Journal of Thermal Analysis and Calorimetry, Vol. 78 (2004) 557–573

CHARACTERIZATION OF *LIPPIA SIDOIDES* OIL EXTRACT–β-CYCLODEXTRIN COMPLEXES USING COMBINED THERMOANALYTICAL TECHNIQUES

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Abstract

Thermoanalytical techniques, being rapid and un-expensive have been used for the investigation of the cyclodextrin inclusion complexes for three decades. The conventional thermoanalytical techniques (TG and DTA/DSC) follow the thermal properties of the uncomplexed compounds. Consequently, the inclusion complex formation as well as the liberation of the entrapped guest cannot be followed. Monitoring the products of the thermal fragmentation of parent cyclodextrin and the included molecule(s), applying TG-MS combined technique provides evidence concerning the inclusion complex formation, and besides, gives selective signal to follow the decomposition of the cyclodextrin inclusion complexes. β-cyclodextrin inclusion complexes of Thymol and *Lippia sidoides Cham* essential oil extract have been prepared and investigated using conventional and combined (TG-MS) thermoanalytical techniques. The evolved gas analysis proved the inclusion complex formation between the host and guests. By the evaluation of the experimental results the elaboration of the entrapped guests from the cyclodextrin cavity could be followed.

Keywords: β-cyclodextrin, essential oil, evolved gas analysis, inclusion complex, *Lippia sidoides Cham*, thermogravimetry–mass spectrometry

Introduction

The essential oils – as complex mixtures of various terpenoids, aromatic substances, aldehydes, ketones, alcohols and esters – are one of the most important raw materials in food, perfumery and also pharmaceutical industries. Usually the fragrance and flavour substances are isolated by hydro-distillation from the dried or fresh plant material (e.g. leaves, seeds, fruits, stems, bark or wood) [1]. The obtained oils have been widely used as antibacterial, anti-inflammatory, antifungal, analgesic or local anesthesic medi-

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1388–6150/2004/ $ 20.00

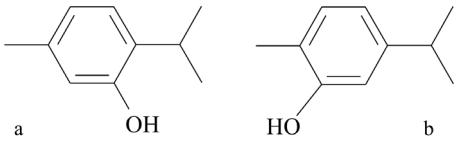
Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

cines and possess also high antioxidant, anti-allergic and anti-depression activity. Probably this is in connection with the content of their phenolic compounds [2].

Lippia sidoides Cham, popularly known in Brazil as alecrim-pimenta, has been used in folk medicine for treatment of gastrointestinal disorders, respiratory and cutaneous diseases. The extract of the leaves and flowers are administered orally or applied on the wounds and burns as compress [3].

Several pharmacological studies on the essential oil have demonstrated 'in vitro' an inhibitory activity against many bacteria, yeasts, dermatophytes, filamentous fungus and mollusc [4–6].

The major compounds of the essential oil from the *Lippia sidoides* leaves are Thymol (50–70%), Carvacol (5–8%), *p*-Cimene (12–27%), *cis*-Cariophyllene (1–10%), γ-Terpinene (6%), Mircene (2%) and other terpens [7, 8]. The antimicrobial and antifungal activities are attributed to the presence of Thymol and Carvacol (for their structure see Scheme 1a and 1b) [4, 9].



Scheme 1 Chemical structures of a – Thymol, b – Carvacol

In order to decrease the volatility of the *Lippia sidoides Cham* (and in general the volatile essential oils) among several others one of the available possibilities is the inclusion complex formation using cyclodextrins.

Cyclodextrins themselves have been known for more than one century [10], however, up to the 1970s a small amount, rather impure and relatively expensive substances could be produced. These (α-1,4)-linked oligosaccharides of α-*D*-glucopyranose are the subject of many papers, reviews [11, 12] and books [13, 14].

At first they were used for pharmaceutical purposes. The aim of the pharmaceutical application of cyclodextrins is to improve the bioavailability to enhance the solubility of the entrapped drug in aqueous solution, to reduce side effects, to eliminate the unpleasant tastes and odours and also to prevent the encapsulated drug against light (UV radiation) and oxidation [15–18]. The effect of additives on complexation both in solid phase and in solution are also reported [19–21].

Cyclodextrins have been widely used for complexation to develop novel dosage forms [22] to modify the drug-release from their solid formulations and turn liquid substances (e.g. essential oils, aromas, flavours) into stable and easy-to-handle powders. This latter one has distinguished importance in case of long term storing and manufacturing [23–27].

Besides, their pharmaceutical application the cyclodextrin inclusion formation has also a great importance in many fields as food, cosmetics, agriculture, chemistry, analytical chemistry [13, 28–32].

For essential oils the inclusion complex formation reduces the loss of active ingredients and protects them against thermally and/or light-induced decomposition, oxidation.

Another advantage of this molecular packing is to provide solid dosage forms instead of a liquid form [33, 34].

Extraction with organic solvents and steam distillation/solvent extraction methods followed by gas chromatography analysis are the most commonly used methods to evaluate and characterize the complex formation between essential oils and cyclodextrins. They involve the identification and determination of the compounds of the original oil and the total amount of the complexed oil as well [27, 35–38], however, Hazra *et al*. reported the application of thermoanalytical methods for the characterization of some essential oils and their key components [39].

For the solid-state characterization of cyclodextrins, as well as their chemically modified derivatives and inclusions, thermoanalytical methods (TG, DTA, DSC), X-ray powder diffractometry, FT-IR spectroscopy and solid-state NMR are frequently used methods. However, due to the relative low costs of measurements, the different thermoanalytical methods are commonly applied as they have been previously reported [40–42].

Despite of the simplicity and usefulness of the application of thermoanalytical techniques, there are some points, which have to be critically considered:

A) The 'conventional' (TG, DTA, DSC) thermoanalytical techniques provide always indirect evidence on the formation of the cyclodextrin inclusion complex. They indicate how the original physico-chemical properties (melting, evaporation, sublimation, decomposition) of the guest compound when it is alone vary upon the technological process and during the thermal run. For example the gradual decrease and finally the disappearance of the melting peak (and consequently the melting enthalpy) of a crystalline compound might be the evidence of the successful inclusion complex formation. This is valid, meanwhile the uncomplexed guest remains in crystalline-state.

B) In some cases, especially, when kneading or grinding techniques for preparation are applied [43], besides the complex formation a certain amorphisation of the originally crystalline guest may occur, too. In such a case, the absence of a melting peak indicates not only the total inclusion complex formation but may represent also a crystalline→amorphous transition due to the mechanical effect upon the process of preparation.

C) For natural (α-, β- and γ-) cyclodextrins, over 270°C gradual thermal decomposition is detected. However, from these mass loss steps one cannot always conclude, whether the sugar derivative or the inclusion complex – including the elaboration of the entrapped guest – decomposes.

D) At last, TG and DSC are not always powerful, when guests with a melting point below 100°C (e.g. Thymol), volatiles (e.g. aromas, flavour components, plant extracts) or easy to sublime compounds (menthol, camphor, etc.) are complexed.

The application of the combined thermoanalytical techniques (TG-FT-IR, TG-MS) is expected to avoid the above listed problems.

This is the reason, why the aim of the present study was the comparative thermoanalytical characterization of the inclusion complex of *Lippia sidoides* oil extract using con-

ventional (TG, DTA, EGD) and combined (TG-MS) techniques. As it was mentioned above, the major constituent of this essential oil is Thymol. So far, besides the inclusion complex of the essential oil, inclusion complex of Thymol with β-cyclodextrin was also prepared. The thermoanalytical results have been completed with X-ray powder diffraction experiments.

Materials and methods

Samples and preparation of inclusions

The essential oil was obtained by steam distillation from fresh leaves of *Lippia sidoides Cham* (Natural Products Laboratoire – University of Ceará - Brazil). The β-cyclodextrin was purchased from Roquette (France) and Thymol standard has been supplied by Fluka (Germany) with 99.99% purity.

According to the literature, the Thymol content in *Lippia sidoides Cham* is between 50–70%. In our case about 68% was determined using GC method.

The samples were prepared starting from 1:8 mass-by-mass ratio, which is equal to about 1:1 molar guest:host ratio. For control, mechanical mixture of 1 g Thymol and (separately) 1 g essential oil were gently mixed with 8 g β-cyclodextrin in an agate mortar.

For the preparation of inclusion complex, 1 g of each guests were added to a sluries of β-cyclodextrin (8 g of β-CD and 6 mL of distilled water) and were kneaded for 40 min to obtain a paste. Then, the samples were dried in a vacuum oven at 70°C for 16 h. The dried products were gently ground and sieved to obtain a fraction with less than 250 μm diameter particles.

Equipments and experimental conditions

For guest content determination Jasco V-550 UV/VIS Spectrophotometer was used. The measurements have been carried out at 276 nm after dissolution an aliquot amount of solids in 1:1 *v*/*v* water–ethanol mixture.

The simultaneous TG-DTA thermoanalytical measurements have been carried out using TA 2960 STA (simultaneous TG-DTA) equipment (TA Instruments Co., Newcastle, Delaware, USA), with a 10 K min^{-1} heating rate starting from room temperature up to 400°C applying a helium purge with 15 L h^{-1} flow rate. The sample amounts were 1–1.5 mg for the pure guests, about 7 mg for cyclodextrin and 8–9 mg for the mechanical mixtures and complexes.

The evolved gas detection experiments have been done using DuPont 916 (Carle 3000) equipment with a built-in hydrogen-air flame ionisation detector, which provides signal for the organic compounds but not for the inorganic ones e.g. water, CO, CO_2, NH_3, etc. The application of this apparatus is advantageous, when the volatile compounds are leaving either from their mechanical mixtures or from their complexes up to 120°C, together with the water content of cyclodextrin. During these experiments 8 K min^{-1} heating rate and 1.8 L h^{-1} nitrogen purging were used between *r.t.*

and 350°C. The initial sample masses were 0.1–0.2 mg for pure guests, 1–1.1 mg for pure cyclodextrin and 1–1.2 mg for all the mechanical mixtures and complexes.

The combined thermoanalytical measurements were carried out using a Balzers Thermostar GSD 300T quadrupole mass spectrometer (Lichtenstein) operating between 1–300 specific mass/charge interval with a heated (200°C) silica capillary transfer tube connected to the gas outlet of the 2960 STA module.

Results and discussion

Guest content determination

The guest content determinations have been carried out as it has been written above. Starting from their molar masses, for Thymol–β-cyclodextrin complex 1:1 molar ratio is equal to 11.7% *w*/*w*%. However, the measured guest content was 10.0%, which corresponds to 0.85:1 (average) guest:host molar ratio. For the essential oil complex 5.6% was measured, which is about 0.48:1 (average) guest:host molar ratio.

Thermoanalytical results

Thymol–β-CD complexes

The TG curves of Thymol–β-CD system are summarized in Fig. 1. As it can be seen, Thymol evaporates completely up to 120°C (dash dot curve), while β-CD looses its water content causing about 14.3% mass loss in this temperature interval (broken double dash curve). Up to 250°C no further mass loss was measured, and the decomposition of β-CD started over 270°C (broken double dash curve). The TG curve of the mechanical mixture (solid curve) behaves as the superposition of the pure (Thymol and β-CD) components, the evaporation of Thymol and the adsorbed water content of cyclodextrin completes up to 120°C in overlapping steps. The measured mass losses support, that no interaction took place between Thymol and β-CD upon heating (solid curve).

On the contrary, the TG curve of the Thymol–β-CD inclusion complex indicates 9.5% of mass loss up to 120°C, which corresponds mainly to the water loss. Between 120–170°C 2.7% further mass loss was detected, belonging to the release of Thymol from its inclusion complex having a lower thermal stability (see EGD curve, Fig. 3) and then a gradual but slow mass loss process (starting from 170 and ending at about 270°C) indicates the release of a tightly bounded part of guest from its inclusion complex (short dash curve).

The DTA curves (Fig. 2) show qualitative picture and confirm the above described findings. The melting endotherm of Thymol (at about 52°C) is well visible both in the DTA curve of the pure compound (dash dot curve) and of the mechanical mixture (solid curve) but does not appear when the complex was investigated (short dash curve) providing indirect evidence on the inclusion complex formation.

The EGD curves of Thymol–β-CD system are summarized in Fig. 3. Due to the different experimental arrangement, approximately 10°C shift towards the lower

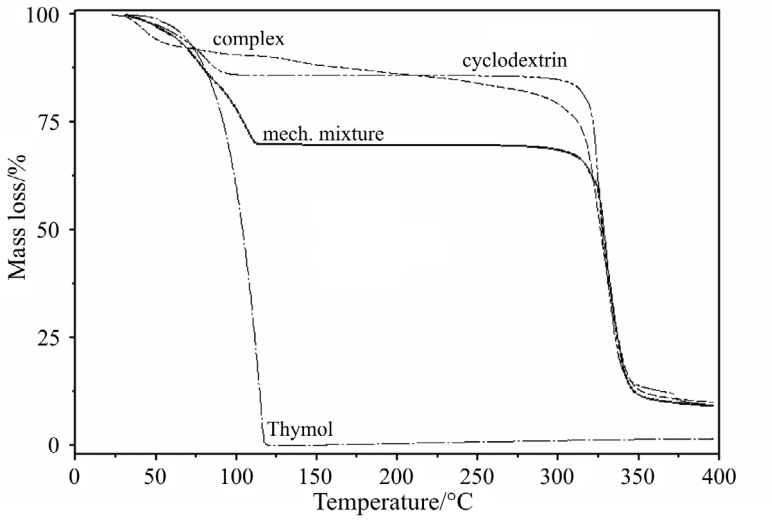


Fig. 1 TG curves of Thymol–β-cyclodextrin samples

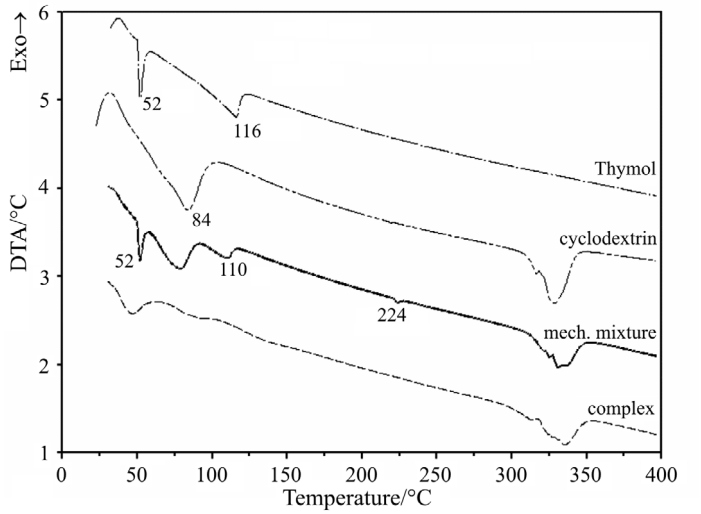


Fig. 2 DTA curves of Thymol–β-cyclodextrin samples

temperatures was observed when the DuPont 916 EGD apparatus was used. That is why the EGD profile of the pure Thymol shows complete evaporation up to 100°C. The EGD curve of β-CD is running on the baseline indicating that no organic compound leaves from the substance up to 270°C, but above this temperature its thermal degradation takes place.

The EGD curve of the mechanical mixture is bearing the thermoanalytical features of the pure components. The peak at 76°C indicates the evaporation of the

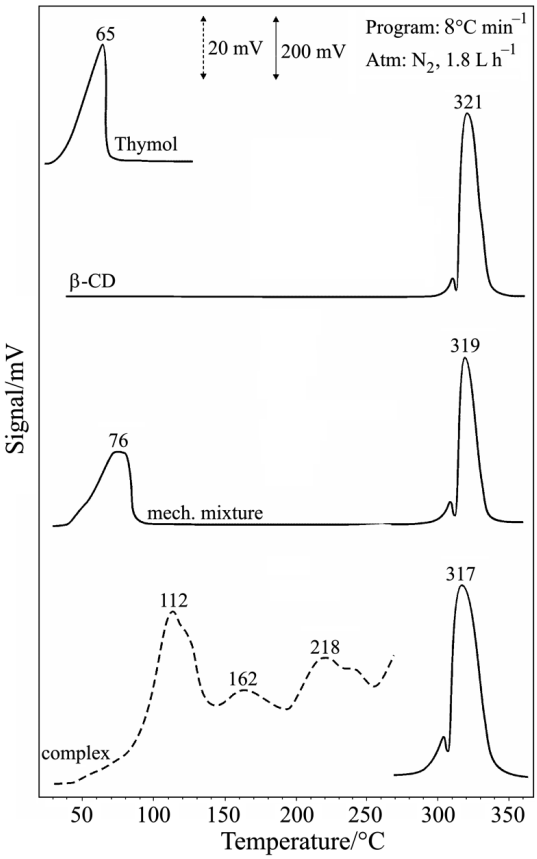


Fig. 3 EGD curves of Thymol–β-cyclodextrin samples

guest, while the peak at 319°C can be attributed to the decomposition of β-CD denying any interaction between the two components upon heating.

According to the EGD measurements, the thermal behaviour of the complex is remarkably different from all previous samples. Between 50 and 250°C three peaks were obtained. They are representative for the thermal decomposition of the Thymol–β-CD inclusion complex. At the same time they indicate the fractions of inclusion complexes with different thermal stabilities, consequently with different stoichiometries.

Essential oil–β-CD complexes

Since the two third of the essential oil is Thymol (and the remaining one third is also volatile) the TG curves of essential oil–β-CD system (Fig. 4) are similar to the TG curves of pure Thymol–β-CD (Fig. 1). Up to 120°C total evaporation of guest was recorded (dash dot curve). The TG curve of the mechanical mixture showed also the superposition of the

thermal behaviour of the pure host and guest indicating multistep evaporation of the essential oil and the adsorbed water content of cyclodextrin up to 120°C. It confirms that heating did not result in interaction between the components (solid line).

The TG curve of the essential oil–β-CD complex is almost the same compared to the TG curve of the Thymol–β-CD complex (short dash curve). 9.4% mass loss was measured at 120°C, then gradual decrease of mass was observed indicating the evaporation/decomposition of the entrapped guest from the cyclodextrin cavity.

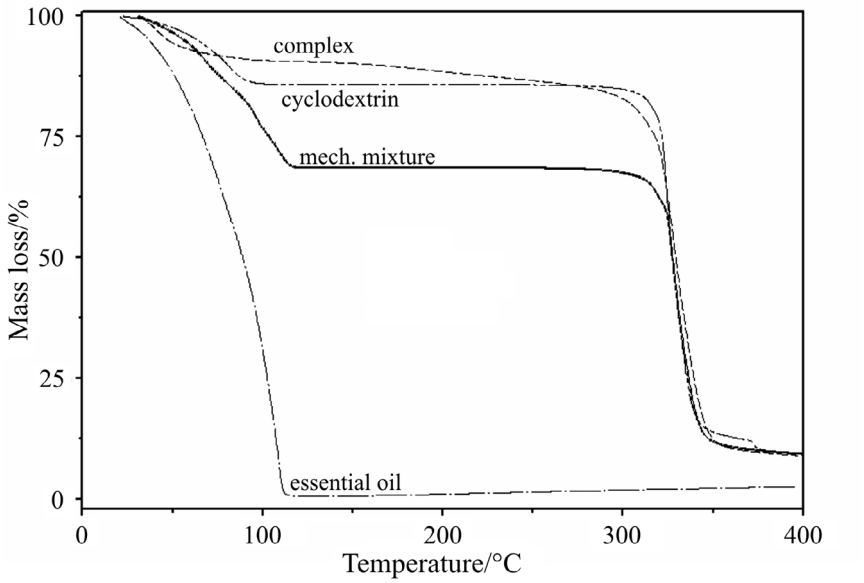


Fig. 4 TG curves of essential oil–β-cyclodextrin samples

Despite of Thymol is the major component of the essential oil, no melting peak was observed in the DTA curves of the pure essential oil and of the mechanical mixture (Fig. 5, dash dot and solid curves). The differences in the DTA curves of the mechanical mixture and the complex of essential oil–β-CD indicate well the complex formation between the components (Fig. 5, solid and short dash curves).

The EGD curves of essential oil–β-CD system are given in Fig. 6. The essential oil evaporates up to 100°C when it is alone and also from its mechanical mixture. The EGD profile of the mechanical mixture indicates that no interaction took place between guest and host upon heating. The comparison of curves of the mechanical mixture and the complex confirms the formation of the inclusion complex. The broad peak with a peak temperature at 170°C is belonging to the decomposition of the formed inclusion complex.

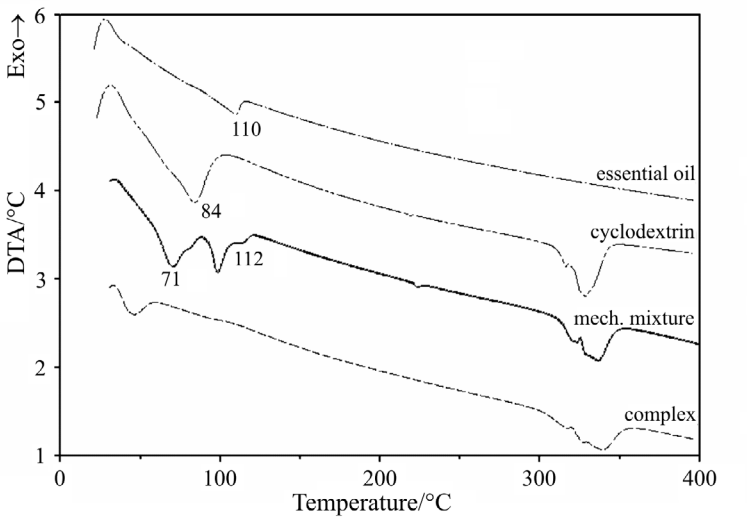


Fig. 5 DTA curves of essential oil–β-cyclodextrin samples

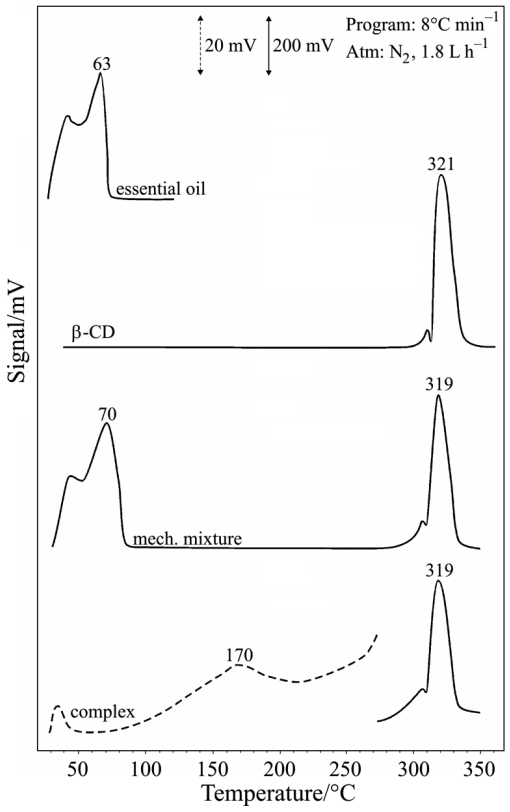


Fig. 6 EGD curves of essential oil–β-cyclodextrin samples

Mass spectrometric results

Thymol–β-CD system

The mass spectrum of Thymol taken from NIST Database [44] is given in Fig. 7. The base peak appeared at *m*/*z*=135, while the second, highest intensity belongs to the molecular peak at *m*/*z*=150.

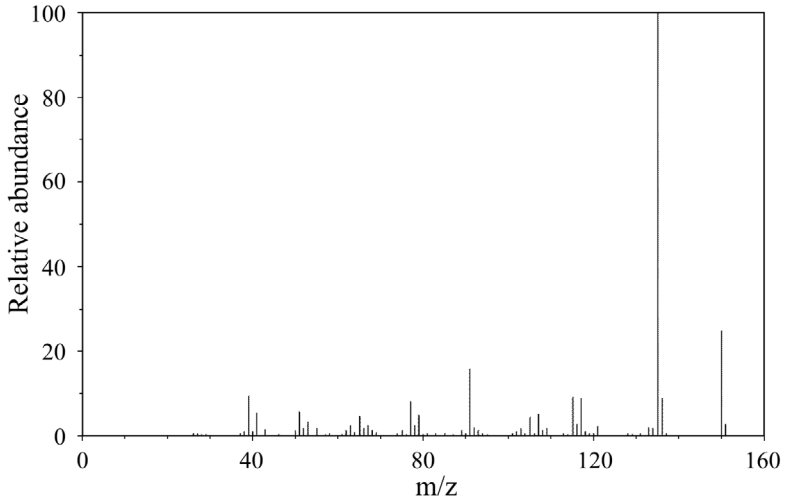


Fig. 7 Mass spectrum of Thymol [44]

Despite of the highly different experimental conditions, very similar and representative three-dimensional mass spectrum was recorded for Thymol using the TG-MS combined technique (Fig. 8).

The *z* axe indicates the number of the recorded cycles, which is proportional to the time. Consequently, along the *z* axe a temperature scale can be drawn, which helps to compare the graph to the corresponding TG curve. Figure 8 also demonstrates that Thymol leaves up to 120°C.

The peaks at *m*/*z*=51, 65, 77 and 91 are representative for the 'tropilium rearrangement' (Scheme 2), which is typical for the aromatic ring containing a methylene group.

On the base of the SAC spectrum of Thymol (Fig. 8), the most representative lines (*m*/*z*=91, 115, 135 and 150) had been selected and were monitored in MID mode resulting a two-dimensional graph, where now the *x* axe is proportional to the temperature of the sample (Fig. 9).

The MID curves of β-cyclodextrin are shown in Fig. 10. The signal at *m*/*z*=44 originates essentially from the thermal fragmentation of β-CD. This is characteristic for the –CH–CH_2–OH part of the glucopyranose units of β-CD [45]. That is, why no remarkably change in the measured ionic current for *m*/*z*=44 can be seen in the MID curves of Thymol (Fig. 9).

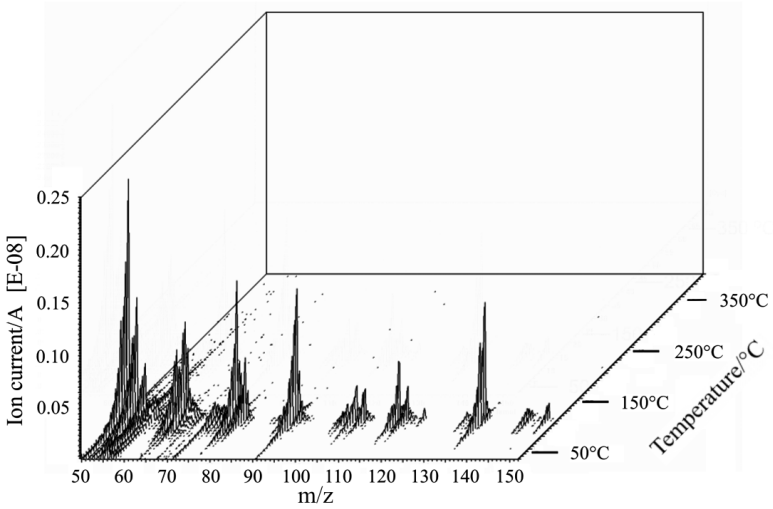
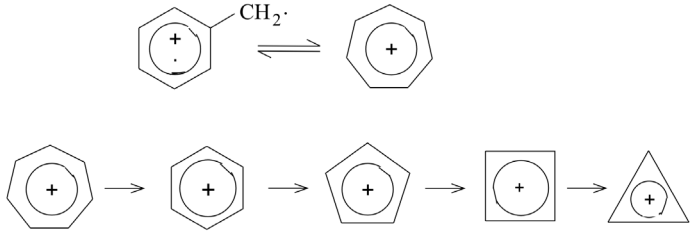


Fig. 8 Three dimensional mass spectrum of Thymol recorded in SAC mode



Scheme 2 Tropilium rearrangement for an aromatic ring containing methylene group

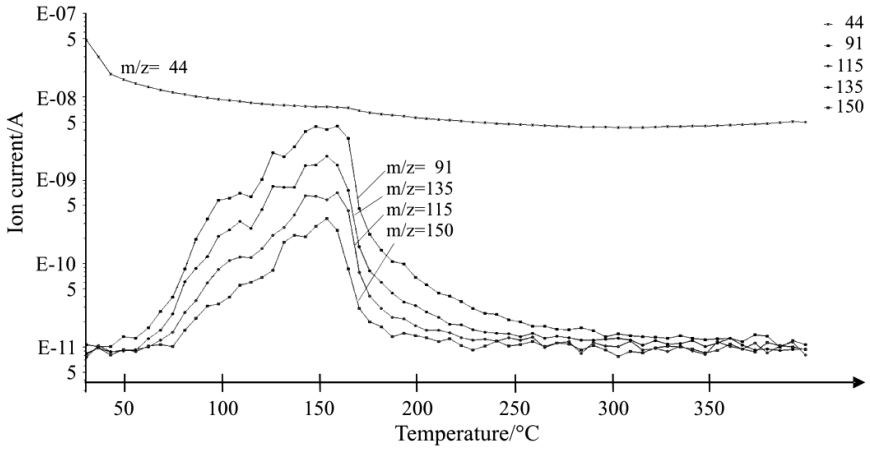


Fig. 9 MID curves of Thymol

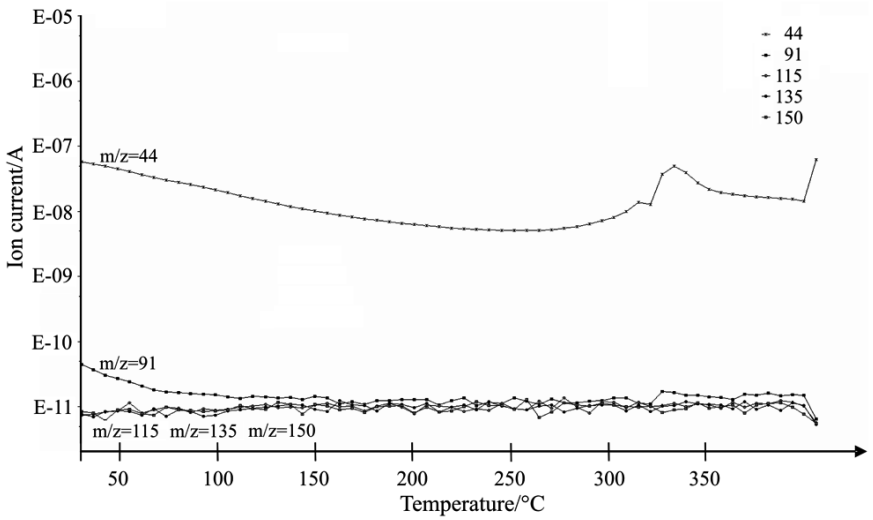


Fig. 10 MID curves of β-cyclodextrin

The MID lines of the mechanical mixture of Thymol–β-CD are summarized in Fig. 11 indicating the separate evaporation of the pure components. In agreement with the TG and EGD curves (Figs 1 and 3) the evaporation of Thymol is complete up to 120°C and then, the decomposition of β-CD starts over 250°C. On the contrary the MID curves of Thymol–β-CD complex show a slow evaporation of the entrapped guest in a wide temperature interval (100–320°C, Fig. 12, *m*/*z*=91).

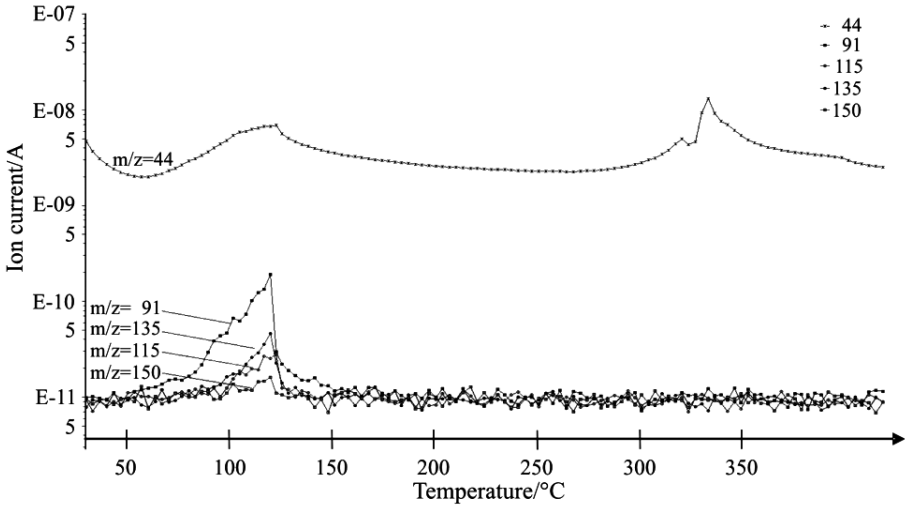


Fig. 11 MID curves of Thymol–β-cyclodextrin mechanical mixture

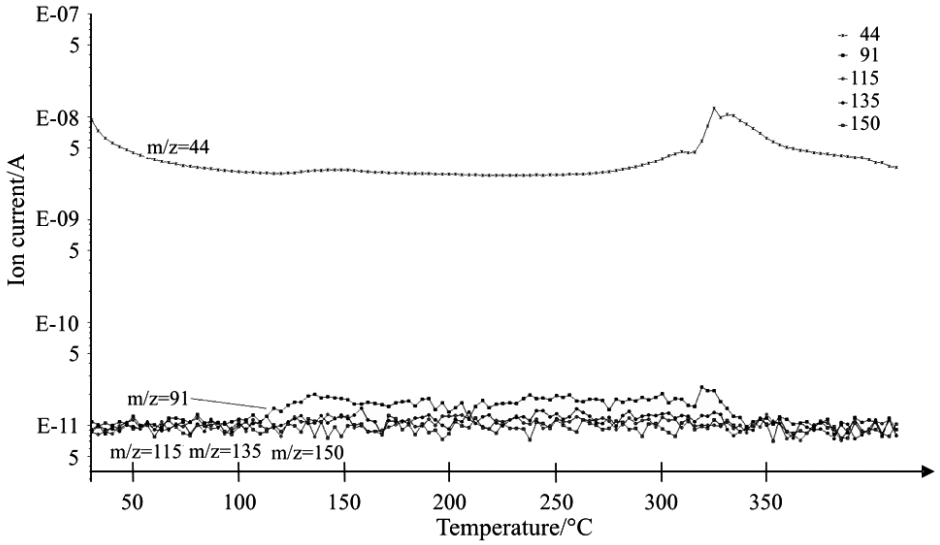


Fig. 12 MID curves of Thymol–β-cyclodextrin complex

Lippia sidoides Cham extract–β-CD system

Figures 13–15 are about the essential oil extract–β-CD system. The pure oil starts to evaporate even at ambient and the major part of the substance leaves up to 200°C, however the process becomes complete at about 300°C. The different shapes of the individual MID curves indicate that the essential oil contains several compounds with different volatility (Fig. 13).

The MID curves of the mechanical mixture are described in Fig. 14. It can be seen, that the process of evaporation also starts at *r.t.* but completes at much lower temperature (150–170°C), due to the larger available surface for the evaporation. The evaporation of the entrapped guest(s) result substantially different MID profiles compared to the thermal behaviour of the mechanical mixture (Figs 14, 15). The different components of the essential oil evaporate slowly from their inclusion complex along a wide temperature interval (100–300°C) proving the complexation overlapping with the decomposition of β-CD itself.

The X-ray powder diffraction patterns are given in Fig. 16. The most intensive diffraction lines for β-cyclodextrin appeared in the X-ray pattern of the mechanical mixture (curve 3) denying any interaction between the components in solid phase after their gently mixing. The X-ray diffraction patterns of both the Thymol–β-CD and essential oil–β-CD are remarkably different from any of the previous ones giving evidence on the inclusion complex formation but they are similar to each other because of the high Thymol content of the essential oil sample (curves 4 and 5).

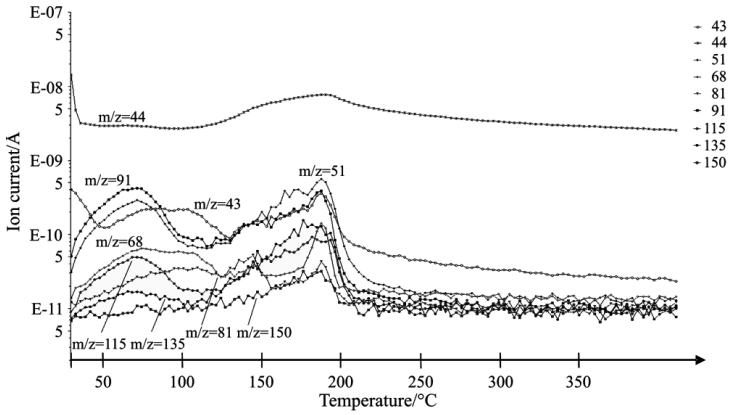


Fig. 13 MID curves of essential oil

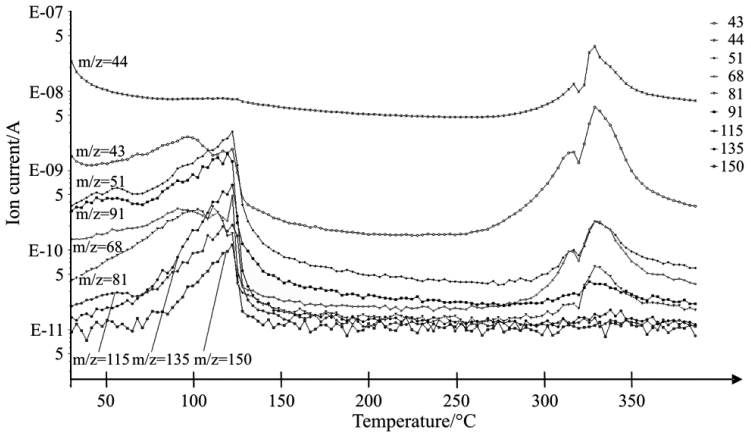


Fig. 14 MID curves of essential oil–β-cyclodextrin mechanical mixture

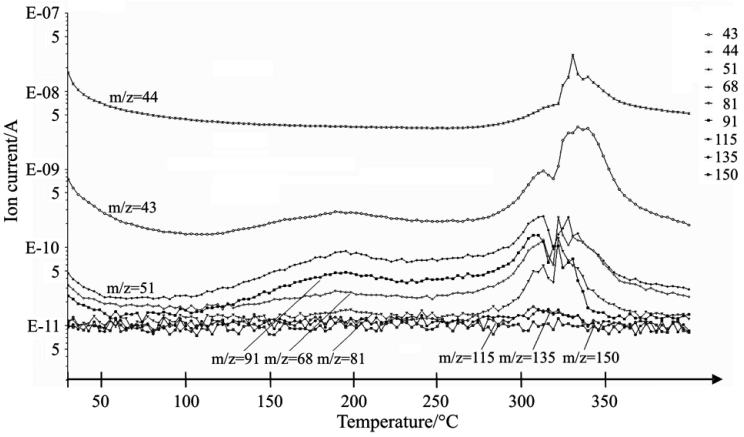


Fig. 15 MID curves of essential oil–β-cyclodextrin complex

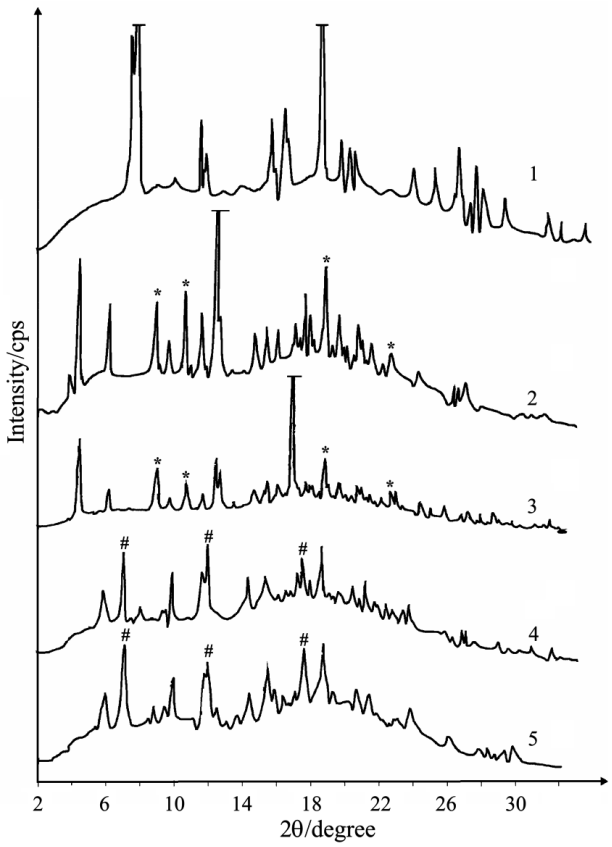


Fig. 16 X-ray diffraction patterns of Thymol–β-cyclodextrin and essential oil–β-cyclodextrin samples. 1 – Thymol, 2 – β-CD, 3 – Thymol–β-CD mech. mixture, 4 – Thymol–β-CD complex, 5 – essential oil–β-CD complex (∗ – β-cyclodextrin and # – inclusion complex)

Conclusions

The aim of the investigations was to work out an experimental method for TG-MS combined technique to identify the inclusion complex formation between β-cyclodextrin, Thymol and *Lippia sidoides Cham.*

All the results of the TG-MS measurements are in a good agreement with the results of the conventional TG, DTA and EGD runs, furthermore, the X-ray diffraction analysis supported also the existence of the inclusion complexes.

The main advantage of the application of combined method is, besides proving the complex formation, the liberation of the entrapped guest can be selectively followed.

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The finantial support of OTKA Grant No. 26459 is gratefully acknowledged.

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