Binary And Quaternary Inclusion Complexes Containing *Lippia Sidoides* Essential Oil

Oliveira W.P.*, Freitas P.N.F., Souza C.R.F.
Fac Ciências Farmacêuticas de Ribeirão Preto, Univ São Paulo Brazil (wpoliv@fcfrp.usp.br)

INTRODUCTION

Lippia sidoides Cham (*L. sidoides*) is an aromatic medicinal shrub natural from Brazil's Northeast. It is source of an essential oil (EO) rich in thymol and carvacrol, which is linked to its larvicidal, bactericidal, fungicidal, antioxidant and insect repellent activities. EO are complex mixtures of aromatic and volatile liquids originated from plant secondary metabolism; and can undergo significant degradation when exposed without protection to light, heat, humidity and oxygen.

EO microencapsution is a technique widely used by industry to inhibit or minimize its volatility and degradation. Among the microencapsulation methods, the molecular inclusion in β-cyclodextrin (β-CD) is extensively used. β-CD are cyclic oligosaccharides composed by seven glucopyranose subunits, a hydrophilic outer layer and a lipophilic cavity. Cyclodextrins are able to form inclusion complex, having the potential to encapsulate hidrophobic molecules. In order to improve the encapsulation efficiency of β-CD, it is suggested to add polymers or surfactants, forming ternary complexes, or adding both, resulting in quaternary complex (QC).

Therefore, the aim of this work was to compare binary and quaternary complexes based on β-CD and *Lippia sidoides* EO in the concentrations β-CD/EO 1:10 and 2:10, with and without the presence of a polymer and surfactant. It was evaluated the encapsulation efficiency, powder properties, product morphology, and drying yield.

MATERIALS AND METHODS

Inclusion complex composition

The slurry method was used in preparation of BC, adding β-CD in 50mL of pure water. To prepare the QC, the polymer Kollidon® VA 64 fine and the surfactant Tween®80 were added to β-CD:water mixture (Table 1). *L. sidoides* EO was then added and the compositions were sonicated for 15 minutes and then mixed for 8 hours in shaker. After an equilibrium period of 24 hours at room temperature, the compositions were submitted to spray drying.

Table1 Binary and quaternary inclusion complexes

Sample	β-CD (g)	Polymer (g)	Surfactant (g)	EO(g)
PLS1	25.0	--	--	5.0
PLS5	25.0	0.5	0.24	5.0
PLS7	25.0	--	--	2.5
PLS11	25	0.5	0.24	2.5

Spray Drying

Drying of BC and QC compositions were carried out in a spray dryer SD-05 (Lab-Plant, Huddersfield, UK), operating in a co-current flow regime. The inlet drying gas temperature and the gas flow rate were set at 160 ºC and 60 m^3/h. The compositions were fed at a flow rate of 4.0 g/min through a double fluid atomizer operated with compressed air at flow rate of 17.0 L/min and pressure 2.5 kgf/cm^2.

Encapsulation Efficiency (EE),

Quantification of the EO in the inclusion complexes was carried out by hydrodistillation in Clevenger apparatus according Reineccius (2004) with some modifications. β-CD/OE complexes (5 g), distilled water (120 mL), and boiling glass pearls were added to a boiling flask. The Clevenger unit was connected to the flask, and 10 mL of water were added into the Clevenger sidearm. The system was allowed in reflux for 2 h. Encapsulation efficiency (EE) was defined as:

$$EE = \frac{Recovered\ OE\ mass}{OE\ mass\ in\ the\ feed\ composition}$$

Flow properties, particle size and morphology

Powder bulk density (ρ_{bd}) and packed density (ρ_{pd}) were determined in a tapped density tester (CALEVA). The powder volume of specified sample masses were determined after 10, 500 and 1250 beats. From the ρ_{bd} and ρ_{pd} (Table 2), it is possible to calculate the Carr Index and Hausner Ratio. Carr Index is an indicator of the powder or granules compactability and compressibility capacity. Values between 5 and 15% are indicative of excellent flow.

$$I_{carr} = \left(\rho_{pd} - \rho_{bd}\right) * 100/\rho_{pd}$$

Hausner Ratio has a more simple interpretation. Values less than1.25 indicate good flow, values higher than 1.5 indicate bad flow properties. Addition of lubricants are necessary to improve flow, for $I_{Hausner}$ ranging from 1.25 to 1.5. Hausner ratio can be estimated by the following Equation :

$$I_{Hausner} = \rho_{pd}/\rho_{bd}$$

Particle size was measured by optical microscopy in an Olympus BX60MIV. Photomicrographs were analyzed with the aid of the Image ProPlus v7.0 software.

Microparticles morphology were monitored in a scanning electron microscope (S.E.M.) Inspect F-50 (FEI, Nederland) set at 12KV.

RESULTS AND DISCUSSION

Encapsulation Efficiency (EE) EE is an important parameter, since indicates the quantity of EO in the inclusion complexes. Table 2 shows that the EE increases conversely with the proportion of EO added for BC and QC samples. Table 2 did not evidenced significant differences in EE for BC (PLS1) and QC (PLS5) products for the samples having the high EO load. However, the retention of EO was almost 10% superior for QC compared to BC system, for compositions having lower OE proportion.

Flow properties, particle size and morphology
BC and QC product have low bulk and packed densities, as can be seen in Table 2. Carr Index higher than 30% and Hausner Ratio superior to 1.5 were obtained, slight higher for QC samples. These results indicates a product with poor flow, compactability and compressibility properties, not promptly viable for tableting and capsules filling, for example.
Mean particle diameter (dp) did not show significant differences for BC and QC product.

Table 2. Encapsulation performance of β-CD

Sample	EE (%)	ρ_{bd} (g/mL)	ρ_{pd} (g/mL)	dp (µm)
PLS1	45.23	0.18	0.28	8.16
PLS5	44.86	0.17	0.31	10.43
PLS7	71.43	0.22	0.34	10.84
PLS11	81.08	0.25	0.43	11.19

The addition of the polymer and surfactant in the QC altered significantly the product shape, generating a more rounded particle. On the other hand, the BC products retain the original shape of the natural β-CD, although considerably smaller, perhaps due to the use of ultrasound (Figure 1). QC particles slightly bigger than BC can be seen in Figure 1, which can be due to differences in encapsulation compositions.

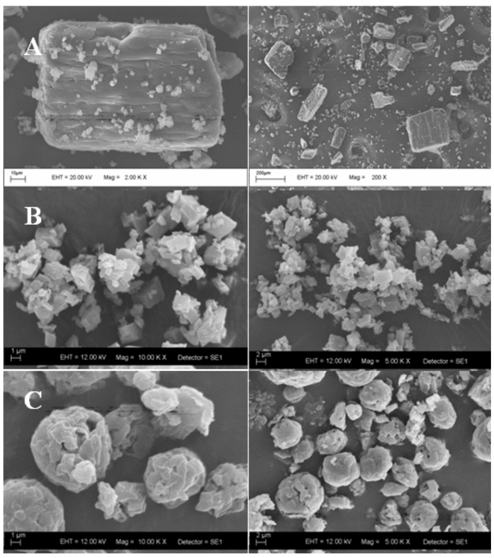

Figure 1. S.E.M. photomicrographs of BC and QC systems. A: pure β-CD, B: BC, C: QC

Appearance of spray dried powders
BC and QC powdered products showed similar macroscopic appearance, independent of the OE proportion, as can be seen in Figure 2. However, product having high EO proportion showed a greasy aspect, which can indicate presence of surface EO.

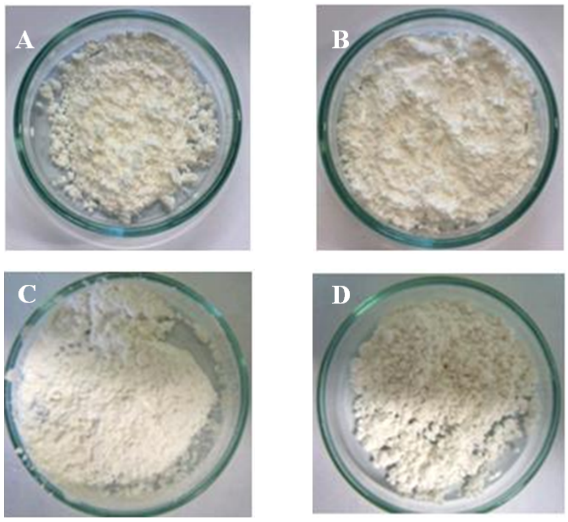

Figure 2. Samples after drying. A: PLS1, B: PLS5, C: PLS7 and D: PLS11

Drying yield Drying yield was defined as the percent amount of powder recovered after spray drying. QC recovered on average 72%, while BC recovered 66%. These are good results, mainly for a lab scale spray dryer. The addition of polymer and surfactant in QC, improved drying recovery at both oil concentrations, perhaps due to the increase in powder density, as can be seen in Table 2.

CONCLUSIONS

The results showed that the addition of polymer and surfactant to β-CD, forming quaternary complexes improves the encapsulation efficiency, drying yield, and modify the particles morphology. Samples with lower EO showed high encapsulation efficiency.

REFERENCES

- Loftsson, T et al. (1998) *The effect of water-soluble polymers on the aqueous solubility and complexing abilities of β-cyclodextrin.* International Journal of Pharmaceutics, 163, 115–121.
- Ribeiro, L et al. (2003) *Investigation and physicochemical characterization of vinpocetine-sulfobutyl ether β-cyclodextrin binary and ternary complexes*. Chem. Pharm. Bull., 51, 914—922.
- Reineccius, T.A et al. (2004) *Utilization of β-cyclodextrin for improved flavor retention in thermally Processed Foods*. Journal of Food Science, 69, 58-62

ACKNOWLEDGMENTS

To FAPESP for financial aid and for a fellowship to C.R.F. Souza (Grants 2011/10333-1 and 2012/03427-2) and to CAPES for the fellowship to P.N.F. Freitas.