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

3

4 Sarah El Masri^{a,b}, Steven Ruellan^a, Maha Zakhour^b, Lizette Auezova^b, and Sophie Fourmentin^{a,*}

5 ^a Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV), EA 4492 SFR Condorcet

6 FR CNRS 3417, Université du Littoral-Côte d'Opale, Dunkerque, France.

7 ^b Bioactive Molecules Research Laboratory, Doctoral School of Sciences and Technologies, Faculty

8 of Sciences, Lebanese University, Fanar, Lebanon.

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- β -
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.

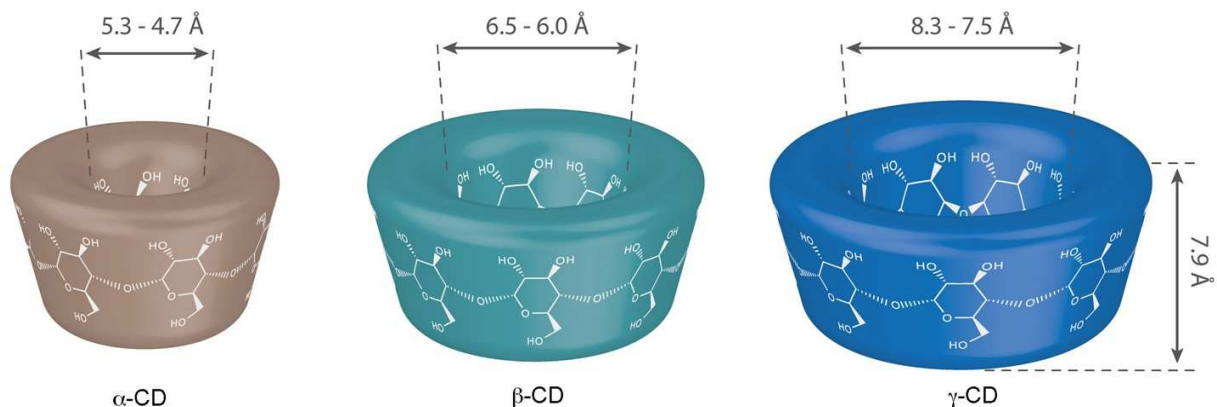
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30 1. Introduction

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 (α -CD), 7 (β -CD) or 8 (γ -CD) (α -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

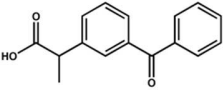
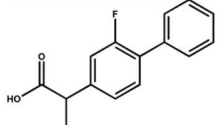
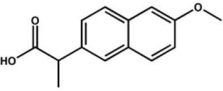
68 natural deep eutectic solvents (NADESs), hold great promise for pharmaceutical applications
69 because of their biodegradability, low toxicity and ability to dissolve chemically diverse
70 compounds, including water-insoluble ones [26–30]. DESs are obtained by mixing organic
71 compounds, generally 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- β -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)		
Ketoprofen		3.12*	0.11 [20]	Rapidly and completely absorbed [34]	
Flurbiprofen		4.16*	0.03 [35] 0.04 [36]	Rapidly and completely absorbed [37]	
Naproxen		3.18*	0.025 [38] 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- β -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.

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

111

112 2.3. Viscosity measurements

113 Viscosity (η) 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 (λ_{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 D₂O 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 D₂O insert were used.

147 The ¹H 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 (BPPLD
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 (Δ) and gradient pulse durations (δ) were optimized for each
157 experiment in order to achieve a 95 % decrease in resonance intensity with respect to the guest

158 molecule; typically, δ has been set between 5 and 1.5 ms and Δ 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
Ketoprofen	189.65 ± 5.74	149.14 ± 4.24	0.12 ± 0.01	1580	1243
Flurbiprofen	135.33 ± 4.89	115.86 ± 4.3	0.027 ± 0.004	5012	4291
Naproxen	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).

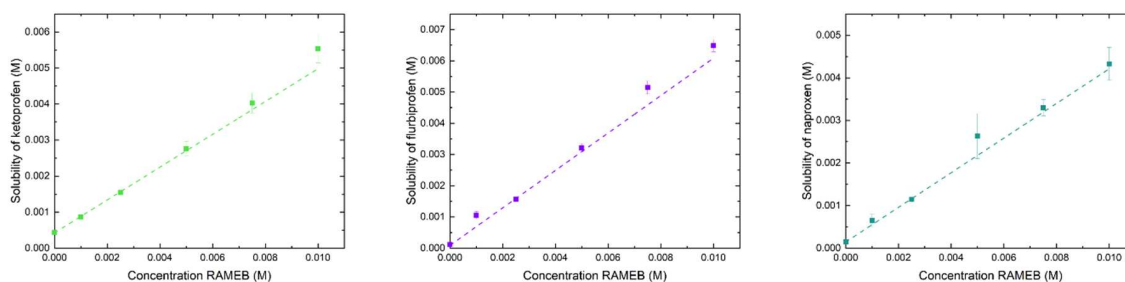
197 This difference in the solubilizing potential taken along with the complexing abilities of
198 RAMEB:Lev raises the question of whether the solubility may be due, in part, to the formation of
199 inclusion complexes.

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 M^{-1} and 6900 M^{-1}) determined by Mura et al. for
219 the complex between methylated- β -cyclodextrin and ketoprofen (Me- β -CD/Ketoprofen), and
220 naproxen (Me- β -CD/naproxen) in the same temperature conditions [46]. The authors also
221 determined the formation constants of the inclusion complexes of ketoprofen with β -CD (810 M^{-1}),
222 hydroxyethyl- β -CD (780 M^{-1}), and hydroxypropyl- β -CD (970 M^{-1}). The formation constants for
223 the inclusion complexes of naproxen with the same CDs were also determined: β -CD (1700 M^{-1}),
224 hydroxyethyl- β -CD (2200 M^{-1}), and hydroxypropyl- β -CD (2600 M^{-1}). The values obtained can be
225 explained by the fact that the methyl groups of Me- β -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 β -CD (1700 M^{-1}), hydroxyethyl- β -CD (2775 M^{-1}), and Me- β -CD (11570 M^{-1}) [47]. This value is

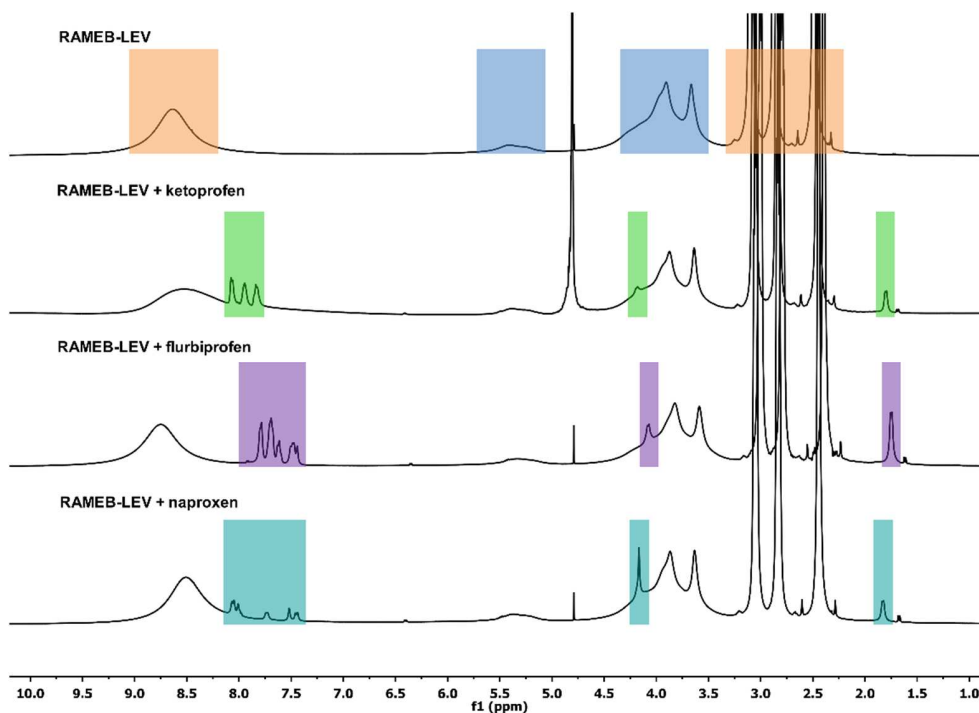
232 consistent with the formation constant found for the complex RAMEB/flurbiprofen in our case
233 ($16300 \pm 485 \text{ M}^{-1}$).

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 ^1H 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 ^1H -NMR spectra of these NSAIDs in the following
244 references [48], [49] and [50].

245

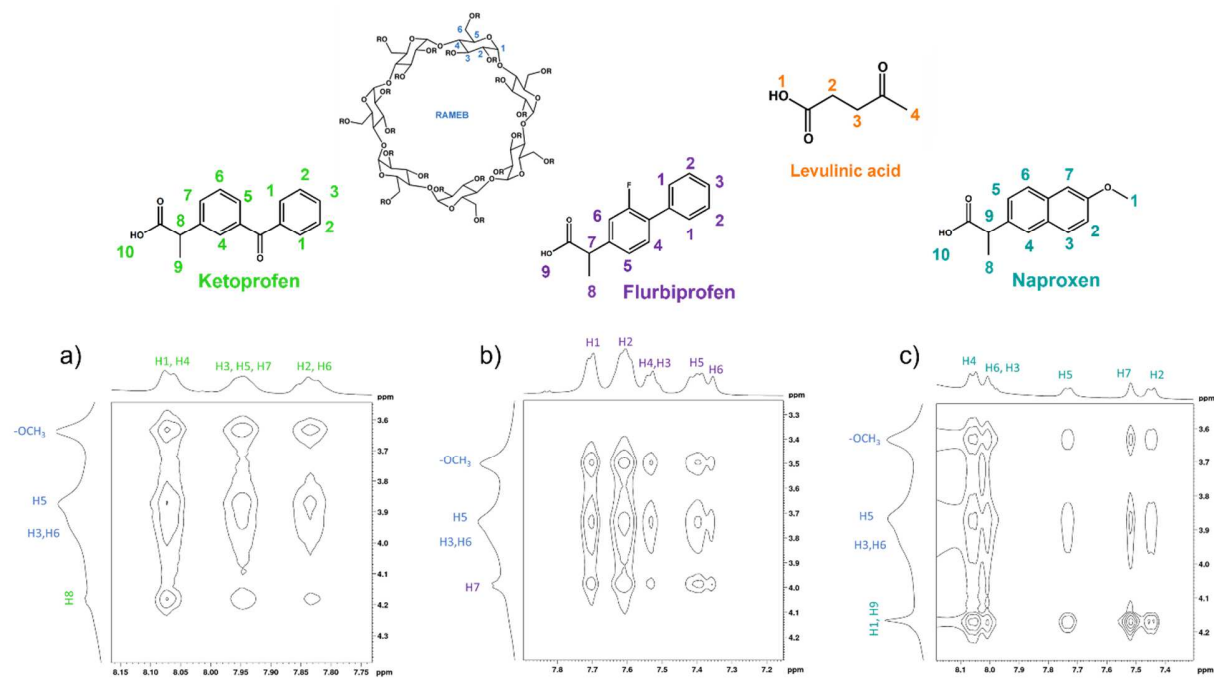


246

247 Figure 3: ^1H 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

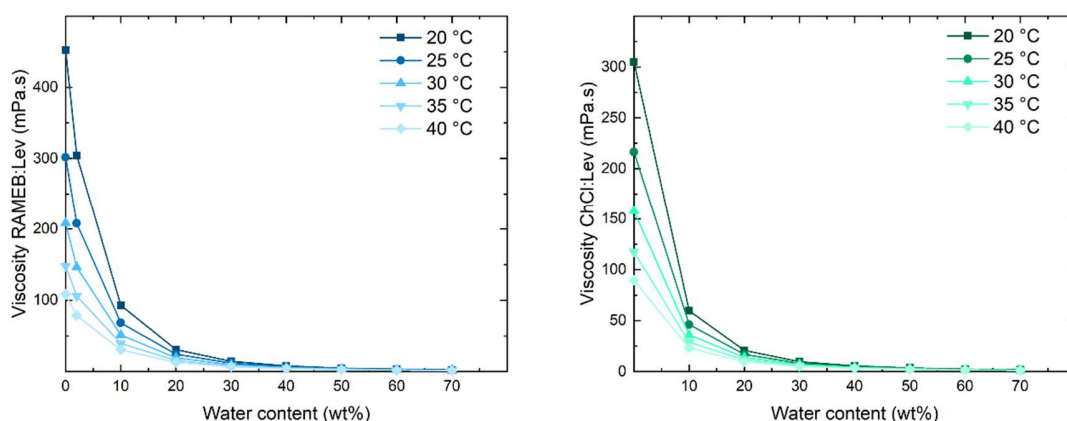
259 Figure 4: ROESY spectrum of RAMEB/ketoprofen (a), RAMEB/flurbiprofen (b) and
 260 RAMEB/naproxen (c) complexes in the RAMEB:Lev solvent (recorded with a D₂O insert).

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

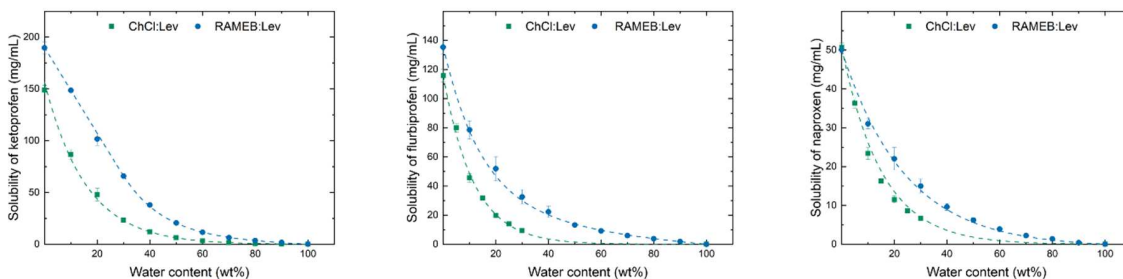
271 The viscosity of the two studied mixtures is highly affected by the changes in water content and
 272 temperature. For example, the addition of 10 wt% of water decreases the viscosity of both
 273 mixtures by 80% at 20°C. The obtained result gives evidence of the tunability of these mixtures.

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
	$\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$	-	-	-	-	-	-	2.23	1.9	1.33	0.99
Flurbiprofen	$\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$	-	-	-	-	-	-	3.48	2.62	1.72	1.17
	$\frac{S_{Rameb:Lev}}{S_{ChCl:Lev}}$	-	-	-	-	-	-	-	-	-	-

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.

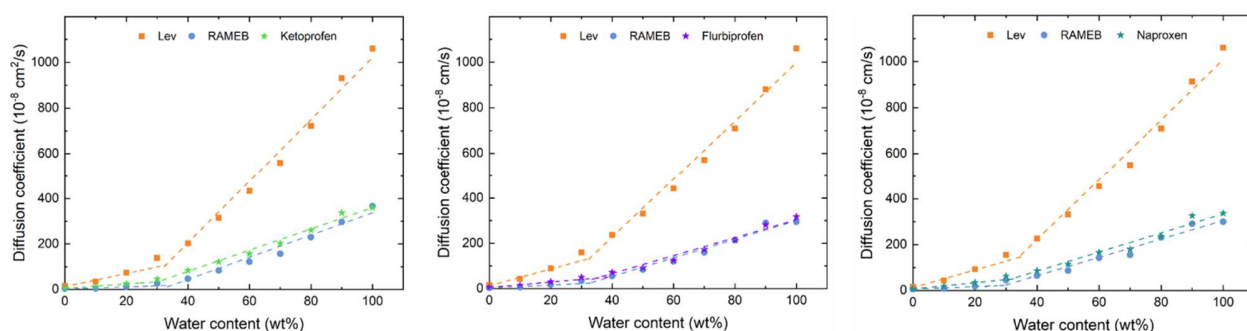
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295 3.3.3. Diffusion coefficients

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 (± 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.

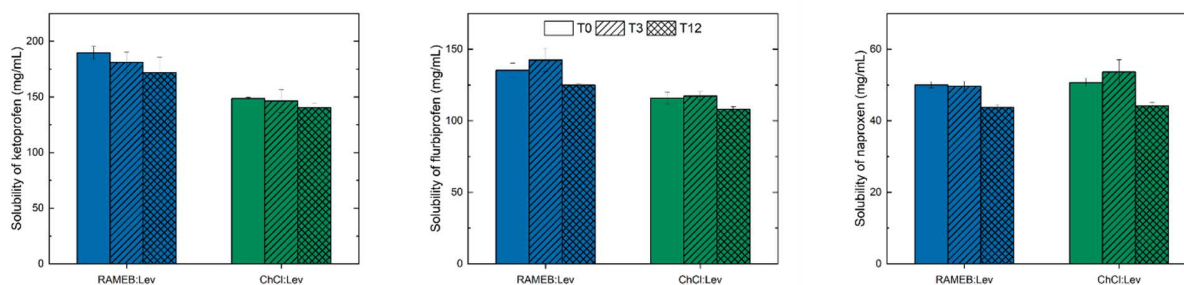
D (10^{-8} cm²/s)	RAMEB	Levulinic acid	Ketoprofen	Flurbiprofen	Naproxen
Water		1180	639	610	650
Water + RAMEB	320 \pm 32	1060	366	317	337

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

