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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 501–505
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Note

Freeze-drying polymeric colloidal suspensions: nanocapsules, nanospheres and nanodispersion. A comparative study

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Received 15 January 2003; accepted in revised form 4 August 2003

Abstract

Different polymeric nanoparticles were freeze-dried and the powders compared to determine the influence of the lipophilic core (Miglyol 810® or benzyl benzoate) and polymeric material (poly(ϵ-caprolactone) or Eudragit S90®) on their drug content and morphology. Diclofenac, a non-steroidal anti-inflammatory drug, was used as a model. To characterize the products, a biological experiment based on the evaluation of the mucosal toxicity of diclofenac was conducted. Nanocapsule and nanosphere suspensions were prepared by nanoprecipitation and freeze-dried after the addition of colloidal silicon dioxide. The powders were examined under scanning electron microscopy (SEM) and gastrointestinal tolerance of products was evaluated in rats. Powders presented drug contents between 90.2 ± 5.5 and 101.1 ± 1.9% (HPLC). SEM analyzes showed non-spherical microparticles and, at higher magnifications, the micro-powder surface presented a homogeneous nanocovering. Regarding the gastrointestinal tolerance, with the exception of benzyl benzoate-loaded formulations, powders presented lesional indexes lower than the diclofenac salt solution. In contrast to the literature, nanocapsules can be dried by freeze-drying without leakage of drug or breaking the capsule wall.

Keywords: Polymeric nanoparticles; Nanocapsules; Diclofenac; Freeze-drying; Benzyl benzoate

1. Introduction

The advantages shown by polymeric nanoparticles as a possible means of delivering drugs by different routes of administration have been previously reported [1]. The industrial development of these systems might be limited due to the problems in maintaining stability of suspensions [2]. Hence, the improvement of the stability of nanoparticle suspensions to reach a longer shelf life has received considerable attention and represents an important field in drug carrier research.

Freeze-drying technique was described to dry nanocapsules prepared with poly(lactic acid) as polymer and benzyl benzoate (BnB) as oil [3,4]. However, after the rehydration of freeze-dried nanocapsules, loss of drug as large as 50% was observed. These results were attributed to changes in the structure of the capsule wall [4] or it was pointed out that water crystallization stress could break the nanocapsules, promoting the leakage of their contents into the continuous phase [3].

In our previous work concerning the spray-drying of nanocapsules prepared with poly(ϵ-caprolactone) (PCL) or poly(lactic acid) and BnB [5], we proposed that these colloidal suspensions prepared with BnB are micelles instead of nanocapsules. In swelling/dissolution experiments, the complete dissolution of both polymers by the BnB was observed. Therefore, the changes in the structure of the wall of nanocapsules [4] would occur before the freezing step by dissolution of the poly(lactic acid) in the BnB, and water crystallization stress [3] would take place on a micellar structure instead of breaking a capsule wall. Consequently, the freeze-drying method cannot be rejected for drying nanocapsule suspensions when the particle structure corresponds to a vesicle with a lipophilic core and a rigid and resistant polymeric wall.

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0939-6411/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0939-6411(03)00139-5

Additives are necessary to obtain freeze-dried redispersible nanoparticles [2,3]. For i.v. administration, particle sizes below 5 μm are required for avoiding embolism [6], which can be achieved by freeze-drying using saccharides as cryoprotectors [2,6]. However, for oral administration this requisite is not necessary. We have shown that colloidal silicon dioxide is useful as drying excipient for spray-drying nanoparticles, resulting in nanocovered microparticles for oral administration [7,8]. In the light of these results, in the present work, silicon dioxide was used as drying excipient for nanoparticles freeze-drying.

The feasibility of producing freeze-dried powders from nanocapsules has been investigated. The freeze-dried products were compared to determine the influence of the lipophilic core (Miglyol 810®, MI or BnB) and polymeric material (PCL or Eudragit S90®, EUD) on their drug content and morphology. Diclofenac, a non-steroidal anti-inflammatory drug, was used as a model drug. In order to characterize the freeze-dried powders, a biological experiment, based on the evaluation of the mucosal toxicity of diclofenac, was conducted.

2. Materials and methods

2.1. Diclofenac free acid [7]

A diclofenac sodium salt (Sigma, St Louis, USA) (1.045 g, 3.28 mmol) aqueous solution (100 ml) was treated with 5 M HCl to final pH 2. The aqueous phase was extracted with ethyl acetate (3 × 30 ml). The organic phase was dried with Na_2SO_4, filtered and evaporated. The free acid was recrystallized from ethanol/water 1:1 (v/v) yielding white crystals (78%) presenting melting point 158–160°C.

2.2. Swelling experiments

Films of PCL (MW = 80,000, Aldrich, Strasbourg, France) or EUD [poly(methyl methacrylate-co-methacrylic acid), Röhm, Altmann, São Paulo, Brazil] were obtained by squeezing of the molten pellet in a heated hydraulic press Paul Weber, PW 10–PW 40 (Grunbach, Germany) for 5 min at 60 or 120°C, 5 or 25 kN, respectively. For each experiment one peace of the film was exactly weighed (about 40 mg) in a glass flask. A quantity of benzyl benzoate (BnB, Delaware, Porto Alegre, Brazil) or Miglyol 810® (caprilic/capric triglyceride, Hulls, Puteaux, France) sufficient to cover the film was added. The flasks were closed and stored at room temperature. After 2, 4, 6, 8 and 13 days the films were sieved and the oil at the surface was removed with absorbing paper. Thereafter the films were weighed.

2.3. Preparation of suspensions

A lipophilic solution consisted of MI or BnB (3.3 ml), diclofenac (0.100 g), sorbitan monostearate (Delaware, Porto Alegre, Brazil) (0.766 g), PCL or EUD (1 g) and acetone (267.0 ml) was added under moderate magnetic stirring into an aqueous phase containing polysorbate 80 (Delaware, Porto Alegre, Brazil) (0.766 g in 533.0 ml of water). Acetone was removed and water was concentrated by evaporation under vacuum. The volume of suspensions was adjusted to 100 ml (1 mg/ml of diclofenac). Nanospheres (PCL or EUD) were also prepared as nanocapsules, omitting the oil. Formulations were made in triplicate.

2.4. Physico-chemical characterization of suspensions

The particle size was measured by laser light scattering observed at 90° (Nanosizer®, Coultronics, Andilly, France) after dilution of the samples. The standard deviations were calculated by the average of three determinations. Diclofenac was assayed by HPLC [7]. The system consisted of Shimadzu (Kyoto, Japan) auto-sampler CIL-10A, pump LC-10AD, UV-detector SPD-10A and a Waters Nova-Pack C18 (3.9 × 300 mm) column. The mobile phase consisted of acetonitrile/water (65:35 v/v) adjusted to pH 5.0 (10% acetic acid). Diclofenac was detected at 280 nm. Non-associated diclofenac was determined by ultrafiltration-centrifugation (Ultrafree-MC 10,000 MW, Millipore) and total diclofenac was determine after dissolution with acetonitrile. The associated diclofenac was calculated from the difference between the total and the free drug concentrations.

2.5. Preparation of freeze-dried powders

Aliquots of 2 ml of each suspension were added of 3 % (w/v) Aerosil 200® (Degussa, São Paulo, Brazil). The samples were slowly frozen at −20°C. Then, the samples were freeze-dried without heating under 0.07 mbar vacuum (condenser temperature of −55°C) for 24 h (Edwards High Vacuum International, Modulyo 4K Freeze-Drier, West Sussex, England).

A diclofenac (free acid) aqueous dispersion (1 mg/ml) containing polysorbate 80 (7.7 mg/ml) and silicon dioxide (3% w/v) and a dispersion of 3% (w/v) Aerosil 200® in water were also freeze-dried in the same conditions for comparison.

2.6. Characterization of powders

After stirring the powders in acetonitrile for 1 h followed by filtration with hydrophilic membrane (Durapore HVLP, 0.45 μm), the drug content of the freeze-dried products was determined by HPLC.

The freeze-dried powders, after gold sputtered, were examined under scanning electron microscopy (SEM) (Jeol Scanning Microscope, JSM-5800).

2.7. Gastrointestinal tolerance

Gastrointestinal tolerance was evaluated on male Wistar rats (200–300 g) ($n = 8$) (Biotério Central UFRGS, Porto Alegre, Brazil). The groups were kept in separate cages and the rats were allowed to eat and drink freely. Three consecutive daily doses (20 mg/kg per day [7]) of drug [diclofenac sodium salt solution (S), freeze-dried diclofenac free acid (FD-D), freeze-dried diclofenac-loaded PCL-nanospheres (FD-NS-PCL), freeze-dried diclofenac-loaded EUD-nanospheres (FD-NS-EUD), freeze-dried diclofenac-loaded PCL-MI-nanocapsules (FD-NC-MI-PCL), freeze-dried diclofenac-loaded PCL-BnB-nanodispersion (FD-ND-BnB-PCL), freeze-dried diclofenac-loaded EUD-MI-nanocapsules (FD-NC-MI-EUD) and freeze-dried diclofenac-loaded EUD-BnB-nanocapsules (FD-NC-BnB-EUD)] dissolved or dispersed in water (2 mg/ml of diclofenac) were administered via gastric probe. Seventy-two hours after the first administration, animals were decapitated following laparotomy. In order to quantify gastrointestinal lesions, the stomach was opened along the greater curvature; and the intestine (duodenum, jejunum, and ileum) was slit open opposite the attached mesenteric tissue. The organs were washed with normal saline and the mucosal surfaces were examined using a magnifying glass according to an arbitrary scale previously reported [7] (Table 1). Experimental data were obtained by multiplying the score by the number of lesions, followed by Mann–Whitney test.

3. Results and discussion

The particle size of colloidal systems were in the range of 200–330 nm, except for the nanosphere suspension prepared with EUD, which presented a particle size of 84 nm. The suspension pH values ranged between 4.81 and 5.63, resulting from the polymers [9] and the colloids presented drug content of 1 mg/ml (Table 2).

The colloidal suspensions were freeze-dried in the presence of silicon dioxide as excipient, furnishing soft agglomerate and re-dispersible powders. These powders presented drug recoveries of 92.6 ± 1.1% (freeze-dried PCL-nanospheres), 99.3 ± 3.2% (freeze-dried PCL-MI-nanocapsules), 97.3 ± 1.1% (freeze-dried PCL-BnB-nanodispersion), 90.2 ± 5.5% (freeze-dried EUD-nanospheres), 101.1 ± 1.9% (freeze-dried EUD-MI-nanocapsules) and 101.1 ± 1.4% (freeze-dried EUD-BnB-nanocapsules).

Table 1
Scale for visible macroscopic mucosal irritation

Lesions	Score
Localized hemorrhages < 1 mm	0.5
Ulcers < 2 mm	1
Ulcers > 2 mm	2
Perforations	3

Table 2
Size, pH and drug content of nanospheres (NS), nanocapsules (NC), or nanodispersion (ND) prepared with poly(ϵ-caprolactone) (PCL), Eudragit S90® (EUD), benzyl benzoate (BnB) and Miglyol 810® (MI)

Formulation	Size (nm)	pH	Drug content (mg/ml)
NS-PCL	195 ± 59	4.81 ± 0.71	0.90 ± 0.05
NC-MI-PCL	327 ± 97	5.00 ± 0.18	0.97 ± 0.04
ND-BnB-PCL	276 ± 82	5.63 ± 0.24	1.02 ± 0.02
NS-EUD	84 ± 36	5.19 ± 0.21	0.95 ± 0.03
NC-MI-EUD	225 ± 76	5.03 ± 0.04	1.02 ± 0.01
NC-BnB-EUD	202 ± 80	5.22 ± 0.07	1.11 ± 0.14

After freeze-drying, all powders presented similar and non-spherical microparticles (SEM). At higher magnifications, nanostructures were observed on the surface of the microparticles for all formulations. Comparing the particle surfaces of freeze-dried raw silicon dioxide (Fig. 1a) and freeze-dried PCL-nanospheres (Fig. 1b) or freeze-dried PCL-MI-nanocapsules (Fig. 1c) in the presence of the SiO_2, nanostructures with different sizes were observed. Nanoparticles were also freeze-dried without excipient and full re-dispersion of the products could not be achieved after re-hydration due to the formation of cake. Regarding the freeze-dried PCL-nanospheres without SiO_2 (Fig. 1d), the product was formed by an agglomerate of nanoparticles presenting diameters (between 150 and 200 nm) similar to those determined in the original suspension (Table 2). In contrast, the freeze-dried powder obtained from the same suspension (PCL-nanospheres) but containing SiO_2 presented lower sized nanostructures onto the microparticle surface (about 80 nm) (Fig. 1b) as previously observed for spray-dried powders using the same excipients [8].

The microparticle surface of the freeze-dried powders, obtained from the nanoparticle suspensions prepared with BnB and EUD or PCL in the presence of SiO_2, showed nanoparticles with size range similar to that observed for the corresponding original suspensions. Over 13 days, the swelling experiments carried out with BnB and PCL or EUD showed that the BnB is a solvent for the former but a non-solvent for the latter. So, the colloid prepared with PCL and BnB corresponds to a micellar structure, while the colloid prepared with EUD and BnB corresponds to a nanocapsule. Despite the different structures for each system, the SEM was not able to differentiate these formulations (data not shown).

Fig. 2 shows the average lesional indexes for each organ (stomach, duodenum, jejunum and ileum), as well as the overall indexes of the eight groups treated. The results show that all products presented lesional indexes independently of the composition of the formulations, which have progressively increased from stomach to jejunum and ileum. These results correlated well with those reported for non-steroidal anti-inflammatory drugs using the same animal model [3,7].

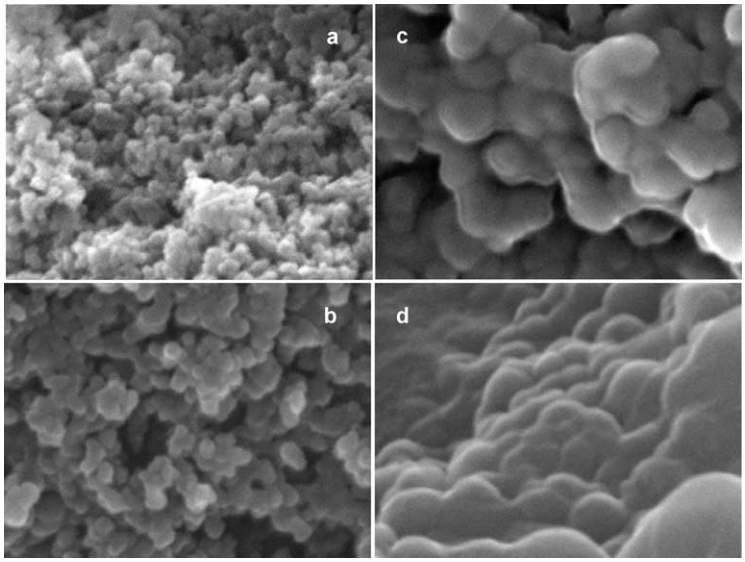

Fig. 1. SEM (width = 1.39 μm) of freeze-dried raw silicon dioxide (a); freeze-dried PCL-nanospheres (b); freeze-dried PCL-MI-nanocapsules in the presence of silicon dioxide (c); and freeze-dried PCL-nanospheres without silicon dioxide (d).

All formulations presented a low lesional index for stomach, as expected for this biological model. As regards the duodenum, low lesional indexes were observed for all formulations. Despite the absence of any statistically significant difference, the duodenum of the animals receiving the aqueous solution of diclofenac sodium salt was thinner and more fragile to manipulation than those of the other groups. Either for jejunum or ileum, the lesional indexes of diclofenac sodium salt aqueous solution were statistically superior to the values determined for freeze-dried diclofenac free acid, freeze-dried diclofenac-loaded nanospheres, and freeze-dried diclofenac-loaded MI-nanocapsules. However, formulations prepared with BnB presented lesional indexes similar to the results determined for diclofenac sodium salt aqueous solution.

The total lesional indexes (Fig. 2) were calculated by the addition of the partial lesional indexes. Excepting formulations prepared with BnB, all powders presented total lesional indexes statistically ($P > 0.05$) lower than the total index presented by diclofenac sodium salt solution. The protective effect observed for the freeze-dried diclofenac free acid (FD-D) showed that SiO_2 has improved the intestinal tolerance of the drug, as previously reported for spray-dried powders [7]. This protection is related to the formation of hydrophobic microparticles that also act as delivery devices. This proposition is supported by the results observed for freeze-dried diclofenac-loaded EUD-nanospheres and freeze-dried diclofenac-loaded EUD-MI-nanocapsules, which polymer is dissolved in the bowel and presented a good protection. For freeze-dried diclofenac-loaded PCL-nanospheres and freeze-dried diclofenac-loaded PCL-MI-nanocapsules the total lesional indexes were not statistically different from the freeze-dried diclofenac free acid (FD-D). However, the powders (PCL or EUD) prepared with colloidal suspension containing BnB presented high total lesional indexes. In order to explain these results, it can be assumed that in both cases the BnB had a direct contact with the gut wall due to the dissolution of the polymers given micellar systems. The BnB can be hydrolyzed in vivo to benzoic acid and benzylic alcohol [10]. In this way, the BnB should be avoided as oil core in colloidal suspensions.

In conclusion, freeze-drying could be used to dry polymeric colloidal suspensions prepared with PCL or EUD by the addition of Aerosil 200®. The powders presented nanocovered non-spherical microparticles. Regarding the gastrointestinal tolerance, with the exception

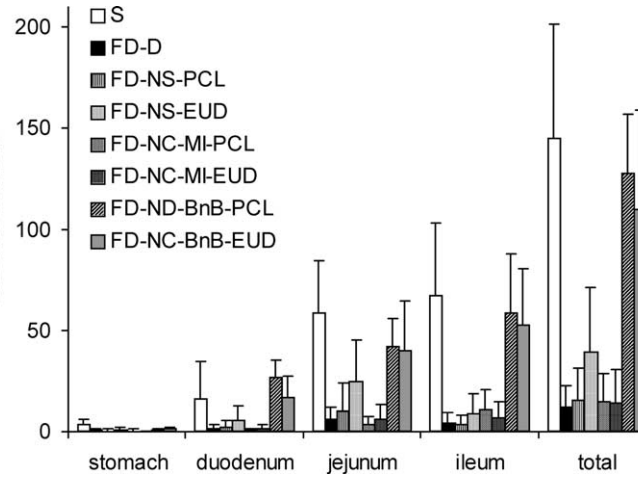

Fig. 2. Mean organ lesional indexes after administration of diclofenac in sodium salt solution (S), freeze-dried diclofenac (FD-D) and freeze-dried powders: diclofenac-loaded PCL-nanospheres (FD-NS-PCL), diclofenac-loaded EUD-nanospheres (FD-NS-EUD), diclofenac-loaded PCL-MI-nanocapsules (FD-NC-MI-PCL), diclofenac-loaded PCL-BnB-nanodispersion (FD-ND-BnB-PCL), diclofenac-loaded EUD-MI-nanocapsules (FD-NC-MI-EUD) and diclofenac-loaded EUD-BnB-nanocapsules (FD-NC-BnB-EUD).

of benzyl benzoate-loaded formulations, powders presented lesional indexes lower than the diclofenac salt solution. In contrast to the literature, nanocapsules could be dried by freeze-drying without leakage of drug or breaking the capsule wall.

Acknowledgements

The authors thank FAPERGS/RS/Brasil and CNPq/MCT/Brasil for financial support; and Dr G. Barratt (Université Paris Sud).

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