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Thermal analysis and gas chromatography coupled mass spectrometry analyses of hydroxypropyl-β-cyclodextrin inclusion complex containing *Lippia gracilis* essential oil

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ABSTRACT

Inclusion complexation with cyclodextrins is an effective way to improve stability and turn liquid materials into re-dispersible and easy-to-handle powders. In the present work, the complexation of *L. gracilis* essential oil, already recognized as a potent larvicide material, with hydroxylpropyl-β-cyclodextrin was performed using slurry and paste procedures and the complexes obtained were evaluated. The gas chromatography coupled to mass spectrometry (GC/MS) analysis showed a total volatile content of 99.24% in the *L. gracilis* oil. The characterization of the complex involved the analysis of the original essential oil, the surface, and the total extracted oils. The major components in *L. gracilis* essential oil were identified as carvacrol (23.52%), *p*-cymene (15.82%), γ-terpinene (14.17%), and thymol (7.27%). GC/MS results showed significant differences between the original oil, the slurry, and paste complexation. Thermal characterization indicates the occurrence of complexation, mainly in paste complexes, which presents a TG–DTA peak at 230–275 °C, probably related to oil loss.

1. Introduction

Lippia gracilis H.B.K. (Verbenaceae), known in Brazil by the name "alecrim-da-chapada", is an herb commonly found in Northeastern Brazil vegetation [1]. This species produces an essential oil (LGEO) which contains as major components: carvacrol, *p*-cymene, γ-terpinene, and β-caryophyllene [2]. LGEO has antimicrobial activity and is used externally to treat cutaneous diseases, burns, wounds, and ulcers, as reviewed by Pascual et al. [3]. Recently, Silva et al. [2] demonstrated a potent larvicidal activity of LGEO against the major dengue vector, the *Aedes aegypti* mosquito, suggesting its use as an ecologically safe alternative larvicide, since LGEO activity was similar to organophosphorate temephos, which is commonly used to control breeding sites of the dengue mosquito.

The demand for new natural larvicides to control the dengue vector is gradually increasing, since reports about temephos toxicity to non-target organisms and the environment, as well as insect resistance, are also increasing [4,5]. Plant essential oils are potential larvicides because they are, in some cases, highly active, economically viable, biodegradable, and appear to have no ill effect on the non-target population [2]. However, the essential oils, in general, may be more rapidly degraded in the environment than synthetic compounds [6] and also present a high volatility. These properties may conduce to low retention at the site of application, therefore decreasing the residual effect and effectiveness of the larvicide. Another important issue related to larvicide application is the handling and transportation to breeding sites of mosquitoes by health agents. Essential oils are applied as dilute aqueous solutions, which need to be transported in high volume to the regions of application with high costs and difficulties of access to remote regions.

Cyclodextrins (CDs) have been widely used to prepare inclusion complexes to improve stability and solubility, modify the release of the drugs, and turn liquid substances into stable and free flowing powders [7–9]. Inclusion complexation of volatile oils with CD has been applied to protect essential oils against oxidation, heat and light degradation, evaporation, and moisture. In this process, every volatile constituent (guest) is tightly held within the cyclodextrin molecule (host), which offers an effective protection against the damaging effects of the environment [6]. Besides, inclusion with cyclodextrins turns liquid essential oils into water dispersible and easy-to-handle powders [10] and allows control of their volatility.

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Cyclodextrins are enzymatically modified starch molecules shaped like a hollow truncated cone [11]. Since they have an internal apolar cavity and hydroxyl groups located on the rims of the molecule, the inclusion of hydrophobic compounds occurs mainly by hydrophobic interactions between guest molecules and the walls of the cavity of cyclodextrin [11,12]. However, other forces may be involved in the binding of the guest, such as van der Walls and dipole–dipole interactions. In spite of the number of factors and different forces involved in the complexation with cyclodextrins, the production of complexes is a rather simple process.

Martin Del Valle reviewed several techniques that are used to form cyclodextrin complexes [13]. Co-precipitation is the most widely used method in the laboratory and complexation occurs in a medium where cyclodextrin is completely dissolved. As the complexation reaction proceeds, or as cooling is applied, the solubility of the cyclodextrin–guest complex will be exceeded and precipitate can be collected by decanting, centrifugation, or filtration. On the other hand, slurry complexation and its variation, paste complexation, are methods that use smaller amounts of water. Thus, complexation occurs in a medium where cyclodextrin is not completely dissolved [13]. The main advantage of slurry/paste methods is the reduction of the amount of water needed and the size of the reactor, which consequently conduces to a more easily scaled-up process and lower production costs [11].

The most common methods used to evaluate and characterize essential oil–cyclodextrin complexes are: the extraction with organic solvents and simultaneous steam distillation/solvent extraction, followed by gas chromatography analysis [10]. Following this procedure, identification of the compounds in the original oil and determination of the total amount of complexed oil can be carried out [10]. Conventional thermoanalytical methods (TG, DSC, and DTA) are routinely used to characterize cyclodextrin inclusion complexes because they can provide fast and reliable assessment of possible interactions between host and guest [14]. Characterization of inclusion complexes by DSC and TG is a widespread approach and in the great majority of cases comprises a qualitative comparison between thermal features of single components, their physical mixture, and inclusion complexes [14,15].

In the present work, it seemed of interest to investigate the complexation of LGEO with hydroxylpropyl-β-cyclodextrin (HPβCD). HPβCD is one of the most commonly used β-CD derivatives in this class, due to its high water-solubility, parenteral safety, and complexation ability [16]. To this aim, LGEO–HPβCD combinations were prepared using different methods under controlled experimental conditions. Slurry and paste methods were used to prepare solid LGEO–HPβCD complexes, and their properties were compared to pure LGEO, HPβCD, and LGEO–HPβCD physical mixtures. The complexes were characterized by differential thermal analysis (DTA), thermogravimetry (TG), Karl Fisher analysis, X-ray powder diffraction (XRD), and gas chromatography–mass spectrometry. This investigation evaluated quantitative and qualitative assessment and comparison of the existing methods, and determined moisture, total oil, surface oil, and volatile profiles in the inclusion complex of HPβCD and LGEO.

2. Materials and methods

2.1. Samples and preparation of inclusion complexes

L. gracilis essential oil (LGEO) was a donation from the Research Farm of the Federal University of Sergipe, Brazil. HPβCD (Carvasol W7HP, batch number 73B011) was obtained from *Nortec Química*, Brazil.

Inclusion complexes were prepared by two different procedures. Slurry complexation was carried out by the addition of water to a beaker containing 6.0 g of HPβCD (3:4, v/w). Five hundred milligrams of LGEO, which is equal to about a 1:1 molar guest:host ratio (based on carvacrol's molecular weight), were added to the slurry and stirred for 40 min by a magnetic stirring device operating at 400 rpm (Quimis Q 261A21, Brazil). Thereafter, the mixture was heated to 70 °C for 2 h in the same device, transferred to an agate mortar, and dried in a desiccator.

Paste complexation was carried out by homogenization of HPβCD (6.0 g) with water (1.2:4, v/w) directly in an agate mortar. In a second step, 500 mg of LGEO (1:1 molar guest:host ratio) were added to HPβCD paste under constant manual agitation. Then, the material was dried at room temperature (in a desiccator) till formation of a glass film, which was removed by manual trituration and stored in airtight glass containers.

A mechanical mixture was prepared by addition of LGEO to an agate mortar containing powdered HPβCD under manual agitation. The LGEO/HPβCD mass ratio was maintained as described for inclusion complex preparation and the mechanical mixture was stored in airtight glass containers.

A standard oil solution was prepared by dissolving 20 mg of LGEO in 1 mL of hexane. The internal standard, menthol, was added to this solution prior to GC analysis.

2.2. Determination of the terpenes inclusion ratios by GC analysis

2.2.1. Extraction of total oil from complexes

Distilled water (8 mL) plus hexane (4 mL) and 0.2 g of the sample were put in a glass tube which was kept in a water-bath at 85 °C for 20 min, with intermittent shaking. The organic phase containing the volatile compounds was decanted. This procedure was repeated 3 times. Then, hexane (1 mL) and the internal standard (2 mg) were added to the decanted sample, and the extract was concentrated to approximately 1 mL using a rotary evaporator and stored at around 4 °C in a vial until the GC/MS analysis [17]. The total oil corresponds to the amount of complexed guest in the HPβCD cavity plus the surface-adsorbed oil.

2.2.2. Extraction of surface-adsorbed oil

The volatile compounds adsorbed on the surface of the cyclodextrin were determined by washing 3 g of powder with 20 mL hexane, which was shaken for 20 min. The suspension was then filtered and the residue was further washed with hexane (10 mL). Then, hexane (1 mL) and the internal standard (2 mg) were added to the extract which was concentrated and stored as it was described above. The difference between the total oil and the surface-adsorbed oil is the amount complexed in the HPβCD cavity and was used to determine the inclusion ratio for each compound.

2.3. Gas chromatography–mass spectrometry

Oil sample analysis was performed in a Shimadzu QP5050A (Shimadzu Corporation, Kyoto, Japan) gas chromatograph interfaced to a mass spectrometer (GC–MS) system comprised of a AOC-20I auto-injector (Shimadzu Corporation, Kyoto, Japan) and a gas chromatograph interfaced to a mass spectrometer (GC–MS) instrument employing the following conditions: column J&W Scientific DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., composed of 5% phenylmethylpolysiloxane). Helium (99.999%) was used as the carrier gas at a constant flow of 1.2 mL min^{-1}, and an injection volume of 0.5 μL was employed (split ratio of 1:83) with an injector temperature of 250 °C, and an ion-source temperature of 280 °C. The oven temperature was programmed from 80 °C (isothermal for 2 min), with an increase of 4 °C min^{-1}, to 200 °C, then 10 °C min^{-1}

to 300 °C, ending with a 10 min isothermal at 300 °C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s, and fragments from 40 to 450 Da.

2.4. Gas chromatography (GC-FID)

Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID), using Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) equipment, under the following operational conditions: capillary ZB-5MS column (5%-phenyl–95%-dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Phenomenex (Torrance, CA, USA), under the same conditions as GC–MS. Quantification of each constituent was estimated by area normalization (%). Compound concentrations were calculated from the GC peak areas and were arranged in order of GC elution.

2.5. Identification of essential oil constituents

Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in the NIST21 and NIST107 mass spectral libraries of the GC–MS data system. Retention indices (RI) for all compounds were determined according to Van Den Dool and Kratz [18] for each constituent, as previously described [19].

2.6. Moisture determination

The moisture contents of the mechanical mixture, slurry, and paste complexes were determined by the Karl Fisher method using a KF 1000 Analyzer (Brazil) and pyridine (Merk) as titrating solution. The analyses were carried out in duplicate.

2.7. Thermal analysis

Thermoanalytical measurements were carried out using TA instruments (Newcastle, Delaware, USA) STD 2960 and simultaneous TG/DTA unit, with a 10 °C min^{-1} heating rate starting at room temperature and going up to 500 °C. A nitrogen atmosphere (100 mL min^{-1}) was used and the sample amount was about 7 mg. Before analysis, the equipment was calibrated using a calcium oxalate standard (99.99%). All analyses were performed in triplicate.

2.8. X-ray diffraction

Powder X-ray diffractometer results were obtained on a Rigaku with a tube of Cu Kα, in the range of 3–65° (2θ) and 1 s of pass time, using the powder XRD method.

3. Results and discussion

Formation of an inclusion complex between a guest molecule and a cyclodextrin depends on a variety of physicochemical parameters and requires a multivariate approach for its description. This study was concerned with the complexation of LGEO with HPβCD using two different methods. We were interested in investigating the occurrence of the complexation as well as in determining the inclusion ratios of the main compounds, in particular, those that showed larvicidal activity against *Aedes aegpti* larvae.

Inclusion complexes obtained by slurry and paste methods, physical mixture, HPβCD, and LGEO were subjected to TG/DTA. Fig. 1 shows the TG curves of the materials and Table 1 lists the mass losses calculated from specific intervals for each material studied in the present work. By their data analysis, it can be seen that the major fraction of LGEO evaporates up to 130 °C. Up to 130 °C the HPβCD loses its water content and no further mass change was observed until 275 °C, when its decomposition started.

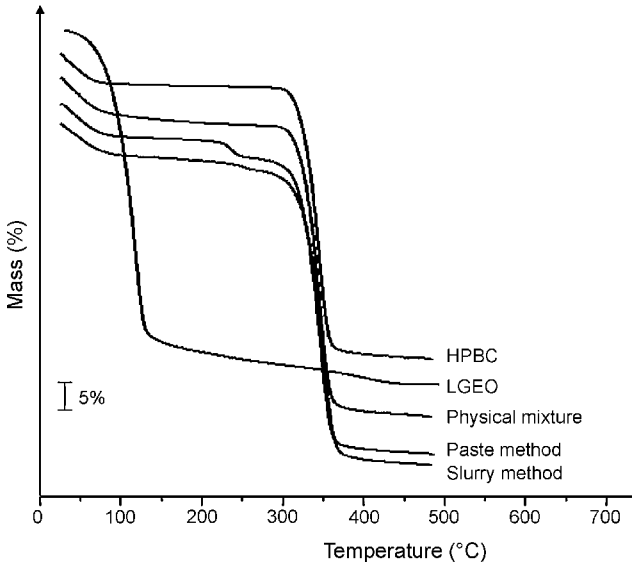

Fig. 1. TG curves of LGEO, HPβCD, physical mixture, slurry complex and paste complex in dynamic nitrogen atmosphere (100 mL min^{-1}) and hate heat 10 °C min^{-1}.

The curve of the physical mixture was a superposition of the guest and host curves, which indicates a lower evidence of inclusion and significant interaction between the host and guest molecules. Two overlapping steps were exhibited as causing 13.3% of mass loss, related to the evaporation of the essential oil and the water release from the HPβCD up to 190 °C (Fig. 1, curve of the physical mixture). The TG curve of the mechanical mixture also showed the superposition of the thermal behavior of the pure host and guest, indicating multi-step evaporation of the essential oil and the adsorbed water content of HPβCD up to 130 °C. This confirms that heating did not result in interaction between the components. By applying thermal analysis to look at the interaction between *Mentha x villosa* Hudson oil and cyclodextrin, Martins et al. [8] explored complexation by co-precipitation and kneading methods. The measured mass losses for the freshly prepared mechanical mixture confirm that the thermal process does not result in complex formation between the pure *Mentha x villosa* Hudson oil and cyclodextrin. In fact, a similar effect was observed by Fernandes et al. [10] in a study characterization of *Lippia sidoides* oil-β-cyclodextrin complexes using combined thermoanalytical techniques.

The curve of the complex prepared by slurry method (SM) showed a water/oil loss event from r.t. up to 130 °C. In the interval from 130 to 275 °C, a gradual mass loss (3.93%) was recorded and can be attributed to oil release. On the other hand, the curve of the complex prepared by paste method (PM) was similar to the SM curve, but showed an important difference between 230 and 275 °C. In this interval, the PM curve presents an acute mass loss

Table 1
Mass losses for LGEO, HPβCD, mechanical mixture and LGEO–HPβCD complexes in different temperature intervals (n = 3)

Sample	Mass loss (%) (±S.D.)		
	25–130 °C	130–275 °C	230–275 °C
LGEO	86.48 (0.41)	13.52 (0.12)	–
HPβCD	9.70 (0.23)	0.09 (0.11)	–
Physical mixture	10.47 (0.79)	2.77 (0.67)	0.12 (0.10)
Slurry complex	9.48 (0.26)	3.93 (0.22)	0.81 (0.19)
Paste complex	9.08 (0.63)	6.01 (0.06)	4.44 (0.09)

Table 2
Moisture contents obtained by Karl Fisher method

Sample	Moisture means (%) ($n = 2$)
LGEO	1.48
HPβCD	12.49
Mechanical mixture	10.44
Slurry complex	8.41
Paste complex	8.40

event, which gives a strong indication of guest inclusion, in contrast to that observed by slurry method. For this method, between 130 and 275 °C, 6.01% further mass loss was detected because of the release of oil from its inclusion complex. The complexation ratio can be attributed to the selected way of preparation (i.e. a part of the oil remained in the solution and/or some evaporation loss took place during the long complexation process) [8].

It is important to note that TG cannot distinguish between oil and water mass losses from mechanical mixture or inclusion complexes. Thus, a volumetric water determination method (Karl Fisher) was used to estimate total oil losses from TG curves. Table 2 lists the percentages of water calculated by the Karl Fisher method. Total oil retention of the complexes was calculated by subtracting total mass loss, up to 275 °C, from the percentage of water amount determined by the Karl Fisher method, and expressed as a function of the theoretical amount of oil added to the complexation medium.

The complex obtained by slurry complexation presented low oil retention, just about 63% of the theoretical value. On the other hand, the paste complex showed almost complete oil retention (99.8%). It seems likely that this difference was caused by heating during the slurry procedure as well as the more prolonged time used in the complexation and drying steps, which can conduce to a relevant evaporation of the volatile components of the LGEO. The mass loss event in TG (230–275 °C) from the paste complex and the corresponding endothermic peak in the DTA curve (Fig. 2) may be a result of the higher amount of oil retained in this complex.

The DTA curves of LGEO, HPβCD, mechanical mixture, slurry, and paste methods (Fig. 2) show a qualitative picture and confirm the above-described findings. The differences in the DTA curves of the mechanical mixture and the complex of essential oil–HPβCD clearly indicate complex formation between the components. The temperature peak at 257 °C (indicated in the graphic) is because of the decomposition of the formed inclusion complex.

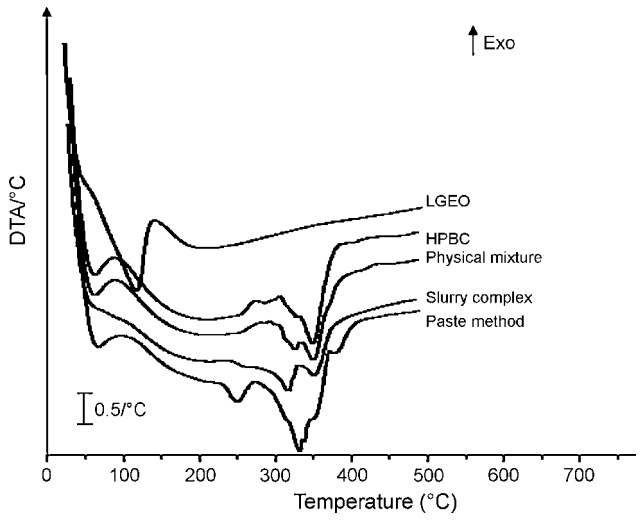

Fig. 2. DTA curves of LGEO, HPβCD, physical mixture, slurry complex and paste complex in dynamic nitrogen atmosphere (100 mL min^{-1}) and hate heat 10 °C min^{-1}.

The standard oil composition was similar to that reported in the literature [2]. The major components in *L. gracilis* essential oil were identified as carvacrol (23.52%), *p*-cymene (15.82%), γ-terpinene (14.17%), and thymol (7.27%). Menthol (10.97%) was used as the internal standard in GC analysis. Twenty-seven compounds, representing 99.24% of the essential oil, have been identified, and their retention indices and percentage compositions are given in Tables 3 and 4. Table 3 also lists the identified compounds extracted from the paste complex, whereas Table 4 lists those extracted from the slurry complex. In all cases, the concentration of the terpenes in complexed oil was calculated by subtraction of the amount of the total oil from the amount of the surface-adsorbed oil.

The chromatograms obtained from each sample in the present work are shown in Fig. 3. The similarity observed in chromatographic profiles of the standard oil and total oil extracted from both complexes indicates the HPβCD capability to include terpenoid compounds of the LGEO, from a qualitative point of view.

A more detailed comparison between complexes is provided by data analysis from Tables 3 and 4. The differences observed between inclusion ratios of the terpenes from both complexes can be attributed to differences between the formulation (water amount) and operational variables (temperature, type, and agitation time) used. Martin del Valle [13] reported that the water amount in complexation media needs to be enough to ensure an appropriate complexation rate that increases host and guest solubility, without excessively diluting these entities in the medium, which could prevent their contact at a sufficiently fast rate. Similarly, improvements in guest and host solubility can be achieved by increasing the temperature level, but heating, at the same time, can also destabilize the complex and contribute to losses of volatile guests by evaporation [13]. Another relevant factor is oil dispersion

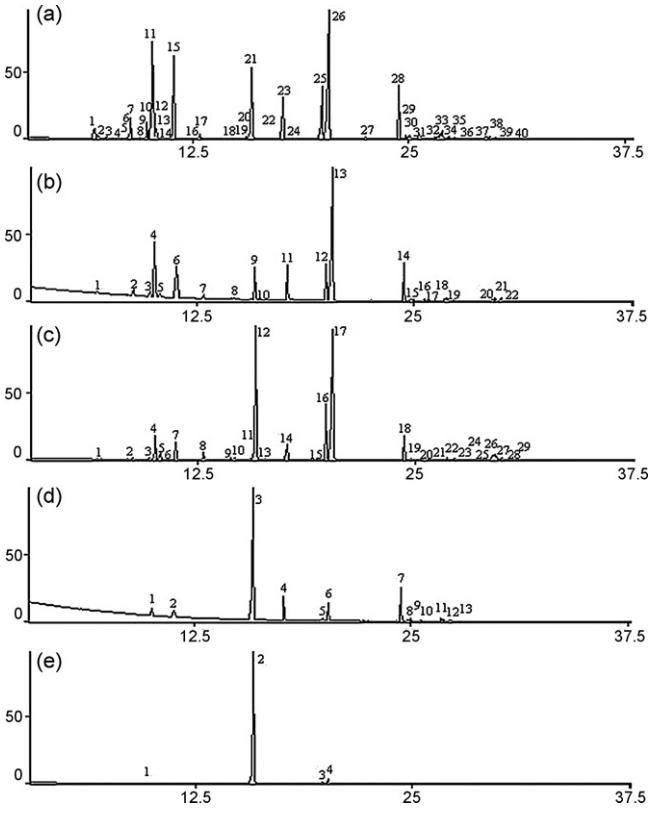

Fig. 3. Total ion chromatogram (TIC) of standard LGEO (a), total oil extracted from paste (b) and slurry complexes (c), surfaced adsorbed oil extracted from paste (d) and slurry (e) complexes.

Table 3
Characterization of *Lippia gracilis* essential oil and its hydroxypropyl-β-cyclodextrin complex obtained by paste procedure

Original oil			Extracts		Conc. % difference of extracts from original *L.gracilis*	
T_R (min)	Compounds	%	Surface oil	Total oil	Compl. oil (%)	Complexation ratio
8.57	Tricyclene	1.17	0	0.36	0.36	Total
8.84	α-Thujene	0.37	0	0	–	–
9.43	α-Fenchene	0.28	0	0	–	–
10.43	NI	0.09	0	0	–	–
10.82	Myrcene	2.20	0	1.20	1.20	Total
11.49	α-Phellandrene	0.19	0	0	–	–
11.57	NI	0.13	0	0	–	–
11.89	α-Terpinene	2.34	0	1.16	1.16	Total
12.18	*p*-Cymene	15.82	3.57	13.24	9.71	1:2.7
12.36	Limonene	0.39	0	0	–	–
12.48	1,8-Cineole	1.32	0	0.85	0.85	Total
13.48	γ-Terpinene	14.17	3.32	8.34	5.02	1:1.5
15.07	Linalool	0.57	0	1.17	1.17	Total
17.94	Borneol	0.34	0	0	–	–
18.15	Menthol	10.97	60.11	6.23	−53.88	–
18.26	Terpinen-4-ol	1.62	0	1.61	1.61	Total
20.05	Methyl thymol	5.39	9.64	7.61	−2.03	None
22.29	Thymol	7.27	0.99	7.07	6.08	1:6.1
22.63	Carvacrol	23.52	4.50	32.21	27.71	1:6.2
27.03	β-Caryophyllene	7.02	12.45	10.49	−1.96	None
27.40	α-*Trans*-bergamotene	0.35	0.78	0.67	−0.11	None
27.66	Aromadrendrene	0.48	1.11	0.69	−0.42	None
28.24	α-Humulene	0.58	0.65	0.67	0.02	None
29.18	NI	0.18	0	0	–	–
29.41	*Cis*-β-guaiene	0.74	1.28	0.91	−0.37	None
29.57	Bicyclogermacrene	1.14	1.00	0.96	−0.04	None
29.88	NI	0.23	0.54	0.48	−0.06	None
30.36	7-*Epi*-α-selinene	0.14	0	0	–	–
32.19	Spathulenol	0.30	0	0.64	0.64	Total
32.39	Caryophyllene oxide	0.34	0	1.29	1.29	Total
32.77	Guaiol	0.22	0	0	–	–

Table 4
Characterization of *Lippia gracilis* essential oil and its hydroxypropyl-β-cyclodextrin complex obtained by slurry method

Original oil			Extracts		Conc% difference of extracts from original *L.gracilis*	
T_R (min)	Compounds	%	Surface oil	Total oil	Compl. oil (%)	Complexation ratio
8.57	Tricyclene	1.17	0	0.19	0.19	Total
8.84	α-Thujene	0.37	0	0	–	–
9.43	α-Fenchene	0.28	0	0	–	–
10.43	NI	0.09	0	0	–	–
10.82	Myrcene	2.20	0	0.23	0.23	Total
11.49	α-Phellandrene	0.19	0	0	–	–
11.57	NI	0.13	0	0	–	–
11.89	α-Terpinene	2.34	0	4.23	4.23	Total
12.18	*p*-Cymene	15.82	0	6.38	6.38	Total
12.36	Limonene	0.39	0	0	–	–
12.48	1,8-Cineole	1.32	0	1.32	1.31	Total
13.48	γ-Terpinene	14.17	0	4.23	4.23	Total
15.07	Linalool	0.57	0	1.41	1.41	Total
17.94	Borneol	0.34	0	0	–	–
18.15	Menthol	10.97	92.89	37.02	−55,87	–
18.26	Terpinen-4-ol	1.62	0	0	–	–
20.05	Methyl thymol	5.39	0	3.36	3.36	Total
22.29	Thymol	7.27	0.70	10.33	9.63	1:13.8
22.63	Carvacrol	23.52	2.47	28.31	25.84	1:10.5
27.03	β-Caryophyllene	7.02	0	4.20	4.20	Total
27.40	α-*Trans*-bergamotene	0.35	0	0	–	–
27.66	Aromadrendrene	0.48	0	0	–	–
28.24	α-Humulene	0.58	0	0.29	0.29	Total
29.18	NI	0.18	0	0	–	–
29.41	*Cis*-β-guaiene	0.74	0	0	–	–
29.57	Bicyclogermacrene	1.14	0	0.40	0.40	Total
29.88	NI	0.23	0	0	–	–
30.36	7-*Epi*-α-selinene	0.14	0	0	–	–
32.19	Spathulenol	0.30	0	0.36	0.36	Total
32.39	Caryophyllene oxide	0.34	0	0.47	0.47	Total
32.77	Guaiol	0.22	0	0	–	–

in the medium. Oils in aqueous media have a tendency to associate with themselves rather than it interact with cyclodextrin. In these cases, good mixing allows better dispersion and a faster rate of complexation [13].

The paste complexation procedure, as performed in the present work, used a lower amount of water, room temperature, and gentle manual agitation. In agreement with that discussed above, the conditions used in the slurry procedure (a higher amount of water, heating, and magnetic stirring for a more prolonged time) are more advantageous to reaching a higher inclusion efficiency than the conditions of the paste method. The results presented here confirmed these tendencies, as can be seen in Tables 3 and 4. Carvacrol, thymol, and γ-terpinene are larvicide compounds in LGEO [2] that were not totally complexed by paste method. Another major compound of LGEO, *p*-cymene, was just partially complexed (see Table 3). Non-oxygenated sesquiterpenes are not complexed at all under paste conditions, probably due to a lower aqueous solubility of these compounds when compared with monoterpenes. Furthermore, it is important to note that sesquiterpenes were not lost, but retained on the surface of the complexes. Nevertheless, oxygenated sesquiterpenes were well complexed by paste method.

On the other hand, slurry complexation conduces to a more efficient complexation of LGEO terpenes. Carvacrol and thymol were complexed in a ratio two times superior to that observed in the paste procedure (Table 4). Besides, γ-terpinene and *p*-cymene were totally complexed by using slurry procedure. Thus, the complexation ratio of active larvicidal constituents was much superior under higher temperatures and higher water amounts, as well as under magnetic agitation for a more prolonged time. Indeed, sesquiterpene hydrocarbons were better included by slurry procedure than paste procedure (Table 4). However, some of them were lost, probably due to the heating during complexation and/or to a more prolonged drying step. The inclusion ratio of the oxygenated sesquiterpenes was similar to that found in the paste procedure.

Inclusion ratio data from GC showed that slurry procedure is superior for including LGEO active terpenes, but it is important to point out that procedure modifications must be done to assure a better retention of the constituents in complex.

X-ray powder diffraction patterns of HPβCD and the complexes were obtained by performing two methods (omitting figure). XRD spectra of both complexes showed typical amorphous substances, as shown by HPβCD.

4. Conclusion

Inclusion complexation of LGEO with HPβCD yields an easy-to-handle powered complex by using a high solubility cyclodextrin derivate. Slurry complexation showed a better inclusion profile of the active and major terpenes, but further work must be done to control losses during this procedure and to evaluate chemical stability of the complex during storage and its effectiveness and residual effect after application at the breeding site of the dengue mosquito.

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