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Carboxymethyl starch mucoadhesive microspheres as gastroretentive dosage form

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ABSTRACT

Carboxymethyl starch microspheres (CMS-MS) were produced from carboxymethyl starch powder (CMS-P) with a degree of substitution (DS) from 0.1 to 1.5 in order to investigate the influence of DS on physicochemical, drug release and mucoadhesion properties as well as interactions with gastrointestinal tract (GIT) epithelial barrier models. Placebo and furosemide loaded CMS-MS were obtained by emulsion-crosslinking with sodium trimetaphosphate (STMP). DS had an impact on increasing equilibrium water uptake and modulating drug release properties of the CMS-MS according to the surrounding pH. The transepithelial electrical resistance (TEER) of NCI-N87 gastric cell monolayers was not influenced in presence of CMS-MS, whereas that of Caco-2 intestinal cell monolayers decreased with increasing DS but recovered initial values at about 15 h post-treatment. CMS-MS with increasing DS also enhanced furosemide permeability across both NCI-N87 and Caco-2 monolayers at pH gradients from 3.0 to 7.4. Mucoadhesion of CMS-MS on gastric mucosa (acidic condition) increased with the DS up to 55% for a DS of 1.0 but decreased on neutral intestinal mucosa to less than 10% with DS of 0.1. The drug release, permeability enhancement and mucoadhesive properties of the CMS-MS suggest CMS-MS with DS between 0.6 and 1.0 as suitable excipient for gastroretentive oral delivery dosage forms.

1. Introduction

Gastric drug delivery can be desirable for certain drugs having local action or absorption window in the proximal part of the gastrointestinal tract (GIT), degrading under intestinal enzymatic activities or neutral/alkaline conditions or when administered in prandial conditions (Bardonnet et al., 2006). Microspheres as oral multiparticulate drug delivery system tend to be dispersed in regions of the GIT ensuring a more reliable and reproducible release profile and thus a more uniform drug absorption (Asghar and Chandran, 2006). Mucoadhesive microspheres, through a high surface to volume ratio and an intimate contact with mucus layers, can improve drug absorption and prolong dosage form residence time thus increasing bioavailability of released drug. Mucoadhesivity of the microspheres is dependent of the intrinsic nature of the polymer (molecular weight, flexibility of polymer chains, spatial conformation, swelling/water uptake, charge), of the dosage form properties (size, shape, surface, density, drug characteristics and loading) and of surrounding environment (pH, applied strength/shear, initial contact time, temperature, mucin surface charge, mucin turnover) (Ahuja et al., 1997; Andrews et al., 2009; Dodou et al., 2005). The most investigated mucoadhesive excipients are hydrophilic macromolecules forming ionic and hydrogen bonds (Peppas et al., 2009; Smart, 2005). Hydrophilic mucoadhesive microspheres can be tailored to adhere to any human ocular, nasal, buccal, pulmonary, GIT, rectal or vaginal mucosa.

Cross-linked starch due to its cost-effectiveness, large availability, resistance to enzymatic degradation by α-amylase, biocompatibility, biodegradability, non-immunogenicity and mucoadhesive nature (Demirgoz et al., 2000) was considered as excipient for mucoadhesive microsphere drug delivery systems (Ahuja et al., 1997; Dodou et al., 2005). Cross-linked starch microspheres were successfully used for nasal or pulmonary drug

Abbreviations: AP, apical; BCS, biopharmaceutics classification system; BL, basolateral; CD, cytochalasin D; CMS, carboxymethyl starch; CMS-MS, carboxymethyl starch microspheres; CMS-P, carboxymethyl starch powder; DS, degree of substitution; FU, furosemide; GIT, gastrointestinal tract; GU, glucose unit; HAS, high amylose starch; HAS-0, non substituted starch; HAS-0-MS, non substituted starch microspheres; *m*, number of repetitions; *n*, number of experiments; NaCMA, sodium monochloroacetate; P_{app}, apparent permeability coefficient; PL, placebo; SDS, sodium dodecyl sulphate; STMP, sodium trimetaphosphate; TEER, transepithelial electrical resistance.

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delivery (Chaudhari et al., 2010; Pereswetoff-Morath, 1998) but their high hydration and swelling in GIT fluids had limited their used for oral drug delivery due to poor mucoadhesivity.

Mulhbacher et al. (2006) have shown that mucoadhesivity of cross-linked starch at neutral pH can be improved by adding small amount of carboxymethyl groups to starch chains. This carboxymethyl starch (CMS) in its uncross- and cross-linked forms was previously used as hydrophilic anionic excipient for tablet matrix able to control the release of active molecules (Lemieux et al., 2009, 2010; Mulhbacher et al., 2001) and of bioactive agents (Calinescu et al., 2007). The properties of CMS are governed by the degree of substitution (DS) defined as the average number of carboxymethyl group per glucose units (GU) constituting the backbone of starch chains and lies between 0 and 3. CMS can be stabilized by hydrogen bonding between hydroxyl groups, between hydroxyl groups and carboxylic (COOH) groups and between carboxylic groups under acidic conditions or between hydroxyl and carboxylate (COO^-) groups and between carboxylate groups under neutral pH conditions (Lemieux et al., 2009). This study also showed that drug release from CMS of DS from 0.9 to 1.2 was pH-dependent.

The objective of this study was to develop mucoadhesive microspheres using CMS with different DS, to evaluate their physicochemical, drug release and adhesive properties under conditions simulating those prevailing in the GIT (acidic and neutral) and to investigate their interactions with model cells of the GIT epithelial barriers (gastric and intestinal) for the oral delivery of small molecules. Furosemide (FU) was used as a model drug since it is classified by Biopharmaceutics Classification System (BCS) under class IV and exhibits relatively low and variable overall oral absorption (50–60%) occurring site-specifically in the stomach and upper small intestine (Kaukonen et al., 2007). The low bioavailability of FU is due to its poor solubility at low pH (5–20 μg/ml) and to the involvement of intestinal efflux proteins (Kaukonen et al., 2007; Pade and Stavchansky, 1998), despite a moderately high solubility at neutral pH (2.25 mg/ml). The narrow FU absorption window would benefit from increased residence time in the stomach or small intestine (Davis, 2005). It was formulated with CMS-MS also because FU alone had a low permeability across gastric (NCI-N87) (Lemieux et al., 2011) and intestinal (Caco-2) (Pade and Stavchansky, 1998) epithelial barrier models. Finally, FU was chosen due to its anionic nature (weak acid, pK_a 3.8 (Klausner et al., 2003)) that can interfere with the interaction forces between the charged polymer chains.

2. Materials and methods

2.1. Materials

High amylose corn starch (HAS) (Hylon VII) was supplied by National Starch (Bridgewater, NJ, USA). Cytochalasin D (CD), FU, sorbitan monooleate (Span 80), sodium monochloroacetate (NaMCA) and sodium trimetaphosphate (STMP) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Other chemicals were reagent or HPLC grade and used as supplied.

2.2. Synthesis of the CMS powder

CMS at various DS was synthesized from HAS as previously described (Lemieux et al., 2009) except that the NaMCA was slowly added into the reactor at a rate of 2.0 g/min and that the reactor was purged with dry nitrogen (about 200 ml/min) over the 4 h carboxymethylation reaction. The CMS slurries were dried by vacuum drying at 50°C and −20 mm Hg for 24 h to obtain several carboxymethyl starch powders (CMS-P). Using a constant glucose unit (GU)/sodium hydroxide (NaOH) ratio of 1.0/2.0 and by varying the NaMCA/GU ratio from 0.15 to 2.00, CMS-P with DS of 0.12 ± 0.01, 0.59 ± 0.04, 1.01 ± 0.06 and 1.45 ± 0.08 ($n = 3$) (determined by back titration (Stojanovic et al., 2005)) were obtained. For the remaining of this study, the DS of the CMS-P and CMS-MS will be referred as 0.1, 0.6, 1.0 and 1.5. As control, non-reacted HAS (hereto called HAS-0) was prepared under the same conditions as CMS but without adding NaMCA to the reactor medium.

2.3. Preparation of cross-linked CMS-MS and drug loading efficiency

The CMS-MS were produced by an inverse emulsion technique (Atyabi et al., 2006; Hamdi et al., 2001) using STMP as cross-linker. For each CMS-MS preparation, the aqueous phase was prepared by dissolving an amount of 5 g of the CMS-P with DS of 0.1 to 1.5 or HAS-0 in 25 g of 1 M NaOH solution and 0.77 g of FU was added to each CMS solution under magnetic stirring at room temperature. Then, 2 g of STMP, dissolved in 5 g of water, was added to each CMS preparation. According to the DS, the CMS/NaOH/STMP ratio varied from 4.7/3.8/1.0 for HAS-0 to 3.1/3.8/1.0 for CMS DS 1.5 where the amount of hydroxyl groups on the GU available for cross-linking was always in excess. Each solution was vigorously stirred for 0.5 min and then degassed for 0.5 min by sonication (Branson 3510, Danbery, CT, USA) under vacuum. The aqueous phase was immediately introduced in 375 ml of an organic phase consisting of cyclohexane/chloroform mixture (80/20 v/v) containing 0.1% w/w of Span 80 at 30°C in a 1 l jacketed reaction glass vessel (Chemglass, Vineland, NJ, USA) equipped with baffles and servo-controlled speed overhead mixer (Cole-palmer 5000-40, Niles, Il, USA) fitted with a Rushton turbine and maintained at 600 rpm to form water in oil emulsion. The cross-linking reaction was continued under these conditions for 4 h. Then, 400 ml of propan-2-ol (isopropyl alcohol, IPA) containing 0.2 M hydrochloric acid (HCl) was added into the emulsion, and the CMS-MS were filtered and recovered on Büchner (grade 50 cellulose filter paper, Whatman, Kent, UK). The loaded CMS-MS (FU-CMS-MS) and unsubtituted high amylose starch microspheres (FU-HAS-0-MS) (control) were washed with IPA and dried in an oven for 12 h at 40°C. Placebo carboxymethyl starch microsphere (PL-CMS-MS) and unsubtituted high amylose starch microspheres (PL-HAS-0-MS) (control) were also produced under the same conditions but without adding FU into the aqueous phase.

The CMS-MS FU loading was determined by quantifying the amount of dissolved FU from precisely weighed microspheres into 50 mM phosphate buffer (pH 7.2). The suspension was kept light protected for one hour at room temperature and periodically vortexed for two min. Finally, the suspension was filtered at 0.45 μm and the FU concentration in filtrate was measured according to Semaan et al. (2005) using an Agilent series 1100 high-performance liquid chromatograph equipped with a reverse-phase ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) with guard column and a diode-array detector at a wavelength of 274 nm (Agilent Technologies, Germany). The drug loading (entrapment) efficiency was expressed against the theoretical FU loading (10% w/w drug load).

2.4. CMS-MS physicochemical properties

2.4.1. Microspheres morphology and particle size distribution

The morphology of CMS-MS was examined with a Hitachi S-4300SE/N scanning electron microscope (SEM) (Hitachi High Technologies America, Pleasanton, CA, USA), at 20.0 kV and under a magnification of 500×. Samples were coated on metallic stubs using electrically-conductive double-sided adhesive tape and then submitted to gold sputter coating.

The particle size and size distribution of the CMS-MS were determined by optical microscopy using a Leica microscope (model DM2500 M, Frankfurt, Germany) interfaced with a color camera