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SOLID STATE STUDIES ON MOLECULAR INCLUSIONS OF *LIPPIA SIDOIDES* ESSENTIAL OIL OBTAINED BY SPRAY DRYING

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Inclusion complexes of *Lippia sidoides* essential oil and β-cyclodextrin were obtained by slurry method and its solid powdered form was prepared using spray drying. The influence of the spray drying, as well as the different essential oil:β-cyclodextrin ratio on the characteristics of the final product was investigated. With regard to the total oil retention 1:10 mass/mass ratio as optimal was found between the essential oil and β-cyclodextrin.

Thermoanalytical techniques (TG, EGD, TG-MS) were used to support the formation of inclusion complex and to examine their physicochemical properties after accelerated storage conditions. It may be assumed that the thermal properties of the complexes were influenced not only by the different essential oil/ β-cyclodextrin ratio but also by the storage conditions. In the aspect of their thermal stabilities, complex prepared with 1:10 *m*/*m* ratio (essential oil:β-cyclodextrin) was the most stable one.

Keywords: *β-cyclodextrin, encapsulation, essential oil, inclusion complex, Lippia sidoides, spray drying, TG-MS, thermal analysis*

Introduction

Lippia sidoides is a Brazilian medicinal aromatic herb widely used in the folk medicine practice as an effective local antimicrobial agent. Its essential oil is obtained by hydro-distillation from the fresh leaves of the plant. The advantageous antimicrobial effect and biological/therapeutic activity of *Lippia sidoides* essential oil might be explained by its large (50–80%) thymol content. Besides thymol, other phenols are also present in the oil and may act synergistically with thymol [1–3].

Essential oil is a complex mixture of lipophilic substances, which are usually odors or liquids. The volatility of the essential oil is one of its main characteristics. However, when it is exposed to oxygen, light and heat, it can be easily oxidized, decomposed or become resinous [4]. Encapsulation of essential oil using β-cyclodextrin (β-CD) was reported by several authors, emphasizing the improvement in the physicochemical stability of terpenoid compounds of the essential oil [5–8].

Molecular encapsulation occurs by the entrapment of the guest molecules into the internal cavity of the cyclodextrin. The ability of cyclodextrin to form inclusion complexes with guest molecules depends on the geometry of guest related to the cyclodextrin and on the thermodynamic interactions among the different components of the system (cyclodextrin, solvent, guest molecule) [9].

Inclusion complexes can be produced by different methods. The selection of the appropriate method depends on the properties of the active compound, the kinetics of the complex formation, etc. Among the various preparation methods, the suspension technique is the most frequently used. Slurry of cyclodextrin (cold, hot, neutral or acidic) together directly with the guest molecule or its concentrated solution is intensively stirred. After reaching the equilibrium the water is removed either by filtration and evaporation or by other convenient techniques, e.g. freeze-drying, spray-drying [10].

Thermal analysis is a frequently used tool in the solid state investigation of cyclodextrin inclusion complexes. The thermal properties of cyclodextrin have been summarized in [11]. In general, thermal studies are more effective by using combined techniques like thermogravimetry–mass spectrometry (TG-MS) and thermogravimetry–Fourier transformed infrared spectroscopy (TG-FTIR). Applications of these techniques on native and chemically modified

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cyclodextrins were reported in [12]. The use of combined TG-MS technique in the investigation of entrapped volatile substances [7–8] as well as the convenience of the TG-MS coupling to investigate the effect of storage [13] was also highlighted.

The aim of the present work was to produce the β-CD inclusion complex of *Lippia sidoides* essential oil using the slurry method. Spray drying technique was applied for solvent removal in order to obtain the inclusion complex in solid state. Influence of spray drying and of the different proportions of essential oil and β-CD on the product characteristics was evaluated. Morphological changes upon complexation and during storage were followed by scanning electron microscopy (SEM) and X-ray diffraction (XRD). Particle size distribution, total oil- and thymol retention were determined in the final product. Thermal analysis was used to provide evidence on the inclusion complex formation and to evaluate the physicochemical properties of the products after accelerated storage conditions.

Experimental

Materials

Lippia sidoides essential oil was purchased from Pronat (Fortaleza-CE-Brazil). Thymol (99.99%) was used as chemical marker and was supplied from VETEC (Brazil). β-cyclodextrin was kindly donated by Roquette (France). The organic chemicals used in the analysis were of analytical grade.

Methods

Inclusion complex preparation

The β-cyclodextrin slurry containing 50% of solid (m/m on the wet basis) was prepared by blending the β-cyclodextrin powder in warm distilled water at 50°C under continuous stirring with a magnetic bar. *Lippia sidoides* essential oil was added to the β-cyclodextrin slurry to reach 3 different (1:10; 1.33:10 and 2:10) essential oil:β-cyclodextrin ratios. The slurry was cooled down to room temperature and left for 12 h under agitation to reach the equilibrium condition, and then fed to a bench-top spray dryer (Lab-Plant SD-05, Huddersfield, U.K.) with concurrent flow regime. The drying chamber had a diameter of 215 mm and height of 500 mm. The main components of the system were a feed system of drying gas, constituted by a blower and air filter; temperature control system of drying gas and product collect system (cyclone). The feed system was constituted by a peristaltic pump, a two- fluid atomizer (inlet orifice of 1.0 mm) and an air compressor. The controlled parameters were: atomizer pressure (5.0 kgf cm^{-2}); compressed air flow rate in the atomizer (17.5 L min^{-1}); flow rate of drying air –60 m^3 h^{-1} and inlet air temperature (160°C).

Storage study

Glass vials (2 cm diameter and 4 cm height) were filled with a thin layer (0.5 cm – nearly 1.0 g) of the samples and then stored in desiccators at relative humidity (RH) of 66.09% and 40°C. Constant RH environment was created using saturated salt solutions of potassium iodine. Samples were removed from the desiccators after 1 and 6 weeks of storage. To avoid degradation, the powdered samples were put into bags to protect them against light and water vapor and then stored at 4°C.

Particle size distribution

Particle size and particle size distribution were determined by laser light scattering pattern (Beckman Coulter LS 13 320) in the range of 0.375–2000 μm.

Scanning electron microscopy (SEM)

The scanning electron microscopic observations were performed on a Jeol JSM 5500LV apparatus after sputtering an Au/Pd conductive layer on the investigated samples.

Retention of total essential oil in the complexes

Retention of encapsulated total oil was determined by hydrodistillation using a Clevenger [14]. 10 g of each sample was steam-distilled with 200 mL of deionized water for 2 h. The volume of distilled oil (recovered oil) multiplied by its density (0.934 mg mL^{-1} at 20°C) gave the mass of the retained oil in the powder. The oil retention in the powder was calculated in percentage related to the amount of oil added originally to the suspensions.

GC-MS analysis

Qualitative analysis of the retained oil as well as the quantitative analysis of the entrapped thymol in the complexes were carried out on a SHIMADZU® GCMS-QP 2010 gas chromatograph coupled to a mass spectrometer equipped with auto sampler mod. AOC-20I SHIMADZU and DB-5 capillary column (30·0.25 mm; 0.25 μ film thickness). The temperature was programmed at 3°C min^{-1} from 60 to 180°C. Other operating conditions were as follows: injector temperature (240°C); carrier gas (H_2) at a flow srate of 1.41 mL min^{-1}; detector temperature (300°C); split ratio (1:100). The components of the oil were identified by comparison of their mass spectra and retention indices (RI) to a series of alkanes (C_{10}–C_{22}) with those published in the

literature [15] and presented in the MS computer library WILEY275.L. The amount of thymol was determined by calibration method (R^2=0.9999) in the 0.125 to 2.5 mg mL^{-1} concentration range.

Thermal analysis

Simultaneous TG-DTA thermoanalytical measurements were carried out using a TA 2960 STD equipment (TA Instruments Co, Newcastle, Delaware, USA). Experimental conditions were: 5°C min^{-1} heating rate starting from 30 up to 350°C and helium purging with 15 L h^{-1} flow rate. The sample amounts were about 7 mg.

Evolved gas detection (EGD) experiments were done using a DuPont 916 (Carle 3000) equipment with a built-in hydrogen-air flame ionisation detector. During these experiments 8°C min^{-1} heating rate and 1.8 L h^{-1} nitrogen purge gas were used between ambient and 350°C. The initial sample masses were around 2 mg.

For the combined thermoanalytical measurements (TG-MS) a Balzers Thermostar GSD 300T quadrupole mass spectrometer (Lichtenstein) operating between 1–300 specific mass/charge range was connected to the outlet of the TG furnace through a heated silica capillary transfer tube.

X-ray powder diffraction (XRD)

XRD patterns of the inclusion complexes samples were recorded by using a PANalytical X'PERT PRO MPD powder diffractometer. X'Pert Data Collector and X'per Highscore Plus softwares (PANalytical, Almelo, The Netherlands) were used for data collection and analysis. The X-ray diffractometer was operated with an anode current of 30 mA and accelerating voltage of 40 kV. Samples were put on obliquely cut silicon crystal zero background holders and exposed to CuK_α radiation at diffraction angles (2θ) from 2 to 42° at a speed of 1.2° min^{-1} followed with a X'Celerator RTMS detector (Real Time Multiple Strip Detection Technology) in scanning mode using an active length of 2.122°.

Results and discussion

Production and characterization of inclusion complexes

The inclusion complexes IC-A, IC-B and IC-C were produced from slurries containing *Lippia sidoides* essential oil and β-CD using 1:10, 1.33:10 and 2:10 mass/mass ratios, respectively. The yields were between 90–92%. Material loss was due mostly to a powder deposit formation on the bottom of the drying chamber caused by the poor collecting efficiency of fine particles in the cyclone.

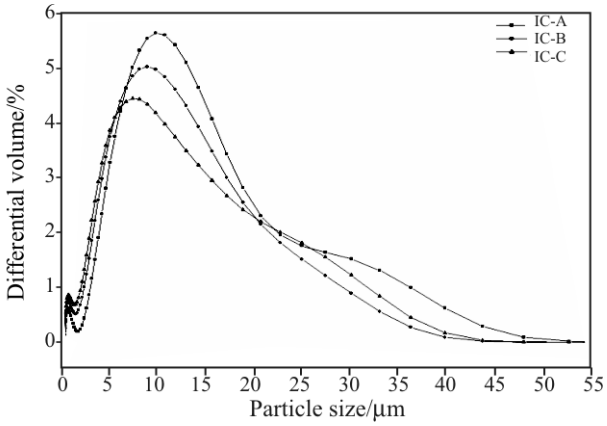

Fig. 1 Particle size distribution of the inclusion complexes

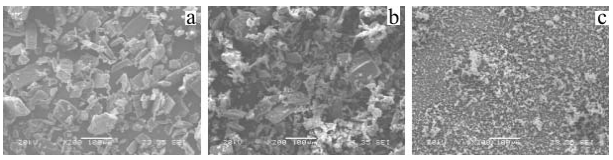

Fig. 2 SEM images of a – native and b – spray dried cyclodextrins and c – spray dried inclusion complex at zero time (N=200)

The particle size distribution of the samples varied from 0.375 to 52.63 mm, however, the representative particle size was about 10–12 mm (Fig. 1).

The SEM images (Fig. 2) of the samples showed that amorphization took place when native cyclodextrin itself was exposed to spray drying. The average particle sizes were below 100 μm. Spray drying resulted also an amorphous product from the uploaded *Lippia sidoides* – β-CD solution. After 6 weeks of storage the morphology of the inclusion complex did not show any changes (so far photo here is not presented).

The amount of the total oil in the samples as a function of the composition is presented in Fig. 3. The best encapsulation efficiency was around 70% and it was reached in sample IC-A. On the other hand, from samples IC-A to IC-C, a decreasing tendency in the total oil content was observed, when the initial amount of added oil was increased. In general, inclusion complex formation means the entrapment of guest compounds inside the β-CD cavity. Volatile compounds, which are not included in the CD cavity, might be lost during the preparation of the complexes (in our case upon spray drying). Thus, when the initial amount of added oil increased, larger guest (essential oil) loss was observed.

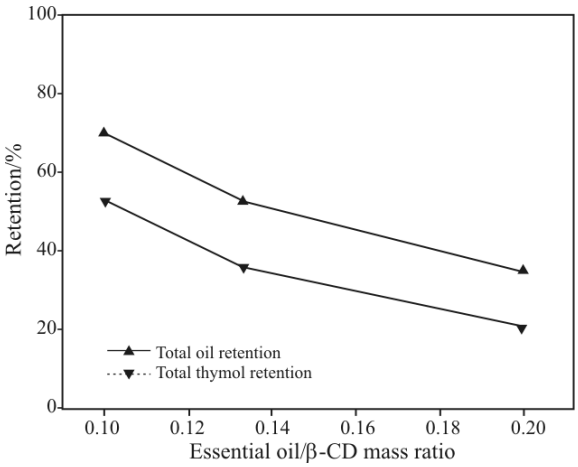

Fig. 3 Retention of the total oil and thymol in the inclusion complexes

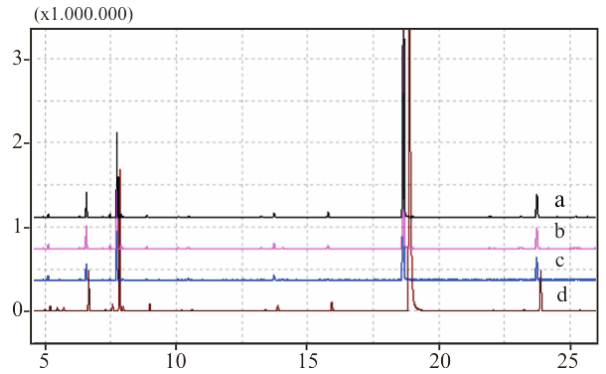

Fig. 4 GC chromatograms of a – IC-A, b – IC-B, c – IC-C and d – original *Lippia sidoides* essential oil

Entrapment of thymol by β-CD was determined from the recovered oil by using GC method (Fig. 3). Thymol retention showed the same tendency as it could be seen for the total oil retention. Thymol content increased when the proportion of β-CD was increased.

The least amount of thymol was observed in sample IC-C, which might be related to the equilibrium relationship and selective diffusion of the free thymol. Liu *et al*. [16] proposed a simple mathematical model for estimating the l-menthol retention during the drying of a single droplet by spray drying. In order to draw some conclusions about the effect of the β-CD content on the thymol retention, similar assumptions to the loss of l-menthol were considered.

It may be assumed for an aqueous cyclodextrin solution that cyclodextrin, thymol and inclusion complex are related to each other according to the following equilibrium equation:

$$Th+CD \leftrightarrow Th\text{-}CD \qquad (1)$$

where Th, CD and Th-CD are the free thymol, cyclodextrin and inclusion complex of thymol and cyclodextrin, respectively.

In the initial step of drying, thymol may be present in included and free state in the droplet. As drying proceeds, free thymol may move to the surface of the droplet by molecular diffusion and then evaporate into the drying air. Subsequently, the equilibrium condition between the complexed and free guest shifts to the left (according to Eq. (1)) and the complexed thymol will be released from the cavity of cyclodextrin. The dissociation rate of the complex is assumed to be proportional to the concentration difference between the equilibrium and current bulk concentration of free thymol.

Essential oils are usually consisted of several volatile compounds. Depending on their polarity, molecular size, etc. the composition of the intact essential oil and the complexed fraction might be different [17].

Identification of the flavor compounds in the *Lippia sidoides* essential oil was accomplished by GC-MS analysis. 25 volatiles, around 99% constituents of the total oil components were identified. Disregard of the minor components, whose proportion in the *Lippia sidoides* oil are less than 0.7%, the major volatiles are: thymol (71.8%); *ortho*-cymene (9.8%); caryophellene oxide (5.4%); β-myrcene (4.1%); γ-terpinene (2.4%); methyl-thymol (1.0%); α-terpinene (1.0%) and terpin-4-ol (0.8%). A number of peaks were absent in the chromatogram of the total oil recovered for all the three samples, which might be due to the loss of minor volatiles during the spray drying (Fig. 4).

Nevertheless, all the major flavor compounds in the original *Lippia sidoides* essential oil were entrapped by β-CD, except γ-terpinene which was not detected in the total oil recovered from IC-C sample. This finding suggests that *Lippia sidoides* essential oil – β-CD inclusion complex can be successfully produced by using slurry method and spray drying.

Thermal behavior of the inclusion complexes was studied by TG (thermogravimetry) and EGD (evolved gas detection). In general, the inclusion of a guest molecule by the cyclodextrin usually causes a shift in evaporation, sublimation and/or decomposition towards higher temperatures, but these features might be as well absent below the decomposition temperature of the cyclodextrin.

TG curve of the essential oil (Fig. 5) showed one step of mass loss corresponding to the total evaporation of the sample between 30 and 120°C.

TG curves of IC-A, IC-B and IC-C inclusion complexes are almost the same (Fig. 5), showing only very slight differences between each other. In the first step up to 105°C around 5% changes in mass (loss of adsorption water) was observed.

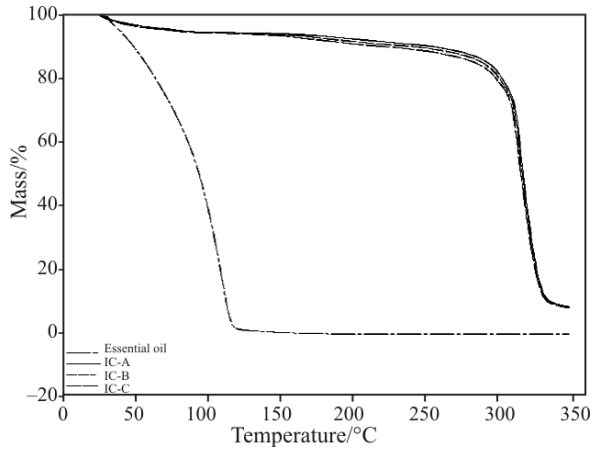

Fig. 5 TG curves of *Lippia sidoides* essential oil and inclusion complexes

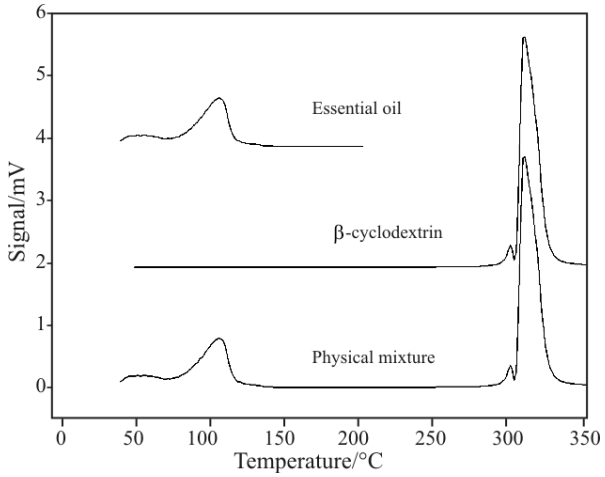

Fig. 6 Comparative EGD curve of: pure essential oil, pure β-CD, physical mixture

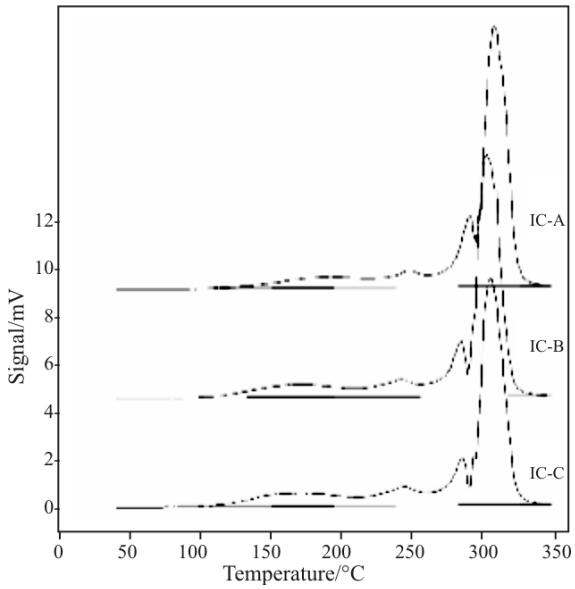

Fig. 7 EGD curves of inclusion complexes

Then, a continuous and slow process was seen between 105 and 270°C, indicating the release of some loosely bounded oil. The corresponding mass losses increased with the amount of added oil in the infeed slurries. The resulting mass losses were: 6.1, 6.9 and 7.7% for IC-A, IC-B and IC-C samples, respectively. Around 270°C the thermal degradation of the complex and β-CD started. 7% of final residue in the samples was detected. Summarizing the above observations, the TG curves confirmed the occurrence of some interaction between the essential oil and the β-CD, protecting the oil *vs*. volatilization.

In order to characterize the inclusion complex formation, the EGD curves of pure essential oil, pure β-CD, their physical mixture and inclusion complex(es) were compared. According to Fig. 6 the pure oil was completely evaporated up to 120°C. On the other hand, the EGD profile of the pure β-CD showed signals above 290°C, which were representative for the β-CD degradation. The EGD curve of the physical mixture of the individual compounds (Fig. 6) is the superposition of the EGD curves of the pure samples. The peak between 30 and 120°C corresponds to the evaporation of the essential oil and the ones over 290°C to the thermal decomposition of β-CD.

EGD curves of the inclusion complexes are presented in Fig. 7. The first peaks between 100–240°C indicate the release of some essential oil. Then, the second peaks in the range of 220–270°C for the three samples show the decomposition of the inclusion complexes. They may be evidences for the formation of the complexes with different thermal stabilities. The peaks above 270°C are related to the thermal decomposition of β-CD.

The amount of released oil from the inclusion complexes were determined by integration of the area under the EGD curve from room temperature up to the starting decomposition of β-CD. According to Fig. 8 one can suppose some relation between the released oil from the complex and the amount of added oil used to prepare the inclusion complexes. Thus, the amount of released oil from the IC-A was less compared to the released amount from IC-B and IC-C samples. These results are in agreement with the TG curves, where crescent mass variations in the same temperature interval were observed for the studied samples. Despite of the very similar thermal behavior among the samples, the different essential oil – β-CD compositions resulted complexes with slightly different retardation properties. Thus, the oil was found to be bonded stronger to β-CD in the IC-A sample than in the IC-B and IC-C samples.

Although classical thermal analysis methods (TG, EGD) are useful to study cyclodextrin complexes, they provide only indirect evidences about the inclusion

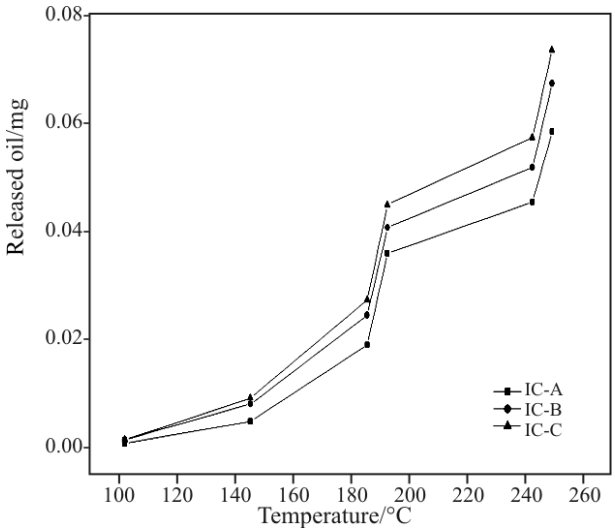

Fig. 8 Release profiles of *Lippia sidoides* essential oil/β-CD complexes

complex formation. Besides, they might be not always enough efficient to characterize the inclusion complexes with multicomponent guests such as essential oils.

Combined thermoanalytical technique (TG-MS) is used to confirm the inclusion complex formation directly [7, 8].

Multiple Ion Detection (MID) mode resulting a twodimensional graph was used to visualize the fragmentation of the samples *vs*. time (so far increasing temperatures). Three representative mass-to-charge units were selected for the fragmentation of the *Lippia sidoides* essential oil (*m*/*z*=91, 135 and 150). The first one (*m*/*z*=91) is related to the tropilium rearrangement, which appears upon the fragmentation of an aromatic ring containing methylene group. The peaks at *m*/*z*=135 and 150 are related to the base peak and to the molecular ion peak of the aromatic compounds, respectively. Finally, *m*/*z*=44 unit was selected to monitor the fragmentation of β-CD.

In the MID curves of the essential oil (Fig. 9a) the lines showing double peak at room temperature and at 115°C are for the evaporation of the essential oil. In the MID curve of β-CD (Fig. 9b), *m*/*z*=91, 135 and 150 are running practically on the baseline. Consequently, they may be considered as selective ones for the presence of any constituents of the essential oil, however, they are not representative for the fragmentation of β-CD. On the contrary, curve at *m*/*z*=44 shows a higher signal intensity around 310°C, which is representative for the thermal decomposition of the glucopyranose unit.

The shapes of the MID lines of the inclusion complexes (Fig. 10) are similar to the EGD curves. Below 105°C they indicate the release of uncomplexed oil and continuous evaporation of the essential oil above this temperature. One can see that the shape of the MID profiles of the studied samples (IC-A, IC-B and IC-C) are very similar to each other.

According to Fig. 10, curves at *m*/*z*=91, 135 and 150 showed a first maximum around 195–200°C and a second one at 265°C, indicating the degradation of the inclusion complex.

Nevertheless, the third maximum around 305–310°C and a forth one at 320°C suggests that a part of the essential oil escapes from the β-CD cavity only when the sugar derivative decomposes. This suggests the high thermal stability of the essential oil – inclusion complex.

Effect of storage on the thermal behavior of the inclusion complexes

In order to know how the environmental conditions might influence the release of entrapped oil from

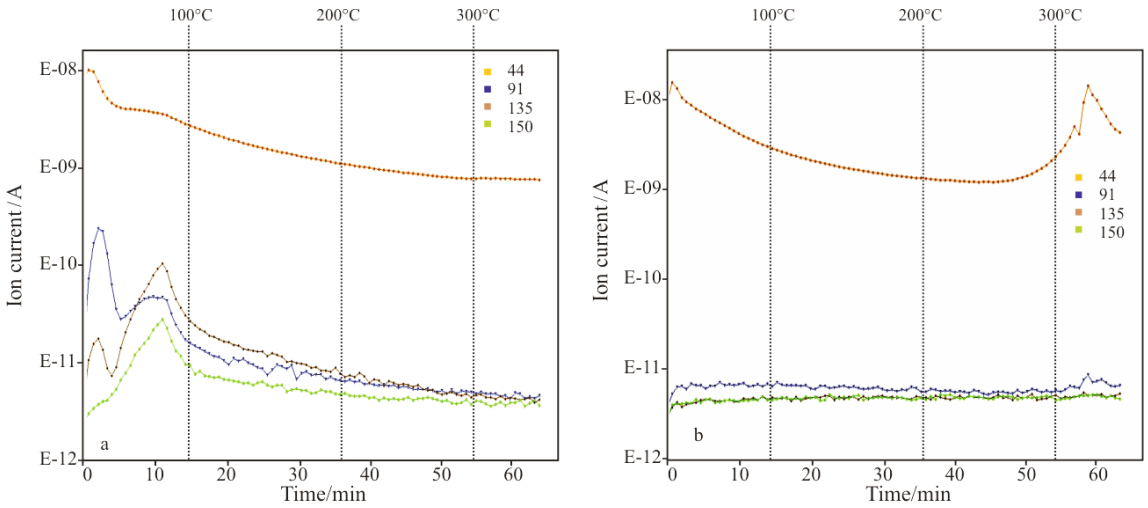

Fig. 9 MID curves: a – *Lippia sidoides* essential oil and b – β-CD

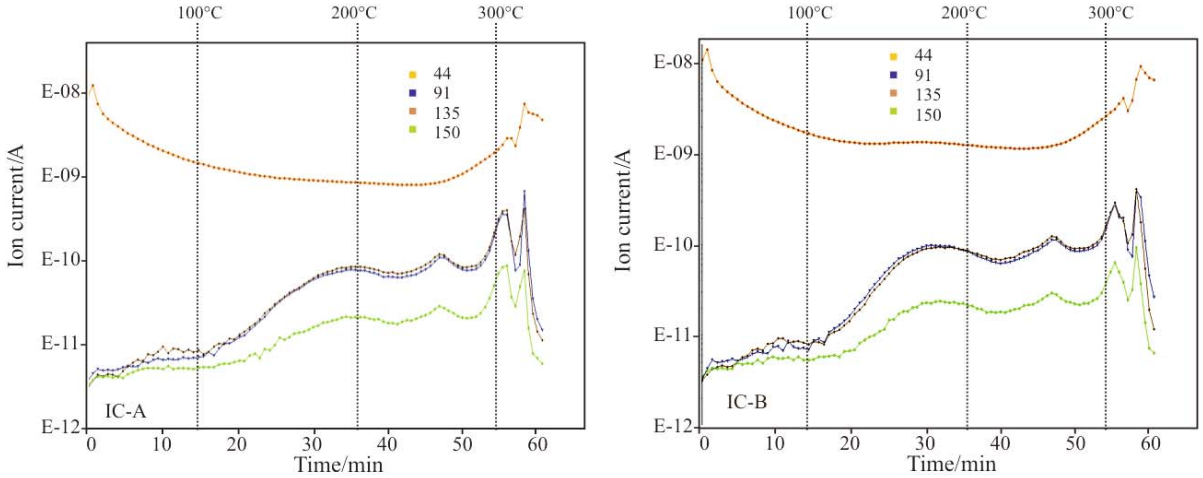

Fig. 10 MID curves of inclusion complexes: IC-A; IC-B and IC-C

β-CD, the samples were stored under 66.09% RH at 40°C for 6 weeks.

TG curve of IC-A (Fig. 11a) right after its preparation shows 5.6% of mass lost at 105°C. After 1 and 6 weeks of storage 6.2–6.3% mass losses were detected. This mainly related to the loss of water (and perhaps to the release of small amount of essential oil). Similar behaviors were found for IC-B and IC-C (Figs 11b and c).

Between 105 and 270°C slow and continuous mass losses were observed, which might be related to the thermal decomposition of the inclusion complexes. The recorded mass losses increased with the storage time. Thus, in the TG curve of the IC-A the mass losses (in a dry mass) increased from 6.9 to 8.4% from zero to 6 weeks of storage. The mass losses for IC-B increased from 7.8 to 9.3% and from 8.7 to 9.4% for IC-C. However, for all the samples the mass loss differences between one and six weeks of storage were not so remarkable.

The thermal stabilities of the complexes might not be influenced by the different essential oil/β-CD ratios (when the different samples at zero time were compared together) or by the storage conditions.

For all studied samples the main decomposition started above 270°C indicating the degradation of the inclusion complex and the β-CD itself leading to 7–8% of final residue.

The amounts of the released oil from the inclusion complexes were determined by integration of the EGD peaks from room temperature up to the decomposition of β-CD. According to Fig. 12 the release profiles of IC-A was different before and after storage. The amount of released oil was slightly increased as a function of the storage time indicating changes in stability for IC-A. These finding are in agreement with those ones from the TG curve at the same temperature interval, where a relation between the mass losses and

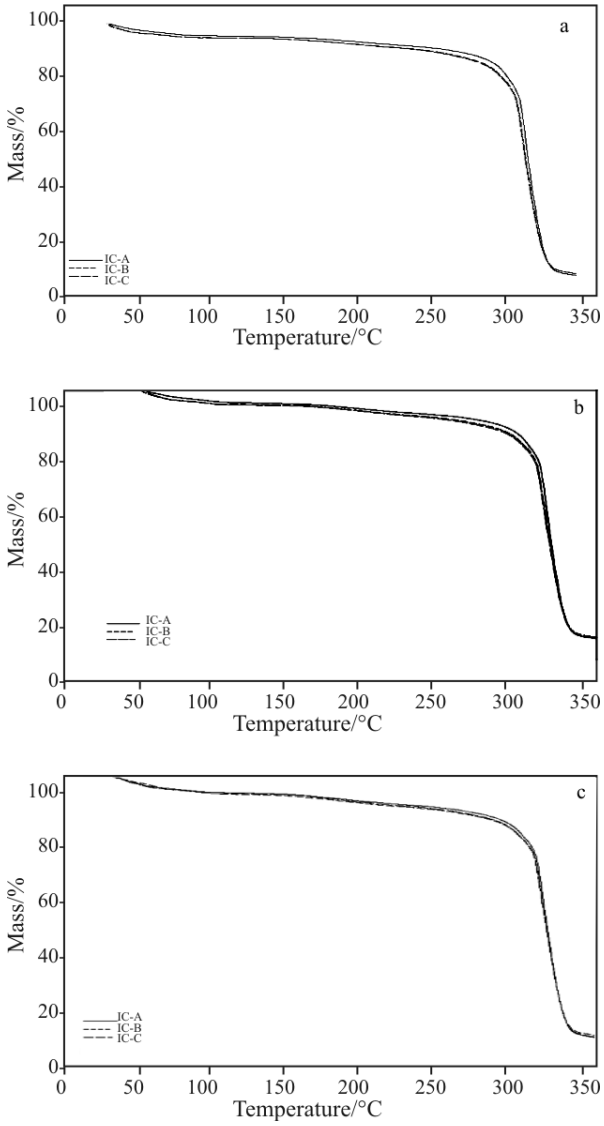

Fig. 11 TG curves of the complexes at zero time and after one and six weeks of storage: a – IC-A; b – IC-B and c – IC-C

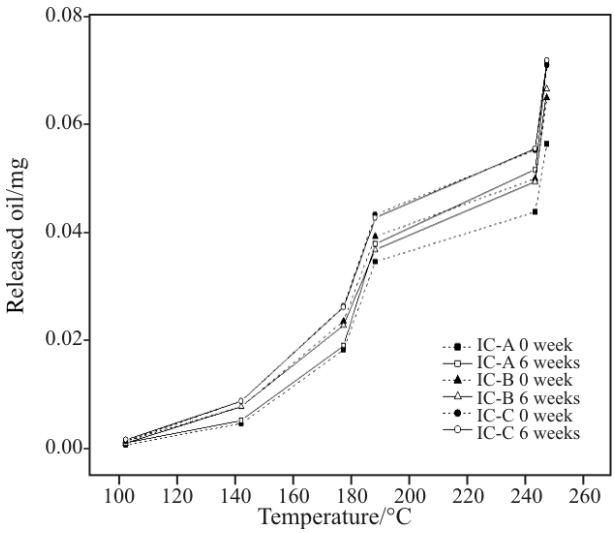

Fig. 12 Release profiles of *Lippia sidoides* essential oil from the complexes at zero time and after 6 weeks of storage

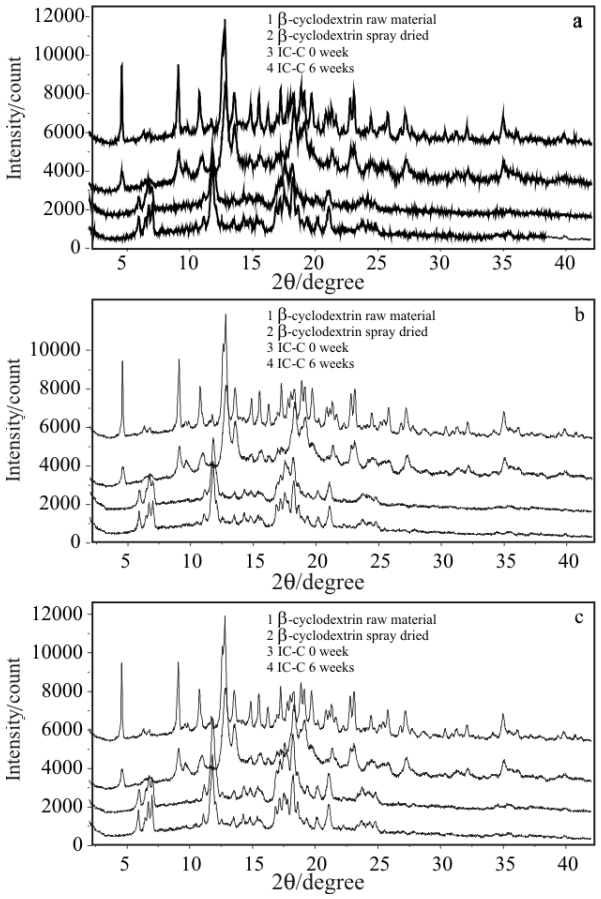

Fig. 13 X-ray patterns diffraction of samples before and after storage: a – IC-A, b – IC-B and c – IC-C

the storage time was found. EGD analysis revealed that the mass losses observed between 105 and 270°C in TG curve might be related to the evaporation of the released essential oil.

For IC-B and IC-C samples the differences in the amount of released oil *vs*. storage time were not so remarkable. Similar behaviors were observed when they were compared before and after storage, which denies the pronounced effect of the storage on the release of the essential oil from those samples.

X-ray diffraction patterns of the samples before and after storage are presented in Fig. 13. For comparison, the XRD patterns of native and spray dried β-CD were also recorded. Spray dried β-CD was found rather amorphous compared to the native crystalline β-CD. Similar to the spray dried β-CD, the complexes were also rather amorphous, only slight traces of crystallinity were found. The diffraction patterns of the spray-dried β-CD and the complexes were different evidencing the inclusion complex formation. According to Fig. 13 the peaks at 4.6, 9.1, 9.7, 10.7, 12.9, 13.6 and 27.3 2θ values belong to the spray dried β-CD and they are absent in the X-ray patterns of the complexes. On the other hand, new peaks appeared in the X-ray patterns of the complexes, e.g. at 5.9, 6.7, 7.0 and 11.8 2θ values. From the XRD patterns one can state that after six weeks of storage, the morphology of all the complexes has changed. Some tendency of crystallization *vs*. time was observed.

Conclusion

Slurry method for the preparation of *Lippia sidoides* essential oil/β-CD inclusion complex and spray drying to produce solid powdered final product was successfully applied. Correlation was found between the essential oil/β-CD ratio and the total oil retention in the powders, resulting an optimal 1:10 *m*/*m* essential oil: β-CD starting ratio. The thermal profiles of the complexes with different essential oil/β-CD proportions were slightly different to each other. With regard to the released amount of essential oil, IC-A sample was the most stable. However, the retardation effect on the encapsulated oil was very similar for all the samples. Combined TG-MS technique was efficient to characterize the inclusion complex formation and to indicate the improvement of the thermal stability of the included oil. The storage conditions might influence the thermal profile of the complexes, affecting the amount of released oil. For IC-A complex, an increase of the released oil amount was noticed as function of the storage time. For IC-B and IC-C complexes, no significant difference in the content of released oil was observed after storage. Subsequently, it might be assumed that the thermal stabilities of the complexes

were influenced not only by the different essential oil/β-CD ratio, but also by the storage conditions.

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