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Microparticles based on chitosan/carboxymethylcellulose polyelectrolyte complexes for colon delivery of vancomycin

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ABSTRACT

The aim of this work was to prepare polyelectrolyte complexes based on chitosan (CH) and carboxymethylcellulose (CMC) for colon delivery of vancomycin (VM). Various batches of polyelectrolyte complexes, using three different CH/CMC weight ratios (3:1, 1:1 and 1:3), were prepared and collected as microparticles by spray-drying process. Microparticles were characterized in terms of yield, encapsulation efficiency, drug loading, morphology and mucoadhesion properties. Microparticles water-uptake and VM release as well as its protection against gastric pepsin degradation were also investigated. Finally, the antibacterial activity against *Staphylococcus aureus*, a Gram-positive model strain, was evaluated. The best formulation CH/CMC 1:3 was selected based on the encapsulation efficiency, water-uptake and drug release rate. Moreover, microparticles were able to prevent VM degradation and showed a good antibacterial activity against *S. aureus*. Finally, to improve the release of VM in the colon the selected formulation was coated with lauric acid.

1. Introduction

Inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease are relapsing and chronic inflammatory disorders of the intestinal mucosa (Carter, Lobo, & Travis, 2004; Coco et al., 2013). Conventional therapy has been extensively used during five decades to treat IBD with, however, serious adverse effects (Grimpen & Pavli, 2010; Rogler, 2010). So, different strategies including microparticulate systems prepared by means polyelectrolyte complexes (PECs) have recently been used to achieve site-specific drug delivery after oral administration (Cota-Arriola, Cortez-Rocha, Burgos-Hernandez, Ezquerra-Brauer, & Plascencia-Jatomea, 2013). One of the most common polysaccharides used for the PECs formation is chitosan (CH), a natural linear polycationic polymer obtained by partial N-deacetylation of chitin; it shows interesting properties such as biocompatibility, biodegradability and mucoadhesion and it has been widely used to prepare various oral drug delivery systems (Luppi, Bigucci, Cerchiara, & Zecchi, 2010; Saladini, Bigucci, Cerchiara, Gallucci, & Luppi, 2013). In particular, in the last years CH particles have been developed as new dosage forms for the treatment of colon diseases (Bagre, Jain, & Jain, 2013; Rehman, Amin, & Muda, 2013), confirming chitosan's ability to enhance drug absorption and to improve drug bioavailability. Chemically, in acid medium, the positive-charged amino groups of CH may react with an anionic group of other polymers, such as sodium carboxymethylcellulose (CMC), leading to the formation of PECs. CMC is a linear, long-chain, water soluble, anionic polysaccharide and its use in pharmaceutical industry is due to non-toxicity and non-allergic properties. Moreover, complexes of CH with CMC have been evaluated as a tool for drug delivery in order to provide the required physochemical properties for the design of specific drug delivery systems (Bigucci et al., 2015; Garcia et al., 2015; Lu, Liu, & Ni, 2010).

Vancomycin (VM) is an antibiotic glycopeptide used in the prophylaxis and treatment of serious infections such as the pseudomembranous colitis caused by *Clostridium difficile* (Rahman & Khan, 2013) and of other pathologies caused by Gram-positive bacteria *e.g. Staphylococcus aureus* and other *Staphylococcus* species that are unresponsive to other antibiotics (Zakeri-Milani, Loveymi, Jelvehgari, & Valizadeh, 2013). Nevertheless, the oral administration pathway of VM is mainly limited by enzymatic and acidic degradation in the environment of the stomach and by rapid clearance from the gastrointestinal tract. For this reason, VM needs to be administered intravenously for systemic therapy and it is associated with severe adverse effects (Rybak et al., 2009).

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To increase VM's oral bioavailability, micro/nanoparticles based on chitosan/pectin (Bigucci, Luppi, Monaco, Cerchiara, & Zecchi, 2009; Luppi et al., 2008) and macrogol-based solid dispersion beads (Rahman & Khan, 2013) have been developed.

The aim of the present work was to prepare and characterize polyelectrolyte complexes CH/CMC for colon-specific delivery of VM. Polyelectrolyte complexes were collected by spray-drying and microparticles were characterized in terms of yield, encapsulation efficiency, drug loading, morphology, water-uptake and mucoadhesive properties. *In vitro* drug release studies were performed in order to elucidate the ability of the developed formulations to release VM at different pH. The ability of microparticles to protect VM against gastric pepsin degradation was characterized by *in vitro* studies as well as their antimicrobial activity against *S. aureus*. Finally, coating of selected microparticles with lauric acid was performed in order to improve the release of VM in the colon.

2. Materials and methods

2.1. Materials

Vancomycin was a kind gift from Hikma Italia (Pavia, Italy). Sodium carboxymethylcellulose (CMC, viscosity 850 mPa, 2%; substitution degree 0.78) was purchased from ACEF (Piacenza, Italy). Low molecular chitosan (CH, Mw ≈ 150 kDa, viscosity 20–300 cP, $T = 20\,^{\circ}C$, 1% in 1% acetic acid; deacetylation degree 97%), pepsin from porcine stomach (4500 U/mg protein), mucin Type II (crude from porcine stomach) as well as all other chemicals and solvents (HPLC grade) were purchased from Sigma–Aldrich (Milan, Italy).

For *in vitro* studies phosphate buffers with different pH were prepared with the following compositions per liter: pH 2.0, NaOH 2.6 g, citric acid 6.43 g and HCl 37% 5.72 ml; pH 7.4, $Na_2HPO_4 \times 12\ H_2O$ 2.38 g, K_2PO_4 0.19 g and NaCl 8.00 g.

2.2. Preparation of CH/CMC polyelectrolyte complex microparticles

The microparticles were prepared by polyelectrolyte complexation of positively charged CH with the negatively charged carboxylate group of CMC. CH and CMC were separately dissolved in acetate buffer at pH 5.0 (CH_3COOH 18 mM, CH_3COONa 32 mM; 50 mM ionic strength) at different concentrations (0.03–0.30 mg/ml). Subsequently, different volumes of CMC solution were added to CH solution in order to obtain a final volume (500 ml) with different CH/CMC weight ratios (3:1; 1:1 and 1:3 w/w).

The conversion of the solution from clear/limpid appearance to opaque indicated polyelectrolyte complexes' formation (Zhao et al., 2009). Finally, opalescent solutions were centrifuged and the pellets were recovered and resuspended in acetate buffer at pH 5.0. The resultant suspensions were spray-dried using Mini Büchi spray dryer B-191 (Büchi Labortechnik AG, Flawil, Switzerland). The drying conditions were as follows: inlet temperature 120 °C, outlet temperature 60 °C, air flow rate 700 NI/h, aspirator 100%, pump feed rate 30%.

For the preparation of VM-loaded microparticles, 200 mg of VM were dissolved in CH solution and the final suspensions (0.4 mg/ml VM concentration) were spray-dried as described before.

2.3. Fourier transform infrared spectroscopy (FT-IR)

Infrared spectra of raw material and unloaded microparticles were recorded with a Jasco FT-IR 4100 spectrophotometer (Jasco Lecco, Italy). The samples were prepared as compressed KBr disks.

2.4. Determination of yield, encapsulation efficiency and loading capacity

The yield percentage (% yield) of the process was calculated as follows (Eq. (1)):

$$\%\ \text{Yield} = \text{Total weight of microparticles} \times 100 / \text{Total initial weight of all components in formulation.} \quad (1)$$

The encapsulation efficiency percentage (% EE) and the drug loading percentage (% DL) of VM were determined by dissolved an accurately weighted amount of microparticles in 10 ml of buffer at pH 2.0 for 24 h. The amount of drug was determined by a HPLC method as described in Section 2.9.

The % EE and % DL were calculated using the following equations (Eqs. (2) and (3) respectively):

$$\%\ \text{EE} = \text{Total amount of released drug} \times 100 / \text{Theoretical amount of drug} \quad (2)$$

$$\%\ \text{DL} = \text{Total amount of released drug} \times 100 / \text{Microparticles weight.} \quad (3)$$

2.5. Morphology

The morphology of microparticles was examined by Scanning Electron Microscopy (SEM). The spray-dried microparticles spread out on carbon tape were coated with a thin layer of gold (around 100 nm) under argon atmosphere using a sputter module in a high vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd, Cambridge, UK) at 15 kV under high vacuum conditions.

2.6. Differential scanning calorimetry analysis (DSC)

DSC thermograms of drug and microparticles were recorded on Netzsch DSC200 PC. About 10 mg of the samples were placed in aluminum pans and then hermetically sealed with aluminum lids. Thermal analyses were performed from 25 °C to 300 °C under a dry nitrogen atmosphere with a flow rate of 25 ml min^{-1} and a heating rate of 10 °C min^{-1}.

2.7. Water-uptake studies

The water-uptake ability of microparticles was investigated in two different media in order to simulate the gastric tract and colon conditions, respectively. In particular, buffers at pH 2.0 and 7.4 were prepared as described in Section 2.1. For this study tablets (25 mg) were prepared by direct compression of spray-dried microparticles (compaction force: 18 kN) with a single punch press (type Korsch, Korsch Maschinenfabrik No. 1.0038.86, Berlin, Germany). Tablets were placed on filter paper (0.45 μm) soaked with buffers at pH 2.0 and 7.4 respectively and positioned on top of a sponge (7 cm × 5 cm × 2 cm), previously placed in a Petri dish filled with the same buffer to a height of 0.5 cm (Bigucci et al., 2015). Water-uptake percentage (% WU) was determined as weight increase of the tablet at predeterminated time points within 3 h, accordingly to the following Eq. (4):

$$\%\ \text{WU} = (\text{Weight of hydrated tablet} - \text{Weight of dry tablet}) \times 100 / \text{Weight of dry tablet.} \quad (4)$$