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Microparticles containing *lemongrass* volatile oil: preparation, characterization and thermal stability

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Lemongrass volatile oil (LVO) is an important ingredient in cosmetics, presenting antimicrobial properties, in particular antifungal activity, and it is a promising raw material for the development of pharmaceutical products. However, its volatility and susceptibility to degradation are the major drawbacks for the use of *Cymbopogon citratus* oil in pharmaceutical compounding. Thus, the aim of this work was to develop and to characterize microparticles containing this oil viewing the stabilization of LVO. Two techniques of preparation were evaluated; spray drying and precipitation, and two encapsulation materials, β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were tested. The microparticles were characterized in terms of content of water, yield, percentage of inclusion, infrared spectroscopy. Morphology was evaluated by scanning electronic microscopy. Studies of stability were also conducted. The content of citral (neral and geranial), major component of the oil, present in microparticles was assayed by a validated HPLC method. The percentage of inclusion of LVO into the microparticles was 56–60% and 26–29% using β-CD and HP-β-CD, respectively. The results showed that the use of the β-CD as encapsulant material was more efficient. Additionally, an increased inclusion of lemongrass oil was observed with the precipitation technique.

1. Introduction

Research and development of microparticulate systems as drug carriers have been conducted in the pharmaceutical field to control release of drugs, to obtain gastroresistent microparticles, to control odour or taste, to protect drugs from degradation, to alter drug solubility, and to prevent pharmaceutical incompatibilities (Ranade and Hollinger 2003; Rawat and Jain 2003; Iaconinoto et al. 2004; Iwata et al. 2009). Microencapsulation is an important process to improve the chemical stability of volatile compounds and to protect them against the oxidation and evaporation, providing controlled release of volatile flavor compounds from microencapsulated flavorant products (Rosenberg et al. 1990; Kim et al. 1996; Krishnan et al. 2005; Baranauskiene et al. 2006; Baranauskiene et al. 2007). Microparticles are generally composed of polymeric materials and can be prepared by several physical and chemical methods like spray drying (Raffin et al. 2008), precipitation (Bhandari et al. 1998), coacervation (Sankar et al. 2009), dissolving (Holvoet et al. 2007), emulsification-diffusion/evaporation (Das and Rao 2007) and solid complexation (Loftsson et al. 2004). Parameters as the nature of drug and polymer, stability, yield and encapsulation efficiency have to be considered in selection of microencapsulation methods (O'Donnell and McGinity 1998). Spray drying has been a widely used technique in the pharmaceutical field, and can be applied to both heat-resistant and heat-sensitive drugs, water-soluble and water-insoluble drugs, or to both hydrophilic and hydrophobic polymers. The main disadvantage of spray drying for many applications is its cost, in terms of both equipment and operation (Ré 2006). Recent studies have focused on spray drying to increase the stability of drugs (Raffin et al. 2008), for producing nanoparticle-coated microparticles that may be used as vehicles for drug encapsulation and delivery (Beck et al. 2004), as well as in microencapsulation of volatile compounds (Bertolini et al. 2001; Krishnan et al. 2005; Baranauskiene et al. 2006).

Different materials are used for the encapsulation of monoterpenes, including arabic gum (Bertolini et al. 2001) and mesquite gum (Beristain et al. 2001), which are commonly used as a food flavor encapsulants, proteins (sodium caseinate, soy protein isolate) (Baranauskiene et al. 2006), colloidal silicon dioxide (Zétola et al. 2002), gelatins (Bruschi et al. 2003), maltodextrin (Pérez-Alonso et al. 2003) and cyclodextrin (Bhandari et al. 1998). Among the polymeric materials with efficient protection of volatile oils and monoterpenes, the cyclodextrins are extensively studied, and β-cyclodextrin is most widely used in the microencapsulation of substances (Bhandari et al. 1998; Waleczek et al. 2003; Jeon et al. 2003; Yuliani et al. 2006). Cyclodextrins (α, β or γ, as well as their commercially available derivatives) are well known for their ability to include apolar molecules or parts of molecules inside their hydrophobic cavity. Most often it is a question of better stability, higher water sol-

Table 1: Yield and water content of the microparticles containing LVO

Microparticles	Yield (%) ± sd[a]	Water content (%) ± sd[a]
βCD-LVO-PR	81.2[b] ± 0.9	9.1 ± 0.1
βCD-LVO-SD	32.7[c] ± 1.9	9.2 ± 0.1
HPβCD-LVO-SD	28.4[c] ± 2.5	8.5 ± 0.1

[a] standard deviation (sd) with n = 3; [b] significant for α = 0.01; [c] not significant for α = 0.05

ubility, increased bioavailability, or decreased undesirable side effects (Duchene et al. 1999).

Cymbopogon citratus Stapf (Poaceae), commonly known as lemongrass, is widely used as an important food ingredient due to its lemon flavor. The volatile oil, obtained from the fresh leaves, is characterized by monoterpene compounds, and citral (a natural mixture of isomeric monoterpenes aldehydes, geranial and neral) is the major component. Bioactivity studies have shown that the *C. citratus* oil possesses antibacterial (Onawunmi et al. 1984; Wannissorn et al. 2005), antifungal (Onawunmi 1989; Wannissorn et al. 1996; Schuck et al. 2001; Silva et al. 2008), antiviral (Minami et al. 2003), and repellent (Oyedele et al. 2002) activities. Due to its antimicrobial properties, the volatile oil is as a promising raw material for the development of pharmaceutical products. In our research, semisolid formulations containing lemongrass volatile oil (LVO) were developed and their properties, stability and activity were evaluated (Rauber et al. 2002; Silva 2005). However, its volatility and susceptibility to degradation are the major drawbacks for the use of *C. citratus* oil in pharmaceutical compounding. Thus, the aim of this work was to develop and to characterize microparticles containing this oil viewing the stabilization of LVO.

2. Investigations, results and discussion

In this work, microparticles containing LVO were prepared using precipitation and spray drying methods for encapsulation, and two encapsulants materials (β-CD and HP-β-CD). The microparticles presented as white powders, and with characteristic odor of volatile oil, however, less intense than pure LVO.

2.1. Yield and water content

Table 1 shows the yield and the water content verified for the microparticles (βCD-LVO-PR, βCD-LVO-SD and HPβCD-LVO-SD). All samples presented a water content inferior to 14%, the maximum limit established for β-CD (USP 32 2009). The microparticles prepared by the precipitation technique presented 81% of yield, a value significantly higher than that observed for the microparticles obtained by spray drying (Table 1). Additionally, in the spray drying technique, β-CD and HP-β-CD were used as encapsulant materials. The yields obtained for the microparticles prepared with β-CD (33%) were similar to that of the HP-β-CD sample (28%).

2.2. Percentage of inclusion

The percentage of inclusion of the oil, represented by the citral content, was satisfactory and better results were obtained for the microparticles prepared with β-CD (inclusion > 50%) in comparison with HP-β-CD (inclusion < 30%) (Table 2).

The results obtained in this work showed that β-CD was more efficient as encapsulant material for the preparation of the microparticles containing LVO by precipitation and spray drying microencapsulation techniques. In addition, the content of the isomers neral and geranial, determined by HPLC, demonstrated that geranial presented higher inclusion with β-CD. The content of the geranial isomer obtained in the microparticles and in the LVO was about 65% and 57%, respectively, calculated in relation to the total citral content. The LVO used in this work had a citral concentration of 76.7% (32.9% and 43.8% for neral and geranial, respectively). This result indicates that the *trans* isomeric form of citral (geranial) fitted more tightly to β-CD than neral (*cis* form).

2.3. Infrared spectrophotometry

IR spectra of β-CD and the complexes (βCD-LVO-PR and βCD-LVO-SD) are shown in Fig. 1. The samples of βCD-LVO-PR and βCD-LVO-SD presented similar profiles, and close to that of the β-CD. However, slight differences between spectra of complexes and of the β-CD can be observed. β-CD spectrum (Fig. 1a) presents a shorter band between 1600-1700 cm^{-1}, and a large band which displays distinct peaks, in the region of 900-1200 cm^{-1}. In Fig. 1, the inclusion of the LVO caused a dislocation in the region of 1650 cm^{-1} (bands 1642.12 cm^{1}, 1653.09 cm^{-1} and 1654.83 cm^{-1} of β-CD, complexes βCD-LVO-PR and βCD-LVO-SD, respectively). This effect can be attributed to the intensive absorption of citral (Fig. 1d) in the region 1670 cm^{-1} (range of carbonyl group). Thus, the microparticles of β-CD containing LVO can be identified using the IR technique.

2.4. Morphological analysis

Microcapsules prepared by precipitation and spray drying techniques of LVO using β-CD (samples βCD-LVO-PR and βCD-LVO-SD) were observed (SEM) for size and shape, and they were compared with the β-CD raw material (Fig. 2).

The morphological analysis of the powders showed differences among the microparticles prepared by different methods. For β-CD (Fig. 2a) the presence of irregular crystals and a broad size distribution were observed. Spherical but breaking particles, also having variable size distribution were obtained from βCD-LVO-SD sample (Figs. 2c and d). At a higher magnification, estimated diameter of the microparticles was 27–64 μm (β-CD), 22–66 μm (βCD-LVO-PR) and 5.5–23 μm (βCD-LVO-SD). Using the spray drying technique for drug microencapsulation, spherical particles are usually obtained. However, parameters as flow, inlet and outlet temperatures, solid concentration and characteristics of the components can be responsible for the differences observed. Besides, micropar-

Table 2: Content of citral (isomers neral and geranial) and percentage of inclusion of the microparticles containing LVO

Microparticles	Neral (%) ± sd[a]	Geranial (%) ± sd[a]	Citral (%) ± sd[a]	Percentage of inclusion (%) ± sd[a]
βCD-LVO-PR	2.25 ± 0.13	4.32 ± 0.06	6.56 ± 0.20	57.2 ± 1.70
βCD-LVO-SD	2.22 ± 0.13	4.29 ± 0.05	6.51 ± 0.18	56.3 ± 1.34
HPβCD-LVO-SD	1.13 ± 0.08	1.97 ± 0.13	3.10 ± 0.21	29.0 ± 1.27

[a] standard deviation (sd) with n = 3

Table 3: Content of citral (isomers neral and geranial) for the microparticles containing LVO in the stability study

Content	Neral (%) ± sd[a]			Geranial (%) ± sd[a]			Citral (%) ± sd[a]		
Time (days)	0	47	110	0	47	110	0	47	110
βCD-LVO-PR	100.0	73.7 ± 1.1	61.0 ± 1.8	100.0	76.3 ± 0.8	65.6 ± 2.1	100.0	75.4 ± 1.5	63.9 ± 1.9
βCD-LVO-SD	100.0	56.6 ± 2.6	40.2 ± 2.3	100.0	57.2 ± 1.0	40.5 ± 0.9	100.0	57.0 ± 1.5	40.4 ± 1.7

[a] standard deviation (sd) with n = 3

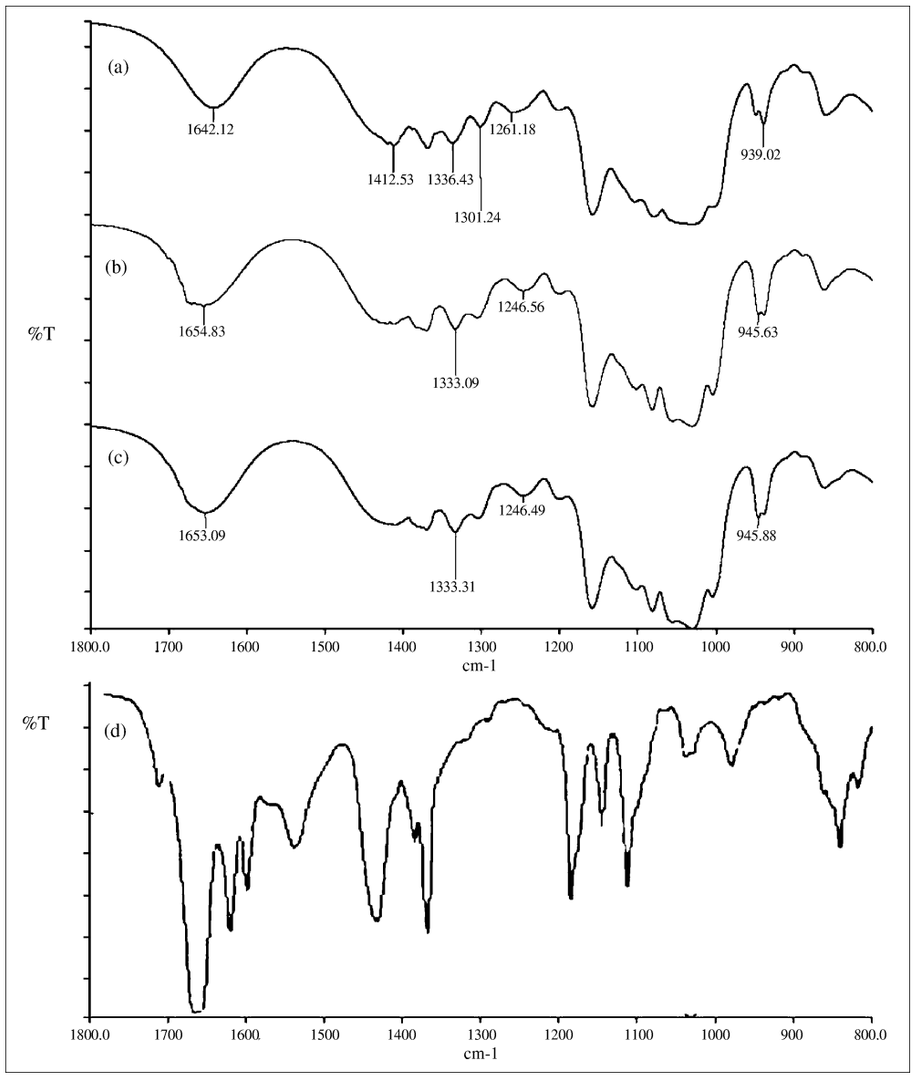

Fig. 1: IR spectra of (a) β-CD; (b) βCD-LVO-SD, (c) βCD-LVO-PR, and (d) citral

ticles prepared by spray drying can present concavity on their structure, and that form is due to the action of capillarity of the dry surface when extracting the liquid from the interior of the particle. This occurs evenly, creating an internal pressure lower than the atmospheric, resulting in bound particles (Walton 2000). Regarding the microparticles obtained in this work by spray drying technique, in which the nucleus material consists of oil, high temperatures may have generated an internal pressure higher than the atmospheric pressure, causing the release of the oil like an 'explosion'. This fact would justify the presence of broken spheres visualized in MEV.

The powders obtained by precipitation technique (Fig. 2b) presented irregular crystals and a wide size distribution. The results obtained were according to the β-CD morphology, and modifications on its structure were not expected, since the microparticles are formed by crystallization in this encapsulation technique.

2.5. Stability of the powders

Table 3 shows stability of microparticles containing the LVO. The content of citral, and the isomers neral and geranial, decreased about 40% and 60% for the βCD-LVO-PR and βCD-LVO-SD, respectively, after 110 days at 40 °C. The samples prepared by the precipitation method (βCD-LVO-PR) showed to be more stable than those prepared by spray drying.

Moreover, to develop topic formulations containing the LVO, we incorporated the microparticles in semisolid formulation in order to assess their stability. This strategy aimed to improve

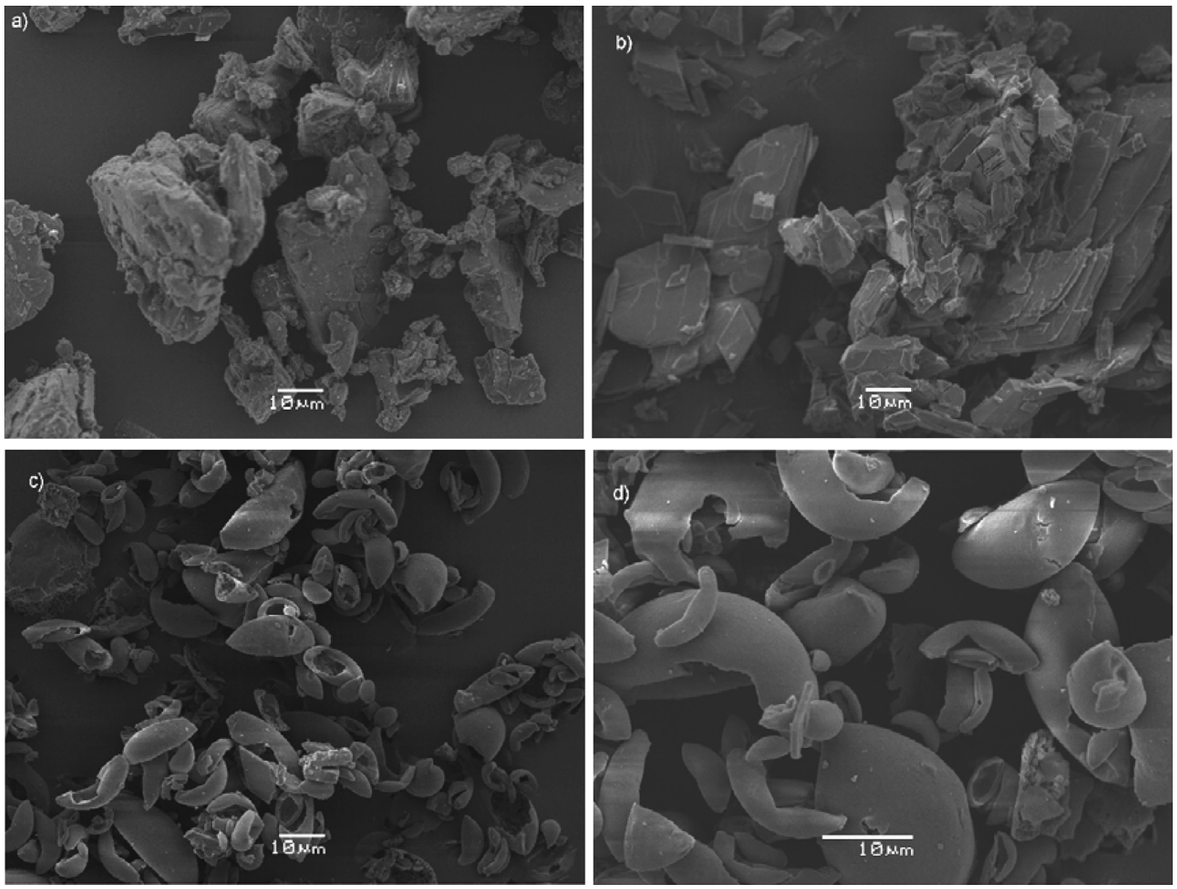

Fig. 2: SEM micrographs of microparticles LVO-βCD by precipitation and spray drying techniques: (a) β-CD; (b) βCD-LVO-PR, and (c) βCD-LVO-SD at magnification 1000 times, and (d) βCD-LVO-SD at magnification 2000 times

the stability of the oil in these formulations. After storage of the formulations containing the microparticles prepared by precipitation and spray drying methods (samples βCD-LVO-PR and βCD-LVO-SD) at 40 °C for 110 days, the content of citral decreased by 13.3 and 27.8, respectively (Table 4). Comparing the content of citral in microparticles and after inclusion of these in a semisolid base, a better stability was verified. In another study, Silva (2005) evaluated the stability of semisolid formulations containing the free oil, under the same conditions. In this study there was a decrease of approximately 14% after 60 days of storage, while a similar result was obtained for the formulation containing microparticles prepared by the precipitation technique after 110 days of storage. Losts of citral on this stability study was related to its volatility since no additional peaks were found on chromatograms. Thus, the inclusion of oil in cyclic oligosaccharides improved its stability, decreasing by hypothesis, its volatility and degradation, obtaining a more stable topical formulation. The higher content of geranial on final results of stability confirms its better affinity to β-CD.

The results obtained show that especially the precipitation technique and the use of β-CD as encapsulant material were efficient in furnishing microparticles with a high process yield and in protecting the volatile oil. Moreover, this method has been shown to be feasible and inexpensive, and the use of low temperature is adequate for inclusion of the volatile compounds.

Table 4: Content of citral (isomers neral and geranial) for the semisolid formulation containing βCD-LVO-SD and βCD-LVO-PR microparticles (formulations NIE-SD and NIE-PR, repectively) in stability study

Content	Neral (%) ± sd[a]		Geranial (%) ± sd[a]		Citral (%) ± sd[a]	
Time (days)	0	110	0	110	0	110
NIE-PR	100.0	79.1 ± 1.9	100.0	90.7 ± 1.3	100.0	86.7 ± 1.6
NIE-SD	100.0	66.4 ± 2.7	100.0	75.8 ± 1.6	100.0	72.2 ± 2.1

[a] standard deviation (sd) with n = 2

3. Experimental

3.1. Materials

Lemongrass volatile oil (LVO, *Cymbopogon citratus*) was obtained from Ferquima (São Paulo, Brasil); citral was purchased from Sigma-Aldrich (Brasil) and used as reference substance (purity of 96.5 %); β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were obtained from Fluka® (Saint Louis, USA) and Roquette® (Lestrem, France), respectively. Sodium lauryl sulfate (SLS) was provided by Synth (São Paulo, Brasil). Acetonitrile (Tedia®, HPLC grade), methanol (Tedia®, HPLC grade), water filtered through a Milli-Q purification system (Millipore) were used for HPLC mobile phase separation. All other chemicals were of analytical grade.)

3.2. Methods

3.2.1. Preparation of the microparticles by precipitation

For the preparation of LVO-βCD complex, the β-CD (11 g) was dissolved in 110 mL of ethanol and water mixture (2:1 v/v) heated at 55 °C on a hot plate. Afterwards, a solution of LVO in ethanol (10%, w/v) was added into the β-CD solution under magnetic stirring for 4 h while cooling down to room temperature. The solution was stored for 16 h at 4 °C and then the precipitate containing the LVO-βCD complex was obtained by vacuum filtration through a membrane nylon filter (0.45 µm). The filtrate was dried on a stove at 50 °C for 24 h. The powders obtained were packed on amber glass

Table 5: Operating conditions to prepare microparticles containing LVO using a Mini Spray Drier equipment

Parameter	Condition
Feed rate	0.3 L h^{-1}
Air flow rate	500 NL h^{-1}
Atomizing air pressure	4 Kgf cm^{-2}
Inlet temperature	120 °C
Outlet temperature	78 °C/90 °C
Nozzle diameter	1.2 mm

containers and stored on a dessicator. The sample was named βCD-LVO-PR (microparticles prepared with β-CD containing LVO by precipitation technique).

3.2.2. Preparation of the microparticles by spray drying

β-CD was dissolved (11 g) in 110 mL of ethanol and water mixture (2:1 v/v) heated at 55 °C on a hot plate. After that, a solution of LVO in ethanol (10%, w/v) was added to the β-CD solution under magnetic stirring for 4 h while cooling down to room temperature. The solution was dried in a Mini Spray Dryer (MSD 1.0, LabMaq, Brazil), operating in the conditions described in Table 5. For the preparation of LVO-HPβCD, the same conditions were used. The microencapsulated products were removed from the dryer and packed on amber glass containers and stored on a dessicator. The samples were named βCD-LVO-SD and HPβCD-LVO-SD (microparticles prepared with β-CD and HP-β-CD, respectively, containing LVO by spray drying technique).

3.2.3. Determination of yield and water content

The yields of the processes were calculated in percentage, by the ratio between the weights of powders obtained in the microencapsulations processes and the weights of substances before of the processes (sum of weight of encapsulant material and weight of oil included). The water content was obtained by titrimetric method (USP 32 2009).

3.2.4. Quantitative analysis of citral

High-performance liquid chromatography (HPLC) analyses were performed with an Agilent instrument (serie 1200), equipped with a photodiode array detector (G1322A, set at the wavelength of 240 nm), a 20 μl loop injection autosampler (G1329A), and Agilent ChemStation software. For analysis, a reversed phase ACE® RP_{18} column (250 mm × 4 mm, 5 μm particle size), and the mobile phase consisted of acetonitrile, water and methanol (50:40:10, v/v) were used. The flow rate of 1.2 mL/min was maintained. Quantification of the citral (and the isomers neral and geranial) was carried out by measuring the peak areas in relation to citral reference substance chromatographed under the same conditions. The method was validated, and the βCD-LVO-PR sample was used for validation of HPLC method. The linearity was evaluated in the range 6.8-20.3 μg mL^{-1} (r = 0.9978) and 7.0-21.1 μg mL^{-1} (r = 0.9980) for the isomers neral and geranial, respectively; the specificity was evaluated with the excipients, and no interference was observed at the detection at 240 nm; the R.S.D. obtained in precision was < 2% for the isomers (neral and geranial); the recovery test resulted in 100.6% of mean recovery, which indicate the accuracy of the method.

3.2.5. Citral content in the microparticles

The percentage of inclusion was determined by the content of citral presented in the microparticles. The powders (50 mg) were dissolved in 10 mL of sodium dodecylsulphate (SDS) 0.5% solution and the concentration was adjusted with the mobile phase. The solutions were filtered through a membrane filter (0.45 μm, Millipore), and then analyzed by HPLC. The results were expressed as percentage of citral inclusion into microparticles and were calculated as follows (Eq. 1):

$$\%\ \text{of citral inclusion} = c_{exp}/c_{theor} \times 100 \quad (1)$$

In which, c_{exp}, content of citral determined by HPLC (sum isomers neral and geranial contents); c_{theor}, theorical content of citral (ratio of 1:1 with the cyclodextrin).

3.2.6. Infrared spectroscopy (IR)

Complex formation was evaluated by comparing the IR spectra of the β-CD and of the solid complexes (βCD-LVO-PR and βCD-LVO-SD). The samples were analyzed on a FT-IR PerkinElmer equipment (model BX, software spectrum GX version 5.3.1.). Blends corresponding to 1.5 mg of samples and 150.0 mg of KBr were produced, compressed and recorded in the region of 4000-400 cm^{-1}.

3.2.7. Morphological analysis

The microparticles were examined under scanning electron microscopy (SEM) using an accelerating voltage of 20 kV (Jeol Scanning Microscope, JSM-6060, Tokyo, Japan), at different magnifications between 500–3500 times. The samples of β-CD, βCD-LVO-PR and βCD-LVO-SD were analyzed after they had been platinum sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan).

3.2.8. Stability studies

The stability of βCD-LVO-PR and βCD-LVO-SD microparticles was evaluated. The powders were placed in open glasses containers and stored at 40 °C. After time intervals of 0, 47 and 110 days, the contents of citral, and isomers neral and geranial were analyzed. In addition, the microparticles were incorporated in semisolid formulations (non ionic emulsion - NIE) at 15% (w/w) concentration, and their stability was evaluated. The samples were placed in plastic containers and stored at 40 °C during 110 days. After time intervals of 0 and 110 days, the content of citral was analyzed by HPLC method.

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