

Bioactive Nanoparticles

The Complexation of Odorant Compounds with α - and β -Cyclodextrin

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This paper presents the molecular encapsulation of some odorant compounds in α - or β -cyclodextrin. The molecular encapsulation of hydrophobic compounds in these natural cyclic oligosaccharides provides powders at nano scale with very good protection against degradative environmental factors (temperature, light, moisture, air/oxygen), controlled release of the bioactive compounds (long life bio-action), easy handling of powdery complexes (containing bioactive liquid compounds), higher water soluble bioactive specialties (containing hydrophobic biocompounds). From this point of view, some odorant bionanoparticles with enhanced bioactive properties (protection against oxidation, enhanced olfactive and long life smelling properties) and possible applications in cosmetic and toiletries were obtained and characterized. Both limonene and linalool enantiomers (d and l), dl-linalyl acetate, β -caryophyllene, benzaldehyde, γ -decalactone, α -ionone, menthol, vanillin, and hydroxycitronellal were used as guest compounds. The encapsulation was achieved by crystallization from the ethanol-water solution. The GC-MS was used for odorant compound analysis and TG for nanoparticle analysis. α -Cyclodextrin release the odorant compounds earlier than the β -cyclodextrin, as indicated by thermogravimetric analysis. The benzaldehyde, menthol and vanillin were decomplexed at lower temperatures than 70°C and the majority of compounds were complexed at 1:1 stoichiometry ratio.

Keywords: cyclodextrins, odorant compounds, nanoparticles, molecular encapsulation, gas, chromatography-mass spectroscopy, thermogravimetry

The aim of using encapsulation in food and cosmetic industries was to provide powdered flavor products and to protect them against degradative environmental factors (light, air, humidity, temperature etc.).

Inclusion complexes are supramolecular systems with adduct-like structures, formed by molecular encapsulation of organic or inorganic compound (e.g. odorant compound), as "guest" molecule, in a "host" molecule (e.g. cyclodextrins) [1-9]. This type of encapsulation (molecular encapsulation) provides the lowest level of encapsulation (nanoencapsulation), affording a quite perfect protection for the bioactive compound [1,4].

The most used macrocyclic compounds for molecular encapsulation are cyclodextrins [1,4]. These compounds were discovered in 1891 by Villiers, but the pioneer for this domain was Schardinger [10]. The named cyclodextrins consists of three natural cyclic oligosaccharides containing six (α), seven (β) or eight (γ) glucopyranosil units (fig. 1), and another few with a higher number of these units [1-9].

Cyclodextrins have structures like truncated cones, with hydrophobic inner cavities, which can accommodate geometric compatible bioactive molecules by van der Waals interactions, providing stable complexes (molecular inclusion compounds, supramolecular systems) with very good protecting properties.

The highest number of studies on the obtaining of the bioactive nanocapsules are in the pharmaceutical area. The latter ones treat the controlled release of various drugs like: propranolol, diclofenac, indomethacin, buformin, clobazimine, ibuprofen, budesonide, pindolol, different vitamins and other bioactive compounds from natural cyclodextrin matrices, or from modified cyclodextrin matrices [11-14].

Attempts on similar studies in the natural compounds and vegetable extracts area have been made. Thus, studies on the molecular encapsulation in cyclodextrins (especially β form) of the lemon, majoran, chamomile, rosemary volatile oils, vanillin and *L*-menthol have been made [15-19].

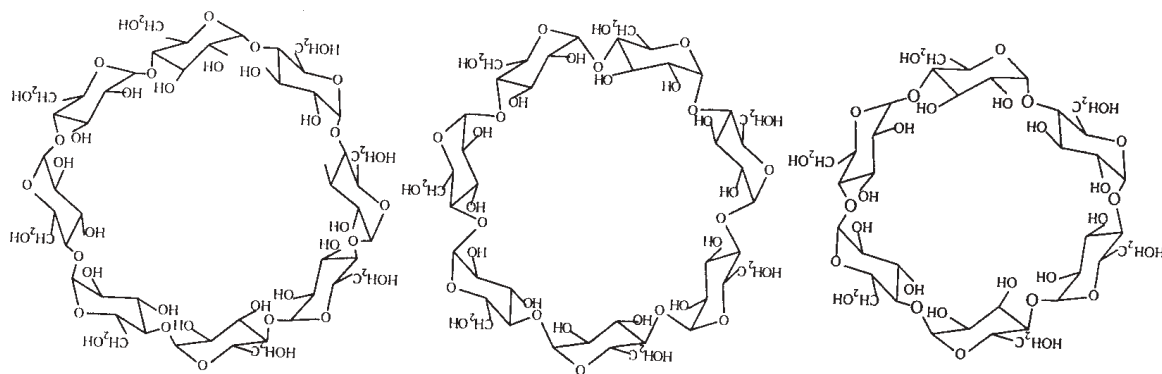


Fig. 1. α -, β -, and γ -Cyclodextrin structures

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Table 1
QUANTITIES AND RESULTS OF MOLECULAR ENCAPSULATION OF ODORANT COMPOUNDS

Host/guest supramolecular system	Molar ratio CD/Odorant	mg CD	mg odorant	mg complex	Yield (%)
α CD/ <i>d</i> -linalool	1:1	100	13	23	23.0
α CD/ <i>l</i> -linalool	1:1	100	13	21	21.0
β CD/ <i>d</i> -linalool	1:1	100	13	36	36.0
β CD/ <i>l</i> -linalool	1:1	100	13	39	39.0
β CD/ <i>d</i> -limonene	1:1	100	12	76	76.0
β CD/ <i>d</i> -limonene	1:2	100	12	86	86.0
β CD/ <i>l</i> -limonene	1:1	100	12	82	82.0
β CD/ β -caryophyllene	1:1	100	18	69	69.0
β CD/benzaldehyde	1:1	100	9	14	14.0
β CD/ γ -decalactone	1:1	100	15	65	65.0
β CD/ α -ionone	1:1	100	17	70.2	70.2
β CD/hydroxycitronellal	1:1	100	15	49.4	49.4
β CD/ <i>l</i> -menthol	1:1	100	14	57.4	57.4
β CD/vanillin	1:1	100	13	24	24.0
β CD/ <i>dl</i> -linalyl acetate	1:1	100	17	63	63.0

18]. Interesting studies were done on the encapsulation of some organic compounds like insecticides or the well known carbon form (C_{60}). Classical chromatographic, spectroscopic, and thermogravimetric analyses were used in almost all cases, but for the characterization of the complex structures and capsules the X ray analysis and scanning electronic microscopy can be used [18].

The aim of this study is to obtain and characterize the complexes of α or β -cyclodextrin with various odorant compound in order to determine the stoichiometry of these supramolecular systems and the possibility of the nanoencapsulation of these hydrophobic compounds. This study continued the team work in the cyclodextrin area [19-23].

Materials and method

d-limonene, *l*-limonene, *d*-linalool, *l*-linalool, β -caryophyllene, benzaldehyde, γ -decalactone, α -ionone, and hydroxycitronellal were purchased from SC Fares SA Orătie (Romania); these odorant compounds were technical grade products and have required a GC-MS analyses for determining the purity. *l*-Menthol and vanillin were food grade products (> 99%), and were purchased from SC Kandia SA Timișoara (Romania). Linalyl acetate was synthesized in our laboratory from linalool and acetic anhydride, in the presence of phosphoric acid as catalyst.

Cyclodextrins (α CD and β CD) were purchased from Merck&Co. Inc., New Jersey, and were reagent grade products (> 99%). Alkane standard solution C_8 - C_{20} used for the determination of Kovats indices for odorant compounds from GC-MS analysis, was purchased from Fluka. Ethyl alcohol (min. 96% purity) was obtained by Chimpar București (Romania).

Molecular encapsulation. α - or β -cyclodextrin were weighted (table 1) and dissolved in a minimum volume of 30% ethanol (about 2 mL) at $50 \pm 1^\circ\text{C}$. Then, 30% ethanolic solutions with various concentrations of different odorant

compounds (table 1), corresponding to 1:1 or 1:2 stoichiometry, were dropped to cyclodextrin solutions in 0.5 h, with continuous stirring; these solutions were slowly stirred for another 15 min. The complex solutions were then cooled at room temperature during 4 h in a water bath, and stored at 4°C for 24 h in a refrigerator. Suspensions formed were filtered, washed with few ml 30% ethanol and dried in exicator.

GC-MS analysis. For the analysis of purity of odorant compounds a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass selective detector (GC-MS) system was used. A capillary HP-5 MS column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used for the GC system. The temperature program was set up to 50°C to 250°C with $4^\circ\text{C}/\text{min}$, both the injector and detector temperatures were 280°C and He as carrier gas.

Ionization energy EI of 70eV was used for mass detector, with a source temperature of 150°C , scan range 50-300 amu, scan rate 1s^{-1} . The mass spectra were compared with the NIST/EPA/NIH Mass Spectral Library 2.0.

TG analysis. A TG 209 NETZSCH thermogravimetric apparatus was used for the thermal analysis of cyclodextrin/odorant compound complexes. The temperature program was 20 to 200°C with $4^\circ\text{C}/\text{min}$ and 200 to 900°C with $10^\circ\text{C}/\text{min}$. All determinations were conducted under nitrogen atmosphere.

Results and Discussion

The GC-MS analysis indicated a maximum concentration for *d*-linalool (93%), the main impurities being linalool - oxide (2.73 %) and camphor (4.75 %); *l*-linalool has a concentration of 71.7% (7% linalool-oxide). Concentrations of 79% and 66.7% were found for *d*- and *l*-limonene, respectively, limonene-oxide being the main impurity in both samples. The concentration of caryophyllene was 81.34% from GC-MS analysis, and the linalyl acetate was a mixture of linalool (48.37%) and its ester (33.65%) (i.e. fig. 2).

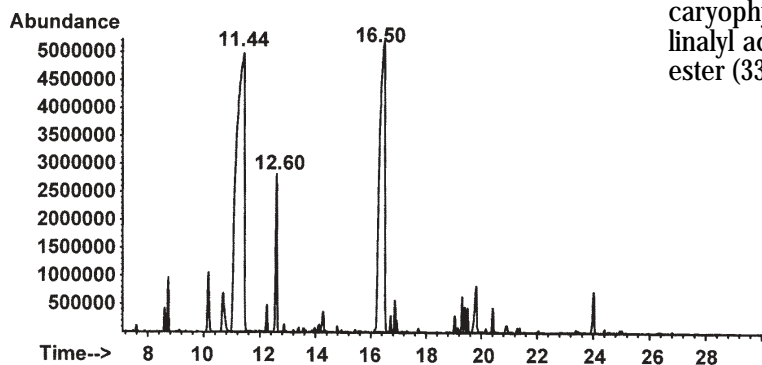


Fig. 2. Chromatogram from the GC-MS analysis of the semi-synthetic linalyl acetate

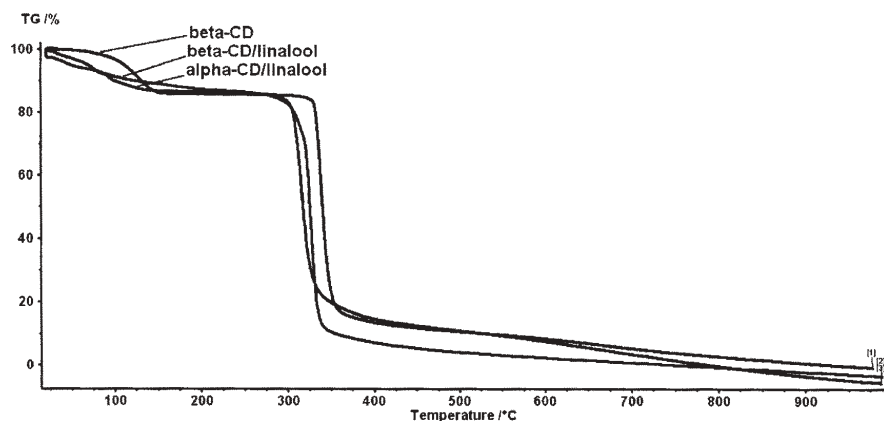


Fig. 3. TG analysis of pure β CD, α CD/*d*-linalool and β CD/*d*-linalool complexes

High yields in the complexation process were obtained for limonene, caryophyllene and α -ionone (> 69%); for α -cyclodextrin as host molecule, the yield was relatively low (up to 25%), but the lowest yield was obtained for the β CD/benzaldehyde complex.

The thermogravimetric analysis clearly indicates the differences between the TG profiles of CDs and CDs/odorant compound complexes. TG analysis of pure β CD/

α CD/*d*-linalool and β CD/*d*-linalool complexes are presented in figure 3.

TG profiles of α CD/linalool and β CD/linalool are almost identical for *d*- or *l*-enantiomers, but significant differences appear between complexes with α CD or β CD (fig. 4). The mass loss of the α CD/linalool complexes are about 11-12.4% in the range of 20-150°C, indicating a stoichiometry of 1:1 for α CD:linalool, and about 10% for β CD complexes and the same stoichiometry.

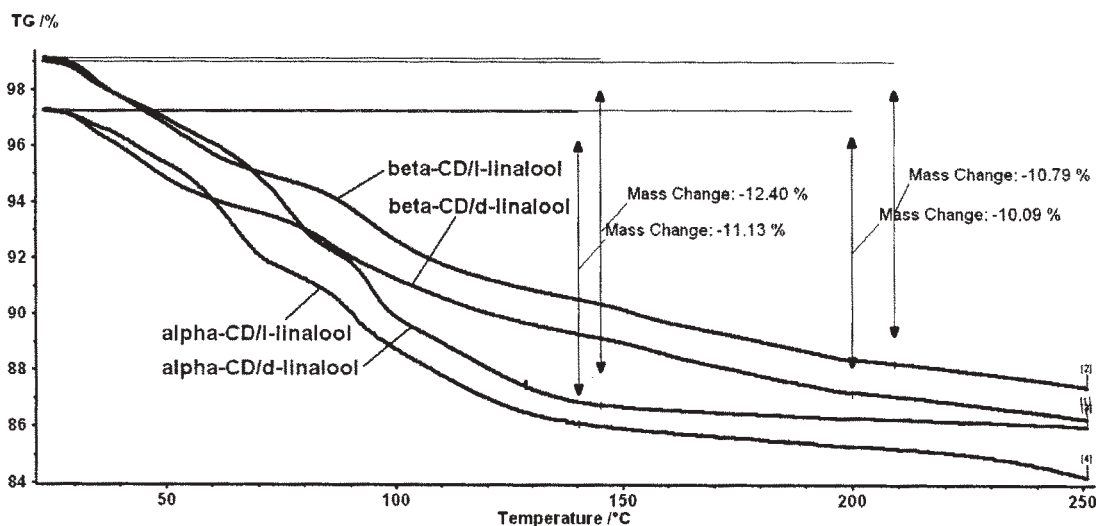


Fig. 4. TG analysis of α CD/linalool and β CD/linalool complexes

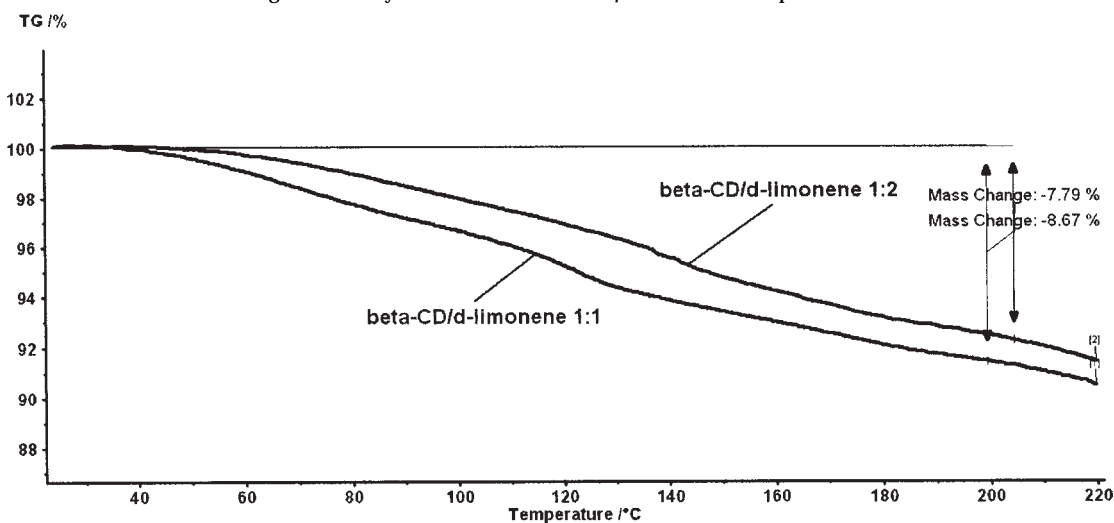


Fig. 5. TG analysis of β CD/*d*-limonene complexes at 1:1 and 1:2 molar ratios

The TG analysis for β CD/*d*-limonene complex at molar ratio of 1:1 and 1:2 indicate almost the same mass change of 8% (fig. 5). The mass change of β CD/*d*-limonene and β CD/limonene were 8.8% and 8.6%, respectively (fig. 6);

probably, the chirality doesn't play any role in molecular encapsulation for these compounds.

TG profiles of β CD/linalool and β CD/linalyl acetate complexes were almost similar, probably due to the large concentration of linalool in linalyl acetate sample (fig. 7).

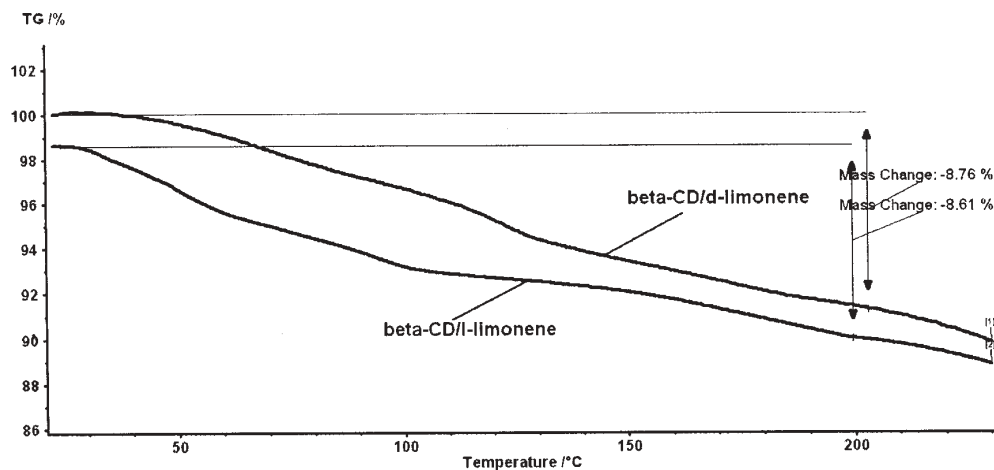


Fig. 6. TG analysis of β CD/*d*-limonene and β CD/*l*-limonene complexes

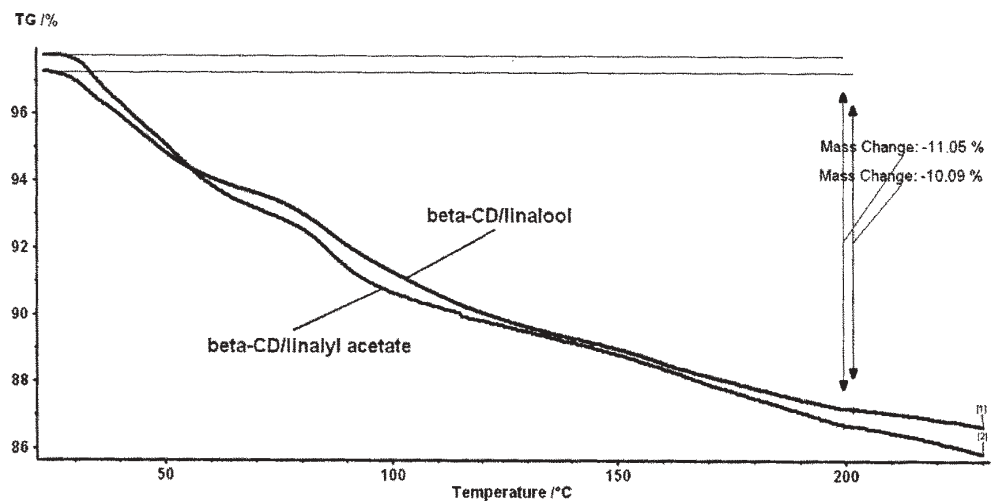


Fig. 7. TG analysis of β CD/*d*-linalool and β CD/linalyl acetate complexes

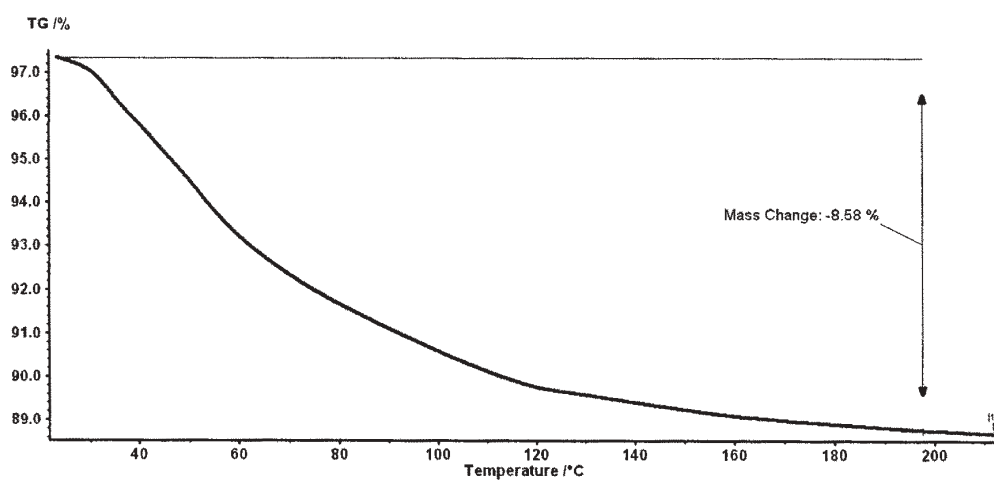


Fig. 8. TG analysis of β CD/ β -caryophyllene complex

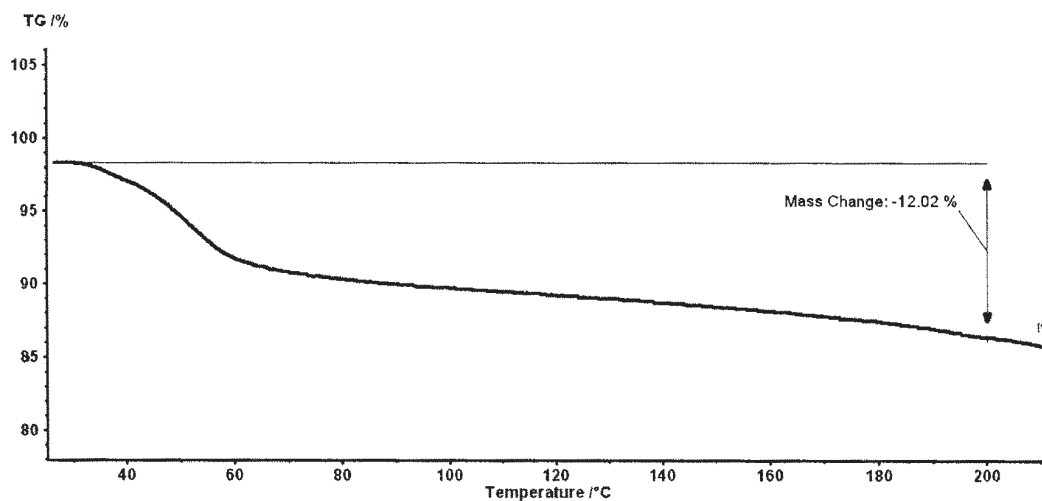


Fig. 9. TG analysis of β CD/benzaldehyde complex

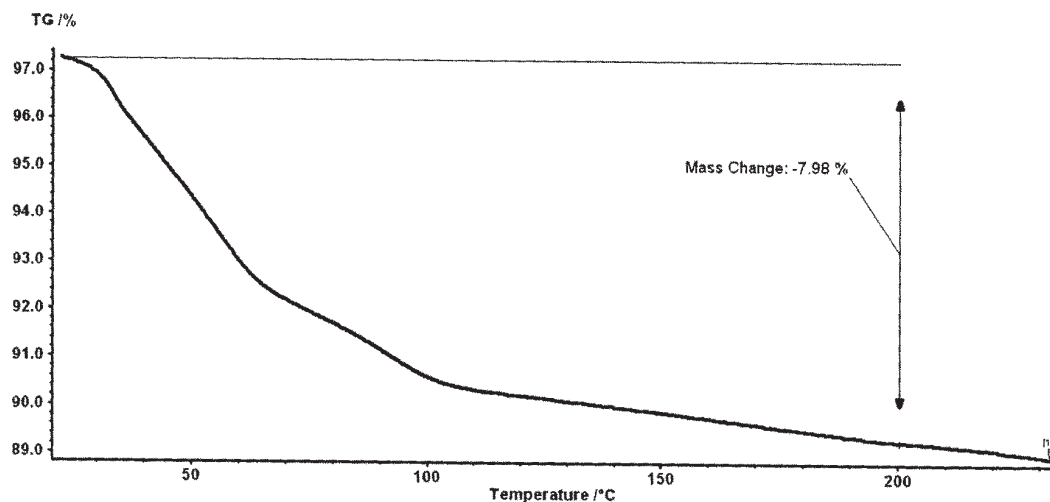


Fig. 10. TG analysis of β CD/hydroxycitronellal complex

Very interesting results were obtained for β CD complexes with β -caryophyllene, benzaldehyde, hydroxycitronellal, *l*-menthol, and vanillin. The mass loss of β CD/ β -caryophyllene complex was 8.6%, almost the whole odorant compound being decomplexed up to 120°C (fig. 8). Almost the whole amounts of odorant compounds were lost up to 75°C for vanillin, and especially for benzaldehyde, which decomplexed in the range of 20-60°C (fig. 9).

Hydroxycitronellal and menthol are released up to 100°C a mass loss of about 7.5% for both complexes (fig. 10).

Conclusion

Fifteen complexes of CDs with different odorant compounds, with medium yields were obtained. Two of these were used α CD as host compound, but the yield in this case was relatively low, probably due to a higher solubility of α CD/odorant compounds in ethanol-water systems. TG profiles clearly differ for complexes containing α CD or β CD; the first complex released the odorant compounds at lower temperatures than the last, due to a weak hydrophobic interaction between odorant compound and α CD.

The CD:odorant compound stoichiometry was 1:1 in most cases, revealed by the TG analysis of β CD/limonene complexes at different molar ratios. The best results were obtained for β CD/benzaldehyde and β CD/vanillin complexes, which released the odorant compounds at lower temperatures than 75°C. The β CD/ β -caryophyllene, hydroxycitronellal, and *l*-menthol complexes released the odorant compounds up to 120°C.

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