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Comparative evaluation of polymeric and waxy microspheres for combined colon delivery of ascorbic acid and ketoprofen

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ABSTRACT

The goal of this work was to combine the ketoprofen anti-inflammatory effect with the ascorbic acid antioxidant properties for a more efficient treatment of colonic pathologies. With this aim, microspheres (MS) based on both waxy materials (ceresine, Precirol® and Compritol®) or hydrophilic biopolymers (pectine, alginate and chitosan) loaded with the two drugs were developed, physicochemically characterized and compared in terms of entrapment efficiency, in vitro release profiles, potential toxicity and drug permeation properties across the Caco-2 cell line. Waxy MS revealed an high encapsulation efficiency of ketoprofen but a not detectable entrapment of ascorbic acid, while polymeric MS showed a good entrapment efficiency of both drugs. All MS need a gastro-resistant coating, to avoid any premature release of the drugs. Ketoprofen release rate from polymeric matrices was clearly higher than from the waxy ones. In contrast, the ASC release rate was higher, due to its high hydro-solubility. Cytotoxicity studies revealed the safety of all the formulations. Transport studies showed that the ketoprofen apparent permeability increased, when formulated with the different MS. In conclusion, only polymeric MS enabled an efficient double encapsulation of both the hydrophilic and lipophilic drugs, and, in addition, presented higher drug release rate and stronger enhancer properties.

1. Introduction

The benefits and potential advantages which can be obtained by colon drug delivery have been widely illustrated and recently reviewed (Yang et al., 2002; Shareef et al., 2003; Van den Mooter, 2006; Kumar et al., 2011). In particular, the colon-targeted drug delivery is highly desirable for the local treatment of different bowel diseases such as ulcerative colitis, cirrhosis, amebiasis, colonic cancer and other colonic pathologies, as well as for the therapy of diseases influenced by circadian rhythms like rheumatoid arthritis or asthma.

Ketoprofen is a poorly water-soluble non-steroidal anti-inflammatory drug (NSAID), extensively used as analgesic and for the acute and long-term treatment of various inflammatory pathologies, including rheumatoid arthritis and colonic adenocarcinoma. Ketoprofen, as other NSAIDs (Chang-Moon et al., 2004; Umadevi et al., 2010; Khandai et al., 2012), would be a good candidate for oral colon delivery, also taking into account its well known gastro-toxicity (El-Gibaly, 2002; Xi et al., 2005; Maestrelli et al., 2008). Moreover, the combined administration of ketoprofen with an antioxidant drug such as ascorbic acid could be advantageous in the therapy of colonic adenocarcinoma and other local inflammatory pathologies, with the additional beneficial effect of reducing gastrointestinal injury typically produced by NSAID drugs (Becker et al., 2003).

Different types of delivery devices have been investigated in the development of colon-targeted delivery systems, including polymeric matrices or hydrogels, beads, micro- and nano-particles (Chourasia and Jain, 2003). Among these, multi-particulate systems present several advantages compared to single-unit forms, like in particular a more predictable and reproducible gastrointestinal transit time, less influenced by food, together with minor local irritation and/or adverse effects, and a more consistent drug absorption (Krämer and Blume, 1994; Singh, 2007; Roy and Shahiwala, 2009).

Several approaches have been utilized with the aim of achieving colon targeting, mainly based on the use of pH-sensitive polymers, time-dependent formulations, or microflora-activated systems. Eudragit S100 is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester and has a pH-dependent solubility. It is insoluble in gastric juice and slowly soluble in the

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region of the digestive tract where juices are neutral to weakly alkaline and it was efficiently used as coating for microspheres aimed for colonic delivery (Oosegi et al., 2008).

Time-controlled erodible devices such as wax-based microspheres have been successfully applied to obtain sustained-release of NSAIDs (Adeyeye and Price, 1991, 1994; Maheshwari et al., 2003).

As for microflora-activated systems, various types of natural polysaccharides are currently under extensive investigation as candidates for the design of solid dosage forms for colonic delivery of drugs, in virtue of their specific biodegradation by colonic microflora (Vandamme et al., 2002; Yang et al., 2002). The combined use of these approaches has been successfully applied to obtain an effective colon targeting (Mura et al., 2003a,b,b; Maestrelli et al., 2008).

Chitosan is a no toxic, muco-adhesive and biocompatible polysaccharide, widely used for the development of sustained-release or colon-targeted drug delivery systems (Paul and Sharma, 2000; Agnihotri et al., 2004; Sinha et al., 2004; Anal et al., 2006; Umadevi et al., 2010). Also the use of pectins, hydrophilic polysaccharides mainly consisting of partially methoxylated poly α-(1,4)-D-galacturonic acids, appears of great interest, due to their nontoxicity, low cost, and variety of types. However, their solubility and swelling properties in aqueous media prevent them from efficiently avoiding drug release during transit through the upper gastrointestinal tract, thus making the combined use of other strategies necessary. The use of divalent cations, which can form intermolecular crosslinks between the negatively charged carboxyl groups of the pectin chains, thus producing a more water-resistant network through formation of "egg-box" complexes, can be exploited (Sriamornsak and Nunthanid, 1998; El-Gibaly, 2002; Das et al., 2010). In particular, Ca-pectinate beads, obtained by pectin gelation in the presence of calcium salts, have been investigated for colon-specific targeting of drugs (Rubinstein and Radaï, 1995; Dupuis et al., 2006), even if some authors reported a lack of activity of pectinolytic enzymes on Ca-pectinate coated pellets, beads and microspheres, probably due to the calcium ions presence (Atyabi et al., 2005; Chambin et al., 2006). As a possible alternative strategy, the combined use of a second polymer has been considered, which could interact with pectin by forming a matrix structure able to retain the drug until to its arrival to the colon and then to undergo to rapid degradation by colonic enzymes (Maestrelli et al., 2012). In particular, Ca-pectinate beads prepared in the presence of chitosan can form polyelectrolyte complexes (PEC) (Luo and Wang, 2014), which exhibit the favorable properties of this latter polymer, such as muco-adhesion and enhancer effect, and maintained colonic selective biodegradation (Munjeri et al., 1997; Chang and Lin, 2000; Atyabi et al., 2005).

Also Ca–alginate microspheres have been used as drug carriers for intestinal drug delivery (Khandai et al., 2012), but the high porosity of the hydrogel structure is responsible for a fast release of the entrapped drug, as well as for a low loading ability, due to drug leakage through the pores during the microspheres preparation (Matricardi et al., 2008). Combination of Ca–alginate with chitosan, by interaction between the free carboxyl groups of the first and the aminic groups of the second one, has proven to be a successful strategy not only to overcome these drawbacks, increasing the hydrogel mechanical properties and reducing its permeability, but also to combine the favorable properties of both polymers, improving their ability as carriers for achieving colon-specific drug delivery (Mennini et al., 2012; Crcarevska et al., 2008; Mladenovska et al., 2007; Wittaya-Areekul et al., 2006).

Based on all the above considerations, the present study was undertaken to evaluate the feasibility of developing a potential colon delivery system consisting in microspheres loaded with a combination of ketoprofen and ascorbic acid. With this aim we prepared different types of microspheres, based on waxy materials (Ceresine, Precirol®, Compritol®) or on natural polysaccharides (chitosan, pectins, alginates, also in combination) and compared them in terms of drug entrapment efficiency and drug release profiles.

2. Materials and methods

2.1. Materials

Ketoprofen (KETO), ascorbic acid (ASC), Chitosan (CS, 150 KDa), sodium alginate (Alg) and tripolyphosphate (TPP) were provided by Sigma–Aldrich (Italy). Ceresine (Cer), Precirol® ATO5 (glyceryl palmitostearate, Pre) and Compritol® 888 ATO (glyceryl behenate, Com) were a kind gift of Gattefossé. Cetyl alcohol was from Galeno (Italy). Low methylester amidated citrus pectin (Pec) (esterification degree 25–31%, amidation degree 19–23%) was a generous gift from Herbstreith & Fox (Neuenbűrg, Germany). According to the manufacturers' data, pectin contained between 30 and 35% of glucose in order to standardize its gelling properties. Calcium chloride was supplied by Carlo Erba (Italy). Eudragit® S100 (methacrylic acid copolymer, type B NF soluble at pH ≥ 7) was kindly gifted by Rofarma-Italia S.r.l. All other reagents were of analytical reagent grade.

2.2. Microspheres (MS) preparation

Waxy MS were prepared according to the method used by Maheshwari et al., 2003, with some modifications. Briefly the drugs (50 mg ASC and 200 mg KETO) were added to a mixture of 2 g of wax and 0.5 g of cetyl alcohol (added as co-surfactant and stabilizer), melted at 65 °C, and then mixed under magnetic stirring to 20 mL of 5% w/v PVP aqueous solution. The emulsion was then warmed with 40 mL of 0 °C water.

Polymeric MS were prepared by adding the drugs (50 mg ASC and 200 mg KETO) to 5 mL of polymer aqueous solution and dropping then the dispersion in the cross-linking agent solution. The experimental conditions were set up based on previous studies (Maestrelli et al., 2006, 2008, 2012; Mennini et al., 2008, 2012). In particular, Calcium pectinate MS (MS Pec) were prepared by dropping the pectin suspension (9% w/v) containing the drugs in 30 mL of 10% w/v $CaCl_2$ aqueous solution under magnetic stirring and allowed to stand 3 min in the cross-linking solution (Maestrelli et al., 2012). Alginate MS covered by CS (MS AlgCS) were prepared in two steps: the 4% w/v sodium alginate solution containing the drugs was dropped in 30 mL of 10% w/v $CaCl_2$ aqueous solution under magnetic stirring and allowed to stand 3 min in the cross-linking solution; then, after filtration, the MS were coated by placing them in 10 mL of a 4% w/v CS solution in 5% v/v acetic acid (Mennini et al., 2012). For preparation of chitosan MS (MS CS), the 2% w/v CS suspension in acetic acid (5% v/v) containing the drugs was dropped in 20 mL of 5% w/v TPP aqueous solution and allowed to stand 3 min in the cross-linking solution (Shu and Zhu, 2000; Maestrelli et al., 2006). All the MS batches, after washing and filtration, were dried overnight in oven at 37 °C.

The enteric coating solution was prepared by dissolving Eudragit® S100 in acetone at 12% (w/v). This solvent was selected based on preliminary experiments which showed that it enabled dissolution of the enteric polymer while maintaining the integrity of the MS without affecting the drug content. Coating was obtained by MS immersion in the solution, followed by complete solvent evaporation in a rotary evaporator (Maestrelli et al., 2008).

2.3. Microspheres (MS) characterization

2.3.1. Mean diameter of microspheres

The mean diameter of polymeric MS was determined with a calibre on 20 MS randomly selected for each batch. This method