INFLUENCE OF DRYING ON THE MORPHOLOGY OF MICROPARTICLES OF B-CAROTENE AND PHBV USING SEDS TECHNIQUE

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Abstract. Nanotechnology is gaining rapid development in several sectors of the global economy, motivated by competition for high quality products coming from the rigid demands of consumers. In food industry, the precipitation/encapsulation of active compounds in polymers in the micrometric range are gaining interest in the area of high quality products, shelf-life increase of perishable foods and incorporation of vitamins and nutraceutical compounds. Throughout the precipitation process, concern about the characteristics of the materials precipitated together with its purity is highly desirable. Drying of the precipited material is an important step which can influence the morphology of the formed particles. Thus, this study aimed to evaluate the influence of drying on the morphology of β-carotene and PHBV precipitated by SEDS technique. The quantity and flow of antisolvent (CO_2) were investigated as follows: 1500 to 2500mL; 10 to 30 mL.min^{-1}, respectively. For PHBV and β-carotene, the results indicated that some drying treatments caused a visual agglomeration and changes in the structure of the microparticles. According to the micrographs, the minor changes in the structure of particles were obtained when the flow of antisolvent was 20ml.min-1 and 2000ml of CO_2. In this study was possible to optimize the drying process of β-carotene and PHBV precipitated by the SEDS technique, identify and minimize changes to the materials and consequently, maximize its applicability.

Keywords: SEDS technique, drying, β-carotene, PHBV

1.   Introduction

Nanotechnology is gaining rapid development in several sectors of the global economy motivated by competition for high quality products coming from the rigid demands of the consumers. In food industry, the precipitation and encapsulation of active compounds in biopolymers in micrometric range are gaining interest in the area of high quality products, shelf-life increase of perishable foods and incorporation of vitamins and nutraceutical compounds.

Specifically, carotenoids are widely used as colorants to recover the color lost during food processing and storage, as well as standardizing the color of some products. In parallel, they act as vitamin A precursors, as antioxidants, and are responsible for aroma, specific color and photoprotection of foods (Sánchez-Contreras et al., 2000 [1]; Miguel et al., 2006 [2]; Martín et al., 2007 [3]). Yet, it is known that the application properties

and the color strength of pigments are strongly dependent on their physical properties such as particle size, particle size distribution and morphology (Suo et al., 2005) [4].

An alternative to enhance the protection and stability of active compounds is their encapsulation in biopolymers. Several polymers can be employed to encapsulate bioactive compounds, due to their biocompatibility and biodegradability. Polyhydroxybutirate (PHB) copolymers with 3-hydroxyvalerate (PHBV) are less stiff, tough and crystalline (Costa et al., 2007 [5]). The use of PHBV in biomedical field has increased mainly due to the possibility to control the drug delivery system in human body (Chen and Wu, 2005 [6]; Pouton and Akhtar, 1996 [7]).

The conventional techniques for micro and nanoparticles production can lead to excessive use of organic solvents, thermal and chemical degradation of solute, high residual solvent concentration, and mainly, difficulty in controlling the characteristics of particles during processing (He et al., 2004 [8]). The use of supercritical or near critical fluids as solvent or anti-solvent for particles precipitation and encapsulation is considered by several researchers as a powerful tool for the modification of material properties such as particle size, particle size distribution and morphology. Another advantage of such techniques is the efficient solute-solvent separation after the precipitation, preventing organic solvent residues in the final product and allowing solvent and anti-solvent reuse (Reverchon et al., 2003 [9]; Rantakylä et al., 2002 [10]).

An important stage in the microparticles production is the reduction of the organic solvent at low levels and for this, an additional drying step should be performed. The dichloromethane is commonly used as the organic solvent and its residual limit allowed (ppm) is no more than 600 ppm (Kim and Shing, 2007 [11]; Kang et al. 2008 [12], Cardoso et al., 2009 [13]). Additionally, Hong et al. (2000) [14] and Kang et al. (2008) [12] studying the precipitation of solids using dichloromethane as organic solvent and CO_2 as antisolvent, reported that drying times ranging from 30 to 120 minutes resulted in a residual amount of dichloromethane particles between 38 and 50 ppm.

In the scientific literature were found only studies about toxicity and residue limit in the final product, however is not mentioned any reference to the product drying time, which is dependent on the flow rate of anti-solvent and the volume of CO_2 used. These two variables have direct influence in the particle morphology and the average particle size of the product precipitated. In this context, the objective of this work was to evaluate the effect of the anti-solvent (CO_2) amount (volume) and flow rate, used for the precipitation, in terms of the morphology of β-carotene and PHBV precipitated by SEDS technique. All other variables (pressure, temperature, PHBV and β-carotene concentration and solution flow rate) were kept constant based on previous works of the group (Priamo et al., 2010 [15]). The morphology of precipitated powders was determined by scanning electronic microscopy (SEM).

2. Experimental Methods

2.1 Materials

Trans-β-carotene, with a purity of 95 %, was purchased from Sigma-Aldrich (USA). Dichloromethane (DCM - 99.5 %) was purchased from Merck (Germany), carbon dioxide (99.9 % in liquid phase) was supplied by White Martins S.A. The co-polymer poly (3-hydroxybutirate-co-hydroxyvalerate) (PHBV), with molar mass (M_w) of 196.000 and polydispersity index of 1.85 (measured by Gel Permeation Chromatography - GPC using a calibration curve obtained from polystyrene standards), was kindly supplied by the PHB Industrial S.A. (Brazil). All materials were used as received.

2.2 Apparatus and Experimental Procedure

Figure 1 presents a schematic diagram of the experimental apparatus using the SEDS technique employed in both steps, i.e., the precipitation of the pure compounds and the co-precipitation of β-carotene with PHBV. Briefly, this apparatus consists of a cylindrical vessel with internal volume of 600 mL and inner diameter of 8 cm, which was used as precipitation chamber (PC), two syringe pumps for CO_2 displacement (ISCO, Model 500D), operated independently by a set of ball valves - V1 to V4 (Swagelok, Model SS-83KS4), and a digital HPLC liquid pump (Acuflow, Series III) used for organic solution delivery (Franceschi et al., 2008) [16].

The organic solution was sprayed into the precipitation chamber at a flow rate of 1mL.min^{-1} through a silica capillary fusing tube, with an internal diameter of 100 μm, connected to a polyetheretherketone tubing (Peek Tubing, Upchurch Scientific). This arrangement was linked to a BPR (back pressure regulator, GO-

Regulator, Series BP-66, Model 1A11QEQ151) and to a tee (T) connector (Swagelok), to link the antisolvent and the solution flows.
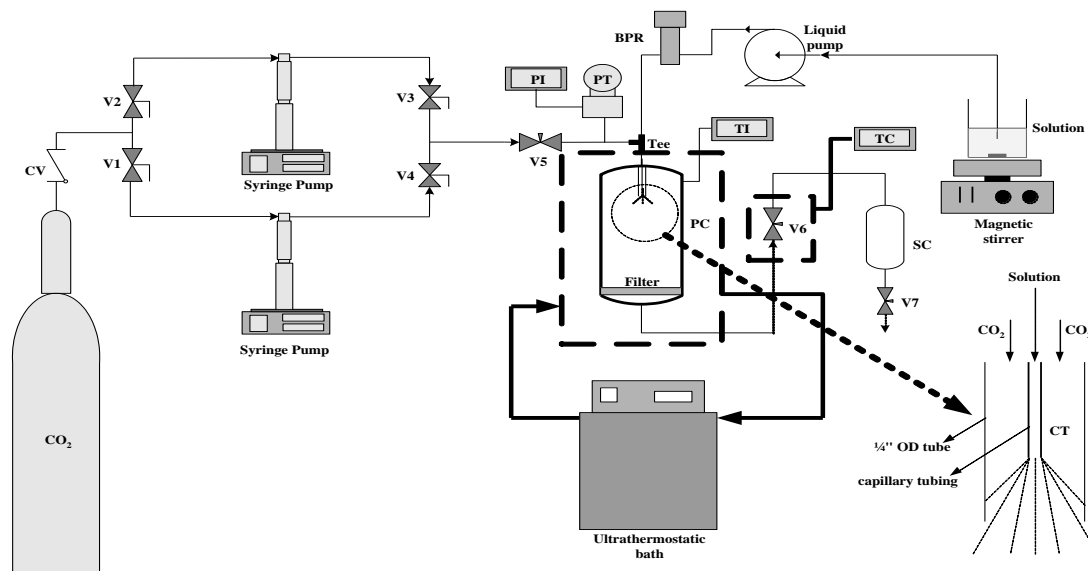

Figure 1. Schematic diagram of the experimental apparatus using the SEDS technique. CV – check-valve; V1, V2, V3 and V4 - ball valve; V5, V6 and V7 - needle valve; BPR - back pressure regulator; PT - pressure transducer; PI - Pressure indicator; TI - temperature indicator; TC - temperature control; PC - precipitation chamber; SC - separation chamber; CT - capillary tube.

During the experiments, the temperature inside the precipitation chamber was kept constant at 313.15 K by an ultrathermostatic bath (Nova Ética, Model 521/2D), while the pressure was controlled at 80 bar by two needle valves (HIP, Model 15-11AF1). The first valve (V5) controlled the antisolvent flow rate while the other one controlled the depressurization. A second vessel (SC) connected after valve V6 was used to keep the stream that leaves the precipitation chamber at a relatively low pressure (about 4 MPa) to prevent the blockage of valve V6. A system for powder collection was located at the bottom of the precipitation chamber, and was composed by sinterized metal filter (superficial porosity of 1.0 μm) as a support to the polytetrafluorethylene membrane filter linked to a high density polyethylene support (Millipore, model FGLP with a porosity of 0.22 μm).

The experimental procedure started with CO_2 filling the precipitation chamber up to the desired pressure. The anti-solvent flow rate was controlled by setting V5 and V6 valves, and monitored by the syringe pump. When the temperature, pressure and anti-solvent flow rate were stabilized, the organic solution was added through the capillary tubing. The pressure for solution spray into the precipitator was controlled by BPR manipulation and monitored by the liquid pump. The solution volume added to the chamber was 30 mL, which enabled the collection of sufficient amount of precipitated powder for analysis. After adding the solution, the anti-solvent flow rate (q_a) was ajusted and a volume of CO_2, according to experimental conditions showed in Table 1 was continuously supplied to dry the precipitated particles. The precipitation chamber was slowly depressurized to atmospheric pressure and particles were collected and stored at appropriate conditions for subsequent analysis. A more detailed description of the apparatus and the experimental procedure can be found elsewhere (Franceschi et al., 2008 [16]; 2008a [17]; 2009 [18]).

2.3 Prepitation and drying conditions

Table 1 showed the experimental conditions for the precipitation and drying of pure compounds. Based on previous work (Priamo et al., 2010) the following variables were maintained fixed: pressure (P) of 40 bar, temperature (T) at 313.15 K, solution flow rate (q_s) at 1 mL.min^{-1}, PHBV concentration (CS) of 30 mg.mL^{-1}, β-carotene concentration (CS) of 8 mg.mL^{-1}. The anti-solvent flow rate (q_a) was ranged, for precipitation of

both compounds, from 10 to 30 mL.min^{-1} and the volume of CO_2, used for drying the precipitated particles, varied from 1500 to 2500 mL.

Preliminary studies carried out indicated that a drying time of 120 minutes was sufficient to perform the particles drying and no change in their structure after this time was observed. Based on this information, a volume of 2000ml for CO_2 and a drying time of 120 minutes was adopted. It is emphasized that these conditions suggest a antisolvent flow rate approximately of 20ml.min^{-1} and therefore, this condition was defined as "standard drying". In paralell, when the flow of antisolvent was lower and the volume of CO_2 used was higher, the drying was denominated as "slow" and on the other hand, when the flow of antisolvent was higher and the volume of CO_2 used was lower, the drying was denominated as "fast".

Table 1. Experimental conditions for the precipitation and drying of β-carotene and PHBV.

Run	Compounds	P [bar]	T [°C]	q_s [ml.min^{-1}]	CS [mg.ml^{-1}]	q_a [ml.min^{-1}]	Volume de CO_2 [ml]	Drying
1						10	2500	Slow
2	β-carotene				8	20	2000	Standard
3						30	1500	Fast
4		80	313.15	1		10	2500	Slow
5	PHBV				30	20	2000	Standard
6						30	1500	Fast

2.4 Analysis and characterization

Precipitated particles were analyzed by a scanning electron microscope (SEM, JEOL, model JSM-6390LV) to determine particle morphology. Basically, the precipitated samples were manually dispersed on conductive tapes arranged on aluminum support, covered with a layer of gold using a metallizer apparatus and then analyzed.

3. Results and discussions

3.1 PHBV and β-carotene drying

Figure 2 and Figure 3 shows the morphology of PHBV and β-carotene, respectively, submitted to different drying conditions.
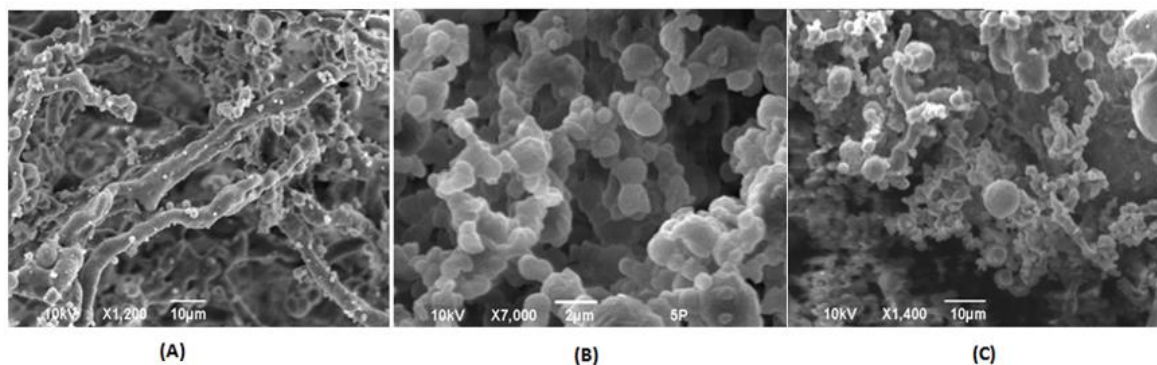

(A)   (B)   (C)

Figure 2. Comparative SEM micrographs of PHBV precipitated. Experimental condition: **(A)** "slow drying" (q_a: 10 ml.min^{-1}; volume of CO_2: 2500 ml and magnification of 1200x); **(B)** "standard drying" (q_a: 20 ml.min^{-1}; volume of CO_2: 2000 ml, magnification of 7000x); **(C)** "fast drying" (q_a: 30 ml.min^{-1}; volume of CO_2: 1500 ml, magnification of 1400x).

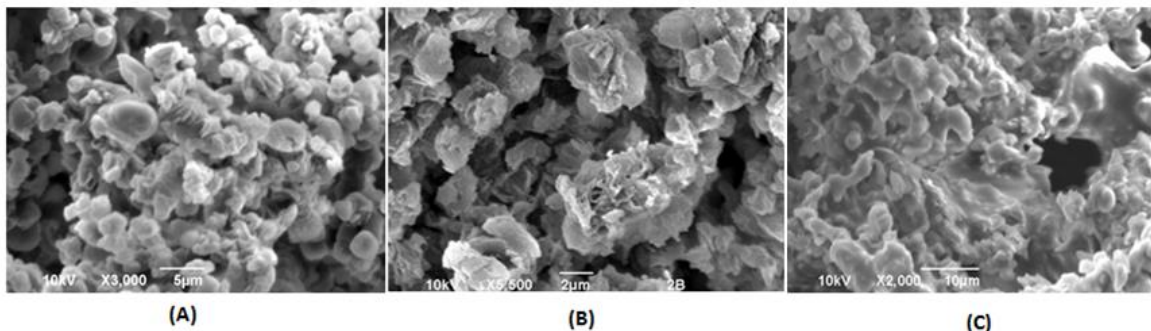**Figure 3.** Comparative SEM micrographs of β-carotene precipitated. Experimental condition: **(A)** "slow drying" (q_a: 10 ml.min^{-1}; volume of CO_2: 2500 ml and magnification of 3000x); **(B)** "standard drying" (q_a: 20 ml.min^{-1}; volume of CO_2: 2000 ml, magnification of 5500x); **(C)** "fast drying" (q_a: 30 ml.min^{-1}; volume of CO_2: 1500 ml, magnification of 2000x).

It can be seen from the figures above that the drying treatments promote alterations in the precipitation process in both pure compounds. Through the micrographs of PHBV (Figure 2) it was found that its morphology has been changed by the different treatments and, in slow and fast drying, apparently, there was a greater agglomeration of the microparticles. Concerning the morphology of β-carotene (Figure 3), no visual differences between the microparticles subjected to standard and slow treatment was found. On the other hand, the fast drying (Figure 3 C) promoted greater agglomeration of precipitated material compared to the other samples (Figure 3 A and B) possibly because with the used volume of CO_2 (1500mL) was not sufficient to promote an efficient drying of the microparticles. According to micrographs obtained for PHBV and β-carotene can be see that lower changes in structures of precipitated particles occurred in standard drying (q_a: 20 ml.min-1; volume of CO_2: 2000 ml).

In general, it appears that this study provides relevant information to be used as support in co-precipitation step for these compounds. In fact, the experimental conditions are factors that need to be considered in co-precipitation step because specifically in SEDS technique, the active principle and the biopolymer were co-precipitated under the same experimental conditions by spraying the solution containing these two compounds in a chamber already with the anti-solvent pressurized.

In order to verify the effect of standard drying step in the morphology of particles (β-carotene and PHBV) subjected to the co-precipitation process, three different assays were conducted with these compounds according to experimental conditions showed in Table 2. Additionally, Figure 4 shows the morphology of the co-precipted particles.

Table 2. Experimental conditions for the co-precipitation of β-carotene and PHBV.

Run	P [bar]	T [°C]	β-Carotene concentration into organic solution (mg.mL^{-1})	PHBV concentration into organic solution (mg.mL^{-1})
1			14.09	30.17
2	80	313.15	16.05	30.24
3			30.11	30.70

Adopting standard drying: q_a = 20 ml.min^{-1} and volume of CO_2 = 2000mL.

Through the micrographs in the step of co-precipitation (Figure 4) it can be inferred that there were no significant visual changes in the morphology of particles compared with those obtained in Figure 2B adopting standard drying. It appears that both particles are close to spherical shape, characteristic of PHBV (encapsulating agent). However, also through Figure 4 it can be seen that some particles containing β-carotene were not encapsulated and remained adhered to the walls of the polymer. This comparison can be performed by visual observation of particles of β-carotene in the plate-like obtained in Figure 3B adopting the standard drying.

Considering the possibility of non encapsulated compound (β-carotene), an additional experimental step was adopted in the present study to evaluate the non-encapsulated remaining particles. This method consists of washing the precipitated material after drying and was presented by Franceschi et al., (2008) [16] and by Priamo et al., (2010) [15] to remove the non-encapsulated β-carotene (excess, free) after the drying of

microparticles by SEDS technique. Briefly, a sample of co-precipitated β-carotene in PHBV was weighed (between 5 and 35 mg) in an analytical balance with precision of 0.00001g (Mettler Toledo, model XS205 DualRange) and added to different volumes of acetone. This solvent was selected because β-carotene presents relatively low solubility in this solvent, compared to other organic solvents (Tres et al., 2007 [19]), hence allowing slow removal of the non-encapsulated material, avoiding possible damages in the polymer wall. The suspensions of co-precipitated into acetone were manually agitated for about 20s at room temperature (~298 K) and then all samples were filtered using a membrane filter with porosity of 0.22μm (Millipore, model FGLP). After filtration, the material retained was dried under controlled temperature (323 K) and vacuum for 24 h.
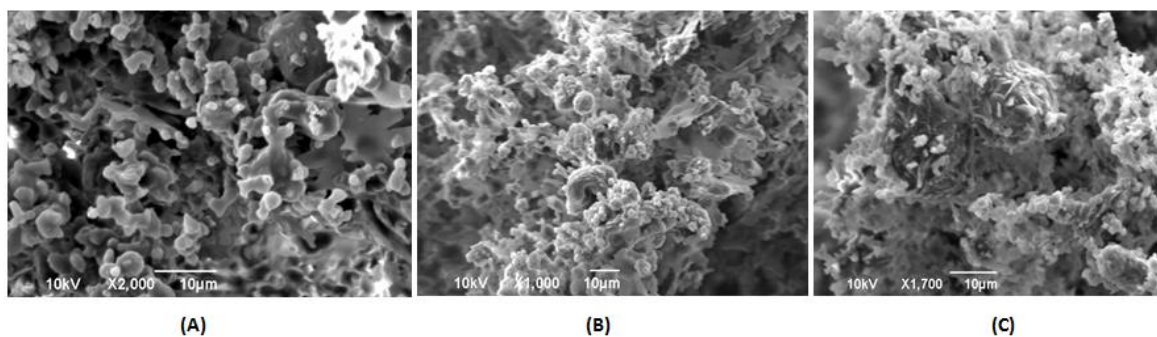

Figure 4. SEM micrographs of PHBV and β-carotene co-precipitated. **(A)** Run 1, magnification of 2000x; **(B)** Run 2, magnification of 1000x; **(C)** Run 3, magnification of 1700x.

Figure 5 shows the micrographs of β-carotene and PHBV co-precipitated obtained in the assays 1, 2 and 3 (Table 2) and submitted to washing (according to the procedure described by above mentioned authors) to remove non-encapsulated material.
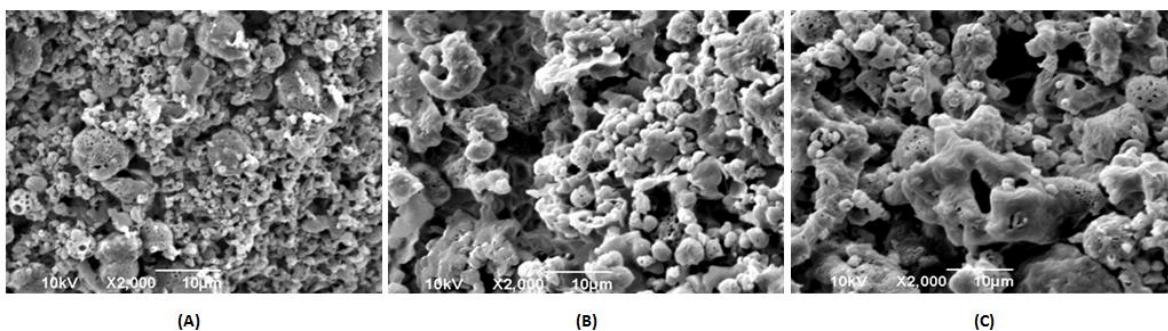

Figure 5. SEM micrographs of PHBV and β-carotene co-precipitated and submitted to washing according to Franceschi et al., (2008) and Priamo et al., (2010). **(A)** Run 1, magnification of 2000x; **(B)** Run 2, magnification of 2000x; **(C)** Run 3, magnification of 2000x.

It can be seen that the washing method was efficient in removing non-encapsulated material, because initially the microparticles (showed in micrographs 4A, 4B and 4C) presented a aspect with higher agglomeration. After the washing treatment (Figure 5A, 5B and 5C) the particles co-precipited showed predominantly spherical morphology (similar to PHBV, encapsulating agent) with porous surface and few connections between the particles.

4. Conclusions

This work investigated the effect of quantity (volume) and flow of anti-solvent (CO_2) in terms of the morphology of β-carotene and PHBV precipitated and co-precipitated by SEDS technique. According to the experimental results it can be seen that in standard drying treatment satisfactory characteristics in particle morphology of the compounds were obtained in both experiment types, precipitation and co-precipitation. Additionally, it was possible to confirm the importance of the washing treatment after co-precipitation aiming

the removal of non-encapsulated β-carotene. Overall, this study showed important information to be used in precipitation and co-precipitation of compounds with potential of applications in food and pharmaceutical products.

Acknowledgements

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