



# Cyclodextrin polymers in combination with water and deep eutectic solvent for the retention of *Eucalyptus citriodora* essential oil

Lamia Nakhle<sup>1,2</sup> · Miriana Kfoury<sup>1</sup> · Isabelle Mallard<sup>1</sup> · H  l  ne Greige-Gerges<sup>2</sup> · David Landy<sup>1</sup>

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## Abstract

*Eucalyptus citriodora* is one of the most widely used essential oils (EOs) because of its various antimicrobial, antioxidant and anti-inflammatory activities. However, its limited aqueous solubility restricts its use. The aim of this study was to develop supramolecular formulations able to retain *E. citriodora* EO in solution. For this purpose, new cyclodextrin (CD) polymers were synthesized and characterized using gel permeation chromatography, FTIR and NMR spectroscopies. Their retention ability toward *E. citriodora* in non-conventional green media (deep eutectic solvent (DES):water mixture), more precisely choline chloride:urea DES:water (70:30 wt%), was evaluated and compared to the corresponding native CD [ $\beta$ -cyclodextrin ( $\beta$ -CD)] or CD derivatives [hydroxypropylated- $\beta$ -cyclodextrin (HP- $\beta$ -CD) and low methylated- $\beta$ -cyclodextrin (CRYSMEB)] using static headspace-gas chromatography (SH-GC). All the studied formulations showed a great capacity to retain and reduce the volatility of *E. citriodora*. The various polymers showed divergent retention efficiencies.

**Keywords** Cyclodextrins polymers · Deep eutectic solvents · Essential oil · *Eucalyptus citriodora* · Retention · Volatility

## Introduction

Essential oils (EOs) are a natural source of aromas and bio-active molecules with a wide range of properties including antioxidant, antimicrobial, anti-inflammatory and anti-carcinogenic. The incorporation of EOs in formulations has always attracted cosmetic, pharmaceutical and food industries. However, their use is challenging due to their volatile nature and their low aqueous solubility. Various methods could be envisaged to reduce their volatility and improve their solubility, including encapsulation in cyclodextrins (CDs) [1, 2] or cosolvency which means adding one or more solvents to the formulation [3, 4].

CDs are natural cyclic oligosaccharides possessing a truncated tridimensional cone shape characterized by a

relatively hydrophobic internal cavity and a hydrophilic external surface [5]. Native CDs composed of 6, 7, and 8  $\alpha$ -D-glucose residues have different cavity dimensions and are called  $\alpha$ -CD,  $\beta$ -CD, and  $\gamma$ -CD, respectively.  $\beta$ -CD is the most interesting host for the encapsulation of EOs components due to its perfect cavity size [6, 7], efficient drug complexation and loading [8, 9], and relatively low cost [10]. However,  $\beta$ -CD is poorly soluble in water (18 g L<sup>-1</sup>) [11]. Various  $\beta$ -CD derivatives with improved aqueous solubility, inclusion capacity and lower toxicity profiles have been synthesized and efficiently investigated in the literature [2]. The hydroxypropylated- $\beta$ -CD (HP- $\beta$ -CD) and the low methylated- $\beta$ -CD (CRYSMEB) are two of the most studied CD derivatives [6, 9].

CD polymers can be also obtained, by copolymerizing CD molecules with other difunctionalized compounds. They can be synthesized using conventional strategies that require the use of organic solvents or sustainable procedures such as the mechanochemical approach [12]. In addition, their synthesis could be conducted in green media such as natural deep eutectic solvents (NADES) [13]. CD polymers also offer the advantages of superior solubilizing and complexing abilities. For example, an increase in the solubility of *Lavandula angustifolia* and *Mentha piperita* EOs was observed

✉ Miriana Kfoury  
miriana.kfoury@univ-littoral.fr

<sup>1</sup> Unit   de Chimie Environnementale et Interactions sur le Vivant (UCEIV, UR 4492), SFR Condorcet FR CNRS 3417, Universit   du Littoral C  te d'Opale (ULCO), 145 Av. M. Schumann, 9140 Dunkirk, France

<sup>2</sup> Bioactive Molecules Research Laboratory, Faculty of Sciences, Lebanese University, Jdeidet el-Metn 90656, Lebanon

when using CD polymers as compared to native CDs ( $\alpha$ -CD and  $\gamma$ -CD) [14, 15].

On the other hand, deep eutectic solvents (DESs) are neoteric class of green solvents defined as a mixture of two or more compounds that become liquid when mixed and heated at a particular molar ratio [16, 17]. A DES is characterized by a great depression of the melting point as compared to its starting materials and to their ideal mixture [18]. The first and most studied DES is reline, composed of choline chloride (ChCl) and urea (U) mixed at a 1:2 molar ratio [19]. DESs are characterized by low volatility, non-flammability and biocompatibility [20]. Few studies used DES to solubilize EOs and their components. A recent study showed a significant increase in the solubility of *trans*-anethole, as well as star anise and fennel EOs in DESs in comparison to water [21].

To the best of our knowledge, the literature lacks any research that considered combining DESs and CD polymers for the solubilization and retention of EOs. The aim of the present study is to develop new formulations composed of DES and CD polymers to solubilize one of the most used EOs, *E. citriodora*. Water-soluble polymers were synthesized using native  $\beta$ -CD or its derivatives (HP- $\beta$ -CD and CRYSMEB) via the cross-linking method. Various crosslinking agents were used including four carboxylic acids (malonic acid, citric acid, glutaric acid and itaconic acid), poly(ethylene glycol) diglycidyl ether-640 (PEGDGE) and sodium trimetaphosphate (STMP).  $\beta$ -CD/malonic acid, HP- $\beta$ -CD/glutaric acid, HP- $\beta$ -CD/itaconic acid, HP- $\beta$ -CD/PEGDGE, HP- $\beta$ -CD/STMP and all CRYSMEB-based polymers were herein synthesized and reported for the first time. The synthesized polymers were submitted to physico-chemical characterization. Different formulations combining ChCl:U (1:2 molar ratio) DES, water and the encapsulating agent ( $\beta$ -CD, CD derivative or CD polymer) were then prepared. Finally, the retention ability of these formulations towards *E. citriodora* was evaluated using static headspace-gas chromatography (SH-GC).

## Experimental section

### Materials

Choline chloride (ChCl, 98%), glutaric acid and trisodium trimetaphosphate were purchased from Sigma-Aldrich, China. Urea (U, 99%) and poly(ethylene glycol) diglycidyl ether (PEGDGE) Mw 640 were purchased from Sigma-Aldrich, USA. Citric acid and sodium hydroxide were purchased from Sigma-Aldrich, France. Itaconic acid was purchased from Sigma-Aldrich, Germany. Malonic acid was provided by Merck group, Germany.  $\beta$ -CD was provided by Wacker-Chemie, Lyon, France. CRYSMEB (DS = 4.9)

and HP- $\beta$ -CD (DS = 5.6) were provided by Roquette Frères, Lestrem, France. *E. citriodora* EO was provided by Herbes et Traditions, Comines, France. Choline chloride was dried at 60 °C for at least 2 weeks before use. All other compounds were used as received. Distilled water was used throughout this work.

### Preparation of deep eutectic solvent

ChCl:U DES was prepared by heating ChCl and U in a 1:2 molar ratio under stirring at 60 °C until the formation of a clear and homogeneous liquid.

### Synthesis and purification of CD polymers

#### Preparation of CD polymers

CD polymers were prepared by reticulation of either  $\beta$ -CD, HP- $\beta$ -CD or CRYSMEB using several crosslinking agents.

Four acids (malonic acid, citric acid, glutaric acid and itaconic acid) were used following the synthetic procedure as previously reported [22]. The aqueous solutions were prepared by mixing the catalyst ( $\text{KH}_2\text{PO}_4$ ), the CD, and the carboxylic acid with respective concentrations of 0.2, 0.09, and 0.45 mol L<sup>-1</sup>. These solutions were then concentrated by evaporation (at 50 °C and 5 mbar) and then treated at 140 °C for 30 min under reduced atmospheric pressure. The polymers were recovered after the addition of 50 mL of distilled water to the round-bottom flask. The flask was kept under agitation overnight to solubilize the reaction products. The fraction was then filtered off using sintered glass funnel (membrane porosity: 40  $\mu\text{m}$ ). Only trace amounts of insoluble products were obtained so these insoluble fractions were discarded.

Another crosslinking agent, the PEGDGE, was also used for polymer synthesis as previously described [23]. 5 g of CD was dissolved in 21 mL NaOH (1.42 mol L<sup>-1</sup>) and the mixture was stirred mechanically for 16 h at room temperature. PEGDGE was then added dropwise during 20 min under stirring, with a molar ratio of PEGDGE/CD equal to 3/1. The reaction mixture was heated to 30 °C for 5 h under stirring. After cooling for 20 min, the mixture was neutralized using HCl (6 mol L<sup>-1</sup>). The volume of water was reduced by evaporation using the rotary evaporator (at 5 mbar and 50 °C). Then polymers were precipitated in acetone three times and one time in ether. The solid was then collected and dissolved in distilled water.

The linker STMP was also used to prepare CD polymers as previously described [24]. 5–6.6 g of CD were dissolved in 10 mL of NaOH solution (2.5 mol L<sup>-1</sup>), under mechanical stirring (300 rpm) at 30 °C. After 210 min, the temperature was raised to 50 °C, and the desired amount of STMP (0.5, 3, 5 or 10 g with identifying codes of STMP1, STMP2,

STMP3 or STMP4, respectively) was added to the solution under vigorous stirring (600 rpm). After 90 min, 50 mL of water were added, and the pH was neutralized by HCl addition ( $6 \text{ mol L}^{-1}$ ).

### Purification of CD polymers

The soluble fractions obtained with malonic, citric and glutaric acids were purified by several passages on a Sephadex gel column (Sephadex<sup>TM</sup> G-25 Medium) using water as eluent. Polymers with itaconic acid, PEGDGE and STMP were purified by dialysis three times at room temperature for 24 h each time using a Spectra/Por<sup>®</sup> Standard grade regenerated cellulose membrane (molecular weight cut-off of 1 KDa). Solvent was then removed by a rotary evaporator and the obtained polymer fractions were dried under vacuum overnight.

### Characterization of CD polymers

The purification process was followed by a physicochemical characterization of polymers using nuclear magnetic resonance spectroscopy (NMR), Fourier-transform infrared spectroscopy (FTIR) and gel permeation chromatography.

### Nuclear magnetic resonance spectroscopy

NMR spectra were recorded at 25 °C in D<sub>2</sub>O using a Bruker Avance III spectrometer, equipped with a multinuclear z-gradient BBFO probe head, operating at 400 MHz and 161 MHz for <sup>1</sup>H and <sup>31</sup>P NMR (when using STMP as the linker), respectively. Standard 5 mm NMR tubes were used.

In addition, using <sup>1</sup>H-NMR spectra, the number of linker molecules (*n*) for one CD molecule was determined by integrating the respective protons peaks. Then, the wt% of CD in the polymer fraction was calculated using the following equation:

$$CD(\text{wt}\%) = \frac{MW_{CD}}{MW_{CD} + (n \times MW_{linker})} \times 100 \quad (1)$$

where  $MW_{CD}$  and  $MW_{linker}$  stand for the molecular weight of the CD and the linker, respectively.

The synthesis yield was also calculated using the following equation:

$$\text{Synthesis yield}(\%) = \frac{m_{polymer}}{\text{apparentMW}} \times 100 \quad (2)$$

where  $m_{polymer}$  stands for the experimentally obtained mass of polymer after purification, apparent MW stands for the calculated MW of the modified monomer unit using the number of linker molecules per one CD molecule from the

<sup>1</sup>H-NMR analysis, and  $n_{CD}$  stands for the initially introduced CD number of moles.  $m_{polymer}$  over apparent MW represents the number of the modified monomer unit.

Results are presented in \*MERGEFORMAT Table 1.

### Infrared spectroscopy

Nicolet iS5 (Thermo Scientific) spectrophotometer was used to obtain the FTIR spectra of all compounds in the range of 600–4000  $\text{cm}^{-1}$  using attenuated total reflection (iD7 ATR) diamond accessory. The spectra are the results of 16 scans with a resolution of 0.482  $\text{cm}^{-1}$ . Before performing the FTIR measurements on the compounds, background scan was carefully filtered.

### Determination of the polydispersity index by gel permeation chromatography

Gel permeation chromatography (PL-GPC50 Plus, Agilent Technologies, Polymer Laboratories, A Varian Inc. Company) was carried out with PL-aquagel-OH 40 column at 40 °C and the flow rate was 1  $\text{mL min}^{-1}$ . Detection was conducted with a refractive index detector. Ultrapure water was used as eluent. The pressure during the analysis was set to 5.69 MPa. Calibration was made using standard pullulan polysaccharide samples from molecular weight: 180–708  $\times 10^3 \text{ g mol}^{-1}$ . Samples were used with concentration of 0.01% and filtered using a 0.2 mm Agilent captiva filter-syringe prior to use.  $M_w$  and  $M_n$  (weight averaged molecular weight and number averaged molecular weight, respectively) were determined and the polydispersity index (PDI) was calculated for all obtained polymers using the following equation [25]:

$$PDI = \frac{M_w}{M_n} \quad (3)$$

### Static headspace gas chromatography

3 mL of ChCl:U (1:2 molar ratio) DES:water (70:30 wt%) mixed with different wt% of native CD, CD derivative or CD polymer were placed in 22 mL headspace vials. Then, an appropriate amount of *E. citriodora* EO (263 ppm) was added to all mixtures. Vials were sealed using silicone septa and aluminium foil and thermostated at 30 °C for 24 h under stirring to reach the equilibrium between liquid and gaseous phases. Subsequently, 1 mL of the headspace was withdrawn from the vial and injected in the chromatographic system for analysis via a heated transfer line (250 °C). Measurements were carried out using

**Table 1** Synthesized CD polymers along with their corresponding molecular weights ( $M_n$  and  $M_w$ ) and polydispersity index (PDI) values obtained using gel permeation chromatography, the number of linkers per CD molecule obtained using NMR spectroscopy, the calculated synthesis yield and the molar concentration of CDs ([CD]) in the headspace vials in retention studies

	Polymer	Linker	$M_n$ (g mol <sup>-1</sup> )	$M_w$ (g mol <sup>-1</sup> )	PDI	Number of linker/ CD molecule	Synthesis yield (%)	[CD] (mmol L <sup>-1</sup> )
β-CD	–	–	–	–	–	–	–	16.19
	P1	Citric acid	263,618	366,551	1.39	2	24.09	14.94
	P2	Itaconic acid	104,536	343,556	3.29	2	6.12	16.08
	P3	PEGDGE	185,786	232,415	1.25	0.5	17.74	15.45
	P4	STMP1	57,113	157,547	2.76	–	–	–
	P5	STMP2	530,153	664,283	1.25	–	–	–
	P6	STMP3	588,408	815,775	1.39	–	–	–
HP-β-CD	P7	STMP4	551,544	804,975	1.46	–	–	–
	–	–	–	–	–	–	–	16.19
	P8	Citric acid	220,401	368,673	1.67	3	45.34	11.44
	P9	Glutaric acid	7355	27,708	3.77	2	83.53	13.56
	P10	Itaconic acid	237,055	809,216	3.41	3	49.42	12.71
	P11	PEGDGE	214,129	332,663	1.55	0.3	14.45	16.02
	P12	STMP1	111,568	214,863	1.92	–	–	–
CRYSMEB	P13	STMP2	142,871	284,844	1.99	–	–	–
	P14	STMP3	217,080	341,469	1.57	–	–	–
	P15	STMP4	64,124	148,639	2.32	–	–	–
	–	–	–	–	–	–	–	18.89
	P16	Citric acid	103,001	117,096	1.14	3	65.10	12.67
	P17	PEGDGE	135,053	222,192	1.64	0.3	10.08	14.19
	P18	STMP1	118,343	187,461	1.58	–	–	–
	P19	STMP2	134,229	228,541	1.70	–	–	–
	P20	STMP3	118,263	245,101	2.07	–	–	–
	P21	STMP4	94,494	185,188	1.96	–	–	–

a Varian Genesis headspace sampler coupled to a Perkin Elmer Autosystem XL gas chromatography equipped with a flame ionization detector and a DB624 column using nitrogen as carrier gas. The GC column temperature conditions were set as follows: initial temperature of 50 °C for 2 min, increased to 200 °C at 5 °C min<sup>-1</sup>, then hold for 2 min, giving a total runtime of 34 min.

### Retention studies

The volatility reduction (%) of *E. citriodora* was expressed as follows:

$$\text{Volatilityreduction}(\%) = 100 - \left( \frac{\sum A_i}{\sum A_0} \times 100 \right) \quad (4)$$

where  $\sum A_i$  stands for the sum of the peak areas of the *E. citriodora* EO volatile components in DES:water mixture (70:30 wt%) alone or in the presence of 2 wt% β-CD,

HP-β-CD, CRYSMEB or CD polymers and  $\sum A_0$  stands for the sum of the peak areas of the *E. citriodora* EO volatile components in water.

### Titration method

Increasing content (wt%) of β-CD, HP-β-CD, CRYSMEB, P1, P8 or P16 was used while the EO concentration was maintained constant (263 ppm). Results were expressed as:

$$\text{Normalizedarea}(\%) = \frac{\sum A_i}{\sum A_0} \times 100 \quad (5)$$

where  $\sum A_i$  stands for the sum of the peak areas of the *E. citriodora* EO volatile components in the presence of β-CD, HP-β-CD, CRYSMEB, P1, P8 or P16 in DES:water mixture (70:30 wt%) and  $\sum A_0$  stands for the sum of the peak areas of the *E. citriodora* EO volatile components in ChCl:U (1:2 molar ratio) DES:water (70:30 wt%).

## Results and discussion

### Characterization of CD polymers

Different crosslinking agents were used to synthesize CD polymers of  $\beta$ -CD, HP- $\beta$ -CD and CRYSMEB (Table 1). Among these studied polymers,  $\beta$ -CD/malonic acid, HP- $\beta$ -CD/glutaric acid, HP- $\beta$ -CD/itaconic acid, HP- $\beta$ -CD/PEGDGE, HP- $\beta$ -CD/STMP and all CRYSMEB/based polymers were synthesized and studied herein for the first time.

Polymers were characterized using  $^1\text{H-NMR}$  and FTIR spectroscopies. Also, their homogeneity was examined using gel permeation chromatography and the corresponding PDI values were calculated.

Figure 1 shows the superposition of  $^1\text{H-NMR}$  spectra of  $\beta$ -CD/based polymers. Results obtained for HP- $\beta$ -CD and CRYSMEB/based polymers are illustrated in Supplementary information in Figures S1 and S2.

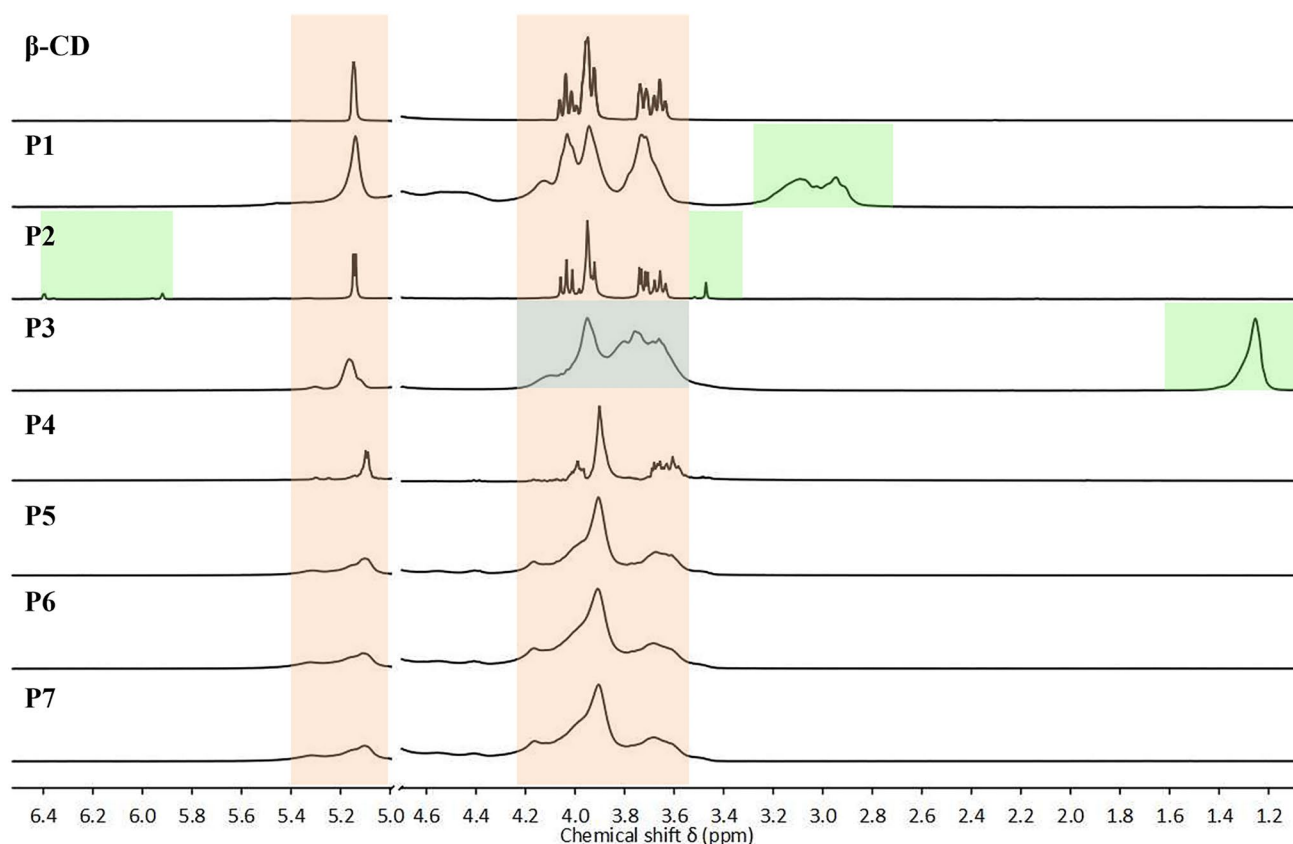
$^1\text{H-NMR}$  results depicted alterations in the form of the CDs peaks as well as modifications in the chemical shifts ( $\delta$ ). This confirmed the modification of all three CDs.

Also, broad signals were observed in the spectra of the new polymers indicating the loss of the symmetry of the CD molecule and changes in the chemical environment due to the polymerization [26].

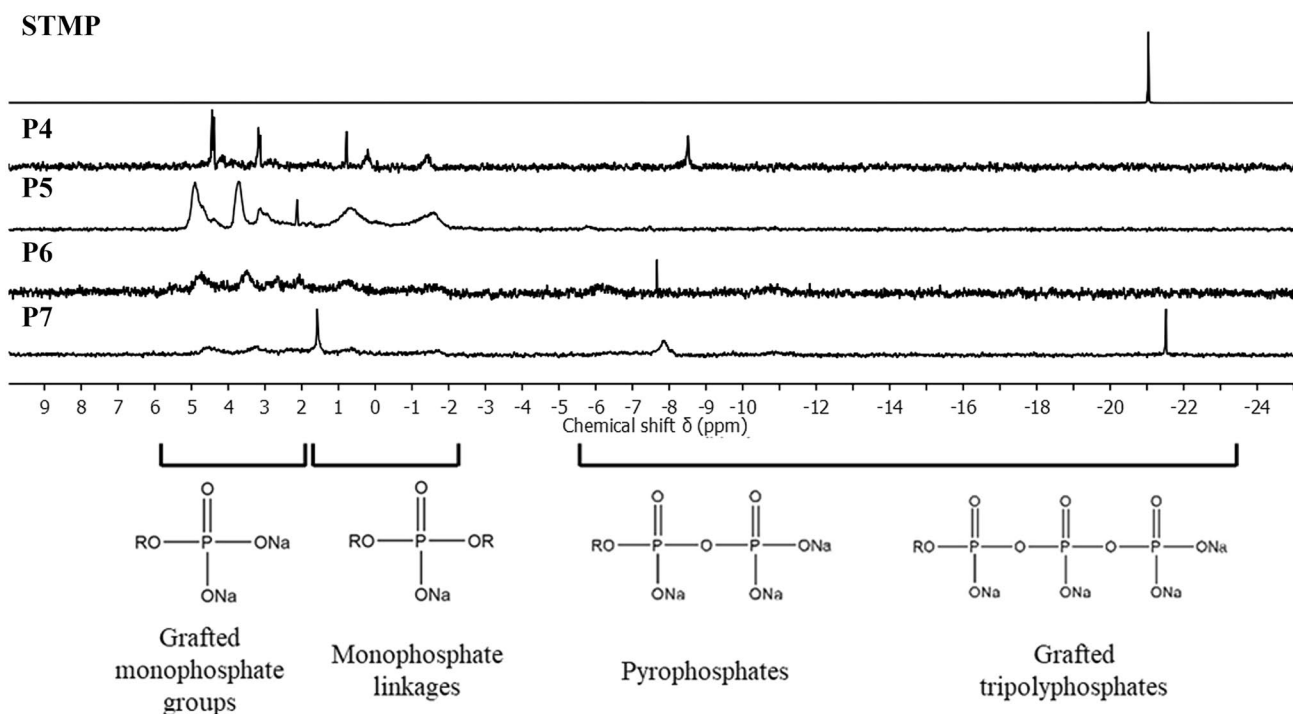
Polymers synthesized with STMP were investigated using  $^{31}\text{P-NMR}$ . Results for  $\beta$ -CD/based polymers are illustrated in Fig. 2. Results for HP- $\beta$ -CD and CRYSMEB/based are presented in Supplementary information in Figure S3.

The peak that corresponds to inorganic phosphates (or unreacted STMP), usually located at  $-21$  ppm, was not detected in the spectra of eight polymers (Fig. 2, Figure S3). This proved that polymers were sufficiently washed before NMR measurements [24]. Peaks located between 2 and 5 ppm,  $-2$  to 2 ppm,  $-10$  to  $-6$  ppm as well as  $-22$  to  $-20$  ppm could be assigned to the grafted monophosphate groups, monophosphate linkages, pyrophosphates and grafted triphosphates, respectively [27]. The corresponding structures of these moieties are found in the polymers and illustrated in \\* MERGEFORMAT Fig. 2. This proved the reticulation and thus the formation of CD polymers.

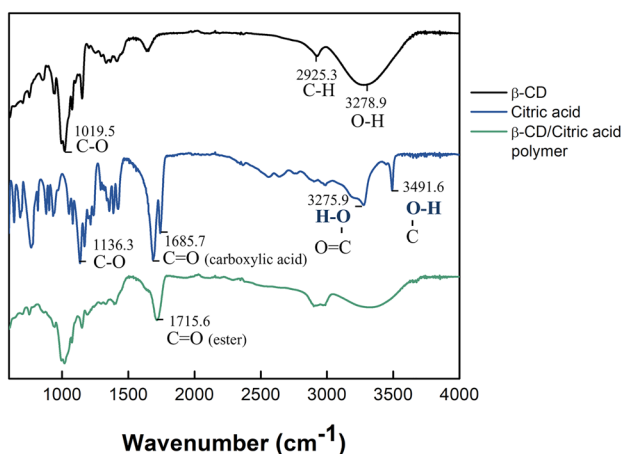
Then, the FTIR spectra were recorded for all polymers and starting materials. The FTIR spectra of  $\beta$ -CD, citric acid



**Fig. 1** Superposition of  $^1\text{H-NMR}$  spectra of  $\beta$ -CD and its corresponding polymers. Orange, green and blue highlighted areas correspond to the peaks of  $\beta$ -CD, linkers and the overlapped  $\beta$ -CD and linker signals, respectively



**Fig. 2** Superposition of  $^{31}\text{P}$ -NMR spectra of  $\beta$ -CD/STMP polymers. Below: Illustration of possible structure of these polymers (R: CD molecule)



**Fig. 3** Overlay of FTIR spectra of  $\beta$ -CD, citric acid and  $\beta$ -CD/citric acid polymer (P1)

and the corresponding polymer (P1) are shown, as an example, in  $\text{\* MERGEFORMAT}$  Fig. 3. Other examples are illustrated in supplementary information in Figures S4 and S5. Results showed the appearance of sharp peaks around  $1700\text{ cm}^{-1}$  in all the polymers spectra. This proved the conversion of the carbonyl groups of the carboxylic acids into ester functions [22, 28].

Finally, gel permeation chromatography was carried out to determine the macromolecular mass of the soluble

polymers and then to calculate the polydispersity index (PDI) values. Results are listed in  $\text{\* MERGEFORMAT}$  Table 1. Examples of the obtained chromatograms are illustrated in supplementary information in Figures S6–S8.

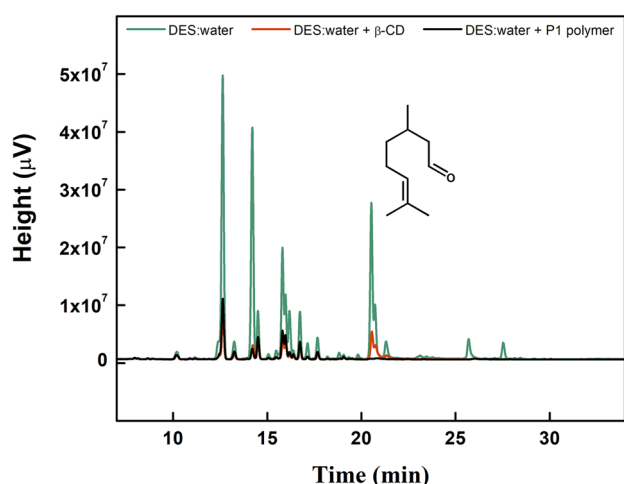
PDI values for all polymers ranged from 1.14 to 3.77 depicting the heterogeneity of the obtained samples.

### Retention studies

Retention studies were carried out in  $\text{ChCl:U DES:water}$  (70:30 wt%) medium. Water was added to  $\text{ChCl:U DES}$  because it was shown that the addition of water decreases the viscosity of this DES [29]. Additionally, the presence of water allows CD experiencing an aqueous like environment and gives the CD macrocycle enough mobility to maintain its encapsulation ability [30].

Retention studies were conducted using 2 wt% of CD or polymer. The final molar concentration of CD in the formulations containing polymers was calculated based on the number of linkers per CD molecule obtained from NMR studies according to Eq. 1 and listed in  $\text{\* MERGEFORMAT}$  Table 1.

The retention ability of native CD ( $\beta$ -CD), CD derivatives (HP- $\beta$ -CD and CRYSMEB) and the synthesized polymers towards *E. citriodora* was evaluated by measuring the reduction in the EO volatility according to Eq. 4. Figure 4 shows, as an example, the effect of  $\beta$ -CD/citric acid (P1) polymer on



**Fig. 4** Overlap of the chromatograms of *E. citriodora* EO in the absence and presence of  $\beta$ -CD or  $\beta$ -CD/citric acid (P1) polymer in ChCl:U (1:2 molar ratio) DES:water (70:30 wt%). Inset the structure of citronellal, the main component of the EO

the chromatographic profile of *E. citriodora* EO in ChCl:U (1:2 molar ratio) DES:water (70:30 wt%). Results obtained with P8 and P16 polymers are illustrated in Supplementary information in Figures S9 and S10.

Results of the volatility reduction of *E. citriodora* EO in the different mixtures containing  $\beta$ -CD/based polymers are presented in Fig. 5. Results for HP- $\beta$ -CD and CRYSMEB/based polymers are presented in Figure S11.

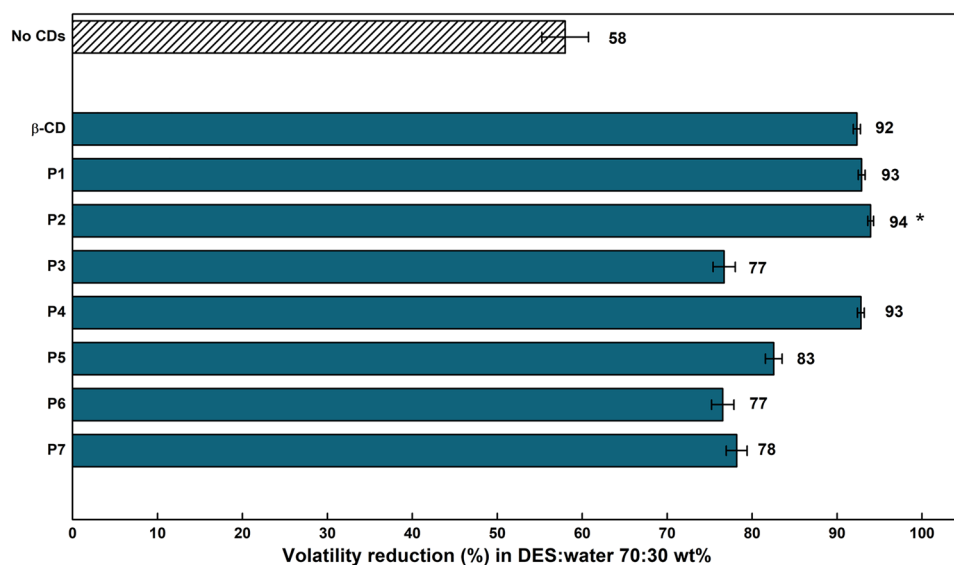
Firstly, it is worthy to note that ChCl:U (1:2 molar ratio) DES:water (70:30 wt%) itself significantly entrapped and reduced up to 58% the volatility of *E. citriodora* compared to water. This can be mainly due to the lower polarity of the mixture compared to water, which allowed higher

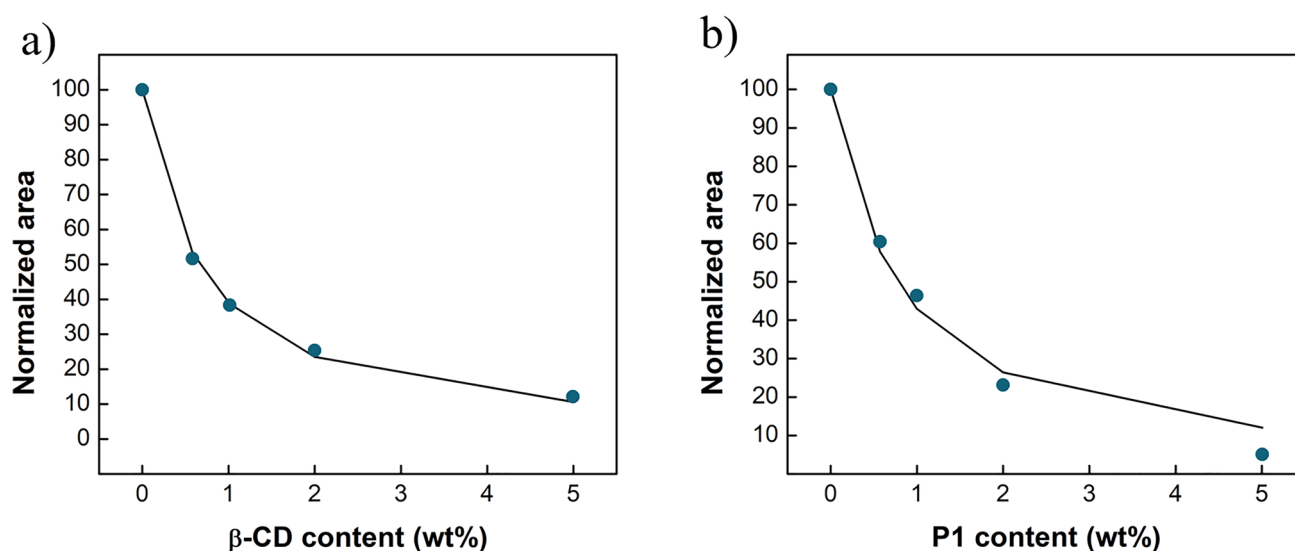
solubilization of the EO [31]. As can be noted in Figures Fig. 5 and S11, the addition of all the encapsulating agents ( $\beta$ -CD, CD derivatives (HP- $\beta$ -CD and CRYSMEB) or their corresponding polymers) led to an additional decrease in the volatility of the studied EO. The volatility reduction observed in the presence of the various hosts in DES:water 70:30 wt% was in the range of 77–94% as compared to water. This important decrease in the volatility directly implies a significant increase in the solubility of *E. citriodora* in the new formulations.

Additionally, from a mechanistic point of view, the better entrapment potential observed in the presence of  $\beta$ -CD, HP- $\beta$ -CD, CRYSMEB and their synthesized polymers indicated that all these structures retained, at least partially, their inclusion capacity in this non-conventional medium (DES:water 70:30 wt%). In addition, results showed that  $\beta$ -CD and its two derivatives (HP- $\beta$ -CD and CRYSMEB) possess a similar efficacy of volatility reduction (Fig. 5). This could be contributed to the fact that these CDs present the same cavity volume and thus pointed out the important role of the cavity in the entrapment potential of EOs components. However, as concerns the polymers, a large discrepancy in their performance was noticed (Fig. 5). This could not be attributed to the difference in the molar concentration of CD in the polymer/based mixtures since the variation in the CD concentration among mixtures was very slight (Table 1). For example, the HP- $\beta$ -CD/glutaric acid polymer (P9) with a molar CD concentration of 13.56 mmol L<sup>-1</sup> showed a better volatility reduction than the HP- $\beta$ -CD/PEDGE polymer (P11) with a molar CD concentration of 16.02 mmol L<sup>-1</sup>, both in the ChCl:U DES:water 70:30 wt%.

Interestingly, some polymers presented significantly better entrapment ability than the parent macrocycle.

**Fig. 5** Volatility reduction of *E. citriodora* EO in DES:water (70:30 wt%) alone or in the presence of native or polymerized  $\beta$ -CD (2 wt%) at 30 °C compared to water. \*Significant, yet slight, difference compared to  $\beta$ -CD (t-test; p-value < 0.05)





**Fig. 6** Representation of the experimental points (filled circles) obtained for *E. citriodora* with a)  $\beta$ -CD and b) P1 in ChCl:U (1:2) DES:water 70:30 wt% at 30 °C compared with theoretical titration curves (solid lines)

$\beta$ -CD/itaconic acid (P2), HP- $\beta$ -CD/glutaric acid (P9), and CRYMEB/STMP1 (P18) polymers showed slightly higher retention ability towards *E. citriodora* EO, in comparison to the corresponding parent CD. All other polymers presented a similar or a less efficiency than the parent CD. This could be due to the highly-branched architecture of CD polymers, that can hinder the CD cavity and lower their encapsulation efficiency [32]. Generally, the lowest retention efficiency was obtained with PEGDGE/based CD polymers. PEGDGE presents a very long chain that could hinder the access to the CD cavity.

### Static headspace-gas chromatography titration method

In order to check out the presence of an inclusion phenomenon, static headspace-gas chromatography SH-GC titration method was carried out. The evolution of the EO volatility as a function of P1, P8 and P16 content in comparison to the relative native  $\beta$ -CD and CD derivatives was evaluated. These polymers were chosen, because they were synthesized using a conventional cross-linking agent, citric acid, and they showed very good synthesis yields (Table 1). Results obtained with  $\beta$ -CD and P1 polymer are presented in Fig. 6. Results obtained with HP- $\beta$ -CD, CRYMEB and their relative polymers are illustrated in Supplementary information in Figures S12 and S13, respectively.

A gradual decrease in the volatility of the EO was observed when increasing the wt% of all studied CDs and polymers. The shape of the titration curves obtained fits

well with the typical curves generally observed for the complexation phenomenon [33, 34]. This suggests that the retention of the EO could be due to the encapsulation of the EO components within the CD cavity. In fact, the geometric fit of citronellal (Fig. 4), the major component of *E. citriodora*, with the cavity of  $\beta$ -CD and its derivatives was already confirmed in the literature [6].

### Conclusion

CD polymers have long been considered as very promising materials targeting different applications including drug carriers, organic pollutants remedies and others in aqueous solutions. Herein, the ability of new ternary mixtures based on ChCl:U DES, water, and CD ( $\beta$ -CD, its derivatives (HP- $\beta$ -CD and CRYMEB), or CD polymers) to entrap and reduce the volatility of *E. citriodora* EO was investigated. Results showed that these novel mixtures efficiently reduced the volatility of EO. This consequently proved their ability to solubilize *E. citriodora* EO.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10847-022-01161-2>.

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