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ORIGINAL ARTICLE

Study of the retention of aroma components by cyclodextrins by static headspace gas chromatography

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Abstract The process of encapsulation is widely employed in the flavour industry to protect volatile and/or labile flavouring materials during storage. A variety of commercial practices are currently followed, but those involving the formation of flavour/cyclodextrin (CD) molecular inclusion complexes afford some of the greatest potential for increased product shelf life. The determination of the stability of inclusion complexes is of critical importance to take advantage of the complexation potential of CDs. Hence, we investigated the interactions between five CDs and thirteen aroma components. Relevant for, the retention of these compounds in presence of different CDs has been determined. The stability constants of the inclusion compounds have been calculated by static headspace gas chromatography in aqueous solution at 30 °C. The results indicate the formation of 1:1 inclusion complex for all the studied compounds. The binding between CDs and the aroma compounds depends on both hydrophobicity of the guest molecule and their geometric accommodation into the CD cavity. The results show that β-CDs are the most versatile CDs for the inclusion of the studied molecules.

Keywords Aroma · Cyclodextrins · Formation constant · Retention · Static headspace

Abbreviations

α-CD	α-Cyclodextrin
β-CD	β-Cyclodextrin
CDs	Cyclodextrins
CRYSMEB	Low methylated-β-cyclodextrin
DS	Degree of substitution
HPBCD	Hydroxypropyl-β-cyclodextrin
HPLC	High-performance liquid chromatography
RAMEB	Randomly methylated-β-cyclodextrin
SHGC	Static headspace gas chromatography

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Introduction

Fragrance materials are often added to industrial products such as cosmetics, drugs and foods. Cyclodextrins (CDs) are known to form complexes with fragrant molecules [1–4]. They are used as solubilizers and/or sustained-release carriers for flavour material in aqueous solution in the cosmetic, food and pharmaceutical fields as they can modify the aroma release and flavour perception [5]. Moreover, microencapsulation is one of the most effective techniques for protecting aroma against oxidation, thermal degradation, and evaporation [6–8]. The main drawback of CDs is a problem encountered with any support, i.e. their selectivity toward some of the compounds that constitute an aroma. As a consequence, some molecules may be poorly retained whereas others are strongly trapped [9]. Hence, the understanding of how the fragrance material interacts with CDs in aqueous solution is essential for perfumers and flavourists.

Some researches regarding the uses of CDs in cosmetic and food industries have been already reported [10–16]. They include solute stability, release control, malodor masking and possible surfactant reduction to produce product irritancy. However, there are a few reports in the literature on the determination of the formation constant between fragrance molecules and CDs. We have recently

developed a modified method using static headspace gas chromatography (SHGC) to determine the stability constant of the inclusion complexes formed between CDs and volatile organic compounds [17–19].

In this paper, the complexation behaviour and the retention capacity of α-CD, β-CD, hydroxypropyl-β-cyclodextrin (HPBCD), randomly methylated-β-cyclodextrin (RAMEB) and a low methylated-β-cyclodextrin (CRYSMEB) for 13 fragrance compounds (Fig. 1) was investigated in aqueous solution by SHGC. These compounds were chosen based on a representative range of molecular sizes and physicochemical properties.

Materials and methods

Chemical

Fragrance molecules (Aldrich) all of analytical reagent grade were used as received. β-CD, HPBCD (DS = 5.6) and CRYSMEB (DS = 4.9) were provided from Roquette Frères (Lestrem, France), α-CD, γ-CD and RAMEB (DS = 12.6) were purchased from Wacker–Chemie (Lyon, France). Distilled deionised water was used throughout this work.

Static headspace

Measurements were conducted with an Agilent headspace autosampler. Sample solutions of 10 mL containing different concentrations of aroma (10–100 ppm) were introduced into 22 mL headspace vials and sealed using silicone septa and aluminium foil. The vials were then thermostated at 30 ± 0.1 °C. After the equilibrium was established (30 min), 1 mL of vapour from the above solution was withdrawn from the vial using a gas-tight syringe and injected directly in the chromatographic column via a transfer line (250 °C). Each sample was then analyzed by gas chromatography (Perkin Elmer Autosystem XL equipped with a flame-ionization detector using a DB624 column). The GC settings were set as follows: detector temperature, 280 °C; column temperature, 120 °C for limonene, linalool, isoeugenol, geraniol; 140 °C for benzyl alcohol, citral neral, citral geranial, β-citronellol and 160 °C for lilial, eugenol, cinnamaldehyde, α-isomethylionone, methyl heptine carbonate.

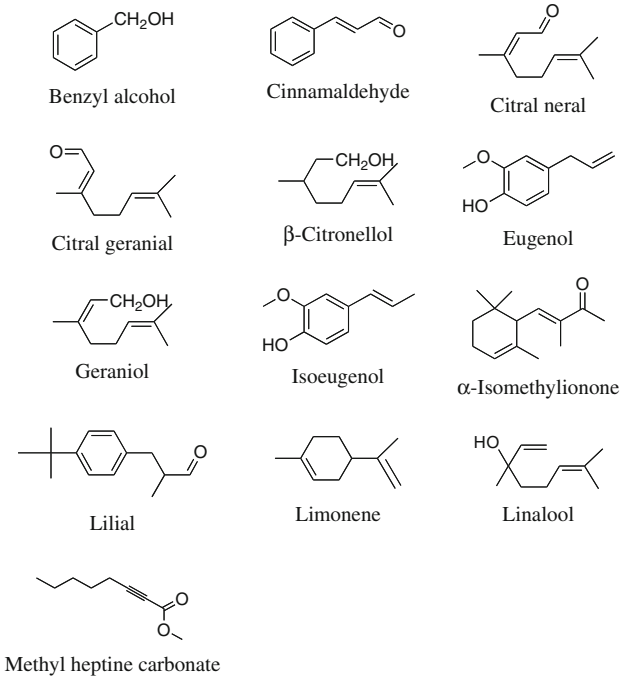

Fig. 1 Molecular structure of the studied aroma compounds

Retention of aroma by cyclodextrins

The presence of chemical agents in solution is known to impact the vapour-liquid equilibria [20, 21], since they can change the solubility of hydrophobic compounds, for example, by forming inclusion complexes as in the case of CDs. The percentage of retention (r) of the studied aroma by the different CDs is expressed as follows:

$$r(\%) = \left(1 - \frac{A_{CD}}{A_0}\right) \quad (1)$$

with A_0 and A_{CD} the peak area of the aroma in the absence and in the presence of CD respectively. The percentage of retention was determined at 30 °C for a 100 ppm solution of the aroma and a concentration of 10 mM for CDs. For each CD and each aroma compound, measurements were done in triplicate.

Formation constants

The host/guest system was studied by a SHGC titration method developed in our laboratory for volatile organic compounds [17–19]. Different concentrations of CD were used at constant guest (G) concentration.

Assuming 1:1 ratio binding, the total concentration of guest in aqueous solution ($[G]_0$) and the total CD concentration ($[CD]_0$) were expressed as follows:

$$[G]_0 = [G] + [CD/G] \quad (2)$$

and

$$[CD]_0 = [CD] + [CD/G] \quad (3)$$

where [CD/G] was the concentration of the associated complex. The $[G]_0$ after equilibrium was determined by subtracting the number of moles of guest in the gaseous phase.

Then, in the presence of CD, the peak area can be expressed as follows:

$$A = \alpha\left([G]_0 - [CD/G]\right) \quad (4)$$

with A the integrated area counts of the GC peak for a given sample, and α a specific parameter of the headspace.

The association constant was given by:

$$K_f = \frac{[CD/G]}{[G][CD]} = \frac{[CD/G]}{\left([G]_0 - [CD/G]\right) * \left([CD]_0 - [CD/G]\right)} \quad (5)$$

Thus, [CD/G] can be estimated by:

$$[CD/G] = -\frac{1}{2}\sqrt{\left[\left(\frac{1}{K_f} + [CD]_0 + [G]_0\right)^2 - 4[CD]_0[G]_0\right]} + \frac{1}{2}\left(\frac{1}{K_f} + [CD]_0 + [G]_0\right) \quad (6)$$

For a given value of K_f, [CD/G] was known, and thus a theoretical value was calculated for the peak area. An algorithmic treatment was then applied to minimize the difference between the experimental and theoretical values of the peak area leading to the adequate formation constant (K_f) [22].

Molecular modelling

The CD hosts were based on a non distorted monomeric β-cyclodextrin with C7 symmetry. Guest molecules were initially retrieved from the data provided by the Structural Data Base System of the Cambridge Crystallographic Data Center, and then energy minimised with MM3 force field. The various structural manipulations were made using the CAChe Library [23] on PC-Computer.

The docking of each studied guest into the β-CD unit has been performed using four dummy atoms as described previously [2, 24].

Results and discussion

Retention of aroma by CDs

The presence of CDs modified the vapour-liquid equilibria for all the studied compounds, which were all are more retained in aqueous solution in presence of CDs (Fig. 2).

No negative retention was observed as reported by Jouquand et al. in the case of aliphatic ketones and β-CD [4], or by Reineccius et al. for α-CD and methyl anthranilate [7]. Except for methyl heptine carbonate, the CD effects were relatively small in the case of α-CD and higher in the cases of β-CDs. The most retained compounds were lilial and α-isomethylionone with an average of 99.9 and 99.1%, respectively, for β-CDs. These results reflected the tendency of the different aroma compounds to form molecular inclusion complexes of different strengths with α-CD and β-CDs.

Formation constant

The determinations of the formation constant were done at 30 °C using four CDs concentration. The effect of an increased concentration of CD on the chromatographic peak of limonene is shown in Fig. 3 in the case of CRYSMEB.

The obtained variations of the peak area are in good agreements with a 1:1 host/guest ratio as can be seen on Fig. 4 for the same compounds. As one can see, the experimental points fit well with the theoretical curve.

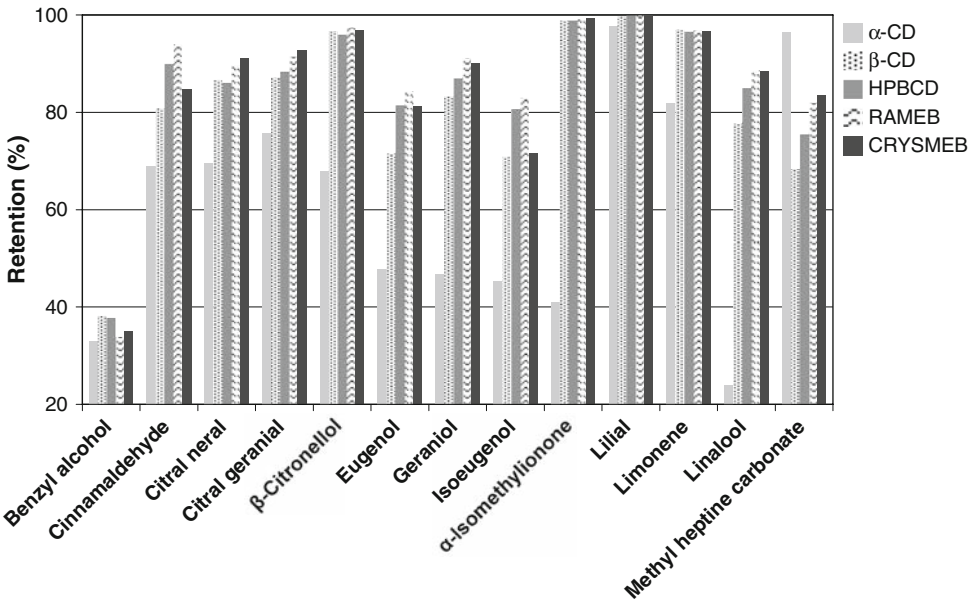

Fig. 2 Retention (%) for the aroma compounds (100 ppm) with the different cyclodextrins (10 mM)

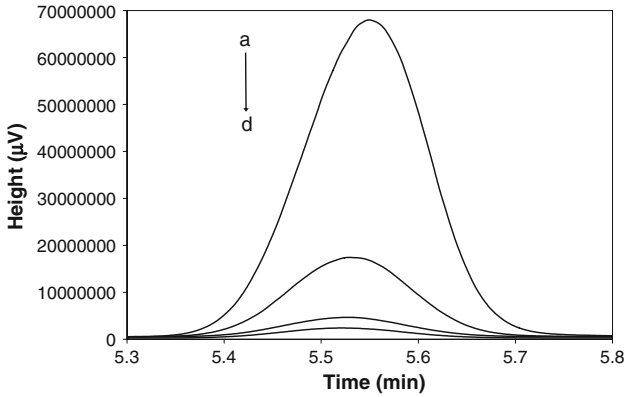

Fig. 3 Representation of the variation of the chromatogram of limonene 10 ppm (**a**) with various concentration of CRYSMEB: 1 mM (b), 4 mM (c) and 10 mM (d)

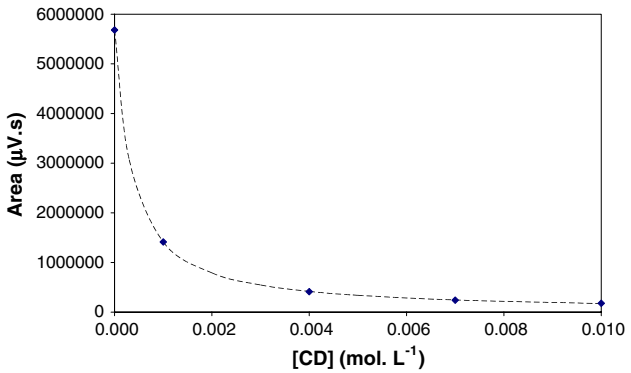

Fig. 4 Representation of the experimental point (◆) obtained for limonene and CRYSMEB compared with theoretical titration curve (- -) for a 1:1 complex (K_f = 3,194 M^{-1})

The formation constants calculated with the algorithmic treatment are reported in Table 1.

To the best of our knowledge, few determinations of the formation constant between aroma and CDs have been performed [1–3, 25–30]. The most studied compounds are limonene, eugenol and benzyl alcohol. Eugenol and benzyl alcohol have been studied by UV–visible spectroscopy and by SHGC [1, 2]. We can observe a good agreement between the values obtained by these two methods (Tables 1, 2). Limonene has been studied mostly with HPBCD (DS = 4.4) by static and dynamic headspace gas chromatography [1, 25, 26, 30] and with α- and *β*-CD by high-performance liquid chromatography (HPLC) [29]. The values obtained in our study are in the same order of magnitude than in previous studies.

Among the studied compounds, lilial presents the highest formation constant. It is well known that the presence of a *t*-butyl phenyl group leads to a good molecular recognition with CDs [31]. Excepted for methyl heptine carbonate, *β*-CDs have more affinity than α-CD for the studied compounds. Therefore, *β*-CDs seem to be the most versatile CDs with regard to the panel of substrate molecules tested in this study. We can observe a good correlation between native *β*-CD and its modified derivatives (0.984, 0.975 and 0.982 for HPBCD, RAMEB and CRYSMEB respectively). An example of this correlation is given in Fig. 5 for HPBCD.

In an analogous family, there is a good correlation between log*P* (*P* is the octanol–water partition coefficient (also referred to as K_{ow})) and logK_f (0.986 for aromatic compounds: benzyl alcohol, cinnamaldehyde, eugenol, isoeugenol and lilial with *β*-CD) but for the overall compounds the correlation is weaker (0.812 with *β*-CD). Furthermore, the two citral isomers have similar K_f but a greater K_f is obtained for limonene, a cyclic compound, although they have similar log*P*. Some compounds (methyl heptine carbonate and eugenol) present similar K_f with *β*-CD but different value for α-CD. From all these results, we can see that the binding between CDs and aroma compounds depends on both hydrophobicity of the guest

Table 1 Log*P* and formation constant (M^{-1}) obtained by SHGC at 30 °C

Compound	Log*P*[a]	α-CD	*β*-CD	HPBCD	RAMEB	CRYSMEB
Benzyl alcohol	1.275	52	64	63	53	56
Cinnamaldehyde	2.484	236	450	969	1,696	595
Citral neral	3.654	93	693	656	922	1,083
Citral geranial	3.654	66	728	805	1,146	1,341
β-Citronellol	3.152	223	3,141	2,578	4,048	3,290
Eugenol	2.100	94	264	462	568	454
Geraniol	3.202	90	528	712	1,100	977
Isoeugenol	2.379	85	255	441	514	263
α-Isomethylionone	4.160	71	9,869	9,789	13,176	15,632
Lilial	4.389	4,387	56,567	11,2205	166,338	147,617
Limonene	3.614	486	3,457	3,074	3,340	3,194
Linalool	3.213	32	366	596	833	816
Methyl heptine carbonate	3.220	2,905	226	325	485	539

[a] http://www.molinspiration.com/cgi-bin/properties

Table 2 Formation constant (M^{-1}) obtained by static headspace, UV–visible spectroscopy or HPLC [1, 2, 25, 26, 29, 30]

Compound	α-CD	β-CD	HPBCD	RAMEB	CRYSMEB
Benzyl alcohol	–	63[a]	54[a]	55[a]	57[a]
Limonene	199[b]	3,920[b]	3,350[c]	–	–
			4,630[d,f]		
			5,630[e]		
Eugenol		322[a]	445[a]	521[a]	401[a]
		270[d]			
Isoeugenol	–	304[a]	452[a]	547[a]	240[a]

[a] UV–visible, see ref [2], [b] HPLC, see ref [29], [c] SHGC, see ref [30], [d] SHGC, see ref [1], [e] SHGC, see ref [25], [f] SHGC, see ref [26]

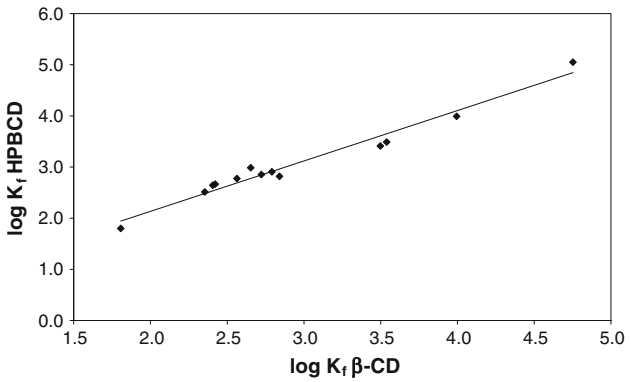

Fig. 5 Relationship between logK_f for β-CD and HPBCD for the studied compounds

(estimated on the basis of log$P$) and on the geometric accommodation of the guest molecule into the CD cavity.

Molecular modelling

To illustrate the importance of the host/guest complementarity we have performed a theoretical study for two compounds with a similar hydrophobicity but from different chemical families, limonene and methyl heptine carbonate. The computed complexation energies (ΔE) obtained for limonene and methyl heptine carbonate with β-CD are −13.1 and −8.6 kcal mol^{-1}, respectively. These results are in good agreements with the lower K_f determined experimentally for methyl heptine carbonate with β-CD.

The weak empty space observed between the β-CD cavity and limonene (Fig. 6) suggests that this CD is more suitable to accommodate such substrate than α-CD and γ-CD ($K_f = 117$ M^{-1}) regarding the respective sizes of their cavities. For methyl heptine carbonate, a linear molecule, the empty space is larger than in the case of limonene. In this case, α-CD is more fitting because of its smaller cavity size.

The influence of the steric complementarity also explains the greater stability generally observed for the modified CDs like HPBCD, RAMEB and CRYSMEB, since hydroxypropylation and methylation extend the cavity, thus increasing van der Waals interactions with the included substrate.

Conclusion

Static headspace gas chromatography has been successfully used to study the interactions between thirteen aroma compounds and five CDs. This study has shown that all CDs reduce the volatility of these compounds and that stable 1:1 inclusion complexes are formed. β-CDs are the most versatile CDs for the studied guest leading to greater retention ability and formation constant. The binding between CDs and the aroma compounds depends on both hydrophobicity of the guest and the geometric accommodation of the guest molecule into the CD cavity. Further studies are under investigation to examine the effect of CDs for fragrance mixtures.

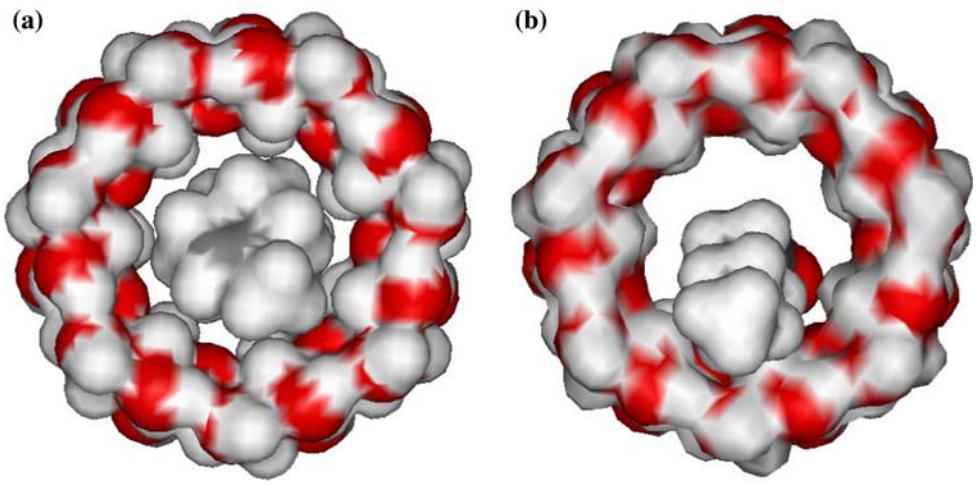

Fig. 6 Van der Waals surfaces of β-CD complexes with limonene (**a**) and methyl heptine carbonate (**b**)

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