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Prilling for the development of multi-particulate colon drug delivery systems: Pectin vs. pectin–alginate beads

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ABSTRACT

This paper proposes a multi-particulate drug delivery system produced by prilling technique in combination with an enteric coating. Optimization of process parameters, such as feed viscosity at nozzle, selection of cross-linker, pH of the gelling solution and cross-linking time, allows to obtain beads with strong gelled matrix. Results showed that dextran/piroxicam beads demonstrated high encapsulation efficiency, very narrow dimensional distribution and high sphericity. Coated beads retained shape and narrow size distribution of the uncoated particles. Moreover, the strength of the produced Zn^{2+}–pectinate beads allows to reduce Eudragit® coating thickness. Piroxicam loaded multi-particulate systems show an interesting prolonged drug release in intestinal fluids. Hence, such platforms could be proposed for the treatment of inflammatory bowel diseases.

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1. Introduction

In the last few years the development of colon-targeted drug delivery systems has attracted significant interest. Particularly, colon local treatment of inflammatory based bowel diseases has well-documented advantages: specific drug targeting after oral administration, reduction of systemic toxicity and increased drug efficacy (Vandamme, Lenourry, Charrueau, & Chaumeil, 2002). In addition such a system may be useful for chronotherapy of diseases which are affected by the circadian rhythms, such as asthma and arthritis (Mastiholimath, Dandagi, Jain, Gadad, & Kulkarni, 2007).

Colon targeting may be achieved by various strategies especially pro-drugs, pH-sensitive or time-dependent therapeutic systems (Sinha & Kumria, 2001; Vandamme et al., 2002). Carbohydrate polymers, such as pectins consisting of linear chains of D-galacturonic acid residues with different degree of methyl ester substituents, are generally considered the most effective regarding colon targeted selectivity. In fact, pectins are degrated by colonic microflora in spite of their resistance to the enzymes present in the stomach and intestine. Moreover, these biodegradable natural carbohydrates appear of great and practical interest due to low cost and wide availability. However, pectins are not efficient in retaining drug during transit through the upper gastrointestinal tract, due to their solubility and swelling properties in aqueous media (Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006). Therefore, a combined strategy simultaneously exploiting microflora-activation and pH-controlled release has been suggested; hydrophilic microparticles have been manufactured by dripping drug–pectin solutions into a gelling bulk (Maestrelli, Cirri, Corti, Mennini, & Mura, 2008) and coated with a pH-sensitive polymer (Ahmed & Ayres, 2011; Maestrelli et al., 2008).

Gelling properties of pectins are mainly due to the carboxyl groups able to engage in coordination bonds with divalent cations forming an "egg-box" structure. However, the type of pectin and its concentration, as well as the cross-linking conditions are key parameters strongly affecting gelled microparticles performance (Sriamornsak & Nunthanid, 1998).

Amidated low methoxy (LM) pectins have been suggested as good candidates for gel-beads formulation (Assifaoui, Loupiac, Chambin, & Cayot, 2010) due to high hydrophobic interactions between pectin chains and internal hydrogen bonding between amide groups.

Moreover, the use of Zn^{2+} as alternative cross-linker with lower hygroscopicity than common Ca^{2+} ions has been reported in few studies (Bourgeois, Gernet, Pradeau, Andremont, & Fattal, 2006; Dhalleine et al., 2011) to produce stronger pectinate network with improved stability in the upper gastro-intestinal tract (Atyabi, Majzoob, Iman, Salehi, & Dorkoosh, 2005; Chambin et al., 2006; El-Gibaly, 2002). The influence of the pH in the reticulation solution on the structure of pectinate network has also been observed. In fact, a reduction in pH of the gelling bulk seems to promote a conformational transition to a more compact threefold pectins structure, due

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not only to dimmer associations described in the "egg-box" model, common in other carbohydrate polymers, but, also, to a combination of hydrogen bonding and hydrophobic interactions (Chambin et al., 2006; Pillay & Fassihi, 1999). Finally, also gelling time has been suggested as another crucial parameter modulating cross-linking of pectin chains (Das, Ng, & Ho, 2010).

In this paper we propose a multi-particulate drug delivery system based on zinc-amidated pectinate beads as colon-targeted delivery system. Beads were produced by prilling or laminar jet break-up, a mild and easy scalable microencapsulation technique, optimizing gelling parameters for LM amidated pectins. Successively, the produced beads were subjected to coating process with a gastroresistant polymer, Eudragit®. A non-steroidal anti-inflammatory drug (NSAID), piroxicam, useful in the treatment of inflammatory-based chronic diseases and as chemopreventive agent in the colon-rectal cancer (Fosslien, 2000), was selected as model drug. Influence of feed concentrations, cross-linking ion (Zn^{2+}) and gelation time on beads micromeritics and drug loading was investigated. Different levels of beads coating was also investigated. Swelling ratio and drug release profiles from the multi-particulate systems were studied in gastric (SGF) and intestinal (SIF) simulated fluids, simulating the physiological gastric pH variation with the aim to predict in vivo drug release in the gastrointestinal tract.

2. Materials and methods

2.1. Materials

Amidated low methoxy pectin (LM pectin called GRINDSTED Pectin LA 415, DE 24% and DA 23%) was kindly donated by Dompè (Dompè s.p.a. l'Aquila, I).

Sodium alginate European Pharmacopoeia X (MW ≈ 240 kDa) was purchased from Carlo Erba (Carlo Erba, Milan, I); zinc acetate dehydrate used as cross-linking agent in aqueous solution ($Zn(CH_3COO)_2{\cdot}2H_2O$ granular) was supplied from Sigma–Aldrich (Sigma–Aldrich, Milan, I). Piroxicam was kindly donated by Sifavitor (Sifavitor srl, Milan, I).

All other chemicals and reagents were obtained from Sigma Aldrich (Milan, I) and used as supplied.

2.2. Drug loaded beads preparation

An appropriate amount of polymer (pectin or pectin/alginate blend) was dissolved in deionized water at room temperature under gentle stirring in order to obtain 100 ml of polymer solution with concentrations ranging between 4.00% and 6.00% (w/w). Different amounts of solid piroxicam (mean diameter 31.06 μm, span 28.36) were suspended into the polymer solution and stirred for 2 h in order to obtain different drug/polymer ratios (between 0.05 and 0.07). The viscosity of piroxicam/dextran and dextrans blend suspensions were measured by rotational rheometer (Bohlin Instruments Division, UK) where a cone-plate combination (CP 4/40) was used as measuring system. Beads were manufactured by a vibrating nozzle device (Nisco Encapsulator Unit, Var D; Nisco Engineering Inc., Zurich, CH), equipped with a syringe pump (Model 200 Series, Kd Scientific Inc., Boston, MA, USA), pumping the drug/polymer solution through a nozzle 400 μm in diameter. Experiments were performed at volumetric flow rate of 10 ml/min. Vibration frequency used to break up the laminar liquid jet was set at 350 Hz, amplitude of vibration 100%. The distance between the vibrating nozzle and the gelling bath was fixed at 25 cm. A stroboscopic lamp was set at the same amplitude as the frequency, in order to visualize the falling droplets. These latter were collected into an aqueous solution 10% (w/v) $Zn(CH_3COO)_2{\cdot}2H_2O$ where they were gelified under gentle stirring. The beads were held into the gelling solution for 5–10 min at room temperature then recovered and thoroughly rinsed with deionized water.

Finally, the beads were dried at room temperature by exposure to air (22 °C; 67% RH) for several hours (12–18 h) until constant weight was reached.

2.3. Coating of beads

The enteric coating solution was prepared by dissolving Eudragit® S100 in acetone at 6% (w/v) concentration; this solvent allowed complete dissolution of the enteric polymer while maintaining the integrity of beads. Coating was obtained by immersion on beads in the coating solution followed by solvent evaporation in a rotary evaporator. The process was repeated until the desired amount of coating was achieved. Microparticles were coated at different levels (weight increase ranging from 10% to 60%). Samples of coated beads were then dried and weighed; the mean coating weight was calculated by difference with respect to the initial beads weight.

2.4. Beads size and morphology

Size distribution of hydrated and dried beads were measured by both an optical microscope (Citoval 2, Alessandrini, Milan, I) equipped with a camera and laser light scattering spectroscopy (Coulter LS 13320, Beckman Coulter Inc., Brea, CA, USA) equipped with a 12 ml micro liquid module. The LS 13320 uses a 5 mW laser diode with a wavelength of 750 nm, reverse Fourier optics and binocular lens systems. During preliminary studies, ethanol was chosen as suspending medium. Beads were suspended into the small-volume cell filled with ethanol to obtain an obscuration between 10% and 12%. The particle size distribution was calculated by the instrument software, using the Fraunhofer model. The analyses were carried out in triplicate for each sample.

Scanning electron microscopy (SEM) was performed using a Carl Zeiss EVO MA 10 microscope with a secondary electron detector (Carl Zeiss SMT Ltd., Cambridge, UK) equipped with a LEICA EMSCD005 metallizator producing a deposition of a 200–400 Å thick gold layer. Analysis was conducted at 20 keV.

Projection diameter was obtained by image analysis (Image J software, Wayne Rasband, National Institute of Health, Bethesda, MD, USA). A minimum of one hundred bead images were analyzed for each preparation in order to calculate length-number mean and relative standard deviation for at least three different prilling processes. Perimeter and projection surface area obtained by image analysis were used to calculate a sphericity coefficient (SC) by the following equation:

$$SC = \frac{4\pi A}{P^2}$$

where A is the projected bead surface area and P its perimeter.

2.5. Calorimetric analysis

Beads thermal characteristics were determined by differential scanning calorimetry (DSC) (Mettler Toledo DSC 822e module controlled by Mettler Star E software, Columbus, OH, USA), and compared with those obtained using both blank beads and drug as raw material. An appropriate amount of dried beads was crimped in a standard aluminium pan that was pierced and heated from 25 °C to 350 °C at a scanning rate of 10 °C/min in nitrogen atmosphere at a flow rate of 150 ml/min. Characteristic peaks were recorded and specific heat of the melting endotherm was evaluated. At least duplicates were carried out for each batch of sample, and the results averaged.

Table 1
Composition, diameter, sphericity coefficient (SC) and encapsulation efficiency (e.e.) of beads manufactured by prilling. Each value represents the mean ± SD ($n = 3$).

Formulation code	Polymer blend specifications	Feed solution (% w/w)	Piroxicam/polymer ratio	Viscosity at nozzle μ (mPa s)	Diameter of hydrated beads (μm)	Diameter of air-dried[a] (μm)	SC	Drug content (%)	e.e. (%)
F1_1	Pct/Alg 10:1	4.0	1:15	422	4380 ± 21	1851 ± 72	0.87 ± 0.01	3.52 ± 0.20	61.53 ± 2.50
F1_2	Pct/Alg 10:1	4.0	1:20	415	4630 ± 19	1973 ± 58	0.90 ± 0.02	2.99 ± 0.18	67.95 ± 1.80
F2_1	Pct/Alg 10:2	4.5	1:15	460	4580 ± 29	2209 ± 73	0.87 ± 0.02	4.92 ± 0.10	85.43 ± 1.56
F2_2	Pct/Alg 10:2	4.5	1:20	455	4821 ± 52	2223 ± 94	0.77 ± 0.06	3.96 ± 0.05	89.39 ± 1.25
F3	Pct	6.0	1:20	472	4956 ± 78	2206 ± 4	0.92 ± 0.01	4.30 ± 0.18	93.21 ± 2.63
F4	Pct	6.0	–	462	4611 ± 18	1975 ± 36	0.90 ± 0.01	–	–

[a] Air-dried: beads dried at room conditions.

2.6. Drug content and encapsulation efficiency

Accurately weighed amounts of beads from each manufactured batch (about 30 mg each) were dissolved under vigorous stirring in PBS buffer (100 mM). Piroxicam content was determined by UV spectroscopy at λ 352 nm (Evolution 201 UV/VIS Spectrometer, Thermo Scientific, Waltham, MA, USA). Encapsulation efficiency was calculated as the ratio of actual to theoretical drug content. Each analysis was performed in triplicate; results were expressed in terms of mean ± standard deviation. Both drug content and encapsulation efficiency were calculated correcting the weight for the residual zinc acetate dehydrate and water contained into the beads, as previously determined by Karl Fischer titration (Tritomatic KF, Crison Instruments, SA, Barcellona, Spain).

2.7. Kinetics of drug release

In vitro dissolution/release tests were conducted in sink conditions on given amounts of beads containing about 10 mg of drug using a USP 27 dissolution apparatus II: paddle, 75 rpm, 37 °C (Sotax AT7 Smart – Sotax, Allschwil, CH) on line with a UV spectrophotometer (Lambda 25 UV/VIS Spectrometer, Perkin Elmer, Waltham, MA, USA). Briefly, dried beads were added to the dissolution medium, 750 ml 0.1 M HCl for 2 h, then 250 ml of 0.20 M Na_3PO_4 was added and pH adjusted to 6.8 as described in the USP 27/NF monograph "Drug release from delayed-release articles". Data were analyzed spectrophotometrically at λ 333 and 352 nm. Dissolution tests were conducted on six different batches of particles; mean values and standard deviation were evaluated.

3. Results and discussion

3.1. Beads production, characterization and micromeritics

Piroxicam loaded hydrophilic particles based on pectin (Pct) or pectin/alginate (Pct/Alg) blends were produced by a Nisco Encapsulator VarD unit equipped with a nozzle of 400 μm diameter using a vibration frequency of 350 Hz and 100% amplitude of the vibration. Several formulations (Table 1) were designed varying polymer total concentration between 4.0% and 6.0% (w/w), pectin to alginate ratio from 10:0 to 10:2, and setting drug/polymers ratio at 0.05 or 0.07.

Taking in account literature data, preliminary experiments were performed to set operative conditions as Zn^{2+} concentration (10%, w/v), pH (1.5) and cross-linking time (8 min) of the gelling solution in order to produce spherical hydrated beads with tough polymer matrix, smooth and regular surface. Cross model equation was used to set other process parameters, as frequency of vibration, polymer solution concentration and volumetric flow rate (10 ml/min), to obtain droplets coming out of the nozzle in narrow size distribution and avoiding satellite droplets formation (Aquino et al., 2012; Auriemma, Del Gaudio, Barba, d'Amore, & Aquino, 2011; Del Gaudio, Colombo, Colombo, Russo, & Sonvico, 2005). In fact, rheological studies conducted on the different feed solutions processed by prilling (F1–F4) showed that a viscosity at nozzle (μ) of 450 mPa s (Table 1) allowed production of polymer beads with desired characteristics. However, owing to the viscoelastic behaviour of both Pct and Pct/Alg blend solutions, the viscosity at nozzle shear rate exhibited exponentially increasing values so that concentration higher than 6% (w/w) did not allow any processing by prilling due to higher μ values (over 550 mPa s) that stacked the nozzle (Auriemma et al., 2011; Del Gaudio, Russo, Rosaria Lauro, Colombo, & Aquino, 2009; Soong & Shen, 1981).

Drying process was conducted overnight by exposing hydrated beads to standard room conditions. As shown in Table 1, mean diameter of dried beads reduced to almost 50% of the hydrated ones (relative standard deviation lower than 3%) because of the shrinking of volume due to the loss of water. Piroxicam loading did not affect morphological properties of the particles after dying process. Water content (over 90% for hydrated beads) was reduced to 1–2% in dried beads depending on the increasing of polymers blend concentration.

Sphericity coefficient (SC) was calculated for all dried beads batches. The highest SC value (0.92) was obtained for formulation F3 (amidated LM pectins alone) whereas the lowest (0.77) for F2 manufactured with Pct/Alg (10:2) blend (Table 1 and Fig. 1). Moreover, detailed SEM analyses pointed out that the increasing in Alg concentration leads to dried beads with few small cracks and irregular shape caused by partial collapsing of polymer network during dehydration process. These phenomena can be explained looking at the different water affinity between the two polymers that lead at an inhomogeneous loss of water from the Pct/Alg matrix compared to beads manufactured by Pct alone. No crystals of piroxicam were visible on the surface, suggesting the drug complete entrapment within the polymeric matrix in all formulations.

High encapsulation efficiency (e.e. ranging from 62% to 93%), correlated to the increase of the polymer concentration in the feed, was achieved for all manufactured formulations, especially for F3. In fact, the higher the polymer concentration, the higher the e.e. (Table 1).

3.2. Particle coating studies

Dried beads were coated by immersion with Eudragit® S100, a copolymer of methylacrilic acid and methylmethacrylate, that dissolves at pH 7. For this reason, it is considered a proper coating material for gastroresistant delivery systems, able to protect the drug core during its passage through the stomach (Shukla & Tiwari, 2012). Preliminary experiments were necessary to set coating conditions as regards processing time and coating level which was gradually varied from 10% to 60% (w/w) of the particles. Weight gained by beads was used as "indicator" of the film thickness.

SEM analysis showed that coating level fewer than 20% leads to a broken Eudragit® shell unable to cover the entire particle surface, as shown in Fig. 2a. The most homogeneous coating layer was obtained with 40% of Eudragit® (batches F1_40, F2_40 and

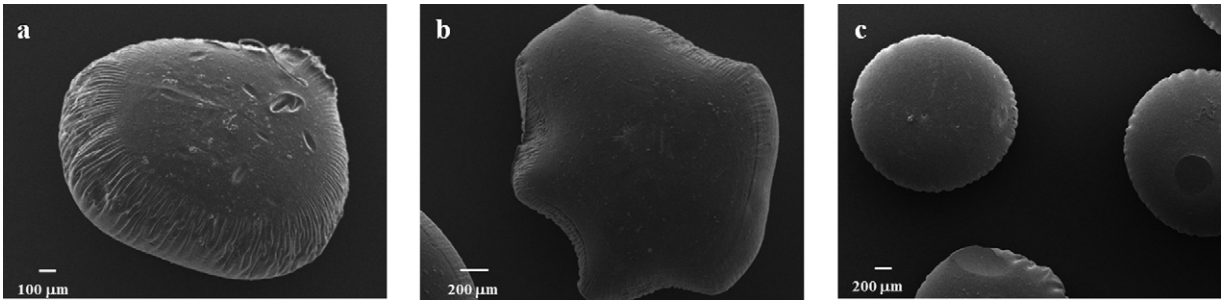

Fig. 1. SEM microphotographs of beads produced by prilling and dried at room conditions: F1_1 (a), F2_2 (b) and F3 (c), respectively.

F3_40) whereas setting the coating at 60% (w/w) produced very sticky material. After the coating process, F3 beads almost retained their SC values (F3_40, SC = 0.91) while both F1_25/40 and F2_25/40 showed an enhancement in sphericity due to the applied film covering the beads irregular surface. SEM analyses of cryofracturated beads showed very homogeneous film thickness of about 115 and 200 μm for F3_25 and F3_40, respectively. F1_40 and F2_40 formulations, even when coated at 40% level, presented a partial irregular layer thickness, probably due to the lower sphericity of the uncoated beads (Fig. 3).

3.3. Thermal analysis (DSC)

The thermal curves of pure materials are reported in Fig. 4. The thermogram of piroxicam raw material exhibited the typical profile of a pure, crystalline drug (melting endothermic peak at 207 °C and broad exothermic peak due to oxidative degradation at 258 °C (Vrecer, Vrbinc, & Meden, 2003). As previously reported in literature (Maestrelli et al., 2008), thermogram of unprocessed LM amidated pectin is characterized by a broad endothermic band due to the water loss and two melting peaks (194 °C and 224 °C), followed by thermal decomposition (exothermic peak at 245 °C). The thermogram of unprocessed Alg showed, after the dehydration band around 80–100 °C, an almost flat profile indicative of its amorphous state, followed by an exothermic event ascribed to a decomposition process. DSC analyses of Eudragit® S100 showed three broad endothermic bands at about 80 °C, 220 °C and 268 °C (Simonoska Crcarevska, Glavas Dodov, & Goracinova, 2008).

The thermogram of blank beads (F4) indicated Zn^{2+}-crosslinked pectinate formation showing broad peak at 155 °C due to enthalpic relaxation (Maestrelli et al., 2008) and depending on the degree of the chelation between Zn^{2+} and pectin. Piroxicam-loaded beads (F1–F3) showed a similar behaviour with F3 peak shifted at higher temperature (T_{peak} = 163 °C) suggesting a solid-state interaction between pectin amide groups of Pct and piroxicam. This phenomenon was not observed for F1 and F2 formulations and might be explained by the formation of a complex between carboxylic groups of Alg and amide groups of Pct. F1–F3 coated formulations showed thermal behaviours similar to F1–F3 (data not shown). DSC thermograms of all drug-loaded beads did not give any evidence of piroxicam melting confirming complete drug entrapment in the polymer matrix (Fig. 5).

3.4. Drug release studies

For in vitro evaluation of colon-specific drug delivery systems, the ideal dissolution testing should closely mimic the in vivo conditions with regard to pH, type of enzymes and enzymes activity, fluid volume and stirring intensity. Such dissolution models are very difficult to be validated; conventional methods reported in the literature (Yang, Chu, & Fix, 2002) are commonly used providing essential information on the robustness of the system design and potential in vivo performance. Therefore, release tests for F1–F3 were performed by a classic pH change assay (USP 27). Results showed that F1_1 was characterized by high release rate leading to a complete liberation of piroxicam in simulated gastric fluid (SGF pH 1.0). On the contrary, F2_2 and F3 released about 50% and 65% of piroxicam in SGF and allowed total drug liberation in SIF after the pH change in about 2 h, in comparison to a lower release rate observed for pure piroxicam (Fig. 6).

No swelling process, widely reported for Ca–pectinate microparticles (Bajpai & Sharma, 2004) was observed during the dissolution experiments in all media and almost entire beads were recovered. The use of zinc as counter-ion agent, instead of calcium, led to a higher degree of cross-linking and aggregation of pectin chains during gelation (Chambin et al., 2006; Sriamornsak & Nunthanid, 1998) inducing higher gel strength and limited or no swelling. However, the high drug release rate in SGF suggested that an enteric coating is necessary to avoid erosion of pectin matrix and protect the drug in acidic medium.

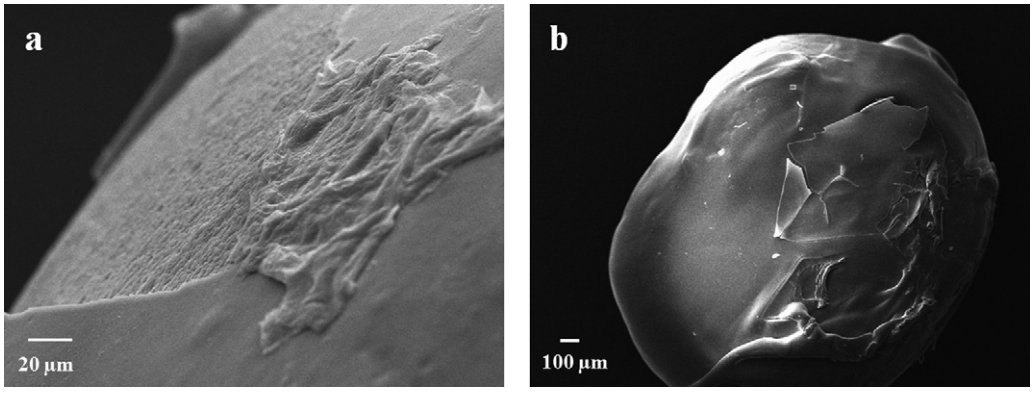

Fig. 2. SEM microphotographs of F3 formulations coated with different amount of Eudragit®: 18% increased weight (a) and 40% increased weight (b) of the beads.

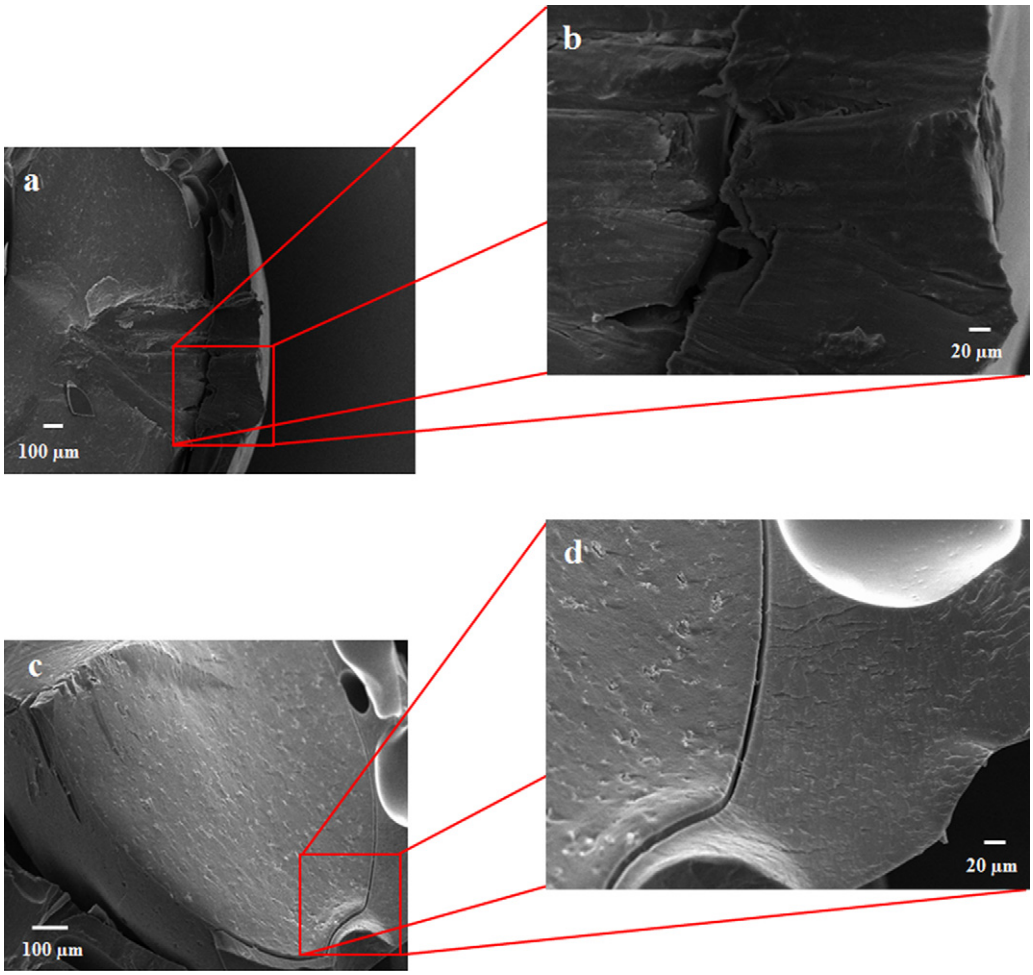

Fig. 3. SEM microphotographs of cryofractured 40% (w/w) Eudragit® coated beads at different magnifications: F3_40 (a and b) and F2_2_40 (c and d).

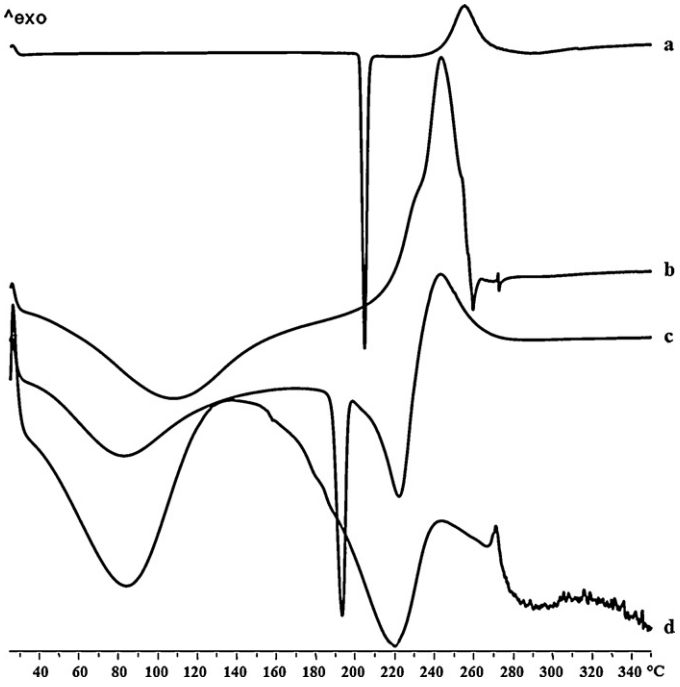

Fig. 4. Differential scanning calorimetry thermographs of pure piroxicam (a), sodium alginate (b), pectin (c) and Eudragit® S100 (d).

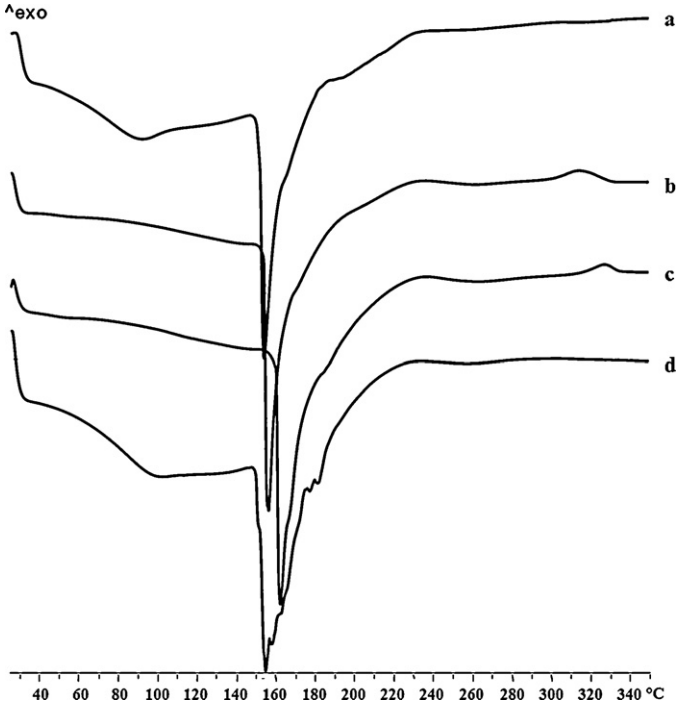

Fig. 5. Differential scanning calorimetry thermographs of different piroxicam loaded beads F1_1 (a), F2_2 (b), F3 (c) and blank pectin beads F4 (d).

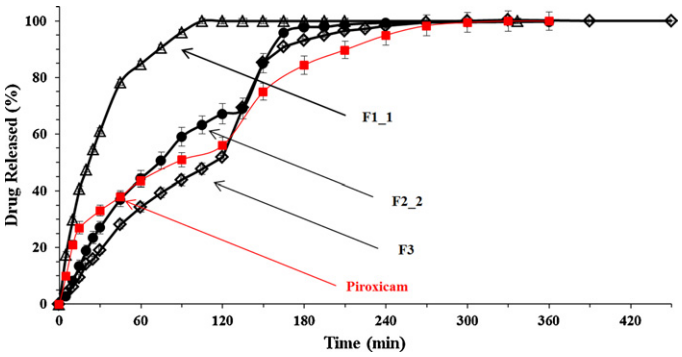

Fig. 6. Release profiles of dried beads formulations F1_1, F2_2 and F3 with respect to pure piroxicam. Mean ± SD (n = 6).

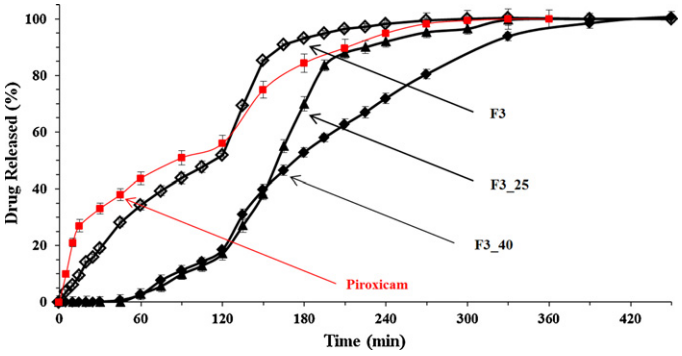

Fig. 7. Release profiles of uncoated F3 and Eudragit® coated beads formulations F3_25–F3_40 with respect to pure piroxicam. Mean ± SD (n = 6).

Batches of F3 coated with different levels of Eudragit® film were tested (Fig. 7). Dissolution profile of F3 coated with both 25% and 40% (w/w) of Eudragit® was typical of gastro-resistant oral dosage form, releasing less than 20% of piroxicam in SGF in 120 min. Moreover, an interesting prolonged residence time of the delivery systems was observed for F3_40. This formulation released about 50% of the drug after 1 h following the Eudragit® dissolution in intestinal simulated fluid (SIF – pH 6.8) whereas F3_25 released 70% of piroxicam in the same time. Moreover, complete drug release was achieved in 3.5 and 5 h for F3_25 and F3_40, respectively.

The interesting prolonged piroxicam release for F3_40 may be explained on the basis of its thicker coating layer (Eudragit® 40%, w/w) and suggests that the disintegration mechanism of the strong Zn^{2+}–pectinate matrix in phosphate buffer medium plays a dominant role during the dissolution process at pH 6.8 (Dhalleine et al., 2011).

All results indicated that mixed LM amidated Pct/Eudragit® platforms should be potentially useful as colonic delivery system of piroxicam.

4. Conclusions

The present study showed that prilling technique in combination with an enteric coating can be used as a simple method to formulate multi-particulate colon-specific drug delivery systems. These platforms are based on gastroresistant and pH-sensitive characteristics of Eudragit® film coating and Zn–pectinate matrix with colonic micro-flora activated properties. Zn-amidated pectinate matrix with regular morphology and homogeneous ultrastructure may be developed using tailored process parameters. The most satisfying multi-particulate system, 40% (w/w) of Eudragit® coating (F3_40), is able to protect piroxicam in gastric environment releasing 50% of NSAID dose in the intestine after 3 h from the oral administration and, interestingly, to prolong drug intestinal residence time until 7 h. This goal was achieved even with a so small film thickness due to smooth and regular surface of prilling beads and strength of Zn^{2+}–pectinate matrix. Results emphasized on the importance of prilling technique as NSAIDs mild encapsulation process easy to scale up; however, optimization of the formulation parameters such as viscosity at nozzle of the feed, selection of cross-linker, pH of the gelling solution, cross-linking time are necessary in order to obtain beads with strong gelled matrix for colon-targeted drug delivery system.

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