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Spray-dried diclofenac-loaded poly(ε-caprolactone) nanocapsules and nanospheres. Preparation and physicochemical characterization

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The aims of the present study were to prepare spray-dried polymeric nanocapsules (NC) and nanospheres (NS) from poly(ε-caprolactone) (PεC) suspensions containing diclofenac (DIC) and to determine the physicochemical properties of the formulations. NC or NS suspensions were prepared by interfacial deposition of the polymer. DSC-thermograms of raw materials and NC or NS suspensions (evaporated or spray-dried) were obtained using a PL-DSC. Spray-dried powders were prepared by addition of 3% (w/v) Aerosil® 200 into suspensions of NC or NS. These mixtures were fed into a spray-dryer. DIC was assayed by HPLC. NC and NS spray-dried powders were examined under SEM (Jeol Scanning Microscope, JSM-5800). NC and NS suspensions had acceptable diameter, 340 and 247 nm respectively. The yields of NC and NS spray-dried powders were 80% and 75% and the recovery of the DIC was 99% and 93%, respectively. The melting peak of PεC in NC and NS was observed at a temperature about 10 °C lower than in the raw material. In the NC thermograms the maximum of the oil (Miglyol 810®) melting peak (+1.6 °C) was lowered about 7 °C. For spray-dried NC formulations, the SEM analyses of powders showed spherical microparticles of silicon dioxide, covered by nanoparticles (300 nm), while for spray-dried NS formulations the microparticles presented a rugged surface at the same magnification.

1. Introduction

Colloidal polymeric suspensions such as nanocapsules (NC) and nanospheres (NC) have been developed as drug targeting delivery systems. Besides the i.v. route, colloidal systems have also been administered orally, either for systemic uptake or local contact within the gastrointestinal tract [1–6]. For peroral administration, the nanoparticles could be administered in the form of an aqueous dispersion as the final dosage form. However, hydrolysis of polymers, poor stability of drugs in aqueous environment or suspension instability, due to the particles sedimentation, may require the incorporation of the colloidal particles into solid dosage forms [2, 4, 5, 7]. This is an important technological problem, which can compromise the industrial feasibility of such systems. Therefore, it is necessary to improve the stability of these forms to reach a shelf-life of several years, although reports in the literature show that freeze-drying is a good method to dry nanospheres [8]. On the other hand, Puisieux et al. [5] have observed that nanocapsules are not easily lyophilized, as a result of their vesicular character, they tend to collapse releasing the oily core. The spray-drying technique exhibit advantages like low price, easiness of industrial transposition and possibility of preparation of powders with better established physico-chemical characteristics than freeze-drying powders [9].

Diclofenac is an anti-inflammatory drug with serious side effects on the gastrointestinal tract, characterized by the formation of lesions and ulcerations. After intragastric administration, nanocapsules containing diclofenac induced a marked protective effect on the gastrointestinal mucosæ as compared to the ulcerative effect of the drug solution [10]. Poly(D,L-lactic acid) nanocapsules containing diclofenac prepared by interfacial polymer deposition following solvent displacement were stable for eight months at room temperature [7].

Recently, we developed the first application of spray-dried technique to dry nanocapsules and nanospheres suspensions with a view to obtaining a solid form of polymeric colloids [11]. This technique was described for drying nanocapsule suspensions of poly(ε-caprolactone) (PεC, 40,000) or Eudragit® S90 containing diclofenac, a phospholipid mixture (Epikuron® 170) and Poloxamer (Synperonic® PE/F68) as surfactants, and caprilic/capric triglyceride (Miglyol® 810) as oil. The colloidal silicon dioxide (Aerosil® 200) was employed as a spray-drying adjuvant. The most interesting characteristic observed in the micrographs was the surface of silicon dioxide covered by nanostructures. For these spray-dried nanocapsules, the nanostructure size was almost the same before and after dehydratation (around 200 nm) [12, 13].

The objectives of the present study were to examine the potential use of others surfactants (sorbitan monostearate and polysorbate 80) in the formulation of spray-dried powders from PεC (80,000) nanocapsules suspensions containing diclofenac (DIC) and to determine the suitability of this technique to dry nanosphere suspensions prepared by deposition of pre-formed polymer. With these purposes in mind, colloidal suspensions of nanocapsules and nanospheres containing DIC or not were spray-dried and characterized, using photon correlation spectroscopy, zeta potential, differential scanning calorimetry and scanning electron microscopy (powders).

2. Investigations, results and discussion

2.1. Particle size and zeta potential of colloidal suspensions

The results of particle size analysis (Table) indicate that the mean sizes of the NC and NS suspensions (empty or containing DIC) are in the sub 400 nm range. Regarding zeta potential, all formulations had a negative charge due to the polymer structure. For NC and NS prepared with DIC (NC-DIC or NS-DIC) the zeta potential was $\zeta = -17$ mV and -18 mV, while the formulations without drug presented values of $\zeta = -15$ mV (NC-EM) and $\zeta = -20$ mV (NS-EM). The Table also shows the loading efficiency defined as the percentage of DIC associated

Table: Characteristics of NC and NS suspensions (empty or containing DIC) and the NC and NS spray-dried powders (empty or containing DIC)

	Colloidal suspensions			
	Size (nm ± SD)	DIC associated (%)	DIC recovery (% ± SD)	Zeta potential (mV ± SD)
NC-EM	281 ± 73	–	–	−15.4 ± 0.2
NC-DIC	340 ± 113	100	105.3 ± 7.6	−17.1 ± 0.2
NS-EM	189 ± 64	–	–	−20.1 ± 0.4
NS-DIC	247 ± 92	100	88.9 ± 9.7	−18.3 ± 0.2

	Spray-dried powders		
	Yield (% ± SD)	Weight loss (% ± SD)	DIC recovery (% ± SD)
NC-EM-P	67	2.06 ± 0.0	–
NC-DIC-P	80	2.05 + 0.1	97.8 ± 7.3
NS-EM-P	80	2.06 ± 0.6	–
NS-DIC-P	75	2.35 ± 0.0	92.6 ± 4.9

with respect to the total amount of DIC used to prepare the colloidal systems. The formulations presented high loading efficiencies (close to 100%). Because the ultrafiltration-centrifugation method is not capable of differentiating between the drug entrapped (dissolved in the oil phase) and the drug adsorbed [14], and the zeta potential values were not significantly different, the association of drug with the colloids could be explained by the chemical structure of DIC. The drug presents aromatic rings (lipophilic character) and a carboxylic acid group which can interact with the oil and/or with the polymer by van der Waals forces and hydrogen bonds.

2.2. Spray-dried powders

In our previous work [13], the impossibility of spray-drying nanocapsule suspensions without adjuvant was demonstrated. The reason is the strong adhesion of the product onto the spray-dryer walls. The presence of colloidal silicon dioxide (3% w/v) avoided this problem and provided homogeneous suspensions with good fluidity to spray-dry. Applying the same conditions, spray-dried products were obtained from NC or NS colloidal suspensions, using colloidal silicon dioxide as adjuvant. The values of bulk densities were 0.268 g/ml, 0.137 g/ml and 0.035 g/ml for NC-DIC-P, NS-DIC-P and silicon dioxide (raw material), respectively. The difference in bulk densities between NC-DIC-P and NS-DIC-P can be attributed to the presence of the oil core (Miglyol® 810) in nanocapsules.

The yields of spray-dried powders were 67% (NC-EM-P), 80% (NC-DIC-P), 80% (NS-EM-P) and 75% (NS-DIC-P), showing a low moisture content, below 2.5% for all formulations (Table). The use of sorbitan monostearate and polysorbate 80 in NC formulations did not influence the spray-dried yields when compared with the similar NC formulation (70%) prepared using phospholipid mixture (Epikuron® 170) and Poloxamer (Synperonic® PE/F68) as surfactants [13]. For NC and NS powders, the DIC recovery was 97.8 ± 7.3% and 92.6 ± 4.9%, respectively.

2.3. Differential scanning calorimetry

DSC analysis performed in the raw materials showed for the PεC only a melting peak at +60 °C, and no glass transition could be detected (Fig. 1 and Fig. 2). The maximum of the melting peak of the sorbitan monostearate was at +58 °C and for Miglyol® 810 it was at +1.6 °C (Fig. 2). The melting point of the PεC in both NC and NS suspensions (evaporated or spray-dried) was observed at a temperature about 10 °C lower, indicating that smaller crystallites were formed during their preparation (Figs. 1–3). For the samples dried by evaporation at room temperature (Fig. 3), it was also possible to observe the glass transition temperature of the PεC at about −55 °C, indicating a lower degree of crystallinity of the polymer in the NC and NS suspensions than in the polymer (raw material). For

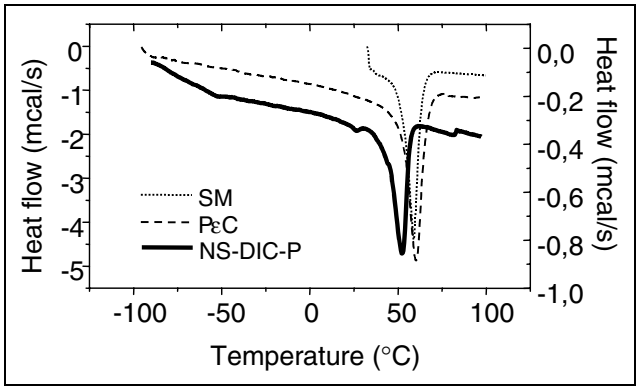


Fig. 1: DSC-thermograms of sorbitan monostearate (SM) and PεC as raw materials and spray-dried diclofenac-loaded PεC nanospheres (NS-DIC-P)

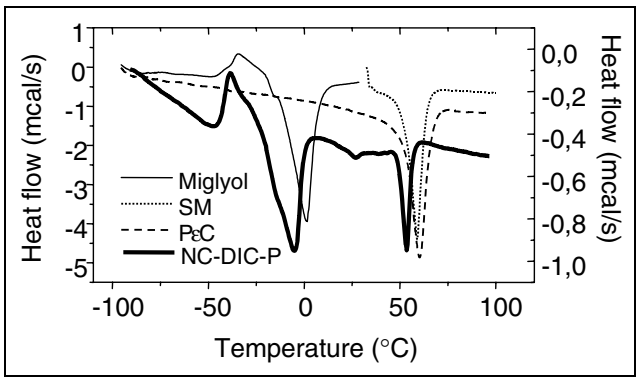


Fig. 2: DSC-thermograms of Miglyol 810®, sorbitan monostearate (SM) and PεC as raw materials and spray-dried diclofenac-loaded PεC nanocapsules (NC-DIC-P)

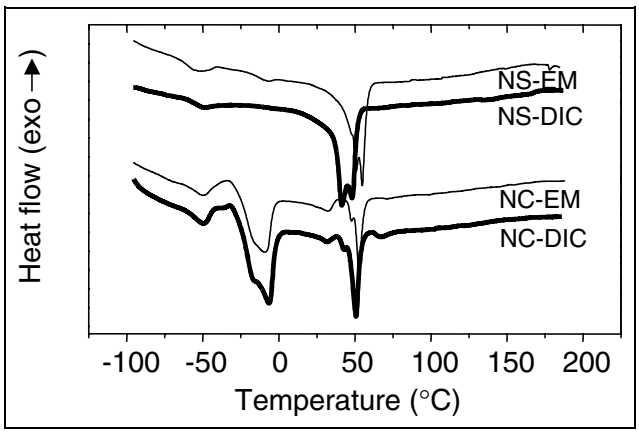
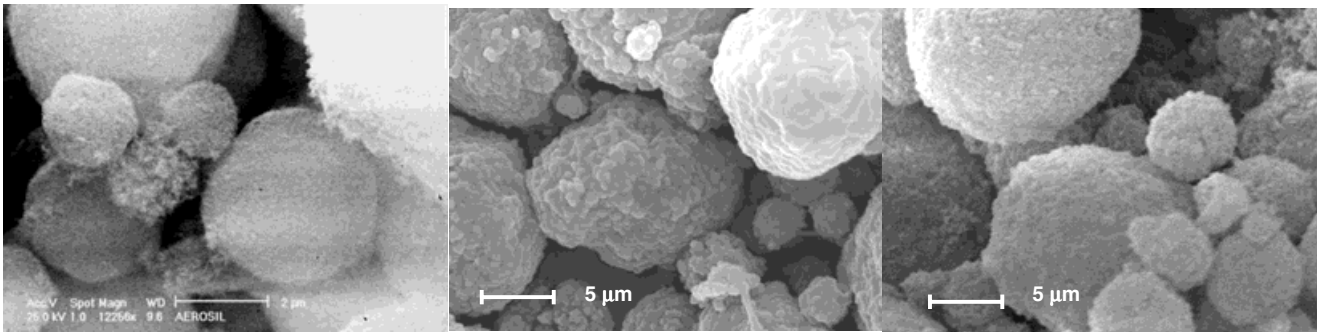


Fig. 3: DSC-thermograms of evaporated suspensions of PεC nanocapsules: empty (NC-EM) and diclofenac-loaded (NC-DIC), and evaporated suspensions of PεC nanospheres: empty (NS-EM) and diclofenac-loaded (NS-DIC)



(a) (b) (c)

Fig. 4: SEM micrographs of (a) spray-dried silicon dioxide (Aerosil 200®); (b) spray-dried diclofenac-loaded nanocapsules (NC-DIC-P) and (c) spray-dried diclofenac-loaded nanospheres (NS-DIC-P)

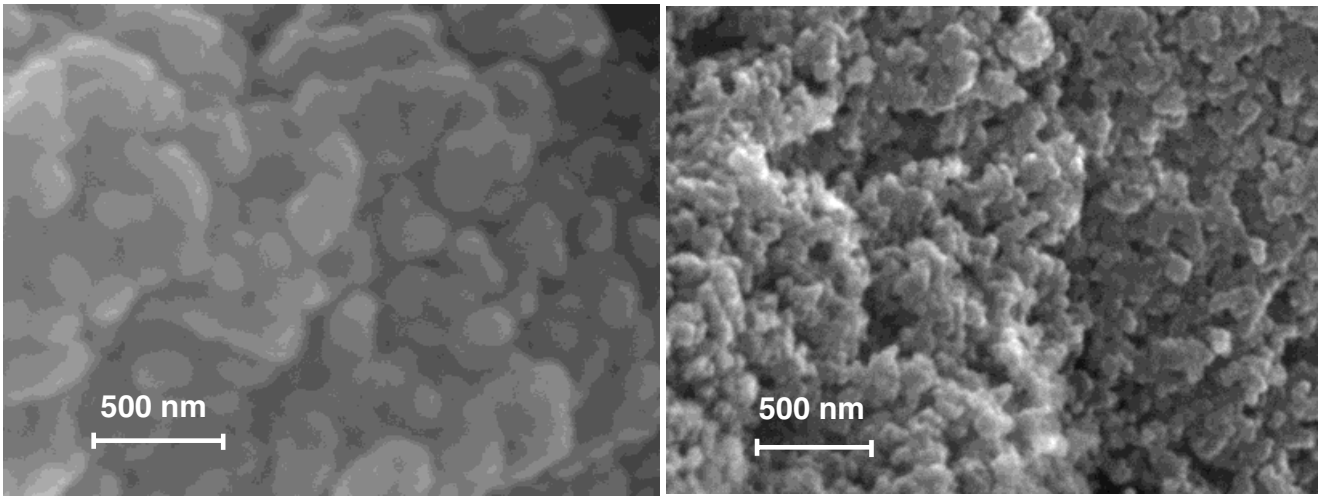
the spray-dried NC and NS no glass transition was observed (Fig. 1 and Fig. 2), probably due to the small amount of polymer in the samples due to the presence of silicon dioxide. In the thermograms of the NC was also possible to observe the melting peak of Miglyol® 810 (Fig. 2 and Fig. 3). However, the maximum of this peak was lowered about 7 °C. This decrease of the melting temperature can be attributed to the presence of the sorbitan monostearate dissolved into Miglyol® 810, by turns the melting peak of this surfactant (+58 °C) is not present in the curve of NC formulations but it is in these of NS formulations.

2.4. Morphological analysis

The scanning electron microscopy analyses of spray-dried powders, obtained from silicon dioxide dispersed in water or aqueous suspensions of NC or NS added of silicon dioxide, showed that microparticles are globally spherical shaped (Fig. 4) and have similar particle sizes. However, the micrographs of each powder, neat silicon dioxide, NC spray-dried or NS spray-dried (Fig. 4 and Fig. 5), showed that formulations obtained from colloidal suspensions presented the silicon dioxide microparticles covered by the material of the NC or NS suspensions (Fig. 5).

The microparticle surface of NC spray-dried powders have clearly shown nanostructures (around 300 nm) (Fig. 5a). These results are in agreement with our previous work in which spray-dried NC have been prepared [13]. To verify the possibility of NC spray-dried powder releasing nanoparticles (300 nm) from the silicon dioxide microparticles, the NC spray-dried powder (NC-DIC-P) was redispersed in water, leading a fine suspension. Then, after separation of silicon dioxide by filtration, the filtrate was analyzed by photon correlation spectroscopy and the average of the particle size was 192 ± 5 nm. The zeta potential of the fine suspension (NC spray-dried powder redispersed in water) was measured, after dilution with NaCl aqueous solution (1 mM), showing a value of $\zeta = -20.1 \pm 0.5$ mV. For spray-dried NS formulations (NS-EM-P and NS-DIC-P), despite the rugged surface of microparticles analyzed by SEM, it was not possible to observe the presence of nanostructures, covering the silicon dioxide, with similar diameters (190–250 nm) to those measured for the respective suspensions (NS-EM and NS-DIC) before the drying process, suggesting that spray-drying causes a structural modification in NS particle arrangement. Nevertheless, after redispersing NS-DIC-P in water, which led to a fine suspension, and separating the silicon dioxide by filtration, the filtrate presented an average of the particle size of 188 ± 12 nm. The zeta potential of the fine suspension diluted with NaCl aqueous solution (1 mM) was $\zeta = -19.4 \pm 0.6$ mV.

In conclusion, the DSC measurements indicated a lower degree of crystallinity of the polymer in all NC and NS formulations than in the raw material. In the case of NC, the sorbitan monostearate, lipophilic surfactant, is dissolved in the oil, whereas in NS it is dispersed in the



(a) (b)

Fig. 5: SEM micrographs of (a) spray-dried diclofenac-loaded nanocapsules (NC-DIC-P) and (b) spray-dried diclofenac-loaded nanospheres (NS-DIC-P)

polymer matrix. The spray-drying technique supplied dried powders from NC and NS colloidal suspensions prepared using sorbitan monostearate and polysorbate 80 as surfactants and PεC (80.000) as polymer. For spray-dried NC formulations, the SEM analyses of powders showed spherical microparticles of silicon dioxide, covered by nanoparticles (300 nm), while for spray-dried NS formulations the microparticles presented a rugged surface at the same magnification. Gastrointestinal tolerance studies in rats are in progress to evaluate the potentiality of these spray-dried products (NC-DIC-P and NS-DIC-P) in protecting intestinal tract, as well as the evaluation of pharmacokinectic profiles of DIC after oral administration of spray-dried NC or NS reconstituted in water.

3. Experimental

3.1. Materials

Diclofenac (DIC, sodium salt) was obtained from Sigma (St Louis, USA) and poly(ε-caprolactone) (PεC, MW = 80,000) from Aldrich (Strasbourg, France). Caprilic/capric triglyceride (Miglyol® 810) was obtained from Hulls (Puteaux, France); sorbitan monostearate and polysorbate 80 were supplied by Delaware (Porto Alegre, Brazil). Colloidal silicon dioxide (Aerosil® 200) was obtained from Degussa (São Paulo, Brazil). All other chemicals and solvents used were of pharmaceutical grade. All reagents were used as received.

3.2. Preparation of NC and NS suspensions

Nanocapsules of PεC containing diclofenac were prepared as described by Fessi et al. [6]. Briefly, the lipophilic solution consisted of Miglyol® 810 (3.3 ml), diclofenac (free acid) (0.100 g), sorbitan monostearate (0.766 g), the polymer (1.000 g) and acetone (267.0 ml). This organic phase was added under moderate magnetic stirring into an aqueous solution containing polysorbate 80 (0.766 g in 533.0 ml of water). Acetone was removed by evaporation under reduced pressure and the final concentration of the suspension was adjusted to 1 mg/ml of diclofenac. Nanospheres were also prepared as described for nanocapsules, omitting the oil. Formulations were made in triplicate.

3.3. Characterization of suspensions

The particle size was measured by laser light scattering (Nanosizer®, Coultronics, Andilly, France). The zeta potential of suspensions was measured using a Zetasizer®4 and correlated with the Malvern® standard solution (Malvern, UK), presenting value of $\zeta = -46.5 \pm 5.9$ mV. From each NC or NS suspensions containing or not DIC (NC-EM, NC-DIC, NS-EM or NS-DIC), 20 µl were added to 8 ml of NaCl aqueous solution (1 mM). After stirring, the mixture was injected and measurements were made in triplicate (25 °C).

DIC was assayed by HPLC. The system consisted of a SPD-10A Shimadzu detector, LC-10AD Shimadzu pump, SIL-10A Shimadzu injector and Nova-Pak® C18 – 3.9 × 300 mm Waters column. The mobile phase consisted of acetonitrile/water (65 : 35 v/v) adjusted to pH 4.0 with acetic acid 10%. Diclofenac was detected at 280 nm with a retention time of about 6.7 min. Free diclofenac (non-associated) was determined in the ultrafiltrate after separation of the nanocapsules by ultrafiltration-centrifugation technique (Ultrafree-MC 10,000 MW, Millipore). Total DIC was measured using HPLC after dissolution of colloidal suspension by acetonitrile. The associated diclofenac with the nanocapsules was calculated from the difference between the total and free drug concentrations measured in the nanocapsule suspension and in the ultrafiltrate, respectively [13].

3.4. Preparation of spray-dried powders

To the suspension of NC or NS was added 3% (w/v) of Aerosil® 200 and the mixture was fed into a mini-spray-dryer Büchi MSD 190 (Flawil, Switzerland) with two component nozzle and co-current flow. The inlet temperature at the drying chamber was maintained at 138 ± 4 °C. The outlet temperature was kept at 90 ± 4 °C [13]. Dispersion of 3% (w/v) Aerosil® 200 in water was spray-dried at the same conditions.

The residual water content of each spray-dried product was determined by weight loss [15]. Each analysis was repeated 3 times.

3.5. Determination of drug content on spray-dried powders

The spray-dried products were dispersed in acetonitrile, filtered with hydrophilic membrane (HVLP, 0.45 µm) and DIC assayed by HPLC.

3.6. Determination of particle size after dispersion of spray-dried powders in water

The NC or NS spray-dried powders were dispersed in water at a final concentration of 1.0 mg/ml of DIC. Then, each sample was stirred during 1 h at room temperature, and filtered through a hydrophilic membrane (HVLP, 0.45 µm) into the cylindrical scattering cells for the photon correlation spectroscopy measurements (PCS) performed at 20 °C with an automatic BI-200M goniometer and a BI-9000AT digital correlator. A Spectra Physics (model 127) He-Ne laser (λ = 632.3 nm) was used as light source. Time correlation functions were obtained in multiple-τ mode at 90°. The time correlation functions were analyzed by the cumulant method.

3.7. Zeta potential after dispersion of spray-dried powders in water

The NC or NS spray-dried powders containing DIC (NC-DIC-P or NS-DIC-P) were dispersed in water Milli-Q (84.4 mg/ml and 54.4 mg/ml, respectively). After stirring, 20 µl of the fine suspension were added to 8 ml of NaCl aqueous solution (1 mM). The measurements were made in triplicate using a Zetasizer® 4.

3.8. Differential scanning calorimetry

DSC-thermograms of raw materials and NC or NS suspensions (evaporated or spray-dried) were obtained using a Polymer Laboratories DSC (England). The scanning temperature ranged between −100 °C and 100 °C or 200 °C with a heating rate of 10 °C/min. All calculations were done for the second scan. Suspensions samples were evaporated at room temperature.

3.9. Morphological analysis of spray-dried powders

The spray-dried powders were examined under scanning electron microscopy (SEM) (Jeol Scanning Microscope, JSM-5800). Samples were analyzed after they had been gold sputtered.

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