International Journal of Pharmaceutics 421 (2011) 293–300

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Layered lipid microcapsules for mesalazine delayed-release in children

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ARTICLE INFO

Article history:
Received 22 July 2011
Received in revised form
22 September 2011
Accepted 27 September 2011
Available online 4 October 2011

Keywords:
Mesalazine
Lipid
Microcapsules
Spray-congealing
Excipient microparticles
Delayed-release

ABSTRACT

The goal was to make available a delayed-release dosage form of mesalazine to be dispersed in water to facilitate swallowing in adults and children. Mesalazine microparticles containing carnauba wax were prepared by spray-congealing technique. A second step of spray-congealing of carnauba microparticles dispersed in liquefied stearic acid gave rise to mesalazine lipid microcapsules in which several carnauba microparticles remained embedded as cores in a reservoir structure. In order to favor their water dispersion, the lipid microcapsules were dry coated by tumbling them with different ratios of mannitol/lecithin microparticles prepared by spray-drying.

Release rate measurements showed a delayed-release behavior, in particular a pH-dependence with less than 10% of drug released in acidic medium and complete release in phosphate buffer pH 7.4 in 4–5 h. The layering with hydrophilic excipient microparticles allowed manufacturing of a pH-dependent dosage form suitable for extemporaneous oral use in adults and children.

1. Introduction

Inflammatory bowel disease is a chronic inflammation affecting the small and large intestine (Kornbluth and Sachar, 2004; Lakatos et al., 2006). Mesalazine or 5-aminosalicylic (5-ASA) is customarily used for the treatment and remission of inflammatory bowel disease (Bergman and Parkes, 2006; Carter et al., 2004; Friend, 2005; Gisbert et al., 2000; Karagozian and Burakoff, 2007; Klotz, 2000). The variability existing in the 5-ASA efficacy is often due to the active drug failing to reach the appropriate site of the intestine where mesalazine acts topically on the mucosa. 5-ASA delivery systems have been developed to avoid drug absorption in the small intestine, thereby delivering maximal amount of drug in the colon (Qureshi and Cohen, 2005; Van den Mooter, 2006).

Current colon delivery systems include: (i) pH-dependent delayed-release formulations, which dissolve at pH ≥ 7.5 (Gupta et al., 2001); (ii) system's enzymatic degradation by colonic bacteria (Fude et al., 2007; Jain et al., 2007; Nunthanid et al., 2008); (iii) time dependent controlled release formulations (Karrout et al., 2009; Jain et al., 2008; Mladenovska et al., 2007; Sandborn et al., 2010; Steed et al., 1997) and (iv) pro-drug carrier systems of 5-ASA (Jung et al., 2006; Minko, 2004).

Due to the high doses, 5-ASA tablet is large (Kamm et al., 2007; Sandborn et al., 2009), representing a problem for administration with respect to swallowing. In addition, delayed-release tablets or capsules are inappropriate for young children and alternative formulations are required (Breitkreutz and Boos, 2007; Nunn and Williams, 2005).

Multiparticulate drug delivery systems, such as granules, pellets (Di Pretoro et al., 2010) and minitablets could be a solution to these problems; they allow to prepare personalized dose for different age patients (Behrens et al., 2003; Breitkreutz and Boos, 2007). Small-sized pellets mixed with food are accepted by children, provided that they are slippery, not gritty and with acceptable taste (Ernest et al., 2007).

The large mesalazine dose is a major problem in designing a pH-dependent multiparticulate dosage form. Due to the large surface area of small particles, unacceptable great amount of polymers for the attainment of drug release control could be used. Lipids are considered more safe as drug delivery excipients; low melting lipids and waxes have already been used for the preparation of multiparticulate controlled-release systems by melt granulation (Passerini et al., 2006), emulsion-solvent evaporation (Chourasia and Jain, 2004; Rodrìguez et al., 1998) or spray-congealing techniques (Albertini et al., 2008; Passerini et al., 2003). However, due to the hydrophobic nature of lipid, the dispersion of these formulations in aqueous media is often difficult due to wetting problems. In order to circumvent this obstacle, a technology capable to increase

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doi:10.1016/j.ijpharm.2011.09.043

the wettability of the lipid microparticle surface, such as agglomeration with hydrophylic microparticles, could be envisaged.

Primary microparticle agglomeration technique has been proposed for manufacturing solid nasal products characterized by rapid water uptake, fast disintegration and reconstitution of the original size of the microparticles (Russo et al., 2004, 2006). Recently, agglomeration by tumbling of pantoprazole gastroresistance microparticles for oral administration was described (Raffin et al., 2007, 2009). The pantoprazole microparticles were agglomerated upon blending them with spray-dried microparticles of mannitol/lecithin. The lecithin was used to improve particle cohesion, to reinforce the internal structure of agglomerates and to favor wettability of system.

The aim of this research was to design and study a new 5-ASA delivery system based on lipid microparticles intended for colonic drug delivery. The goal was to make available a delayed-release dosage form of mesalazine that was easily dispersed in water to facilitate swallowing in adults and children. The microparticles were manufactured using a spray-congealing technique. Since the performance of the spray-congealing process strictly depends on the atomization efficiency of the molten mixture (Killeen, 1996), in this work a wide pneumatic nozzle was used. In order to improve microparticles wettability and dispersion in water, mannitol/lecithin spray-dried microparticles were employed for agglomerating mesalazine microparticles. The delayed-release drug delivery system was characterized with respect to morphology, solid state properties and drug release rate.

2. Materials and methods

2.1. Chemicals

Mesalazine (batch 0822, $d_{v,0.5}$ 7.0 μm ± 0.1) was kindly supplied by Doppel (Cortemaggiore (PC), Italy). Stearic acid (Carlo Erba Reagenti, Rodano (MI), Italy), carnauba wax (Roche Products S.A., Neuilly sur Seine Cédex, France), Eudragit® L100 (Evonik Röhm GmbH, Darmstadt, Germany) were used for microparticle manufacturing. Mannitol was donated by Lisapharma (Erba (CO), Italy) and lecithin (Lipoid S45) was supplied by Lipoid AG (Ludwigshafen, Germany). All other chemicals were of analytical grade.

2.2. Manufacturing of microparticulate powders

The 5-ASA lipid microparticles were manufactured by a two steps spray-congealing technique on an apparatus equipped with a new pneumatic nozzle, identified with the acronym WPN (Wide Pneumatic Nozzle) (Albertini et al., 2008).

In the first step, 5-ASA microparticles were produced. Briefly, mesalazine was dispersed in a solution of Eudragit® L100 in isopropanol under stirring at a temperature of 70 °C. Carnauba wax (m.p. 85 °C) was added to the dispersion and the temperature was raised up until 95 °C to melt the lipid and evaporate isopropanol. Then, the melted mass was sprayed through the WPN nozzle at 3.0 bar pressure. The use of congealing chambers with integrated bag filters allowed the cooling and separation of product in one unit (Albertini et al., 2008).

In the second step, stearic acid microparticles embedding the mesalazine carnauba microparticles were prepared. The 5-ASA carnauba microparticles were dispersed in a low melting point lipid i.e., stearic acid (m.p. 69–70 °C), at the temperature of about 70 °C. At this temperature the carnauba microparticles did not melt and remained homogeneously dispersed in the fluid mass. The dispersion was sprayed with the WPN nozzle at 1.2 bar pressure. The compositions of 5-ASA carnauba microparticles and stearic microparticles are summarized in Table 1.

Table 1
Composition of 5-ASA carnauba microparticles and stearic microparticles (%).

	5-ASA	Carnauba wax	Eudragit® L100	Stearic acid
Carnauba microparticles	31	68	1	–
Stearic microparticles	17.05	37.4	0.55	45

Mannitol was dissolved in 92 mL of water. Lecithin was dissolved in 8 mL of ethanol at 40 °C and mixed with mannitol solution giving an opalescent mixture. The total solid concentration was 4% (w/v) at a mannitol/lecithin ratio of 85:15 (w/w).

The solution was spray-dried using a Buchi Spray Dryer B-191 (Büchi Labortechnik, Flawil, Switzerland) under the following conditions: inlet temperature 130 °C; outlet temperature 65–68 °C, feed rate 5 ml/min, nozzle diameter 0.7 mm, drying air flow 600 L/h.

5-ASA stearic microparticles and excipient microparticles blended at different ratios (2:1, 4:1, 6:1 and 8:1, respectively) were tumbled in a bakelite cylindrical jar (diameter 5.0 cm, length 4.4 cm) rotating on the major axis at 30 rpm. The tumbling process, which lasted 45 min, was carried out introducing into the jar two stainless steel spheres (diameter 1 cm).

Morphology and appearance of the microparticles were examined both under an optical stereomicroscope (Citoval 2, Jena, Germany) connected to a video camera (JVC, Tokyo, Japan) and by Scanning Electron Microscopy (SEM).

2.3. Drug loading determination

To determine the 5-ASA content in the microparticles, an accurately weighed amount of powder was added to simulated intestinal fluid at pH 7.4 and heated up to 85 °C to melt the lipophilic excipients. The process was carried out under magnetic stirring for 5 h to quantitatively extract 5-ASA. Finally, the solution was filtered with microcellulose filter (Minisart RC, 0.45 μm, Sartorius, Goettingen, Germany) and then assayed by UV spectroscopy (UV–vis spectrophotometer, Lambda25, PerkinElmer®, Waltham, Massachusetts, USA) at λ 330 nm. The analysis was performed in triplicate.

2.4. Solid state analysis

2.4.1. Hot stage microscopy

Physical changes in the 5-ASA stearic microparticles during heating were monitored by Hot Stage Microscopy (HSM). A hot plate (FP 52 Mettler, Grefensee, Switzerland) connected to a temperature controller (FP 5 Mettler) was used. A small amount of the stearic microparticles was placed on the sample holder and heated in the temperature range of 30–100 °C at 2 °C/min. Changes in the samples were observed via an optical microscope (Nikon Eclipse E400) (magnification 10×).

2.4.2. Thermal analysis

Temperature and enthalpy measurements of raw materials and microparticles were performed by Differential Scanning Calorimetry (Mettler DSC 821e STARe, Mettler Toledo, Greifensee, Switzerland). Samples of about 5–10 mg in pierced aluminum crucibles were subjected to a thermal program from 30 °C to 300 °C, at a scan rate of 10 °C/min under a dynamic nitrogen atmosphere (100 ml/min). Instrument calibration was performed with standard indium and zinc samples (purity > 99.99%) of known temperatures and enthalpies of melting.

2.4.3. X-ray powder diffraction studies

Powder diffraction data were obtained with a Miniflex X-ray Diffractometer (Rigaku, Tokyo, Japan) with a graphite

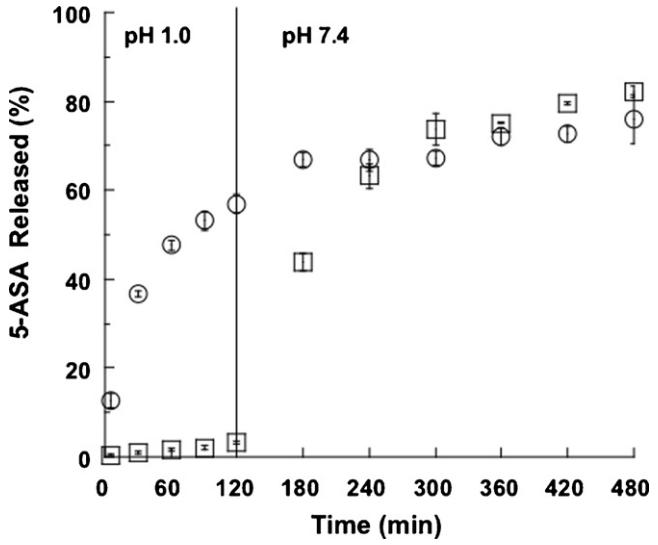

Fig. 1. Release profiles of cores (◯) and microcapsules (□) in variable pH medium: 2 h in acid medium and then in phosphate buffer pH 7.4.

monochromator in the diffracted beam-path (Cu Kα radiation, $K_{\alpha1}$ 1.5406 Å; $K_{\alpha2}$ 1.5443 Å). A system of diverging, receiving and anti-scatter slits of 0.58, 0.58, and 0.2 mm, respectively, was used. The patterns were collected with 30 kV of tube voltage and 15 mA of tube current in the angular range of $2^\circ \leq 2\theta \leq 50^\circ$ in a step scan mode (step width, 0.05°; counting time, 2 s/step).

2.5. In vitro drug release

Drug release tests were performed using the USP XXXI apparatus II with paddle rotating at 100 rpm at a temperature of 37 °C. In order to evaluate whether the system was gastro-resistant at pH 1.0 and released the drug at pH 7.4, the microparticles were tested at different pH. A weighed amount of the sample was introduced in 300 mL of 0.1 M HCl for 2 h. After this period, 600 mL of an aqueous solution containing 2.6 g of NaOH and 6.12 g of KH_2PO_4, were added to the acidic medium to raise the pH to 7.4 (Raffin et al., 2007). 0.01% of sodium lauryl sulphate (SLS) was added to the aqueous medium to wet the formulation. The samples were collected at pre-determined time points from 0 min to 480 min and filtered with 0.45 μm filter. 5-ASA concentration was determined by UV spectrophotometry at 301 nm and 330 nm in acid and neutral medium, respectively. Drug release studies were performed in triplicate.

The drug release data of the microparticles were statistically analysed and compared using the Fit Factor described by Moore and Flanner (1996). The similarity factor (f_2) i.e., the logarithmic reciprocal square root transformation of the sum of squared errors, is a measurement of the similarity in the percentage released between curves. f_2 can be calculated comparing different pairs of dissolution profiles using the following equation:

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum (R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

where n is the number of dissolution sample times, R_t and T_t represent the mean percentage of drug released at each time point (t) for the reference and the test dissolution profiles, respectively. An f_2 value larger than 50 indicates that the two dissolution profiles are similar.

3. Results and discussion

It was problematic to prepare a delayed-release polymeric drug delivery system of mesalazine mainly due to the drug large dose and unfavorable physico-chemical properties. 5-ASA raw material showed crystalline solid state with needle-shape morphology. This habitus did not favor the microencapsulation process. Therefore, the drug was micronized in order to modify the aspect ratio of particles. However, since the drug is very soluble in acidic medium, a very thick polymeric coat was required to avoid drug release in the stomach.

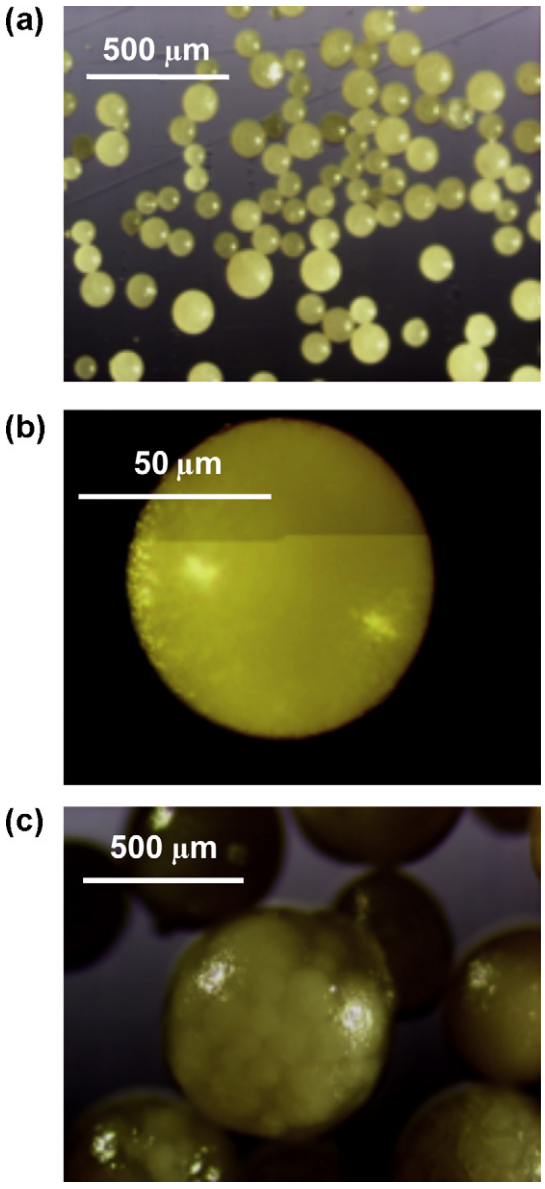

Fig. 2. Optical microscopy images of the Eudragit® L/carnauba wax cores ((a) 4× and (b) 20×) and of lipid microcapsules ((c) 4×).

Then, we attempted the manufacturing of pH-dependent multiparticulates by lipid spray-congealing technique (Passerini et al., 2003; Rodrìguez et al., 1998). By atomizing at a temperature lower than the carrier melting point a dispersion of the drug in a liquefied lipid carrier, droplets quickly solidified and drug loaded microparticles were obtained. These microparticles containing 5-ASA micronized crystals were prepared from a dispersion of the drug in melted carnauba wax containing a small amount of Eudragit® L100. The metacrylic polymer was added to the dispersion to contribute to the gastro-resistance by creating a pH dependency of particle dissolution. Dissolution test at pH 1.0 revealed that the carnauba microparticles released 60% of the drug in the acidic medium in 2 h (Fig. 1). Therefore, we decided to modify

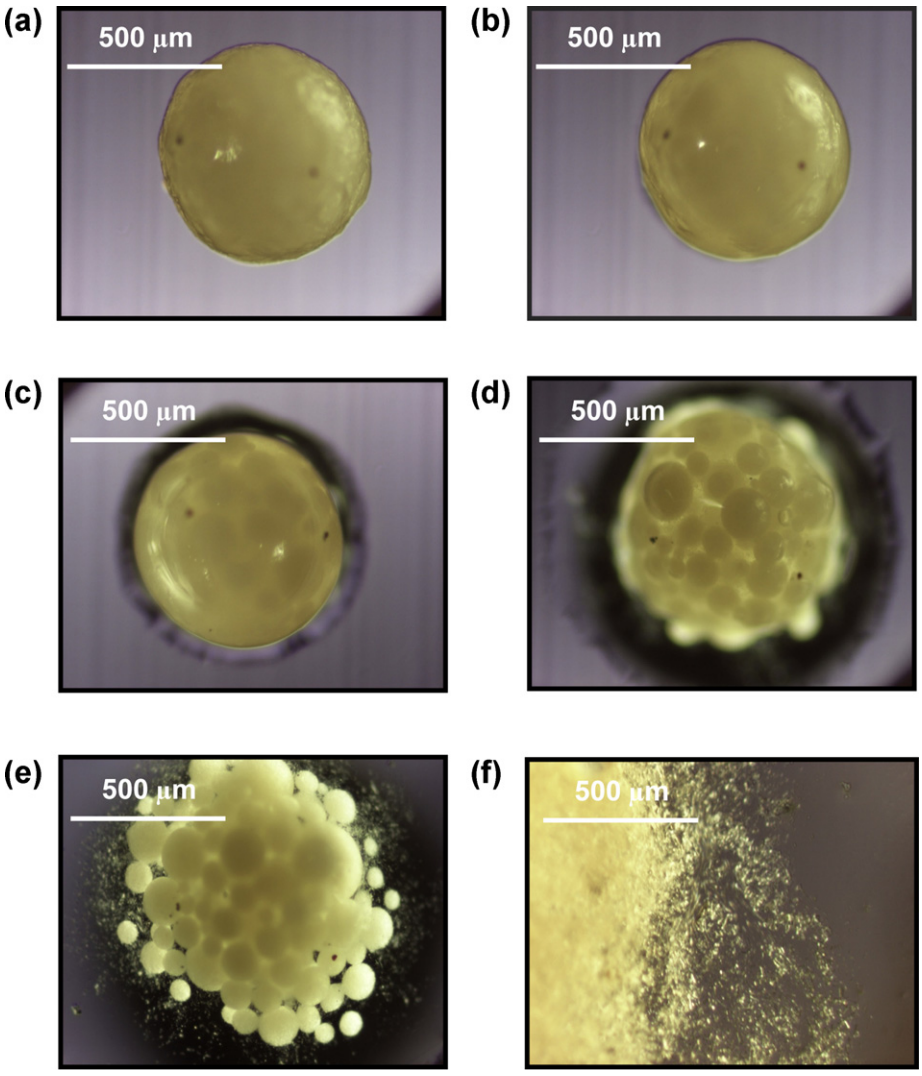

Fig. 3. Hot Stage Microscopy of the lipid microcapsule at different temperatures: (a) 35 °C, (b) 56 °C, (c) 56.5 °C, (d) 57 °C, (e) 80 °C and (f) 90 °C.

these microparticles by introducing them in a stearic acid matrix. Consequently, the 5-ASA carnauba microparticles were dispersed in melted stearic acid (m.p. 56 °C), whose melting point was lower than that of carnauba wax (m.p. 85 °C). The dispersion was again sprayed through the same nozzle in order to obtain the two-lipid containing microparticles.

5-ASA loading in the carnauba microparticles and stearic microparticles was measured as 29 ± 0.02% and 18 ± 0.16% (w/w), respectively. The morphology of both microparticles was assessed by optical and scanning electron microscopy. The carnauba microparticles had spherical shape and smooth surface and the size ranged between 50 μm and 75 μm (Fig. 2a and b). No needle crystals of 5-ASA were visible on the carnauba microparticles surface. Surprisingly, the Fig. 2c revealed that several carnauba microparticles were completely embedded as cores in a stearic particle leading to the formation of a reservoir structure i.e., a microcapsule. The stearic microcapsule (lipid microcapsule) still presented a globular shape, but the size increased to 500–700 μm range.

The release behavior of these lipid microcapsules was tested, discovering that less than 10% of 5-ASA was released in acidic medium after 2 h. When the pH was set at 6.0 no significant variation in release rate was observed (data not shown). The drug release then raised to 80% in 5 h after the dissolution medium change to pH 7.4. Indeed, the two-steps spraying procedure with two different lipids allowed obtaining a pH-dependent microparticulate product (see Fig. 1).

Despite in spray-congealing technique the melt hardening is very fast, the surface of the lipid microcapsules was quite smooth with few fissures. Nonetheless, there is the possibility to bring along solid-state transition of the lipid excipients during the process. Drug can also change from the original crystalline form to different polymorphs or amorphous state. Therefore, HSM (Hot Stage Microscopy), DSC (Differential Scanning Calorimetry) and PXRD (Powder X-ray Diffraction) were used to detect modifications of the physico-chemical properties of drug and excipients and possible interactions. The HSM analysis confirmed the already observed encapsulation of 5-ASA carnauba cores within the stearic acid matrix. During the melting at 56 °C of lipid microcapsule, the carnauba cores remained intact (Fig. 3). Continuing the heating, the carnauba cores started to melt at about 79 °C. After the complete melting of the wax, floating of the 5-ASA crystals in the molten carrier was visible. The color of microcapsules was pale yellow due to the excipient color masking the original dove gray color of raw material. No brownish change was observed during storage over 1 year at room temperature.

Fig. 4 reports the DSC thermograms of 5-ASA, stearic acid, carnauba wax, Eudragit® L100 raw materials and 5-ASA lipid microcapsules. In the case of 5-ASA (curve *a*), an endothermic peak was

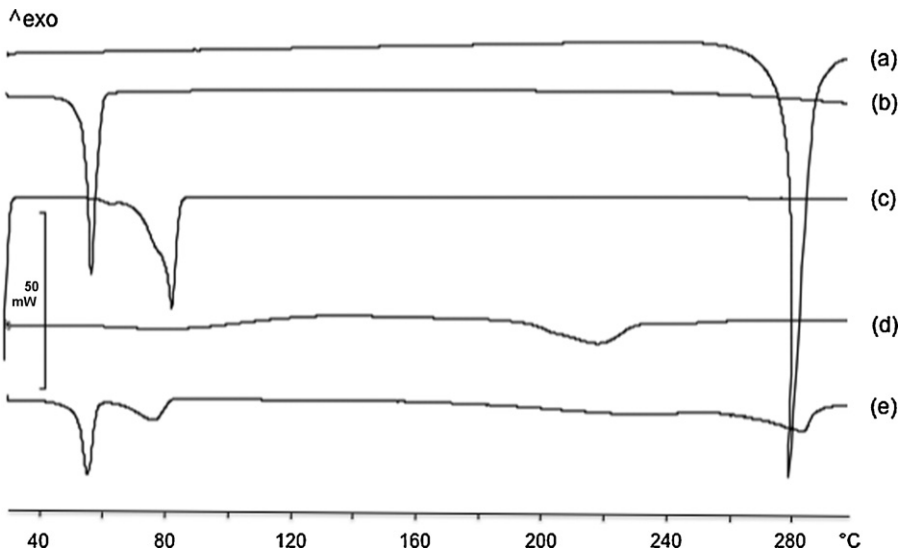

Fig. 4. Thermograms of (a) 5-ASA raw material, (b) stearic acid, (c) carnauba wax, (d) Eudragit® L100 and (e) lipid microcapsules.

observed at around 280 °C in agreement with the melting point of the drug. Stearic acid (curve *b*) showed a melting point around 57 °C, while the carnauba wax (curve *c*) exhibited an endothermic peak at 83 °C with a shoulder at lower temperature, which may indicate the presence of more than one crystalline form. Eudragit® L100 (curve *d*) showed glass transition at about 220 °C. The thermogram of the lipid microcapsules (curve *e*) showed two endothermic peaks at about 59 °C and 77 °C, due to the melting of stearic acid and carnauba wax, respectively. A further endothermic peak of small entity at around 288 °C corresponded to the melting point of the drug. In summary, the DSC analyses did not show any modifications of the drug crystalline state in the lipid microcapsules as a consequence of the spray-congealing process.

The PXRD patterns of lipid microcapsules and the raw materials are depicted in Fig. 5. The diffractogram of 5-ASA raw material (Fig. 5a) showed high intensity reflections at 2θ angle of 7.85°, 14.15°, 15.10°, 27.05° and 28.15°. These diffraction peaks assigned to 5-ASA were still present in the lipid microcapsule diffraction pattern (Fig. 5e). Stearic acid (Fig. 5c) showed intense peaks at 2θ angle of 21.70°, 24.10° and 36.50° and further diffraction peaks of minor intensity. The carnauba wax (Fig. 5d) also showed some very intense signals (2θ angle of 21.45°, 23.75° and 36.20°). In contrast, the diffractogram of Eudragit® L100 (Fig. 5b) is typical of an amorphous structure. The diffraction pattern of lipid microcapsules displayed the intense signals of microcrystalline carnauba wax and stearic acid, while the main signals of the drug were less intense because of a dilution effect due to the small amount of active ingredient in the lipid microcapsules. The PXRD results confirmed the lack of modification of the drug crystalline state as observed with the DSC analysis.

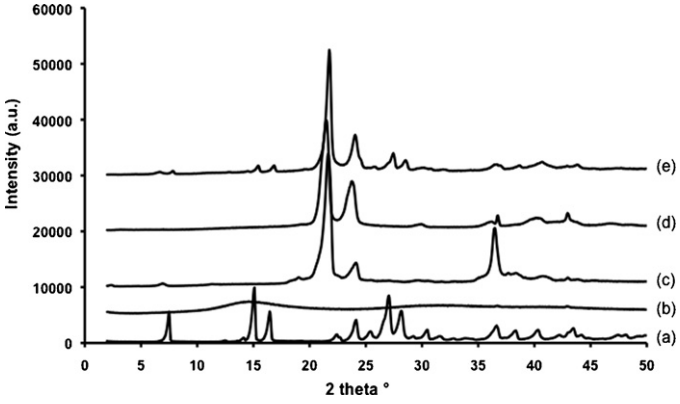

Fig. 5. X-ray pattern of (a) 5-ASA, (b) Eudragit® L, (c) stearic acid, (d) carnauba wax and (e) lipid microcapsules.

During the dissolution test we observed that the pH-dependent lipid microcapsules were not easily dispersed in the aqueous medium as, due to their hydrophobic nature, they floated on water surface. To overcome this problem, we embedded the lipid microcapsules in hydrophilic materials by tumbling them with spray-dried excipient microparticles made of mannitol and lecithin. Similarly to the previous case of pantoprazole microparticles (Raffin et al., 2007), tumbling particle mixtures in a rolling cylindrical container could lead to microparticle agglomeration. However, the two populations of particles here processed, i.e., the large 5-ASA microcapsules and the small spray-dried mannitol/lecithin microparticles, remained separated after tumbling, without any agglomerate formation. Therefore, for improving the tumbling process effectiveness, overcoming the cohesion tendency of spray-dried excipient microparticles, two stainless-steel spheres were introduced in the rotating cylinder. This modification improved the mixing and promoted the adhesion of layers of spray-dried excipient microparticles on the surface of larger lipid microcapsules. The composition of the layered lipid microparticles is reported in Table 2.

Optical microscopy images show that by increasing the amount of spray-dried excipient microparticles from 11.1% up to 33.3% (see Table 2), lipid microcapsules were progressively coated (Fig. 6a–d). SEM analysis allowed to deeply explore the deposition of excipient microparticles on lipid microcapsules (Fig. 7a–d). The surface of the lipid microcapsule in the 8:1 blend (8 parts of lipid microcapsules and 1 part of excipient microparticles corresponding to 11.1%) appeared not completely covered, due to the low amount of excipient microparticles (Fig. 7b). On the contrary, in the 2:1 blend (33.3%

Table 2
Layered lipid microcapsules made by different ratio of spray-dried excipient microparticles.

Ratio	Excipient microparticles (%)	5-ASA loading (%)
2:1	33.3	11.9 ± 0.4
4:1	20.0	13.8 ± 0.2
6:1	14.3	14.8 ± 0.1
8:1	11.1	15.9 ± 0.2

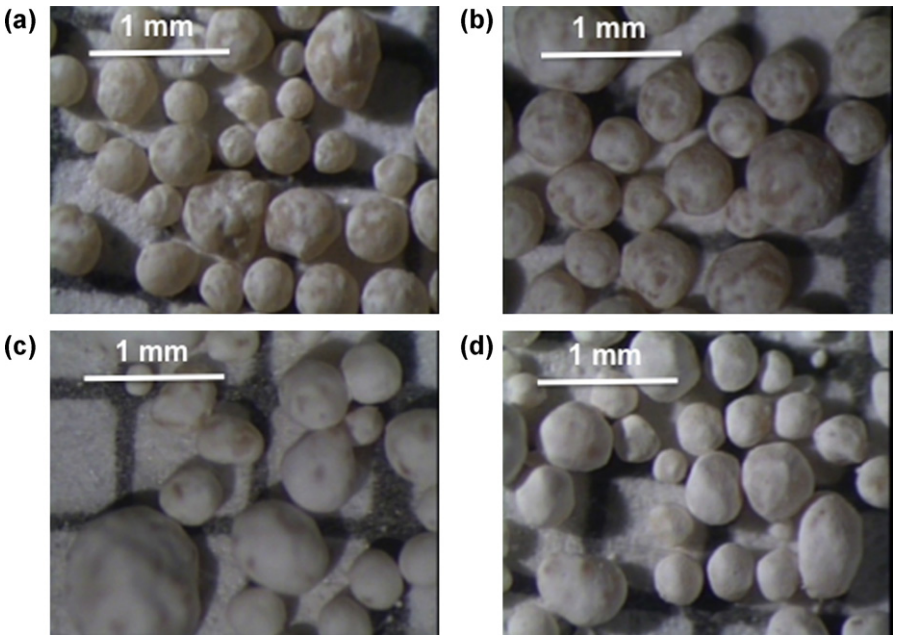

Fig. 6. Optical microscopy pictures of the lipid microcapsules layered with excipient microparticles in different ratio: (a) 8:1, (b) 6:1, (c) 4:1 and (d) 2:1 (magnification 40×).

of excipient microparticles), a uniform layer of excipient microparticles was deposited on lipid microcapsule surface (Fig. 7d). In summary, from the tumbling of the two particle populations with the help of the stainless steel spheres, instead of agglomerates dry-coated lipid microcapsules were obtained, as illustrated in the schematic drawing (Fig. 8). Lipid microcapsules were differently dry-coated depending on the amount of spray-dried excipients.

Differential scanning calorimetry and powder X-ray diffractometry were performed also on the dry-coated lipid microcapsules. The thermograms of lipid microcapsules, spray-dried excipient microparticles and layered lipid microcapsules are compared in Fig. 9. The thermogram of lipid microcapsules (Fig. 9, curve *a*) showed the 5-ASA sharp endothermic peak around 290 °C and the stearic acid and carnauba wax melting peaks at 56 and 78 °C, respectively. The DSC curve of the spray-dried mannitol/lecithin microparticles (curve *b*) showed an endothermic peak around 170 °C, corresponding to the melting of mannitol. In the case of the DSC thermograms of 8:1, 6:1, 4:1 and 2:1 layered lipid microcapsules (curves *c–f*), the melting peak of 5-ASA shifted to around 262 °C. From the comparison of the thermograms of the layered microcapsules, we observed that, as the percentage of the excipient microparticles in the mixture decreased, the area of the mannitol peak around 170 °C was reduced. This is reasonably due to the

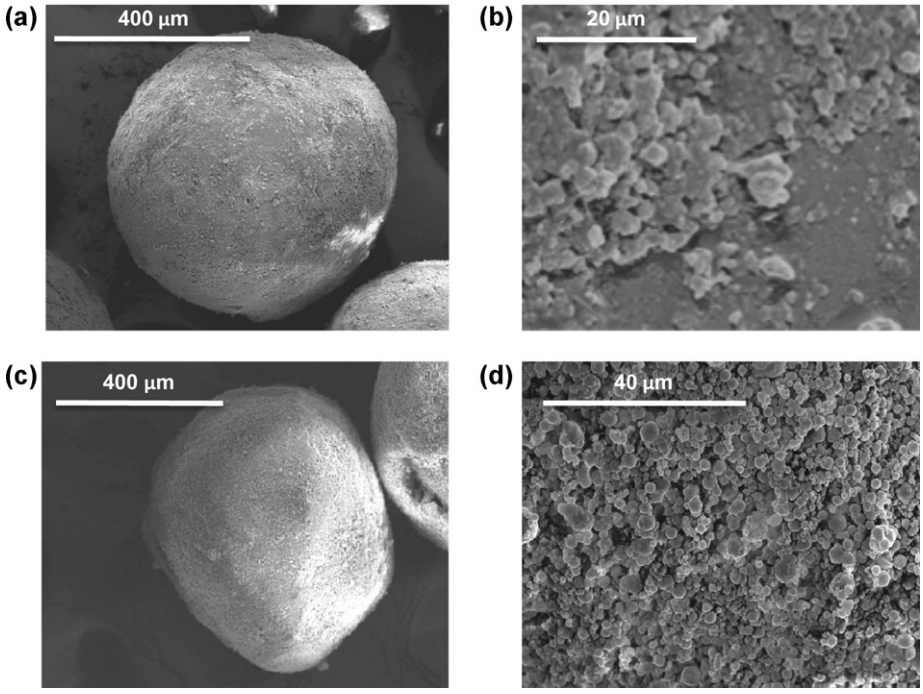

Fig. 7. SEM images of (a) layered lipid microcapsules 8:1 and (b) a relative detail of the surface; (c) 2:1 layered lipid microcapsules and (d) a relative detail of the surface.

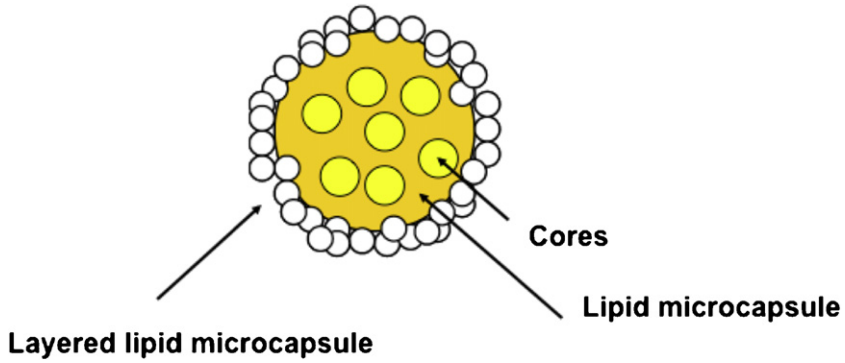

Fig. 8. Schematic sketch of the layered lipid microcapsules.

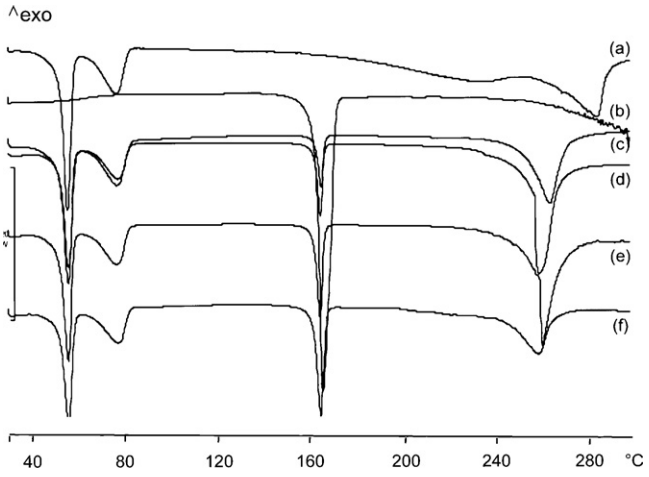

Fig. 9. DSC thermograms of (a) lipid microcapsules, (b) spray-dried excipient microparticles and (c) layered lipid microcapsules 8:1, (d) 6:1, (e) 4:1 and (f) 2:1.

amount of excipient microparticles on lipid microcapsule surface, decreasing from 2:1 to 8:1 ratio.

In order to evaluate if the pH-dependence of the lipid microcapsules was affected by the tumbling, release rate measurement was carried out on the layered lipid microcapsules (Fig. 10) and compared with the release profile of the uncoated lipid microcapsules (see Fig. 1). Less than 10% of 5-ASA was released at pH 1.0 by the layered microcapsules containing 11.1%, 14.3% and 20.0% of excipient microparticles, respectively. However, the layered lipid

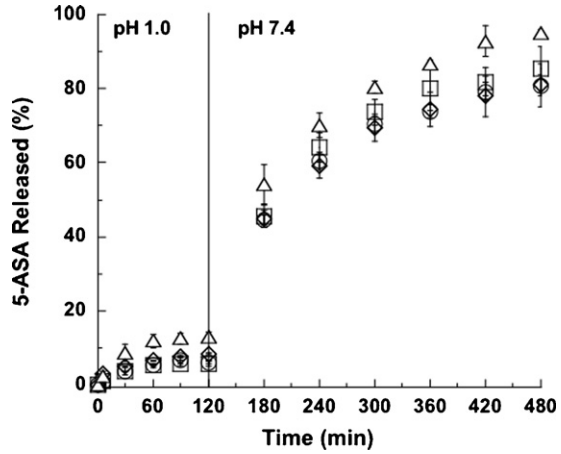

Fig. 10. Release profiles of lipid microcapsules layered with mannitol/lecithin microparticles in ratio 8:1 (◊), 6:1 (□), 4:1 (○), 2:1 (△) (mean± s.d.; $n = 3$).

microcapsules with 33.3% of excipient microparticles showed a release around 15% after 2 h in acidic medium. The highest amount of mannitol and lecithin microparticles, deposited on the lipid microcapsule surface, increased the wettability of this system and more drug was released in the acidic and neutral environment. The analysis of dissolution data by similarity factor f_2 confirmed that the layered lipid microcapsules with 11.1%, (f_2 69.6) 14.3% (f_2 63.7) and 20.0% (f_2 73.2) of excipient microparticles and the lipid microcapsules had similar dissolution profiles. In contrast, by comparing the dissolution profile of the layered lipid microcapsules covered with 33.3% of excipient microparticles with that of the lipid microcapsules, a value of f_2 of 45.9 was obtained, supporting different dissolution profiles.

The theoretical number of excipient microparticles forming a continuous layer on microcapsule surface was calculated from the particle size of lipid microcapsules and excipient microparticles. Using the Hatch–Choate equations, the values of surface number diameter (d_{sn}) of lipid microcapsules (171.04 μm) and length diameter (d_{ln}) of excipient microparticles (2.67 μm) were calculated (Hatch and Choate, 1929). It was found that the theoretical amount of excipient microparticles for covering one gram of lipid microcapsules was around 0.22 g. Thus, a single layer of excipient microparticles on the lipid microcapsules could be approximately reached at 4.5:1 ratio.

The microscopy observation confirmed that the 6:1 lipid microcapsule/excipient microparticle ratio was not enough for obtaining a single coating layer onto the lipid microcapsule surface. In the case of 4:1 ratio, uniform layer of microparticles was deposited, whereas with 2:1 ratio the excipient microparticles were excessive and part of them remained as powder in the tumbling container.

In summary, the tumbling process for microcapsule layering did not affect the gastro-resistance and drug release profile of uncoated lipid microcapsules. The presence of excipient microparticles layered on the surface of lipid microparticles did not significantly change the drug release profile in both mediums. However, for the 2:1 layered lipid microcapsules, the excessive amount of excipient microparticles increased the drug release, likely due to the higher amount of lecithin acting as surface active agent.

4. Conclusions

Mesalazine pH-dependent lipid microcapsules could be prepared by spray-congealing technique through a two-step process. 5-ASA cores preliminarily made with a mixture of Eudragit® L/carnauba wax were embedded in a matrix of stearic acid to form microcapsules.

Thermal and X-ray diffraction data showed that the encapsulation process did not affect the crystalline state of the drug. The pH-dependent behavior of the lipid microcapsules was demonstrated by the in vitro release profiles. In fact, less than 10% of

mesalazine was released in acidic medium. However, due to the lipid nature of the excipients, the lipid microcapsules tended to float on the medium surface. This problem was overcome by layering the lipid microcapsules with spray-dried mannitol/lecithin microparticles. The lipid microcapsules were dry coated by the excipient microparticles. An amount of the spray-dried excipient microparticles between 15% and 20% respect to lipid microcapsules, provided a uniform hydrophilic layer deposition on the lipid surface. The release profiles showed that the layering process did not modify the gastro-resistance. The wettability of the layered lipid microcapsules was significantly improved due to the presence of lecithin acting as surface active agent.

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