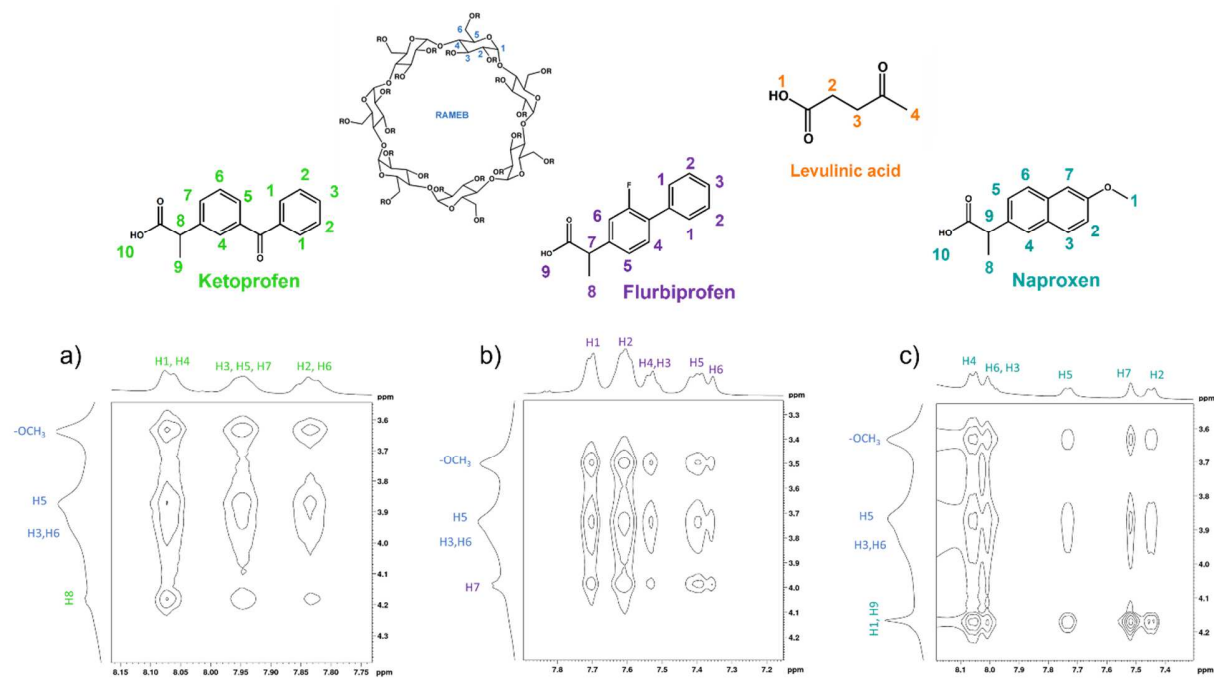
246

247 Figure 3:  $^1\text{H}$  NMR spectra of RAMEB:Lev alone and in the presence of ketoprofen, flurbiprofen  
 248 and naproxen respectively. (Blue box covers the peaks of RAMEB, orange box covers the peaks  
 249 of levulinic acid, green box covers the peaks of ketoprofen, purple box covers the peaks of  
 250 flurbiprofen, and turquoise box covers the peaks of naproxen).

251

252 Figure 4 shows a section of the ROESY spectrum of ketoprofen, flurbiprofen and naproxen  
 253 respectively in RAMEB:Lev. Significant correlations were observed between the aromatic  
 254 hydrogens of the studied NSAIDs and H3 and H5 of RAMEB. These two latter protons are located  
 255 inside the CD cavity. Such correlations confirm the formation of inclusion complex between  
 256 RAMEB and the studied NSAIDs, proving that the CD is still capable of forming inclusion  
 257 complexes despite being part of the solvent.





258

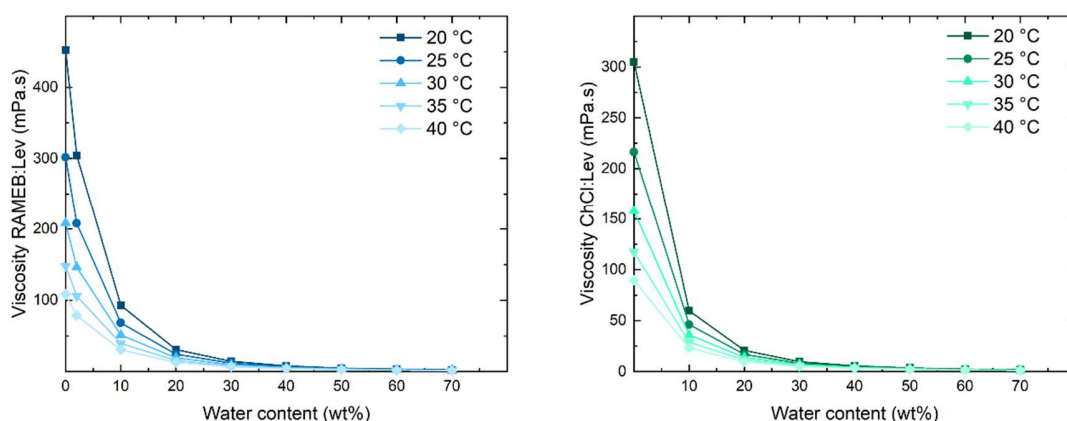
259 Figure 4: ROESY spectrum of RAMEB/ketoprofen (a), RAMEB/flurbiprofen (b) and  
 260 RAMEB/naproxen (c) complexes in the RAMEB:Lev solvent (recorded with a D<sub>2</sub>O insert).

261

### 262 3.3. Effect of water

#### 263 3.3.1. Viscosity measurements

264 As mentioned repeatedly, the high viscosity of DESs is a serious practical problem. This problem  
 265 can be solved by adding some water, since water addition is frequently employed to modulate  
 266 the viscosity, polarity and conductivity of DESs [51]. Here we report the effect of water addition  
 267 on the viscosity of the studied mixtures at different temperatures (Figure 5).



268

269 Figure 5: Effect of water content and temperature on the viscosity of RAMEB:Lev and ChCl:Lev.

270

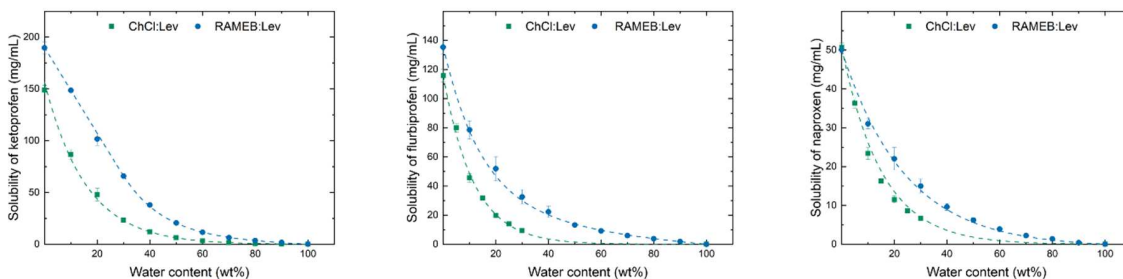
271 The viscosity of the two studied mixtures is highly affected by the changes in water content and  
 272 temperature. For example, the addition of 10 wt% of water decreases the viscosity of both  
 273 mixtures by 80% at 20°C. The obtained result gives evidence of the tunability of these mixtures.

274

### 275 3.3.2. Solubility of NSAIDs in the binary systems

276 It is known that DESs lose their identity and properties upon excess water addition and the  
 277 system becomes an aqueous solution of its constituents [51,52]. Herein, we studied the effect of  
 278 water addition on the solubilizing properties of RAMEB:Lev and ChCl:Lev.

279



280

281 Figure 6: Solubility of ketoprofen, flurbiprofen and naproxen in the binary systems (n=2).

282

283 As can be seen in Figure 6, the solubility of ketoprofen, flurbiprofen and naproxen decreases as  
 284 the water content in the binary system increases. This decrease does not follow the same pattern  
 285 for the two systems; it tends to be sharper in the case of ChCl:Lev, and the ratio of the NSAIDs'  
 286 solubility in these two systems ( $S_{RAMEB:Lev}/S_{ChCl:Lev}$ ) is always in favor of RAMEB:Lev (Table 4).  
 287 This ratio has not been calculated for the whole range because experimental values of  
 288 flurbiprofen and naproxen solubility could not be determined.

289

290 Table 4: Ratio of NSAIDs' solubility in the binary systems.

| NSAID        | Water wt%                            | 90  | 80   | 70   | 60  | 50   | 40   | 30   | 20   | 10   | 0    |
|--------------|--------------------------------------|-----|------|------|-----|------|------|------|------|------|------|
| Ketoprofen   | $\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$ | 5.7 | 4.59 | 4.02 | 3.5 | 3.15 | 3.09 | 2.73 | 2.43 | 1.78 | 1.27 |
|              |                                      | -   | -    | -    | -   | -    | -    | 2.23 | 1.9  | 1.33 | 0.99 |
| Flurbiprofen | $\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$ | -   | -    | -    | -   | -    | -    | 2.23 | 1.9  | 1.33 | 0.99 |
|              |                                      | -   | -    | -    | -   | -    | -    | 3.48 | 2.62 | 1.72 | 1.17 |
| Naproxen     | $\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$ | -   | -    | -    | -   | -    | -    | 3.48 | 2.62 | 1.72 | 1.17 |
|              |                                      | -   | -    | -    | -   | -    | -    | -    | -    | -    | -    |

291 (-): not determined.

292 Taken together, these results demonstrate the efficiency of RAMEB:Lev as solubilizer more  
293 resistant to dilution thanks to its complexing abilities.

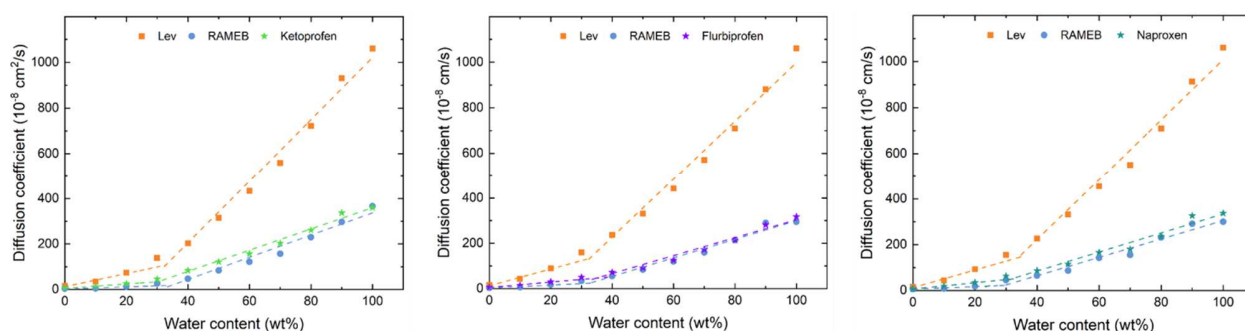
294

### 295 3.3.3. Diffusion coefficients

296 We were also interested in studying the effect of water addition on the diffusion coefficients of  
297 the individual components of the mixture for many considerations. First, this experiment, among  
298 others, allows us to prove the formation of inclusion complexes [53]. In addition, it is useful to  
299 determine the type of system (water-in-DES) or (DES-in-water) dominant at a given amount of  
300 water in the binary system [54]. Moreover, and since the diffusion of molecules is highly  
301 dependent from their interactions with the surrounding environment, this technique can also be  
302 useful for predicting which component in the eutectic mixture is governing the interactions with  
303 the solute and therefore the solubilizing potential.

304 The variation of diffusion coefficient values are reported in Figure 7.

305



306

307 Figure 7: Plot of the diffusion coefficients versus water content (wt%) in the binary systems.

308

309 As the water content in the binary system increases, the values of diffusion coefficients increase.  
 310 This increase does not follow the same pattern over the entire dilution range. The 31 ( $\pm 3$ ) wt%  
 311 water content is a turning point. Above and below this critical point, the variation of the diffusion  
 312 coefficient values of the three components follows different linear correlations which from their  
 313 intersections this point was determined. The observed increase is dependent on the molecular  
 314 weight of the molecules and their interactions with the surrounding environment. It is  
 315 noteworthy that the diffusion coefficient values of RAMEB and the studied NSAIDs increase  
 316 almost at the same rate, highlighting the interactions between these two entities and thus  
 317 providing another evidence of the inclusion complex formation. The same conclusions can be  
 318 reached by considering the diffusion coefficient values of levulinic acid and the studied NSAIDs  
 319 in water and in aqueous solution of RAMEB. As can be seen in Table 5, the diffusion coefficients  
 320 of the studied NSAIDs are highly impacted by the presence of RAMEB, while no significant  
 321 changes were observed for levulinic acid.

322

323 Table 5: Diffusion coefficients (D) of levulinic acid, ketoprofen, flurbiprofen and naproxen in  
 324 water, in absence or in presence of RAMEB.

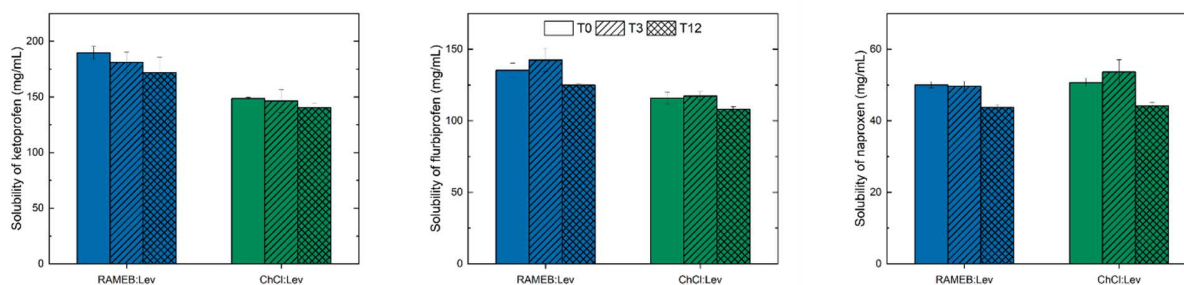
| <b>D (<math>10^{-8}</math> cm<sup>2</sup>/s)</b> | <b>RAMEB</b> | <b>Levulinic acid</b> | <b>Ketoprofen</b> | <b>Flurbiprofen</b> | <b>Naproxen</b> |
|--|--------------|-----------------------|-------------------|---------------------|-----------------|
| <b>Water</b>                                     |              | 1180                  | 639               | 610                 | 650             |
| <b>Water +<br/>RAMEB</b>                         | 320 $\pm$ 32 | 1060                  | 366               | 317                 | 337             |

325

326 3.4. Stability studies

327 The stability of ketoprofen, flurbiprofen and naproxen in RAMEB:Lev and ChCl:Lev was checked  
328 after a period of three months, also after a year at 25°C. It was evaluated by comparing the  
329 amount of NSAID determined in the freshly prepared samples (T0) to its amount after 3 months  
330 (T3) and one year (T12) in the same samples.

331 The studied NSAIDs showed a relatively good stability, up to 90% after a year of storage. It is  
332 important to note that the stability profiles were comparable in RAMEB:Lev or ChCl:Lev (Figure  
333 8).



334  
335 Figure 8: Ketoprofen, flurbiprofen and naproxen solubility in RAMEB:Lev  
336 determined in freshly prepared samples (T0), after a 3-month (T3) and one year (T12) storage at  
337 25°C.

338  
339 Summarizing the results obtained on viscosity, solubility and stability, we can say that RAMEB:Lev  
340 represents a stable and tunable mixture with good solubilizing properties and hence considered  
341 promising for future applications in formulation sciences.

## 343 4. Conclusion

344 This work highlighted the advantages of using the new emerging class of CD-based low melting  
345 mixtures that have demonstrated promising solubilizing potential and stability. In this study, we  
346 confirmed that, in addition to their role as components of the solvent, CDs are able to interact  
347 with guest molecules to form inclusion complexes, using three poorly water-soluble NSAIDs:  
348 ketoprofen, flurbiprofen and naproxen. The inclusion complex formation is a highly beneficial  
349 property of the CD-based LMM which provides efficient drug solubilization and stability.  
350 Interestingly, the addition of a small amount of water leads to a sharp decrease in the viscosity  
351 of the studied solvents while maintaining their high solubilizing ability. Therefore, such systems  
352 have great potential for future applications; in particular, they can be used as a formulation  
353 strategy for the administration of NSAIDs ensuring their good solubility as well as protection  
354 against gastrointestinal irritation caused by these drugs.

355

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359

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# Graphical abstract

