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Amidated pectin/sodium carboxymethylcellulose microspheres as a new carrier for colonic drug targeting: Development and optimization by factorial design

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ABSTRACT

The colon is a promising site for drug targeting owing to its long transit time and mild proteolytic activity. The aim of this study was to prepare new low methoxy amidated pectin/NaCMC microspheres cross-linked by a mixture of Zn^{2+} and Al^{3+} ions and test their potential for colonic targeting of progesterone. A 2^4 factorial design was carried out to optimize the preparation conditions. High drug entrapment efficiency (82–99%) was obtained and it increased with increasing drug concentration but decreased with increasing polymer concentration. Drug release rate was directly proportional to the microsphere drug content and inversely related to Al^{3+} ion concentration. Drug release was minimal during the first 3 h but was significantly improved in the presence of 1% rat caecal contents, confirming the microsphere potential for colonic delivery. The microspheres achieved >2.3-fold enhancement of colonic progesterone permeability. These results confirm the viability of the produced microspheres as colon-targeted drug delivery vehicle.

1. Introduction

The colon is an attractive site for local and systemic delivery of conventional and labile drugs due to its unique features which include a near neutral pH, much longer transit time, reduced enzymatic activity and great response to absorption enhancers (Maestrelli, Cirri, Corti, Mennini, & Mura, 2008; Oliveira, Ferrari, Carvalho, & Evangelista, 2010; Sinha & Kumria, 2003). Typically, drugs which are destroyed by stomach acidity or metabolized by pancreatic enzymes are minimally affected in the colon (Vervoort, Rombaut, Van den Mooter, Augustijns, & Kinget, 1998). Further, drugs which suffer low oral bioavailability due to poor dissolution and metabolism in the gut wall, such as progesterone might enjoy enhanced bioavailability due to reduced colon enzymatic activity and enhanced dissolution achieved by the colon long transit time (Gadalla, Soliman, Mohammed, & El-Sayed, 2015).

There are several strategies to achieve colon targeting, such as the use of prodrugs (McLeod, Friend, & Tozer, 1994), pressure-based systems (Leopold, 1999; Muraoka et al., 1998), pH-sensitive polymers (Klein, Stein, & Dressman, 2005) and time-dependent formulations (Del Curto et al., 2009). However, prodrugs are new chemical entities requiring extensive pharmacological and toxicological characterization prior to commercialization (Sinha & Kumria, 2001) while time-dependent formulations lack reproducible drug release due to high variability of GI transit times (Lorenzo-Lamosa, Remunan-Lopez, Vila-Jato, & Alonso, 1998). The most promising strategy relies on the use of polysaccharides, which

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are specifically hydrolyzed by the colonic eco-systems. Polymers such as pectin, chitosan and cellulose derivatives are excellent candidates for colonic drug targeting due to their biodegradability, biocompatibility and low cost of production (Mennini, Furlanetto, Maestrelli, Pinzauti, & Mura, 2008; Shukla & Tiwari, 2012).

Pectin is a naturally-occurring anionic polysaccharide consisting of linear chains of α-(1–4) linked D-galactopyranosyluronic acid units (with varying degrees of carboxyl groups methyl esterification) and rhamnogalacturonan units (Thakur, Singh, Handa, & Rao, 1997). It is derived from the cell wall of different types of plants, such as citrus, apple and sugar beet (Rockwell, Kiechel, Atchison, Toth, & Schauer, 2014). Pectin is completely degraded by pectinolytic enzymes produced by the colonic microflora, while it is not affected by gastric or intestinal enzymes making it an excellent candidate for colonic drug targeting (Auriemma et al., 2013). However, using pectin alone is challenged by its high aqueous solubility and swelling which result in rapid drug release in the upper GIT (Prezotti, Cury, & Evangelista, 2014). To overcome this problem, various approaches have been postulated. These included pectin cross-linking with divalent cations, such as Zn^{2+} or Ca^{2+} (El-Gibaly, 2002; Sriamornsak & Nunthanid, 1998), formation of polyelectrolyte complex with polycations, such as chitosan (Birch & Schiffman, 2014; Gadalla et al., 2015; Oliveira et al., 2010), gelatin (Saravanan & Rao, 2010) and pectin amidation (Ahrabi, Madsen, Dyrstad, Sande, & Graffner, 2000). Low methoxy amidated pectin (LMAP), in which some of the carboxylic acid groups are reacted with ammonia, is more resistant to pH variations of the GIT. This makes it more preferable than conventional pectin for colon targeting (Munjeri, Collett, & Fell, 1997b; Mura et al., 2015; Oliveira et al., 2010). Another strategy used combinations of pectin with other polymers, such as ethyl cellulose (He, Du, Cao, Xiang, & Fan, 2008), hydroxypropyl methylcellulose (Hodges et al., 2009), Eudragit® S-100 (Jain, Khare, Agrawal, & Jain, 2009), amylose (Munjeri, Collett, & Fell, 1997a), dextran (El-Gibaly, 2002; Munjeri, Collett & Fell, 1997a) and inulin (Ravi & Kumar, 2008).

Sodium carboxymethylcellulose (NaCMC) is a linear polysaccharide consisting of β-(1-4) linked D-glucopyranose residues in which hydroxyl group protons are replaced by carboxymethyl groups (Banerjee et al., 2012; Ninan et al., 2013). NaCMC consists statistically of 8 different monomeric components (one unsubstituted, three monosubstituted, three disubstituted and one trisubstituted) (Baar, Kulicke, Szablikowski, & Kiesewetter, 1994). It is a water soluble, anionic polymer which is degraded by cellulase enzyme produced by a number of bacteria in the colon (Darvari & Hasirci, 1996). Owing to its slow swelling/erosion properties, NaCMC could be used to delay drug release until reaching the colon. Additionally, it is a cheap polymer that makes the added cost of constructing a controlled-release system more tolerable.

This study aims at the development and optimization of novel LMAP/NaCMC microspheres (MS) for colonic targeting of progesterone (PG) as a model hydrophobic drug. PG is a BCS Class II drug with poor aqueous solubility and high permeability. The extent of absorption of this class depends mainly on the drug dissolution rate and aqueous solubility (Amidon, Lennernäs, Shah, & Crison, 1995). The MS were based on a combination of two colon-targeting strategies; LMAP as bacterially-degradable polymer and NaCMC as a slow-swelling, time-dependent one. We hypothesize that the simultaneous use of these two strategies could efficiently enhance drug delivery to the colon. Blending LMAP and NaCMC might also overcome their individual shortcomings and modulate swelling/erosion properties. Further, cross-linking of LMAP/NaCMC using Zn^{2+} and Al^{3+} ions could improve the MS mechanical and physical strength, as well as their drug-retaining properties.

To optimize the preparation conditions, the experimental approach of 2^4 factorial design with multiple regression analysis was adopted. A great advantage of this model is that all factors are varied simultaneously allowing interaction terms to be postulated. In contrast, the standard procedure of changing one factor at a time is both time- and material-consuming as it requires a large number of experiments and ignores the detection of interactions between the investigated variables.

2. Materials and methods

2.1. Materials

Progesterone was obtained from Pharco pharmaceuticals Inc. (Alexandria, Egypt). Low methoxy amidated pectin type GENU® pectin LM-101 AS with degree of esterification (DE) of 35% and degree of amidation (DA) of 15% was purchased from CPkelco (Copenhagen, Denmark). The viscosity average molecular weight (Mv) of this pectin grade was reported to be 59.3 ± 1.2 kDa (Cheng & Lim, 2004). Sodium carboxymethylcellulose (Mw∼90 kDa, low viscosity grade) with degree of substitution (DS) of 0.65–0.90, degree of polymerization ∼400 was purchased from Sigma Chemical Co. (St. Louis, Mo). Zinc acetate dihydrate, aluminum sulfate, sodium chloride, monobasic sodium phosphate, disodium hydrogen phosphate, hydrochloric acid, Tween 80 and dehydrated ethanol were obtained from Prolabo Chemicals Co. (Cairo, Egypt). All chemicals used were of analytical grade and were used as received.

2.2. Experimental design

LMAP/NaCMC gel microspheres were prepared based on 2^4 full factorial design. The independent variables investigated were the polymer concentration (LMAP/NaCMC ratio 1:1, w/w) (X_1), drug concentration (X_2), $Zn(CH_3COO)_2$ concentration (X_3) and $Al_2(SO_4)_3$ concentration (X_4) (Table 1). The cross-linking agents were investigated as separate independent parameters to elucidate their individual influences on the microsphere properties. The response parameters were the release rate constant in simulated small intestine fluid (K, mg h^{-n}, Y_1) and drug entrapment efficiency (EE, % w/w, Y_2). The matrix of the prepared formulations and their response parameter values are shown in Table 2. Fitting a multiple linear regression model to the response parameters gave a predictor equation which was a first-order, polynomial, having the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots\ldots + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + \ldots\ldots + b_{123}X_1X_2X_3 + \ldots \quad (1)$$

where Y is the level of a given response (dependent variable), b_1, b_2, b_3, ... are the regression coefficients for the independent variables and X_1, X_2, X_3, ... are the coded levels of the corresponding independent variables.

2.3. Preparation of LMAP/NaCMC gel microspheres

Micropheres were prepared by the modified ionotropic gelation technique (El-Gibaly, 2002). Briefly, PG (0.5–1%, w/v) was dispersed in LMAP/NaCMC blend aqueous solution (total polymer concentration:1.25–1.5%, w/v, LMAP/NaCMC ratio: 1:1, w/w) under constant magnetic stirring until a uniform dispersion was obtained. The homogenous, bubble-free slurry was added drop-wise with a disposable syringe (nozzle inner diameter: 1 mm) at a rate of 1 ml/min and falling distance of 5 cm into 25 ml of a gently stirred solution of $Zn(CH_3COO)_2$ and $Al_2(SO_4)_3$ at different concentrations (Table 2). The produced MS were allowed to cure in the cross-linking solution for 2 h and then removed, washed three times with deionized water and dried at room temperature for 48 h. All batches were prepared in triplicate.