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Fabrication and in vivo evaluation of highly pH-responsive acrylic microparticles for targeted gastrointestinal delivery

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ABSTRACT

Acrylic enteric microparticles for oral drug delivery were prepared by an oil-in-oil emulsion solvent evaporation process. The novel use of sorbitan sesquioleate as a surfactant produced Eudragit L55, L and S (pH thresholds of 5.5, 6 and 7, respectively) microparticles of good morphology (spherical, smooth surfaced), size (<100 μm) and size uniformity. The process was efficient (yield approximately 90%) and the encapsulated model drug (prednisolone) was in the amorphous form. The Eudragit L and S microparticles showed excellent pH-responsive drug release in dissolution studies (negligible drug release at pH 1.2; rapid drug release above the polymers' pH thresholds). In contrast, Eudragit L55 particles aggregated in fluid and showed poor control of drug release. In vivo in rats, Eudragit L microparticles released their drug load rapidly (T_{max} < 1 h) and the C_{max} and AUC were higher than those of a control suspension of prednisolone. Drug absorption from Eudragit S microparticles was low which was attributed to the fact that the threshold pH of Eudragit S was not reached in the rat intestine and drug release was therefore incomplete. It was concluded that although the rat is an inappropriate model for the investigation of Eudragit S microparticles, the positive results seen with the Eudragit L microparticles indicate its potential use in pH-targeted drug delivery.

1. Introduction

Enteric polymers are commonly applied to conventional solid dosage forms to modify drug release, exploiting the aboral increase in gastrointestinal pH (Evans et al., 1988) to manipulate the dissolution of pH-sensitive polymeric coatings. The small intestine can be targeted with polymers having a dissolution threshold in the region of 5.0–6.0 while the distal gut requires polymers which dissolve around pH 7.0–7.5. Dissolution in the small intestine is generally used for systemic absorption, whilst protecting the drug from the conditions in the stomach, or protecting the stomach from the effects of the drug. Colon-specific targeting is used for the topical treatment of local disorders e.g. inflammatory bowel disease. However, due to the inherent inter- and intra-individual variability in the gastrointestinal physiology of man (McConnell et al., 2008a), the targeting efficacy of conventional pH-responsive systems is variable and often poor. For example, enteric formulations for targeting the small intestine (coated with acrylic-, cellulose- or polyvinyl-based polymers) are often observed to disintegrate 1.5–2 h post-gastric emptying, rather than immediately after gastric emptying (Hardy et al., 1987; Cole et al., 2002) resulting in delayed release or reduced bioavailability. The variability in time, site and extent of drug release and absorption is attributed to limited free fluid (Schiller et al., 2005), and highly variable transit times (Fadda et al., 2009). Colon-targeted systems are even more complicated. These systems are reliant, not only on the highly variable pH at the ileocaecal junction (Fallingborg et al., 1989; Ibekwe et al., 2008), but their residence time at this site, feeding status of the subject (Ibekwe et al., 2008) and the limited fluid in the colon (Schiller et al., 2005). This variability is reflected in the fact that single-unit enteric dosage forms for colonic targeting are sometimes voided intact (Ibekwe et al., 2006, 2008; Sinha et al., 2003; Safdi, 2005; Schroeder et al., 1987).

One approach to overcome the limitations of single-unit modified release dosage forms is size reduction. Multi-unit systems, such as pellets, granules or beads have been proposed, but even pellets of 0.5–1 mm diameter do not show reliable and fast gastric emptying (Clarke et al., 1995) and enteric coated pellets have shown the same failure to release drug in the colon as single-unit dosage forms (McConnell et al., 2008b). It is possible that further size reduction to microparticles less than 100 μm may overcome the limitations of larger pellets. Microparticles can be dosed in the form of liquid suspension, which may improve gastric emptying, and the increased surface area:volume ratio of the microparticles would enable rapid drug release at the desired location in the gastrointestinal tract. Thus, there has been great interest in the manufacture of enteric

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microparticles, but the major issues to date has been the use of toxic solvents, retention of high levels of solvents in the products (Squillante et al., 2003), the use of overly complicated methodology (Kilicarslan and Baykara, 2004), poor microparticles morphology (Amorim and Ferreira, 2001a; Yuksel et al., 2000; Jain et al., 2006), relatively large size (Amorim and Ferreira, 2001a,b; Obeidat and Price, 2005, 2006; Kilicarslan and Baykara, 2004; Yuksel et al., 2000), changes upon storage (Rassu et al., 2008), non-uniformity of size (Rattes and Oliveira, 2007) and poor control of drug release (Obeidat and Price, 2005, 2006; Kilicarslan and Baykara, 2004; Scarfato et al., 2008).

The aim of the work described in this paper was therefore to develop a simple, safe, and universal method for the fabrication of pH-responsive microparticles for site-specific release in the gastrointestinal tract. Specifically, we report the preparation and subsequent in vitro and in vivo characterisation of uniform and spherical microparticles (less than 100 μm in size) of Eudragit L55 (polymethacrylic acid, ethyl acrylate 1:1; dissolution threshold pH 5.5), and Eudragit L (polymethacrylic acid, methyl methacrylate 1:1; dissolution threshold pH 6.0) for proximal small intestinal targeting, and Eudragit S (polymethacrylic acid, methyl methacrylate 1:2; dissolution threshold pH 7.0) for ileo-colonic targeting. Due to the small particle size and hence ease of suspension in liquid medium, we were able to easily dose the enteric microparticles to rats and the drug levels in the plasma were measured to assess the drug release and targeting behaviour in vivo. Prednisolone was used as a model drug.

2. Materials and methods

2.1. Materials

Eudragit L55, Eudragit L and Eudragit S were gifts from Evonik (Darmstadt, Germany), prednisolone was purchased from Sanofi-Aventis (Romainville, France), sorbitan sesquioleate (Arlacel 83) was purchased from Sigma Aldrich (Poole, UK). Liquid paraffin BP was supplied by JM Loveridge Plc. All other materials were reagent grade.

2.2. Animals

Male Wistar rats were obtained from Harlan, UK. All procedures were approved by The School of Pharmacy's Ethical Review Committee and were conducted in accordance with Home Office standards under the Animals (Scientific Procedures) Act, 1986.

2.3. Preparation of Eudragit S, L and L55 microparticles by emulsification/solvent evaporation

The following method was developed after extensive investigations into the choice of solvents, polymer concentrations, nature and concentration of emulsifying agent, method of mixing, and in-process stability. Solvents with low toxicity potential, such as acetone, methanol and ethanol, on their own and in binary mixtures in different ratios were tested and ethanol was chosen as it was the least toxic. A variety of non-ionic emulsifying agents was tested at concentrations of 1, 2 and 3% (w/w) (in liquid paraffin), and sorbitan sesquioleate (Arlacel 83) at 1% was chosen for the stability of the emulsion, quality of the microspheres produced and for the method's transferability to the different Eudragit polymers.

Prednisolone (100, 300, or 600 mg) as a model drug and either Eudragit S, L or L55 (3 g) were dissolved in 30 ml ethanol to prepare microparticles with a drug to polymer weight ratio of 1:30, 1:10 and 1:5, respectively. The resultant solution was emulsified into 200 ml liquid paraffin containing 1% (w/w) of sorbitan sesquioleate as an emulsifying agent, using a Heidolph RZR1 stirrer (5 cm diameter propeller) at 1000 rpm. Stirring was conducted for 12 h at room temperature to allow solvent evaporation. Subsequently, the microparticles formed were recovered by vacuum filtration through a Pyrex sintered glass filter (pore size 4; 5–15 μm), washed three times with 50 ml portions of *n*-hexane, and placed in a vacuum oven overnight (room temperature, 1000 mbar). Microparticles were prepared in triplicate for all formulations and characterised as described in Section 2.4.

To determine the influence of the nature of the Eudragit polymer on particle properties, such as size, the viscosity of the ethanolic solutions of prednisolone and Eudragit L-55, L or S (at a drug to polymer weight ratio of 1:5) was assessed using a U Tube Viscometer (Rheoteck, Essex, UK) according to the British Pharmacopoeia 2008. The U-tube was placed in a waterbath and all the measurements were conducted on the same day at room temperature (which was measured to be $29 \pm 0.5\,^{\circ}C$).

2.4. Particle morphology, size, and yield

The shape and surface topography of microparticles were examined by scanning electron microscopy (SEM). Samples of microparticles were mounted onto aluminium stubs using double-sided carbon adhesive tape, sputter coated with gold in a high-vacuum evaporator for 3 min at 30 mA (Emitech K550, Ashford, England) and photographed using a scanning electron microscope (Philips XL30, Eindhoven, Holland).

The volume median diameter of Eudragit L and S microparticles was measured using laser light scattering using a Malvern Mastersizer X with a 45 mm lens (Malvern Instruments Ltd., Malvern, UK). The microparticles were suspended in 0.1 M HCl by vortex mixing for 30 s, which was then added dropwise into the magnetically stirred small volume diffraction chamber, also containing 0.1 M HCl until an obscuration of 10–15% was achieved. The volume median diameter of Eudragit L55 microparticles was measured similarly except that hexane was used as a suspending agent instead of HCl as Eudragit L55 tended to agglomerate in aqueous medium. Particle size analysis of each formulation was carried out in triplicate, and the polydispersity (span) was calculated as $[D(v, 0.9) - D(v, 0.1)]/D(v, 0.5)$.

The yield of particle production was calculated using the following formula:

$$\text{yield} = \frac{\text{observed mass of microparticles produced}}{\text{theoretical mass of microparticles expected}} \times 100$$

2.5. Loading, encapsulation efficiency and physical state of drug

The particles were assessed for crystalline prednisolone using X-ray powder diffraction. Prednisolone powder, Eudragit L/S powder and prednisolone-loaded Eudragit L and S microparticles (at 1:5 drug:polymer ratio) were compressed into a round disc sample holder, smoothed with a Perspex block and analysed using a Philips PW3710 Scanning X-ray Diffractometer (Philips, Cambridge, UK) with a Cu Kα filter generated at 30 mA and 45 kV, at a scan rate of 0.02°/s from 5° to 85° 2θ for prednisolone, and 5° to 25° 2θ for microparticles (as the peak of interest was found to lie at 15° 2θ). Each scan was repeated three times, to minimize the effects of preferred orientation, with the sample being repacked between repeats. Data was analysed using X'Pert HighScore data analysis software (Version 2.0a).

Drug loaded microparticles (30 mg) were dissolved in 10 ml methanol with the aid of brief sonication. The resulting methanolic solution was made up to 100 ml with 0.1 M HCl which caused precipitation of the pH-sensitive Eudragit, after which the sam-