International Journal of Pharmaceutics 420 (2011) 11–19

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Mucoadhesive platforms for targeted delivery to the colon

Felipe J.O. Varum[a,b], Francisco Veiga[a], João S. Sousa[a], Abdul W. Basit[b,*]

[a] Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[b] The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, United Kingdom

ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form 3 August 2011
Accepted 4 August 2011
Available online 11 August 2011

Keywords:
Mucoadhesion
Large intestine
Colonic delivery
Enteric coatings
pH-sensitive polymers
Methacrylic acid and methyl methacrylate
Carbomers
Transit time
Multiparticulates

ABSTRACT

A novel platform system, comprising a mucoadhesive core and a rapid release carrier, was designed for targeted drug delivery to the colon. Prednisolone pellets containing different carbomers, including Carbopol 971P, Carbopol 974P and Polycarbophil AA-1, with or without organic acids, were produced by extrusion–spheronization. Mucoadhesive pellets were coated with a new enteric double-coating system, which dissolves at pH 7. This system comprises an inner layer of partially neutralized Eudragit® S and buffer salt and an outer coating of standard Eudragit® S. A single layer of standard Eudragit S was also applied for comparison purposes. Dissolution of the coated pellets was assessed in USP II apparatus in 0.1 N HCl followed by Krebs bicarbonate buffer pH 7.4. Visualization of the coating dissolution process was performed by confocal laser scanning microscopy using fluorescent markers in both layers. The mucoadhesive properties of uncoated, single-coated and-double coated pellets were evaluated *ex vivo* on porcine colonic mucosa. Mucoadhesive pellets coated with a single layer of Eudragit® S release its cargo after a lag time of 120 min in Krebs buffer. In contrast, drug release from the double-coated mucoadhesive pellets was significantly accelerated, starting at 75 min. In addition, the mucoadhesive properties of the core of the double coated pellets were higher than those from single-coated pellets after the core had been exposed to the buffer medium. This novel platform technology has the potential to target the colon and overcome the variability in transit and harmonize drug release and bioavailability.

1. Introduction

The complexity and variability in gastrointestinal physiology presents a challenge for oral drug delivery, particularly in the case of modified release dosage forms (McConnell et al., 2008a). One such physiological parameter, which is subject to marked variability, is gastrointestinal transit. Total transit through the gut can be as short as a few hours or as long as a several days (Varum et al., 2010a). For example, the total gastrointestinal transit time of the osmotic-pump system (Oros®) ranged from 5.1 to 58.3 h in healthy volunteers (John et al., 1985). Transit through the colon makes the greatest contribution to overall variability in gastrointestinal transit. For instance, it has been reported that the transit time of dosage forms through the colon can range from 0 to 72 h (Coupe et al., 1991; Wilding, 2001) and in a recent study, pellets were still present in the colon 5 days after oral administration (Basit et al., 2009).

In a study designed to evaluate the *in vivo* behavior of a bacteria-sensitive colonic delivery system, in one subject the coated capsule was voided intact due to rapid transit through the gut (Tuleu et al., 2002). A failure to disintegrate was also observed with pH-sensitive polymer coated tablets (Schroeder et al., 1987; Sinha et al., 2003; Ibekwe et al., 2006, 2008). This is not restricted to tablets, as enteric coated pellets are also not immune to the pass-through effect (McConnell et al., 2008b). Therefore, the impact of colonic transit variability on formulation performance is independent of the type of dosage form and the trigger mechanism.

Considering this variability, an increased or harmonized residence time in the colon would be beneficial. This can be achieved using the mucoadhesion approach. This concept would offer significant therapeutic advantages, such as an increase in drug absorption or an improvement in topical efficacy (Smart et al., 1984; Ch'ng et al., 1985). Acrylic acid polymers have been recently suggested as potential candidates for the development of mucoadhesive dosage forms intended to target the lower gut (McGirr et al., 2009; Varum et al., 2010b). However, these materials present technical problems when formulated in solid dosage forms if a wetting step is required, due to the gelling and swelling properties, resulting in tacking. This problem has been addressed by using strong electrolytes, such as calcium chloride solutions (Neau et al., 1996, 2000) or high levels of spheronization aid (Awad et al., 2002; Mezreb et al., 2004). However, a significant reduction in mucoadhesive properties was observed *in vitro* (Gómez-Carracedo et al., 2001).

The combination of the mucoadhesion concept with a colon-specific drug delivery vehicle would contribute to a more efficient colonic targeting and avoid the pass-through effects. Recently, a novel enteric double-coating system with accelerated drug release

* Corresponding author. Tel.: +44 020 7753 5865; fax: +44 020 7753 5865.
E-mail address: abdul.basit@pharmacy.ac.uk (A.W. Basit).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.08.006

in the intestine was developed (Liu et al., 2009a; Liu and Basit, 2010). This concept was further developed to target the ileo-colonic region (Liu et al., 2010). This double-coating system successfully accelerated drug release from tablets compared to the standard Eudragit® S single-coating. Furthermore, rapid coating dissolution may trigger an earlier exposure of a mucoadhesive platform in the ascending colon, where the mucus turnover and colonic motility are lower (Lehr et al., 1991; Rubinstein and Tirosh, 1994) and mucus is thicker compared to the small intestine (Varum et al., 2010b).

The aim of this work was to investigate the combination of an ileo-colonic drug delivery platform with mucoadhesive characteristics for improved colonic targeting. In order to attain this, the usefulness of organic acids in reducing tack of carbomer polymers during the extrusion–spheronization process was assessed. Further developments were made towards the application and optimization of a novel double-coating system based on Eudragit® S and its implications in the mucoadhesive properties after exposure to media resembling the lower gut. The mechanisms underlying the acceleration process in this system were also elucidated.

2. Materials and methods

2.1. Materials

Prednisolone was purchased from Aventis Pharma., Antony France. Microcrystalline cellulose (Avicel® PH101) was obtained from FMC Biopolymer, Philadelphia, USA. Lactose monohydrate and polyvinylpirrolidone (K30) and citric acid anhydrous were purchased from VWR International Ltd., Poole, UK. Carbopol 974P NF, Carbopol 971P NF and Polycarbophil AA-1 were kindly donated by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium. Eudragit® S was kindly donated by Evonik Röhm GmbH, Darmstadt, Germany. Eudragit® S is a methacrylic acid and methyl methacrylate copolymer in the ratio 1:2 with a dissolution pH threshold of 7. It is composed of 27.6–30.7% methacrylic acid units on dry substance and has an acid value equivalent to 180–200 mg KOH/g polymer. Potassium dihydrogen phosphate and polysorbate 80 were purchased from Sigma–Aldrich Co. Ltd., Dorset, UK. Triethyl citrate was supplied by Lancaster Synthesis, Lancashire, UK. Glyceryl monostearate (Inwitor 900) was purchased from Hüls AG, Witten, Germany. Fluorescein and rhodamine B were purchased from Sigma–Aldrich Co. Ltd., Dorset, UK.

2.2. Rheological analysis

0.5% (w/v) carbomer (Carbopol 974 NF, Carbopol 971 NF and Polycarbophil AA-1) solutions were prepared by dispersing polymer into 20 ml of distilled water and stirring overnight. Carbomer solutions with citric acid were prepared at different carbomer:citric acid ratios (1:0, 1:1, 1:0.5, 1:0.25) as described above. Viscosity of carbomer aqueous solutions was determined using a rotational rheometer (Bohlin Instruments, Cirencester, UK). Briefly, 2 ml of carbomer dispersion were gently spread on the base of the rheometer and the cone and plate geometry was used with a radius of 50 mm and angle of 2° for frequency measurements. All measurements were performed at 25 °C, controlled by a thermostatic system. Viscosity of carbomer dispersions was measured in the shear rate range of 1–200 (1/s), in triplicate. The pH of these carbomer dispersions was measured with a pH meter (Hanna Instruments, Bedfordshire, UK).

2.3. Pellet manufacture

Prednisolone was chosen as a model drug. Microcrystalline cellulose was used as a spheronization aid and citric acid as a pH modifier (5%, w/w). Three different grades of carbomer polymers were used, namely, Carbopol 971P NF, Carbopol 974P NF and Polycarbophil AA-1, at concentrations ranging from 0 to 20% (w/w) of the total powder batch (50 g). Distilled water was used as a binding liquid and the required amount of water was optimized in order to achieve a high yield of pellets within a size range of 1.0–1.4 mm and acceptable sphericity. Briefly, dry powders were mixed in a planetary mixer (Kenwood Major) for 10 min, the required amount of distilled water was then added dropwise and the mixing continued for an additional 15 min.

The resultant wet mass was tightly packed into the ram extrusion assembly and extruded at 200 mm/min through a 1 mm diameter multiple hole die. The equipment used was the Instron® (Instron, High Wycombe, United Kingdom) equipped with a 10 kN load cell which was connected to an *in*-house designed device to fit the piston inserted into the ram extrusion assembly. The extrudate was spheronized until spherical pellets were obtained using a spheronizer (Caleva model 120, G.B. Caleva Ltd., Sturminster Newton, UK). Pellets were dried overnight at 40 °C (Gallenkamp, Weiss-Gallenkamp, United Kingdom) sieved using a nest of standard sieves (Endecott, Endecott Ltd., London, UK) on a $\sqrt{2}$ progression (500, 710, 1000, 1400, 1700, 2360 μm aperture). The size range of pellets between 1000 and 1400 μm was used for further characterization.

2.4. Eudragit® S double-coating of pellets

2.4.1. Inner coating

The 1.0–1.4 mm pellet fraction was used for the coating and 1.2 mm was considered as a mean diameter for coating calculations. The inner coating comprises a partially neutralized Eudragit S dispersion and a buffer agent. Triethyl citrate (TEC 50%, w/w) and potassium dihydrogen phosphate (10%, w/w), both based on polymer weight, were dissolved in water under mechanical stirring (Heidolph RZR1 stirrer, Heidolph Instruments, Schwabach, Germany) for 15 min. Eudragit® S was dispersed into the above solution under stirring and then the dispersion was neutralized to pH 8 using 1 M NaOH and stirring continued for 60 min. Glyceryl monostearate (GMS, 10%, w/w, based on polymer weight) was added into the Eudragit® S solution and mixed for 15 min prior to coating. The final dispersion contained 10% (w/w) of total solid contents. The coating level was set to 5 mg polymer/cm^2. Batches of 30 g of pellets were coated using a Strea-1 bottom spray fluidized bed coater (Aeromatic AG, Bubendorf, Switzerland) and the coating conditions were: inlet air temperature 50 °C, outlet air temperature 40 °C, fan capacity 15, atomizing pressure 0.2 and flow process rate 1.0 ml/min. Coated pellets were further fluidized for 15 min and cured at 40 °C for at least 2 h, before applying the outer coating.

2.4.2. Outer coating

TEC (20%, w/w, based on polymer weight) was dissolved into 90% ethanol for 10 min and Eudragit® S was slowly added into the ethanolic solution under stirring and mixing continued for 60 min. GMS (10% based on polymer weight) was added to the above solution and mixed for 15 min. The final dispersion contained 10% (w/w) of total solid contents. The coating level was controlled by the amount of polymer applied onto the pellets surface. The coating was performed as reported above but using a slower flow rate (0.8 ml/min). Pellets were further air dried for 15 min in the coating equipment and cured in the oven at 40 °C for at least 2 h.

For comparison purposes, single-coated pellets were prepared as reported here at the same coating level (5 mg/cm^2).

2.5. In vitro drug release

In order to closely resemble the conditions of the ileo-colonic region, Krebs bicarbonate buffer pH 7.4 was used as a dissolution medium after the acid stage (Fadda and Basit, 2005; Fadda

et al., 2009). Drug release from the coated pellets was assessed using USP II paddle apparatus (Model PTWS, Pharma Test, Hainburg, Germany). The tests were conducted in 900 ml of 0.1 N HCl for 2 h followed by Krebs buffer pH 7.4 maintained at 37 ± 0.5 °C, at least in triplicate. A paddle speed of 50 rpm was employed. The tests were conducted under sink conditions. The amount of prednisolone released from the pellets was determined at 5 min intervals by an in-line UV spectrophotometer at a wavelength of 247 nm. Data were processed using Icalis software (Icalis Data Systems Ltd., Berkshire, UK). Due to the inherent instability of bicarbonate buffers, as a result of carbon dioxide loss and pH rise, Krebs buffer was gassed continuously with an oxygen/carbon dioxide mixture (95%/5%) at a constant flow in order to keep the pH at 7.4 ± 0.05 as reported elsewhere (Fadda et al., 2009).

2.6. Confocal laser scanning microscopy testing of coated pellets

2.6.1. Coating of pellets with fluorescent probes

Rhodamine B (2%, w/w, based on dry polymer weight) was dissolved in the inner coating formulation, whereas fluorescein (1%, w/w, based on dry polymer weight) was dispersed into the outer coating and single coating formulations. The coating formulations and conditions used here were the same as reported above in Sections 2.4.1 and 2.4.2. The rationale behind this strategy is that rhodamine B is very water-soluble, hence it is completely solubilized in the inner coating aqueous formulation. In contrast, fluorescein is not soluble in the acidic conditions of the outer coating and single coating ethanolic formulations, thus it would not interfere with the dissolution properties of these coatings (O'Neil et al., 2006).

2.6.2. Dissolution of coated pellets for confocal testing

Dissolution of coated pellets intended for further visualization of coating dissolution kinetics under the confocal microscope was carried out in a USP II paddle dissolution apparatus (Model PTWS, Pharma Test, Hainburg, Germany) in the darkness. To avoid pellet aggregation during the dissolution testing, an in-house built device was used (Liu et al., 2009b). This device consists of a round net (8 cm diameter) with 20 chambers (1 cm diameter) supported at the bottom for a 250 μm mesh. Twenty pellets were placed in each device (one in each chamber) and each device was placed in each dissolution vessel. The distance between the paddle and the bottom of the chambers was set to 25 mm. The coated pellets were placed in 900 ml of 0.1 N HCl for 2 h and subsequently in Krebs buffer pH 7.4. Pellets were retrieved from the dissolution medium at predetermined time points and dried at room temperature before analysis.

2.6.3. Confocal laser scanning microscopy analysis

Coated pellets were cross-sectioned and the fluorescence distribution was assessed under a Zeiss LSM 510 Meta Laser Scanning Confocal Microscope (Zeiss, Jena, Germany), equipped with a Plan-Neofluer 5×/0.15 air lens. Cross-sectioned pellets were placed in circular cell culture chambers and analysed under the single track configuration (single coated pellets), using an Argon laser with 488 nm line or under multi-track configuration (double coated pellets), using an argon laser with 488 nm line and a helium-neon laser with 543 nm line. Images were stored as 1024 × 1024 pixel boxes.

2.7. Scanning electron microscopy analysis

The morphology of the surface and cross-section of pellets was analysed by scanning electron microscopy. Samples were placed on SEM stubs and fixed using carbon discs before being gold coated using an EMITEC K 550 sputter coater for 3 min at 40 mA. The samples were then transferred to a Phillips XL20 Scanning Electron Microscope for imaging.

2.8. Mucoadhesion testing

The mucoadhesive performance of uncoated, single-coated and double-coated pellets formulated with different grades of carbomer and at different loads was assessed, *ex vivo*, on porcine mucosa from the ascending colon, using a tensile strength method (Instron, High Wycombe, United Kingdom). Mucus thickness has been shown to change along the large intestine of the pig. Differences in mucus thickness have been reported play a role in the mucoadhesion process (Varum et al., 2010b). The pig was chosen as animal model due to the similarity of its alimentary canal to the human gut, namely in terms of anatomy (Kararli, 1995), intestinal transit (Davis et al., 2001; Snoeck et al., 2004), mucin structure (Kararli, 1995) and drug absorption (Hildebrand, 1994; Oberle and Das, 1994). Due to difficulties in directly testing pellets using a traditional tensile test setup, a cylindrical HDPE adaptor (height: 10 mm; diameter: 10 mm) was designed in-house and attached to the probe of the tensile tester using double-face tape. The surface of the HDPE adaptor was fully covered by pellets (40) by means of double-face tape. Briefly, the probe with the sample was moved down towards the porcine colonic mucosa and a force of 0.5 N was applied for 10 min. It should be noted that the mucosa under testing was not pre-hydrate with simulated physiological fluids in order to not change the mucosa natural moist microenvironment. Moreover, the fluid volumes available in the large intestine of humans are very low (Cummings et al., 1990; Schiller et al., 2005). After the contact, the probe was brought to its initial position at constant speed (20 mm/min) and the mucoadhesion was expressed as the energy at break, usually referred to as work of adhesion. Each pellet sample was tested on a different sample of mucosa and 5–7 replicates were performed for each batch of pellets. The mucoadhesion performance of single- and double-coated pellets, after exposure to the dissolution medium was evaluated as described above. Due to limitations of the method, wet pellets could not be used. Therefore, after the dissolution stage, pellets were dried at room temperature before testing.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 17.0 for Windows®. One-way analysis of variance (ANOVA) was used to compare the different variables in the mucoadhesion experiments. Tukey's test was used for post hoc comparisons between groups. Results were considered statistically significant when $p \leq 0.05$.

3. Results and discussion

3.1. Assessment of the organic acid approach

Polyacrylic acid polymers (carbomers) are weak acids with a reported pK_a of 6.0 ± 0.5 (Lubrizol, 2008). These polymers are in the coiled state when dry and slowly uncoil when dispersed in water, increasing their viscosity, which is highly sensitive to pH (Taberner et al., 2002). Neutralization above the pK_a results in an extensive ionization, bursting the swelling, which is higher in the pH range of 5–9 (Singla et al., 2000). Below pH 5, less than 10% of the carboxylic acid groups are ionized, limiting the polymer swelling (Lubrizol, 2008). Considering this, a polymer swelling modulation during processing (extrusion–spheronization) could be used to overcome the tackiness issue, when wet processes are required. Organic acids have been widely used as pH modifiers in formulation development for different purposes. For instance, they have been used to

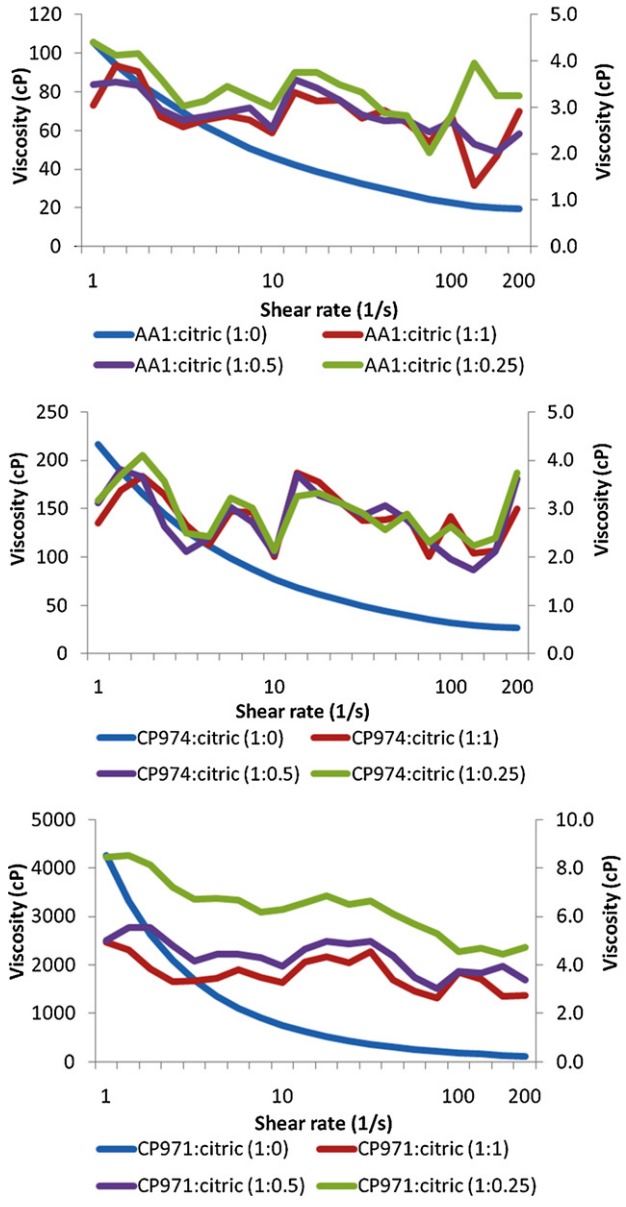

Fig. 1. Rheograms of Polycarbophil AA-1 (AA1), Carbopol 974P (CP974) and Carbopol 971P (CP971) aqueous dispersions (0.5%) in the absence (primary y-axis) and in the presence (secondary y-axis) of citric acid at ratios representing the ratios in the pellet formulations.

decrease the microenvironmental pH during dissolution, promoting a pH-independent drug release of weakly basic drugs in alkaline media (Varma et al., 2005; Guthmann et al., 2007; Ploen et al., 2009; Tran et al., 2010). Here, we attempt to use organic acids to modulate the swelling of carbomer polymers during wet mixing. This would facilitate mixing, extrusion and spheronization, allowing the possibility to increase polymer load.

The proof of concept of using organic acids, as swelling modifiers of carbomer dispersions was confirmed by the rheology measurements (Fig. 1). All carbomer and citric acid combinations showed a very low viscosity, independent of the shear rate (Newtonian behavior), contrasting to the high viscosity and thinning behavior for high shear rates (non-Newtonian, pseudoplastic) of pure carbomer dispersions (Taberner et al., 2002). This lower viscosity observed for citric acid and carbomer mixtures is accompanied by the lower pH attained with these combinations (Table 1). Furthermore, in the case of Carbopol 971P, which is less cross-linked,

Table 1
Effect of citric acid on the pH of aqueous carbomer dispersions.

Ratio polymer:citric acid	1:0	1:1	1:0.5	1:0.25
Polycarbophil AA-1	3.47	2.40	2.65	2.84
Carbopol 971P	3.34	2.40	2.62	2.76
Carbpol 974P	3.39	2.40	2.59	2.78

some discrimination in the rheology profile between different citric acid concentrations was observed. As the citric acid concentration increased the viscosity of the combinations decreased, following the same trend observed for pH (Fig. 1 and Table 1). This contrasts to the cases of Carbopol 974P and Polycarbophil AA-1 which have a higher cross-linking density and a lower viscosity at 0.5% (w/v), where no differences were observed between different citric acid concentrations (Fig. 1).

Pure carbomer dispersions showed a pH between 3.34 and 3.47, depending on the carbomer grade (Table 1), which is well below the pK_a of these polymers (6.0 ± 0.5). Taking Carbopol 974P as an example and using the Henderson–Hasselbalch equation (Eq. (1)), it is possible to estimate the ratio between nonionized and ionized groups at a predetermined pH.

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{\mathrm{A}^-}{\mathrm{HA}} \quad (1)$$

Without citric acid, 0.5% (w/v) Carbopol 974P aqueous dispersion has a pH of 3.39, which corresponds to a ratio $[A^-]/[HA]$ of 2.45×10^{-3}. A further reduction in pH (2.40), given by the citric acid (1:1) decreases this ratio to 2.51×10^{-4}, which is approximately 10-fold lower than the ratio obtained without citric acid. Therefore, citric acid is effectively further reducing the ionization degree of the polymer, constraining its uncoiling and swelling. The ionization of citric acid dramatically increases hydrogen ions in solution resulting in the lower pH observed (pH 2.40). This shifts the aqueous acid–base equilibrium of carbomer (weak acid) towards a lower ionization of the polymer (Eq. (2)) (Schosseler et al., 1991).

$$-[CH_2CH(COOH)]_n - + H_2O \leftrightarrow -[CH_2CH(COO^-)]_n - + H_3O^+ \quad (2)$$

From the evidence provided here, organic acids, such as citric acid, can successfully reduce the viscosity of carbomer aqueous dispersions as it has been demonstrated elsewhere with electrolytes, such as calcium chloride. However, in the case of the latter approach the mucoadhesive properties of the obtained products are often reduced (Bonner et al., 1997; Gómez-Carracedo et al., 2001).

3.2. Properties of the pellet core

The results obtained from the rheology experiments led to the development of pellets by extrusion–spheronization containing an organic acid (citric acid) as a microenvironmental pH modifier. The incorporation of citric acid into the pellet formulation effectively decreased the tackiness. Spherical pellets with a smooth surface containing up to 20% of the tested carbomer grades were obtained (Fig. 2). However, remarkable differences were noticed in the amount of water required and in the yield of pellets produced. For instance, less water (45–60%, w/w) was used to formulate pellets loaded with 5–20% Carbopol 971P NF compared to Carbopol 974P NF (60–80%, w/w) and Polycarbophil AA-1 (50–70%, w/w). The 971P grade is less crosslinked than the 974P grade, therefore it hydrates much faster and at a higher extent, which is a disadvantage during the extrusion–spheronization-process. Hence, the amount of water required was lower for this grade in order to provide a suitable formulation.

An increase in carbomer load, after water content optimization, resulted in a lower fraction of pellets within the size range of 1.0–1.4 mm (Table 2), whereas the fraction of pellets with size

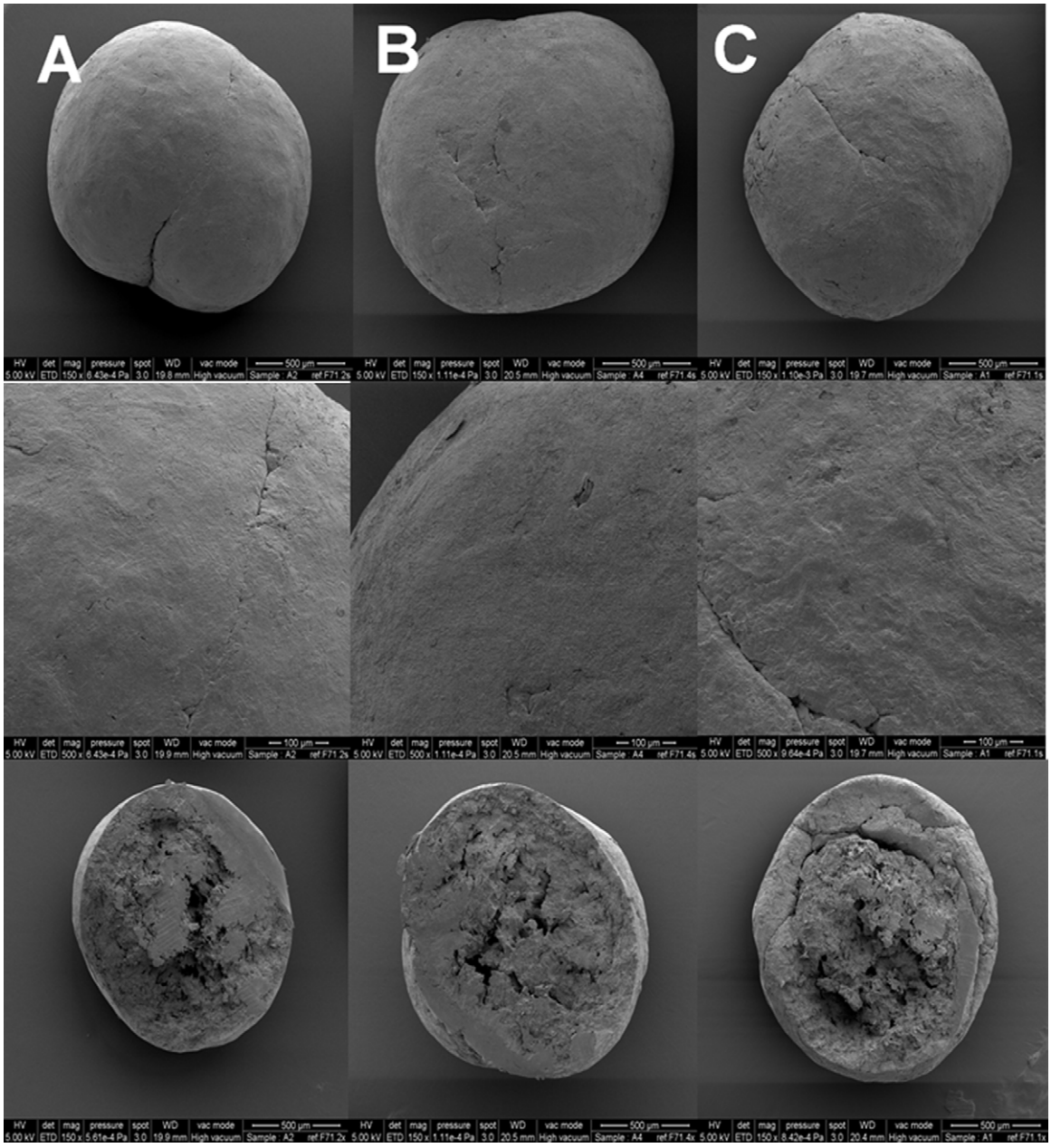

Fig. 2. Scanning electron micrographs of the surface and cross-section of pellets containing 20% of (A) Polycarbophil AA-1, (B) Carbopol 971P and (C) Carbopol 974P.

above 1.4 mm increased (Table 2). This effect was more pronounced for Carbopol 971P NF. This may be explained as a follow up of the reasons reported above; the lower crosslinking nature and higher water uptake by this polymer makes the swelling control by the organic acid (mediated by pH) less efficient as the carbomer load increases. Therefore, during the spheronization process, particles tend to bind to each other, resulting in larger pellets.

The incorporation of carbomer polymers into pellet formulations clearly provided mucoadhesive properties on porcine colonic mucosa (Fig. 3). The work of adhesion, a parameter considered more representative of mucoadhesion (Hägerström and Edsman, 2001), increased as carbomer concentration increased, for all polymer grades ($p \leq 0.05$). The work of adhesion could not be determined for control pellets as it was below the limit of quantification of the equipment, indicating a lack of mucoadhesion. Pellets containing 20% Carbopol 971P NF exhibited stronger binding properties to the mucus of the colonic mucosa than pellets containing 20%

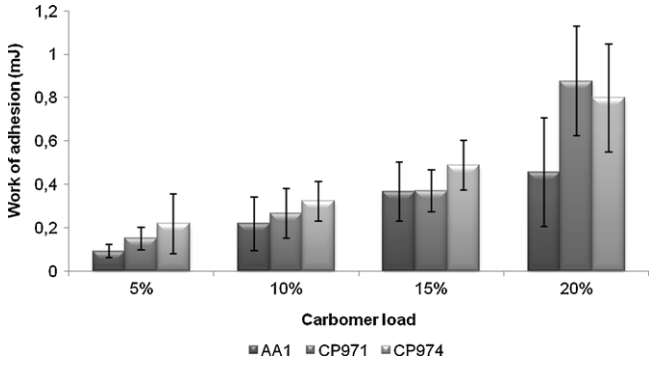

Fig. 3. Effects of the type and load of carbomer (AA1 – Polycarbophil AA-1, CP971 – Carbopol 971P, CP974 – Carbopol 974P) on the mucoadhesive properties of pellets on porcine colonic mucosa. Results are expressed as the mean and standard deviation of 5–7 replicates.

Table 2
Size analysis of carbomer pellets containing citric acid prepared by extrusion–spheronization.

	Carbomer load (%)	Yield (1.0–1.4 mm)
Control (without acid)	0	55.99
Control (with citric acid)	0	51.74
Polycarbophil AA-1	5	94.20
	10	77.06
	15	62.52
	20	51.98
Carbopol 971P	5	90.19
	10	74.90
	15	40.88
	20	17.44
Carbopol 974P	5	80.53
	10	75.31
	15	59.74
	20	52.51

of Polycarbophil AA-1 ($p \leq 0.05$). No differences in mucoadhesion were detected between different polymer grades at lower polymer concentrations ($p > 0.05$).

Despite the inherent limitations of the tensile strength method to evaluate the mucoadhesion performance in the *in vivo* resembling conditions context, it is a valuable tool to screen potential mucoadhesive formulations. However, some important physiological features cannot be reproduced, such as the gastrointestinal motility and mucus turnover, which can affect the *in vivo* fate of oral mucoadhesive dosage forms (Lehr et al., 1991; Rubinstein and Tirosh, 1994). Also, perpendicular forces are not likely to take place, *in vivo*, in the human gastrointestinal tract.

Laulicht et al. (2009) attempted to correlate the *in intro* and *in vivo* mucoadhesion measurement of a bioerodible polymers using a tensile strength method in the stomach of the rat. The fracture strength values obtained were higher when measurements were performed *in vitro*, using excised gastric mucosa, than *in vivo*. It was suggested that, *in vivo*, the mucoadhesive probe does not reach the adherent mucus layer (Atuma et al., 2001) and only the loosely bound mucus layer interacts with the probe due to the continuous movement of the gastric mucosa. Therefore, the fracture strength observed *in vivo* is lower and reflect more the weak nature of the loosely bound mucus layer (Laulicht et al., 2009). Polymers that interact with mucus by chain entanglements, such as carbomers, may be able to reach the firmly adherent mucus layer due to the polymer chain diffusion, strengthening the mucoadhesive bond (Tobyn et al., 1996; Varum et al., 2010b). Also, multiparticulate mucoadhesive formulations, such as pellets, afford an augmented surface area, increasing the exposure to the mucus layer, compared to single-units.

3.3. Properties of the novel mucoadhesive colonic drug delivery system

3.3.1. Assessment of the double-coating system concept

Ideally, a mucoadhesive material would be specific to a target region in the gut, such as the colon. However, this would require a specific physiological trigger to promote mucoadhesive properties once the region is reached. Most of the mucoadhesive materials available lack specificity; therefore a coating system with colonic-specific delivery characteristics is required. This also avoids the adhesion of the core in the upper gastrointestinal tract while maintaining its properties until the coating is completely dissolved in the ileo-colonic region. A novel double-coating system, based on Eudragit® S (dissolves at pH 7), was successfully applied onto mucoadhesive pellets formulated by the organic acid approach.

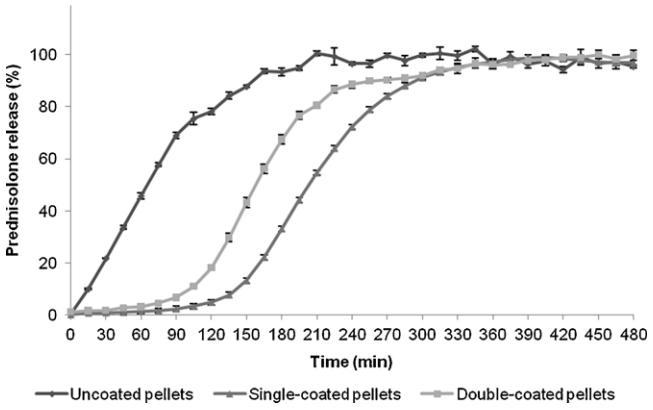

Fig. 4. *In vitro* prednisolone release from single-coated and double-coated pellets loaded with 20% of Carbopol 974P in Krebs buffer pH 7.4 (after pre-exposure to 0.1 N HCl for 2 h). Drug release from uncoated pellets in Krebs buffer (without pre acid exposure) is presented for comparison purposes.

Prednisolone release from Eudragit® S single- and double-coated pellets loaded with 20% Carbopol 974P in Krebs buffer pH 7.4 after exposure to 0.1 N HCl for 2 h is presented in Fig. 4. The single- and double-coated pellet formulations were able to withstand the acid conditions without releasing any drug (data not shown). After transition to Krebs buffer pH 7.4, the onset of drug release (5% drug released) from single-coated pellets occurred after 120 min. In contrast, release from the double-coated pellets is initiated after 75 min exposure to the buffer medium (Fig. 4). The faster release attained with double-coated pellets may avoid the pass-through effects often observed with enteric coated formulations intended to target the colon (Ibekwe et al., 2006; McConnell et al., 2008b). The faster dissolution of the coating, once the trigger is initiated (ileo-colonic region) may result in drug release occurring in the ascending colon, where more fluid is available for drug dissolution and the viscosity is lower, contrasting to the more viscous environment of the descending colon.

The coating dissolution process of both single- and double-coated pellets was visualized by Confocal Laser Scanning Microscopy (CLSM). After pre-exposure in 0.1 N HCl for 2 h, no change in the single- and double-coated pellets was noticed (Figs. 5 and 6). A slow dissolution of the single-coating was observed when pellets were transferred to Krebs buffer pH 7.4. At 60 min, the single coating started to thin as a result of the dissolution in the media with pH above the pH threshold of the polymer. The coating only breaks at 120 min and completely dissolves after 180 min exposure to Krebs buffer pH 7.4 (Fig. 5). This correlates with the late onset of release (120 min) observed for the single-coated pellets (Fig. 4).

In contrast, the confocal micrographs showed a faster dissolution of the coating in the double-coated pellets. After 30 min in Krebs buffer pH 7.4, there was a discontinuity in the inner coating, indicating that it had started to dissolve, while the outer coating became thinner, but still covering most of the pellet surface (Fig. 6). However, at 50 min the outer coating ruptured and the inner and the outer coating continued to dissolve until the outer layer completely disappeared after 70 min in Krebs buffer pH 7.4. The complete dissolution of the outer coating coincided with the onset of release of the double-coated pellets (Fig. 4).

Upon exposure to Krebs buffer pH 7.4, fluid starts to diffuse through the outer coating of the pellets. Since the inner coating is composed of partially neutralized Eudragit® S, the uptake of buffer promotes a faster dissolution of the inner coating when compared to the dissolution of the outer coating. The dissolution of the inner coating can generate a buffer system due to the presence of potassium dihydrogen phosphate. Therefore, the buffer capacity of

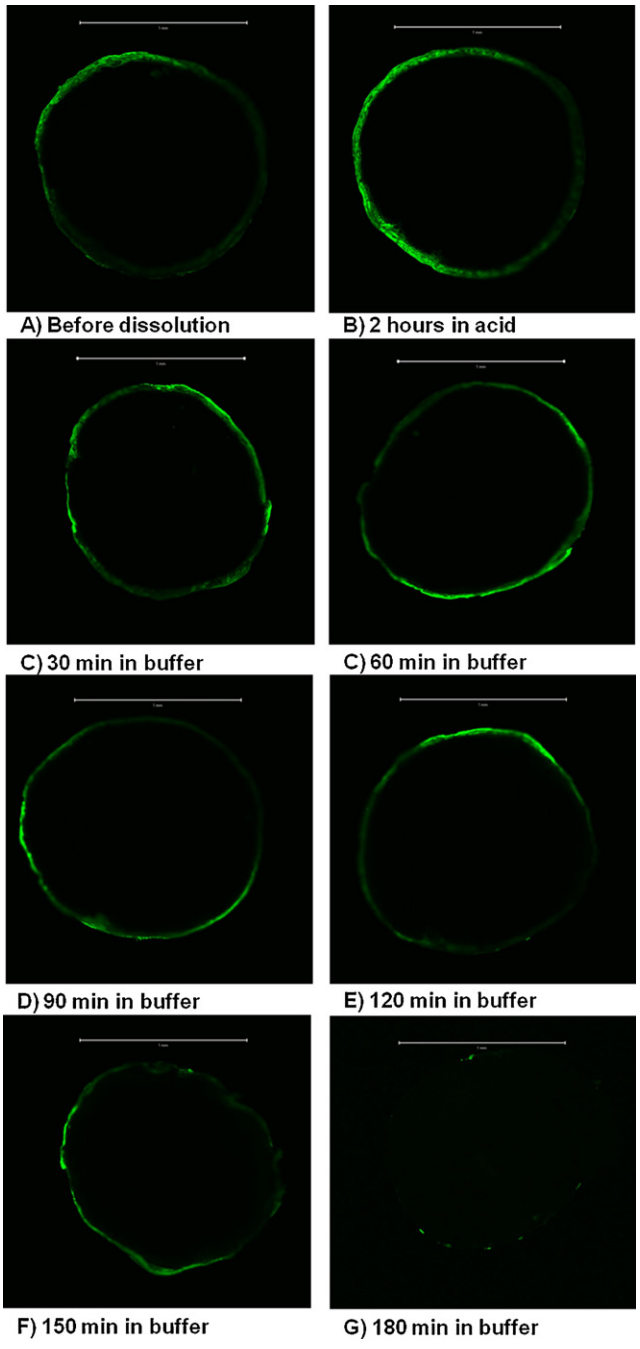

Fig. 5. Confocal micrographs of single-coated pellets containing 20% Carbopol 974P after exposure to 0.1 N HCl and Krebs buffer pH 7.4.

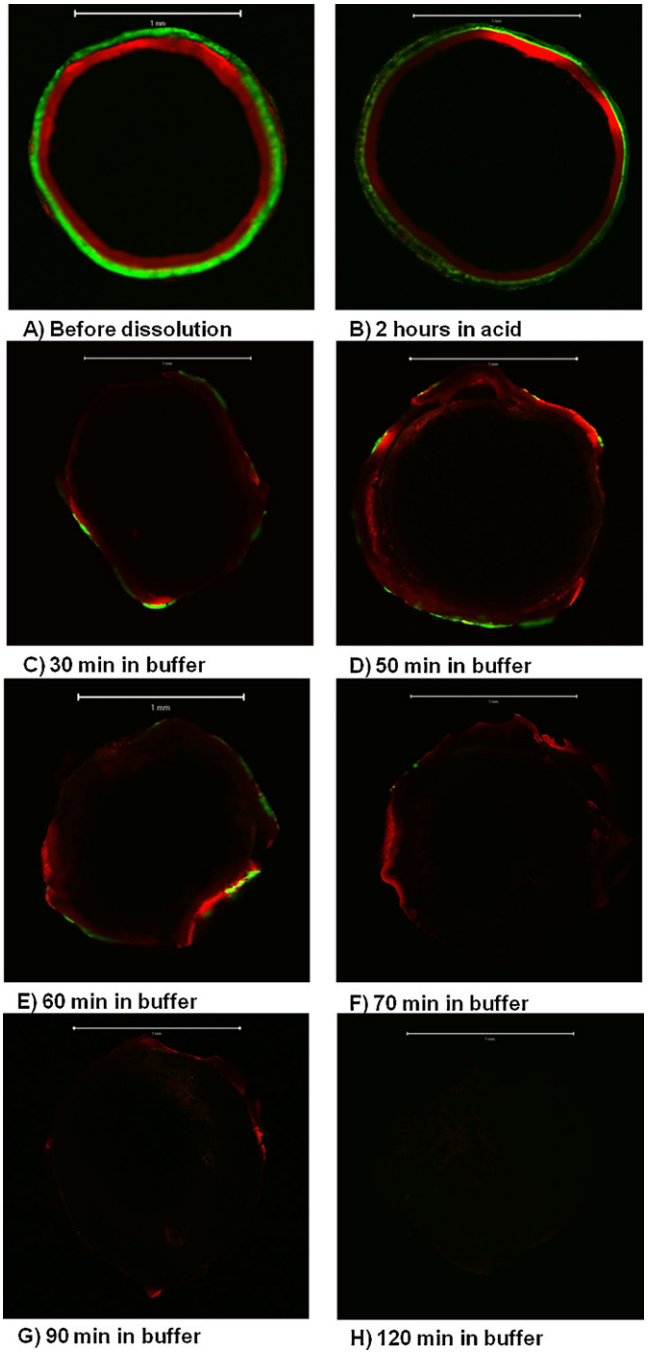

Fig. 6. Confocal micrographs of double-coated pellets containing 20% Carbopol 974P after exposure to 0.1 N HCl and Krebs buffer pH 7.4.

the dissolving inner coating, along with the high pH can assist the dissolution of the outer coating, which is immediately adjacent. Simultaneously, the outer surface of the outer coating undergoes dissolution. The combined effect of the dissolution of the inner side and the outer side surfaces of the outer coating results in drug release acceleration observed with the double-coated pellets. In contrast, the standard single-coating can only dissolve from the outer surface due to the high pH of the buffer medium and its buffer capacity.

3.3.2. *Mucoadhesive and controlled release features*

The incorporation of either 5% or 20% carbomer into the pellets core did not affect the onset of drug release when compared to pellets without this polymer. This shows that the performance of the coating system is independent of the core composition. Both formulations containing carbomer, and the control formulation, had a lag time of 75 min after exposure to Krebs buffer pH 7.4. Prednisolone pellets (based on microcrystalline cellulose) without carbomer did not disintegrate and the release was very slow and incomplete after 8 h (Fig. 7). Non-disintegrating microcrystalline cellulose based pellets often display prolonged and incomplete drug release (Schroder and Kleinebudde, 1995), which is a disadvantage if a complete drug release is required in a short gastrointestinal absorption section, such as the ascending colon. This is further complicated due to the large colonic transit variability. Several excipients have been studied in order to replace microcrystalline cellulose as a spheronization aid, such as

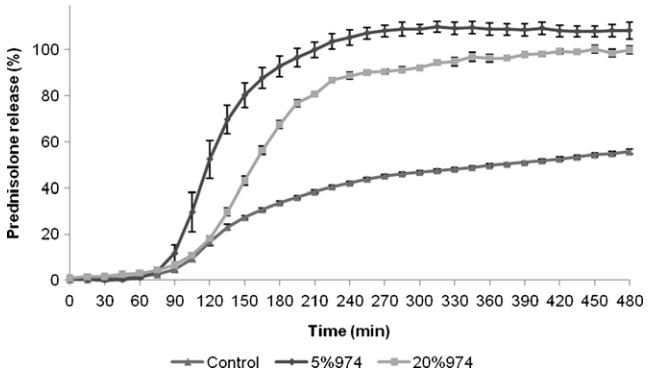

Fig. 7. *In vitro* prednisolone release from double-coated pellets, containing different levels of Carbopol 974P, in Krebs buffer pH 7.4 (after pre-exposure to 0.1 N HCl for 2 h).

colloidal silica (Podczeck, 2008), carrageenan (Bornhöft et al., 2005), pectin (Tho et al., 2003) or glyceryl monostearate (Basit et al., 1999). However, none of these excipients have been widely recognized as a better option for extrusion–spheronization. Interestingly, pellets formulated with carbomer, even at 5% load, provided complete drug release after the double-coating had dissolved (Fig. 7).

Double-coated pellets loaded with 5% Carbopol 974P NF exhibited complete drug release after 275 min in Krebs buffer pH 7.4. An increase in carbomer concentration up to 20% showed a more extended drug release profile. After 480 min (8 h) in Krebs buffer pH 7.4, drug release was still incomplete, providing an extended release for approximately 7 h after the onset of release (Fig. 7). The high cross-linking density presented by Carbopol 974P NF is responsible for the formation of regions of microviscosity (between swollen particles) and regions of macroviscosity (surrounding particle resins), forming channels from which drug can diffuse. An increase in polymer concentration decreases the regions of microviscosity, as individual resin particles are more packed, limiting drug release.

In order to efficiently increase residence time in the colon, the pellet core must retain some mucoadhesive capacity after the dissolution of the coating. Double-coated pellets showed negligible adhesion on porcine colonic mucosa (Fig. 8). After 60 min exposure to Krebs buffer pH 7.4, the outer coating is ruptured (as confirmed by CLSM) but still not completely dissolved (Fig. 6). Therefore, the complete surface of the mucoadhesive core is not exposed, which failed to increase the mucoadhesive properties of the system ($p > 0.05$). As the coating dissolution progresses, a higher area is being exposed which is available to establish mucoadhesive interactions. This was confirmed by the increase in mucoadhesion ($p \leq 0.05$) as the dissolution progressed, until the core was completely exposed after 150 min in buffer (Fig. 8). However, the mucoadhesive performance of uncoated pellets was not achieved for the double-coated pellets after the coating had completely dissolved (≤ 0.05).

Conversely, single-coated pellets only exhibited mucoadhesive properties after a longer exposure time in Krebs buffer pH 7.4 (Fig. 8). Negligible mucoadhesion was observed after dissolution in 0.1 N HCl and up to 120 min in buffer. However, after 150 min exposure to Krebs buffer, single-coated pellets showed higher mucoadhesion ($p \leq 0.05$). From the CLSM micrographs (Fig. 5), the partial rupture of the single coating can be seen after 150 min. However, as dissolution progressed, no further improvement in mucoadhesive properties was observed ($p > 0.05$), although the core surface area is more exposed. This may be explained by the continuous and prolonged medium uptake by the core, which has been demonstrated to decrease mucoadhesive properties of

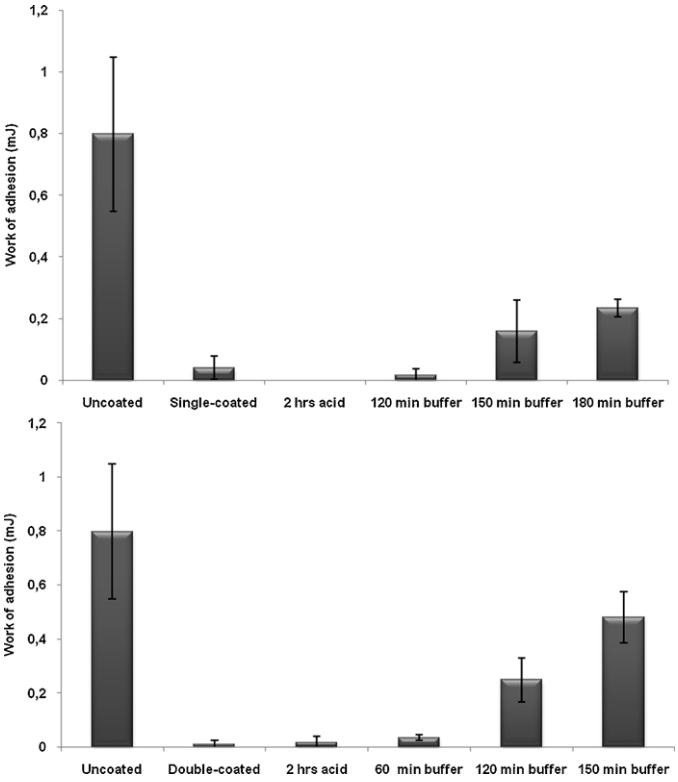

Fig. 8. Influence of dissolution of single-coated (top) and double-coated (bottom) pellets containing 20% Carbopol 974P on their mucoadhesive properties on porcine colonic mucosa. Results are expressed as mean and standard deviation of 5–7 replicates.

carbomer polymers (Mortazavi and Smart, 1995; Varum et al., 2010b).

4. Conclusion

A novel targeted mucoadhesive colonic drug delivery platform with accelerated drug release in conditions resembling the lower gut was successfully developed. Engineering carbomer loaded pellets by the extrusion–spheronization process, using the organic acid approach proposed here, clearly reduced tackiness and offers a promising strategy to formulate mucoadhesive multiparticulates. The system described presents key features for efficient colonic drug delivery: fast dissolution of the colon-specific delivery carrier, good mucoadhesive performance associated with prolonged drug release and disintegrative properties. These features in combination may result in a longer colonic residence time, overcoming the pass-through effects often seen with non-disintegrating pellets and ultimately an improvement in oral drug bioavailability.

Acknowledgements

The authors would like to acknowledge David McCarthy for his contribution with the SEM images, Cheale Meats Ltd., Orchard Farm, Essex, UK for proving the biological samples for the mucoadhesion studies and Fundação para a Ciência e a Tecnologia for their financial support (SFRH/BD/30160/2006).

References

Atuma, C., Strugala, V., Allen, A., Holm, L., 2001. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am. J. Physiol. Gastrointest. Liver Physiol. 280, 922–929.

Awad, G.A.S., Charrueau, C., Allain, P., Chaumeil, J.C., 2002. Formulation and evaluation of bioadhesive pellets containing different carbomers made by extrusion–spheronization. S. T. P. Pharma Sci. 12, 157–162.
Basit, A.W., Newton, J.M., Lacey, L.F., 1999. Formulation of ranitidine pellets by extrusion–spheronization with little or no microcrystalline cellulose. Pharm. Dev. Technol. 4, 499–505.
Basit, A.W., Short, M.D., Mcconnell, E.L., 2009. Microbiota-triggered colonic delivery: robustness of the polysaccharide approach in the fed state in man. J. Drug Target. 17, 64–71.
Bonner, M.C., Jones, D.S., Woolfson, A.D., 1997. Rheological characterization of carbopol gels as electrically-conducting drug delivery interfaces. J. Pharm. Pharmacol. 4, 74.
Bornhöft, M., Thommes, M., Kleinebudde, P., 2005. Preliminary assessment of carrageenan as excipient for extrusion/spheronisation. Eur. J. Pharm. Biopharm. 59, 127–131.
Ch'ng, H.S., Park, H., Kelly, P., Robinson, J.R., 1985. Bioadhesive polymers as platforms for oral controlled drug delivery II: synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. J. Pharm. Sci. 74, 399–405.
Coupe, A.J., Davis, S.S., Wilding, I.R., 1991. Variation in gastrointestinal transit of pharmaceutical dosage forms in healthy subjects. Pharm. Res. 8, 360–364.
Cummings, J.H., Banwell, J.G., Segal, I., Coleman, N., Englyst, H.N., MacFarlane, G.T., 1990. The amount and composition of large bowel contents in man. Gastroenterology 98, A48.
Davis, S.S., Illum, L., Hinchcliffe, M., 2001. Gastrointestinal transit of dosage forms in the pig. J. Pharm. Pharmacol. 53, 33–39.
Fadda, H.M., Basit, A.W., 2005. Dissolution of pH responsive formulations in media resembling intestinal fluids: bicarbonate versus phosphate buffers. J. Drug Deliv. Sci. Tech. 15, 273–279.
Fadda, H.M., Merchant, H.A., Arafat, B.T., Basit, A.W., 2009. Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. Int. J. Pharm. 382, 56–60.
Gómez-Carracedo, A., Alvarez-Lorenzo, C., Gómez-Amoza, J.L., Martínez-Pacheco, R., Souto, C., Concheiro, A., 2001. Extrusion–spheronization of blends of Carbopol 934 and microcrystalline cellulose. Drug Dev. Ind. Pharm. 27, 381–391.
Guthmann, C., Lipp, R., Wagner, T., Kranz, H., 2007. Development of a multiple unit pellet formulation for a weakly basic drug. Drug Dev. Ind. Pharm. 33, 341–349.
Hägerström, H., Edsman, K., 2001. Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method. J. Pharm. Pharmacol. 53, 1589–1599.
Hildebrand, M., 1994. Interspecies comparison of pharmacokinetic parameters of an oral sustained release preparation of Iloprost. Drug Dev. Ind. Pharm. 20, 1367–1376.
Ibekwe, V.C., Liu, F., Fadda, H.M., Khela, M.K., Evans, D.F., Parsons, G.E., Basit, A.W., 2006. An investigation into the *in vivo* performance variability of pH responsive polymers for ileo-colonic drug delivery using gamma-scintigraphy in humans. J. Pharm. Sci. 95, 2760–2766.
Ibekwe, V.C., McConnell, E.L., Fadda, H.M., Khela, M.K., Evans, D.F., Basit, A.W., 2008. Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive ileo-colonic release systems. Pharm. Res. 25, 1828–1835.
John, V.A., Shotton, P.A., Moppert, J., Theobald, W., 1985. Gastrointestinal transit of Oros drug delivery system in healthy volunteers: a short report. Br. J. Clin. Pharmacol. 19, 203S–206S.
Kararli, T.T., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos. 16, 351–380.
Laulicht, B., Cheifetz, P., Tripathi, A., Mathiowitz, E., 2009. Are in vivo gastric bioadhesive forces accurately reflected by in vitro experiments? J. Control. Release 134, 103–110.
Lehr, C.-M., Poelma, F.G.J., Junginger, H.E., Tukker, J.J., 1991. An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. Int. J. Pharm. 70, 235–240.
Liu, F., Basit, A.W., 2010. A paradigm shift in enteric coating: achieving rapid release in the proximal small intestine of man. J. Control. Release 147, 242–245.
Liu, F., Lizio, R., Meier, C., Petereit, H.-U., Blakey, P., Basit, A.W., 2009a. A novel concept in enteric coating: a double-coating system providing rapid drug release in the proximal small intestine. J. Control. Release 133, 119–124.
Liu, F., Lizio, R., Schneider, U.J., Petereit, H.-U., Blakey, P., Basit, A.W., 2009b. SEM/EDX, confocal microscopy analysis of novel and conventional enteric-coated systems. Int. J. Pharm. 369, 72–78.
Liu, F., Moreno, P., Basit, A.W., 2010. A novel double-coating approach for improved pH-triggered delivery to the ileo-colonic region of the gastrointestinal tract. Eur. J. Pharm. Biopharm. 74, 311–315.
Lubrizol, 2008. Formulating controlled release tablets and capsules with Carbopol® Polymers, Lubrizol.
McConnell, E.L., Fadda, H.M., Basit, A.W., 2008a. Gut instincts: explorations in intestinal physiology and drug delivery. Int. J. Pharm. 364, 213–226.
McConnell, E.L., Short, M.D., Basit, A.W., 2008b. An *in vivo* comparison of intestinal pH and bacteria as physiological trigger mechanisms for colonic targeting in man. J. Control. Release 130, 154–160.
McGirr, M.E.A., McAllister, S.M., Peters, E.E., Vickers, A.W., Parr, A.F., Basit, A.W., 2009. The use of the InteliSite® Companion device to deliver mucoadhesive polymers to the dog colon. Eur. J. Pharm. Sci. 36, 386–391.
Mezreb, N., Charrueau, C., Boy, P., Allain, P., Chaumeil, J.C., 2004. Production of Carbopol1 974P and Carbopol1 971P pellets by extrusion–spheronization: optimization of the processing parameters and water content. Drug Dev. Ind. Pharm. 30, 481–490.
Mortazavi, S.A., Smart, J.D., 1995. An investigation of some factors influencing the in vitro assessment of mucoadhesion. Int. J. Pharm. 116, 223–230.
Neau, S.H., Chow, M.Y., Durrani, M.J., 1996. Fabrication and characterization of extruded and spheronized beads containing carbopol(R) 974P, NF resin. Int. J. Pharm. 131, 47–55.
Neau, S.H., Chow, M.Y., Hileman, G.A., Durrani, M.J., Gheyas, F., Evans, B.A., 2000. Formulation and process considerations for beads containing Carbopol® 974P, NF resin made by extrusion–spheronization. Int. J. Pharm. 199, 129–140.
O'Neil, M.J., Heckelman, P.E., Koch, C.B., Roman, K.J., 2006. The Merck Index, 14th ed. Merck Research Laboratories, New Jersey.
Oberle, R.L., Das, H., 1994. Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs. Pharm. Res. 11, 592–594.
Ploen, J., Andersch, J., Heschel, M., Leopold, C.S., 2009. Citric acid as a pH-modifying additive in an extended release pellet formulation containing a weakly basic drug. Drug Dev. Ind. Pharm. 35, 1210–1218.
Podczeck, F., 2008. A novel aid for the preparation of pellets by extrusion/spheronization. Pharm. Tech. Eur. 20.
Rubinstein, A., Tirosh, B., 1994. Mucus gel thickness and turnover in the gastrointestinal tract of the rat: response to cholinergic stimulus and implication for mucoadhesion. Pharm. Res. 11, 794–799.
Schiller, C., Frohlich, C.P., Giessmann, T., Siegmund, W., Mönnikes, H., Hosten, N., Weitschies, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic ressonance imaging. Aliment. Pharmacol. Ther. 22, 971–979.
Schosseler, F., Ilmain, F., Candau, S.J., 1991. Structure and properties of partially neutralized poly(acrylic acid) gels. Macromolecules 24, 225–234.
Schroder, M., Kleinebudde, P., 1995. Influence of formulation parameters on dissolution of propyphenazone pellets. Eur. J. Pharm. Biopharm. 41, 382–387.
Schroeder, K.W., Tremaine, W.J., Ilstrup, D.M., 1987. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N. Engl. J. Med. 317, 1625–1629.
Singla, A.K., Chawla, M., Singh, A., 2000. Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. Drug Dev. Ind. Pharm. 26, 913–924.
Sinha, A., Ball, D.J., Connor, A.L., Nightingale, J., Wilding, I.R., 2003. Intestinal performance of two mesalamine formulations in patients with active ulcerative colitis as assessed by gamma scintigraphy. Pract. Gastroenterol. 27, 56–69.
Smart, J.D., Kellaway, I.W., Worthington, H.E.C., 1984. An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery. J. Pharm. Pharmacol. 36, 295–299.
Snoeck, V., Huyghebaert, N., Cox, E., Vermeire, A., Saunders, J., Remon, J.P., Verschooten, F., Goddeeris, B.M., 2004. Gastrointestinal transit time of non-disintegrating radio-opaque pellets in suckling and recently weaned piglets. J. Control. Release 94, 143–153.
Taberner, T.S., Martin-Villodre, A., Pla-Delfina, J.M., Herraéz, J.V., 2002. Consistency of Carbopol 971-P NF gels and influence of soluble and cross-linked PVP. Int. J. Pharm. 233, 43–50.
Tho, I., Sande, S.A., Kleinebudde, P., 2003. Disintegrating pellets from a water-insoluble pectin derivative produced by extrusion/spheronisation. Eur. J. Pharm. Biopharm. 56, 371–380.
Tobyn, M.J., Johnson, J.R., Dettmar, P.W., 1996. Factors affecting in vitro gastric mucoadhesion. II. Physical properties of polymers. Eur. J. Pharm. Biopharm. 42, 56–61.
Tran, T.T.-D., Tran, P.H.-L., Choi, H.-G., Han, H.-K., Lee, B.-J., 2010. The roles of acidifiers in solid dispersions and physical mixtures. Int. J. Pharm. 384, 60–66.
Tuleu, C., Basit, A.W., Waddington, W.A., Ell, P.J., Newton, J., 2002. Colonic delivery of 4-aminosalicylic acid using amylose–ethylcellulose-coated hydroxypropylmethylcellulose capsules. Aliment. Pharmacol. Ther. 16, 1771–1779.
Varma, M.V.S., Kaushal, A.M., Garg, S., 2005. Influence of micro-environmental pH on the gel layer behavior and release of a basic drug from various hydrophilic matrices. J. Control. Release 103, 499–510.
Varum, F.J.O., Merchant, H.A., Basit, A.W., 2010a. Modified-release formulations in motion: the relationship between gastrointestinal transit and drug absorption. Int. J. Pharm. 395, 26–36.
Varum, F.J.O., Veiga, F., Sousa, J., Basit, A.W., 2010b. An investigation into the role of mucus thickness on mucoadhesion in the gastrointestinal tract of pig. Eur. J. Pharm. Sci. 40, 335–341.
Wilding, I.R., 2001. The Enterion Capsule: a novel technology for understanding the biopharmaceutical complexity of new molecular entities (NMEs). Drug Deliv. Technol. 1, 8–11.