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Spray drying of lipid nanosystems (SLN and NLC) loaded with *Syzygium aromaticum* essential oil

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Abstract

A quality by design approach was used to investigate the influence of formulation composition and spray drying conditions on physicochemical properties of redispersable lipid based nanosystems loaded with Syzygium aromaticum essential oil. Four critical independent variables were studied: presence or absence of the liquid lipid oleic acid (0% - 1%), of the cationic surfactant CTAB (0% and 1%), inlet drying temperature (60 ºC -80 ºC), and ratio of the drying aids (ADJ) regarded to total formulation constituents weight (1:1 and 2:1). Resuls showed the production of spray dried redispersable lipid systems loaded with essential is feasible under very restrict conditions.

Keywords: *Encapsulation; lipid systems; essential oil; spray drying; redispersable.*

1. Introduction

Currently, significant attention has been addressed towards the use of essential oils (EOs) in food, pharmaceutical and cosmeceutical sectors, mainly due to their broad spectrum of proven biological activities, particularly antifungic, antibacterial insecticide, antiviral, antioxidant, among others.[1] EOs are hydrophobic, volatile and odoriferous liquids, produced by the plant secondary metabolism, and consist of complex mixtures of chemicals.[2] Several limitations exhibited by the EOs such as the high volatility, insolubility in aqueous systems and propensity to degraded due to environmental factors, would deter they use in more elaborated products. Encapsulation processes can modulate and improve the EO physicochemical properties, thus expanding its potential of use in a high variety of products. In this way, the incorporation of essential oils in lipid systems such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) can be a promising strategy to modify its physicochemical properties; which might positively alter its stability, volatility, water solubility, bioavailability and biological activity.[3] Since the SLNs and NLCs are generally presented in liquid forms; the drying of these systems can furnish a redispersible powdered product with higher shelf-life; which can be reconstituted when exposed to aqueous solution or be used in more refined applications.[4-6] The drying process as well as the constituents of the encapsulating composition affects directly the physicochemical product properties, such as product granulometry, microstructure, redispersability capability, retention of bioactive compound, and so on. These characteristics affect strongly the product functionality, stability and other technological and biopharmaceutical properties. Several drying technologies can be used to dehydrated these systems, including freeze drying and spray drying.[7] Although spray drying is one of the most used method for drying and encapsulation of thermosensitive materials, its use for drying of emulsions and other lipid systems are barely reported in the current literature.[8,9] Most of the lipids and surfactants used to develop these systems (and the EOs as well), are liquid at ambient temperature or present low melting temperatures, turning their dehydration process challenging. On the other hand, *Syzygium aromaticum*, popularly knowed as clove, is an important aromatic plant rich in phenolic antioxidants, and has been used for centuries as a food preservative and pain reliever.[10] Clove buds contains an essential oil rich in eugenol, substance usually linked to its biological properties, such as antioxidant, antimicrobial, larvicide, and anti-inflammatory. So, clove EO is a promising active phytopharmaceutical ingredient. Therefore, the aim of this work was to investigate the spray drying of lipid based nanosystems (SLNs and NLCs) loaded with *Syzygium aromaticum* EO, evaluating the effects of formulation composition and spray drying temperature on physicochemical properties of the dried product.

2. Materials and Methods

Syzygium aromaticum EO (from a clove essential oil producer located in Valença, BA, Brazil), solid lipid Precirol® ATO 5 (glyceryl palmitostearate - Gattefossé, France – Melting point ~56 °C), liquid lipid oleic acid (cis-9-octadecanoic acid - LabSynth, Brazil), anionic surfactant Kolliphor P 188 (Poloxamer® 188 - BASF, Brazil), cationic surfactant CTAB (cetyl trimethylammonium bromide - Sigma-Aldrich, Germany - Melting point 237-243 °C), and reverse osmose water were the materials used for the development of primary SLNs and NLCs. Arabic gum (AG - Nexira, Brazil), Aerosil® 200 (SiO_2 - Evonik Degussa, Germany), Maltodextrin DE-10 (MD - Ingredion, Brazil), were used as spray drying aid (ADJ). Eugenol and eugenyl acetate with purity of 99.0 % and 98.0 % respectively (Sigma-Aldrich, Germany), HPLC grade solvents (Sigma-Aldrich, Germany), and milliQ® water were used in the quantification of Eugenol (EU) and Eugenyl acetate (ACT) by high performance liquid chromatography with diode array detection (HPLC-DAD).

2.1 Preparation of SLNs and NLCs formulations

The preparation of the SLNs and NLCs loaded with *Sygyzium aromaticum* EO was conducted by the phase inversion method. Solid lipids were melted at 10 ºC above their melting temperature, and mixed with 1 % of the liquid lipid oleic acid (only for NLCs). EO at concentration of 3 % (wet basis) was added to this lipid phase and maintained at same temperature. 3% of Poloxamer 188 and 1% of CTAB (when used) were solubilized in the reverse osmose water and kept at the same temperature of the lipid phase. Then, the aqueous phase was slowly dispersed in the lipid phase under magnetic stirring, and then submitted to a ultraturrax (UltraTurrax T-18 IKA WORKS, Inc., Wilmington, NC, EUA) at 21.500 rpm during 3 minutes.[10] To reduce the particle size of the NLCs, the lipid system was submitted to ultrasonic processing (US), using a VCX-750 (SONICS Vibracell, Newtown, EUA) with a 13 mm probe at amplitute of 45%, during 30 minutes at cycles of 5 minutes on and 2 minutes off.[11] The total amount of solid lipid, plus liquid lipid and CTAB were set constant at 10% (wet basis). Hence, the ADJ (SiO_2:GU:MD - 1:3:6), hydrated overnight, were added to the primary lipid compositions at 1:1 or 2:1 ratio regarded to the total amount of formulation components (dry basis). After a stabilization period of 24 hours, the SLNs and NLCs formed were characterized, to determine the effects of composition constituents on selected physicochemical properties. Differential scanning calorimetry of the formulations prepared (freeze-dried) were conducted in a PerkinElmer calorimeter (mod. Jade-DSC), running from 20 to 250 °C, heating rate at10 °C/min, and N_2 atmosphere (3.0 kgf/cm^2) to estimate their melting and crystallization temperatures.

2.2 Spray drying of the SLNs and NLCs loaded with *Sygyzium aromaticum* EO

The drying runs were performed in a SD05 spray dryer (Lab-Plant UK Ltd, Huddersfield, UK), operating in a concurrent flow regime. First, the system was fed with distilled water to

stabilize the SD temperature and humidity profiles. The outlet gas temperature was measured each 5 minutes to detect the instant when the process attains steady state, after which the feed of encapsulating composition began. Operating conditions were: feed rate 4 g/min, diameter of the atomizer nozzle 1 mm, drying gas flow rate 60 m^3/h, pressure and flowrate of atomizing air of 3 bar and 17 L/min, respectively; and inlet drying temperatures (Tgi) of 60 and 80 °C. Solids concentration (Cs) was set at 26%. Samples of the spray dried powders were reserved, and their physicochemical properties determined.

2.2.1 Experimental planning

A 2^{4-1} fractional factorial design with addition of a central point[12] was used to study the effect of following variables: presence or absence of the liquid lipid oleic acid (OA - 0% - 1%), of the cationic surfactant CTAB (0% and 1%), inlet spray drying temperature (60 ºC and 80 ºC), and ratio of ADJ regarded to total formulation constituents (1:1 and 2:1 – dry basis). The set of experiments conducted are presented in Table 1 (coded variables). Regression analysis were performed to evaluate the significance of the effects of processing variables on product properties and drying performance.

Table 1. Processing conditions used according to the 2^{4-1}experimental design.

Run	T_{gi} (-)	CTAB (-)	OA (-)	ADJ (-)
F1	-1	-1	-1	-1
F2	1	-1	1	-1
F3	-1	-1	1	1
F4	1	-1	-1	1
F5	-1	1	1	-1
F6	1	1	-1	-1
F7	-1	1	-1	1
F8	1	1	1	1
F9	0	0	0	0

2.2.2 Determination of SD product properties and drying performance

The moisture content, water activity, content and retention of marker compounds were measured for the SD powders. The moisture content was determined by Karl Fischer titration, using a Karl Fischer 870 Titrino Plus (Methrom, Switzerland). The water activity was determined in an Aqua Lab 4Tev® water activity meter (Decagon devices, USA) using the capacitance electrode. Results were expressed as mean and deviation of triplicate mesurements. The concentrations of eugenol (EU) and eugenyl acetate (ACT) in liquid and spray dried samples were determined by a validated high performance liquid chromatography method (HPLC).[9] Retention of EU (R_{EU}), and ACT (R_{ACT}) were defined as the percentual ratio of the amount of marker compounds in the SD product related to the values in original liquid formulation (dry basis). Particle sizes (dp) and the polydispersity index (PDI) of the liquid and redispersed SD samples were determined by dynamic light scattering (DLS) using

a Zetasizer Nano ZS90 (Malvern, UK). Zeta potential (Z) was measured in the same equipment, using the specific measurement cell. The SD samples were redispersed with reverse osmose water at the original concentration and stirred for 30 minutes. Then, the original and redispersed samples were diluted to 1:200 (v/v) before the measurements (triplicate assays). Microphotographs of selected SD samples were acquired by scanning electronic microscopy (S.E.M. - Zeiss mod. EVO 50). Product recovery (R_{EC}), defined as the percentual ratio of the mass of SD product collected by the cyclone by the total mass fed (dry basis), was used as a measure of spray drying performance. The outlet spray drying gas temperatures were monitored during the drying.

3. Results and discussion

Nine distinct lipid compositions were produced according to the experimental planning (Table 1). After 24 hours, the compositions were characterized through determination of dp, PDI, Z, and the contents of eugenol (EU) and eugenyl acetate (ACT). The pH and electrical conductivity of compositions ranged from 4.23 to 4.93 and from 359.0 to 1,068.2 (µS/cm), respectively (data not shown). The amount of marker compounds in the liquid compositions are linked to the ADJ ratio; decreasing conversively with this composition variable.

3.1 Effects of processing variables on product properties and drying performance

The moisture content of the SD product varied slightly with processing variables, reaching a mean value of 5.3 ± 0.4 (% w/w d.b). The water activity (a_w) of the SD powders ranged from 0.357 to 0.578. Values of water activity lower than 0.5 are desired to avoid microbial spoilage of dried powders, resulting in longer shelf-life.[13]. Most of SD powders reach water activity bellow this value, except compositions F4 and F6 that show a_w of 0.578 $\pm$ 0.003 and 0.568 $\pm$ 0.003, respectively, although none of the variables studied showed statistical significant effect on a_w. The physicochemical properties (EU, ACT, R_{EU}, R_{ACT}, dp, PDI and Z) of the SD product and of the redispersed samples were submitted to a regression analysis (Table 2). Table 2 shows similar effects of processing variables on the responses EU, ACT, R_{EU}, R_{ACT}, with $R^2 \geq 0.95$. The increase in T_{gi} leads to a decrease in these responses ($\alpha \leq 0.05$), perhaps due to the increase of drying energy available, which can contribute to the increase of volatilization of EO constituents. The effect of ADJ on EU and ACT are expected (dilution effect), but its effects on R_{EU} and R_{ACT} were in an opposite direction to the normal reasoning, seemingly due to the interactions between ADJ and the main lipid formulation constituents (CTAB, OA, poloxamer 188, and precirol). The addition of OA in the lipid systems increased EU, ACT, R_{EU}, R_{ACT}, as expected for NLCs, compared to SLN, but this effect only show statistical significance for ACT ($\alpha \leq 0.05$). Surfaces responses of EU as a function of CTAB and ADJ for SLNs and NLS are presented in Fig. 1 (T_{gi} = 60°C). Spray dried products with 7% of EU were formed at selected processing conditions.

***Table 2*. Regression coefficients and their statistical significance for selected process responses.**

FACTORS	PROCESS RESPONSES							
	EU (mg/g)	**ACT (mg/g)**	**R_{EU} (-)**	**R_{ACT} (-)**	**dp (nm)**	**PDI (-)**	**Z (mW)**	**R_{EC} (%)**
Mean	35.836*	2.910*	34.96*	44.88*	1680.7**	0.54*	-29.33*	46.67**
T_{gi}	-8.309*	-0.448*	-8.14*	-6.70**	66.6	0.05**	1.567	-3.45
ADJ	-11.334*	-0.790*	-3.86**	-4.86**	-77.1	0.00	-0.367	-2.48
CTAB	8.586*	0.388*	9.61*	7.56*	-43.2	-0.06**	3.892**	3.32
OA	1.516	0.160**	1.37	2.56	-313.1	0.05**	-1.642	1.48
R^2	0.956	0.959	0.984	0.973	0.258	0.918	0.725	0.715

* Effect significant at $\alpha \leq 0.01$ - ** Effect significant at $\alpha \leq 0.05$

Fig. 2 shows graphs comparing data of dp, PDI and zeta potential of the original lipid systems with the corresponding values for redispersed SD samples. It can be seen that nanosized liquid SLNs and NLCs were engineered (F1 to F4), with small polydispersity index (PDI) and adequate |Z|. Significant increase of the droplets size for formulations containing CTAB were observed, perhaps due to interaction between the negatively charged[14] Arabic gum with the cationic lipid CTAB. This interactions were strong enough to invert the zeta potential of the CTAB primary lipid systems (without ADJ),[11] from highly positive values (+32.97 to +44.03 mW) to negative (from -30.8 ± 0.1 to -21.0 ± 1.0), linked to ADJ amount and OA addition. Therefore, a previous scrutiny of the charges of the composition constituents is highly recommended to reduce the likelihood of occurrence of unwanted interactions. The graphs also show good redispersion properties of SD powders; although some compositions exhibit dp slightly higher than the initial value, except for F6 to F8 products (containing CTAB). The redispersed SD powders with CTAB (F5 to F9) showed a reduction of PDI comparatively to initial composition. Similar values of [Z] were observed for the redispersed SD product and the original liquid lipid systems. These results give robust evidences that our initial goal, the production of redispersible SLNs and NLCs loaded with *Sygyzium aromaticum* OE, is feasible. Fig. 3 shows S.E.M. micrographs of selected spray dried SLNs and NLCs; showing predominance of spherical somewhat wrinkled particles. The addition of OA caused a slight decrease in dp (Figs. **c** and **d**). Strutural changes in the SLNs and NLCs are linked to their thermal and crystalline properties. DSC analysis show small differences in temperatures and enthalpies of fusion and crystallization, linked to systems composition (data not shown).

Fig. 4 shows the experimental results of R_{EC} as a function of the outlet SD temperature. The solid line shows linear fit of the experimental data, indicating a tendency of decrease of R_{EC} conversely with T_{gs}. Traced lines shows the mean values of melting and crystallization

temperatures of lipid compositions. Drying at temperatures at left side of these lines show positive effects on R_{EC}.

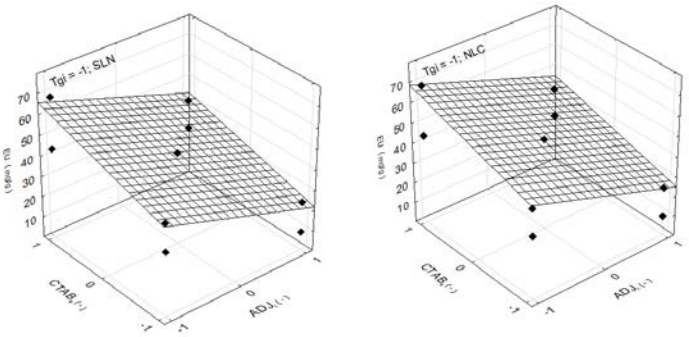

Fig. 1 Effects of CTAB and ADJ on Eugenol content in spray dried SLN and NLC (T_{gi} = 60 °C).

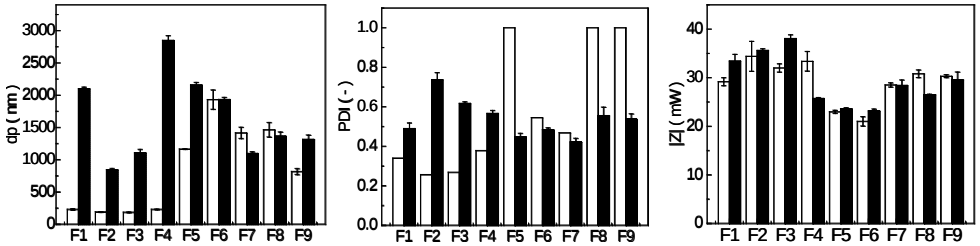

Fig. 2. Comparison of particle size (dp), Polidispersity index (PDI) and zeta potential (|Z|) of the original liquid lipid system and of the redispersed SD product (respectively, open and solid columns).

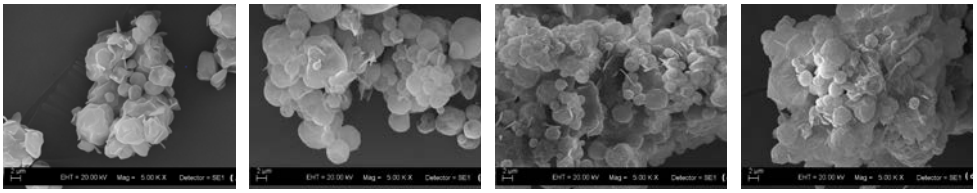

Fig. 3. S.E.M. images of the SD powder. a: SLN (T_{gi} = 60 °C, ADJ = 1:1; CTAB = 0%); b: SLN (T_{gi} = 80 °C, ADJ = 1:1; CTAB = 1%); c: NLC (T_{gi} = 80 °C, ADJ = 1:1; CTAB = 0%); d: NLC (T_{gi} = 60 °C, ADJ = 1:1; CTAB = 1%).

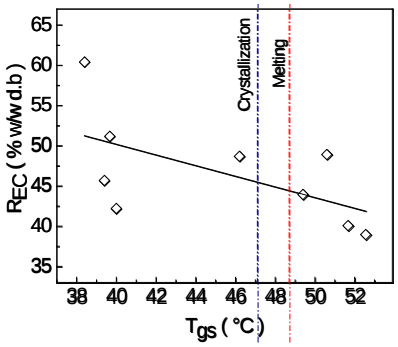

Fig. 4. R_{EC} as a function of T_{gs}

4. Conclusions

The properties of the nanosized lipid systems loaded with EO are affected by processing conditions. EU and ACT in SD products depend on drying temperature and formulation constituents. R_{EU} was higher (~50-60%) for systems with CTAB and OA, at lower SD temperature. Dried SLNs and NLCs are easily redispersed, indicating the potential of this technology.

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6. References

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