European Journal of Pharmaceutics and Biopharmaceutics 92 (2015) 112–119

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Chitosan based micro- and nanoparticles for colon-targeted delivery of vancomycin prepared by alternative processing methods

T. Cerchiara [a,*], A. Abruzzo [a], M. di Cagno [b], F. Bigucci [a], A. Bauer-Brandl [b], C. Parolin [a], B. Vitali [a], M.C. Gallucci [c], B. Luppi [a]

[a] Department of Pharmacy and Biotechnologies, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy
[b] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark
[c] Department of Chemistry and Chemical Technologies, University of Calabria, Via P. Bucci, 87036 Arcavacata di Rende (CS), Italy

ARTICLE INFO

Article history:
Received 3 July 2014
Revised 26 November 2014
Accepted in revised form 3 March 2015
Available online 11 March 2015

Keywords:
Microparticles
Nanoparticles
Drug delivery
Chitosan
Vancomycin

ABSTRACT

The aim of this work was to prepare chitosan (CH) based particulate formulations for colon delivery of vancomycin (VM). Chitosan microparticles (MPs) and nanoparticles (NPs) loaded with VM were prepared using different CH/tripolyphosphate (TPP) molar ratios and different technological processes. In particular, nanoparticles were prepared by ionic gelation and freeze-drying to recover these particles, or, alternatively, by spray-drying method. Microparticles were prepared using a different spray-dryer. Micro- and nanoparticles were characterized in terms of size distributions by photon correlation spectroscopy (PCS), while encapsulation and drug loading efficiencies were studied using a dialysis method. Fourier Transform Infrared Spectroscopy (FT-IR) was employed to determine the surface composition of the micro- and nanoparticles respectively, and the morphologies of the developed systems were studied by scanning electron microscopy (SEM). Water uptake as well as drug release profiles were also measured. Antibacterial activity against *Staphylococcus aureus*, a Gram-positive model strain, was evaluated. FT-IR results suggested an electrostatic interaction between VM and CH/TPP particles. Moreover, the particles were found to hold a positive zeta-potential, indicating the presence of CH on the particle surfaces. Particle size and encapsulation efficiency were mainly influenced by the different manufacturing processes employed. Nanoparticles obtained by spray-drying showed the best results in terms of water uptake and drug release rate. Moreover, they showed a good bactericidal activity against *S. aureus*.

1. Introduction

It has recently been demonstrated that micro/nanoparticles based on chitosan can be suitable delivery systems for oral site-specific drug delivery [1]. Chitosan (CH) is a natural linear polycationic polysaccharide 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 [2]. In particular, in the last years chitosan particles have been developed as new dosage forms for the treatment of colonic diseases such as ulcerative colitis, Crohn's disease, pseudomembranous colitis and irritable bowel syndrome [3,4], confirming chitosan's ability to enhance drug absorption and to improve drug bioavailability. Vancomycin (VM) is an antibiotic glycopeptide used in the prophylaxis and treatment of serious infections such as the pseudomembranous colitis caused by *Clostridium difficile* [5] and of other pathologies caused by Gram-positive bacteria e.g. *Staphylococcus aureus* and other Staphylococcus species that are unresponsive to other antibiotics [6]. Nevertheless, the oral administration pathway of VM is mainly limited by degradation in the acidic environment of the stomach, by enzymatic degradation, by low epithelial permeability, 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 [7].

To increase VM's oral bioavailability, micro/nanoparticles based on chitosan/pectin [8,9] and macrogel-based solid dispersion beads [5] have been developed. In particular, nanoparticles possess several advantages over other forms of conventional delivery systems such as tablets and capsules. The size of particles plays an important role in colon-targeted delivery: small particles can better attach to mucus layers due to their easier penetration and their relatively small mass. Moreover, nanoparticle accumulation could

* Corresponding author at: Dpt. Pharmacy and Biotechnology, Bologna University, Via San Donato 19/2, 48127 Bologna, Italy. Tel.: +39 (0)51 20 95615.
E-mail address: teresa.cerchiara2@unibo.it (T. Cerchiara).

http://dx.doi.org/10.1016/j.ejpb.2015.03.004
0939-6411/

locally deliver higher amounts of entrapped drugs to the colon, thus leading to a better therapeutic efficacy. Finally, drugs are much stable in the gastro-intestinal tract when encapsulated within nanoparticulate systems [10,11].

The aim of the present work was to prepare CH micro/nanoparticles for colon-specific delivery of VM. CH particles were prepared with different CH/TPP molar ratios and different technological processes. In particular, particles were obtained by ionic gelation and subsequent freeze-drying or, alternatively, by using different types of spray-drying apparatus. Subsequently, CH particles were characterized in terms of size, encapsulation efficiency, drug loading 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, and the antimicrobial activity was estimated.

2. Materials and methods

2.1. Materials

Vancomycin was a kind gift from Hikma Italia (Pavia, Italy). Low molecular chitosan (CH, $M_w \approx 150$ kDa, viscosity 20–300 cP, $T = 20$ °C, 1% in 1% acetic acid; deacetylation degree 95%), penta-sodium tripolyphosphate (TPP), 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, NaOH 2.6 g; citric acid 6.43 g; HCl 37% 5.72 mL and pH 7.4, $Na_2HPO_4 \times 12H_2O$ 2.38 g; K_2PO_4 0.19 g; NaCl 8.00 g.

2.2. Preparation of chitosan nano- and microparticles

2.2.1. Preparation of chitosan-particulates by dispersion method

CH nanoparticles were prepared by an ionic gelation method as reported by Calvo et al. [12] with some modifications. CH was dissolved in acetic acid (0.5% v/v) obtaining the cationic phase (60 mL) in two respective final concentrations (0.16 and 0.20% w/v). Upon addition of TPP solution (0.15% w/v, drop wise) to the cationic phase under constant agitation at room temperature for 10 min chitosan nanoparticles were obtained. The conversion of the solution from clear/limpid appearance to opaque indicated nanoparticles' formation. Two formulations (A and B) with different CH/TPP molar ratios were prepared as shown in Table 1. For the preparation of VM-loaded nanoparticles, 400 mg of VM was dissolved in the cationic phase (60 mL) and the final nanoparticle suspension was prepared as described before.

2.2.2. Obtaining of solid state chitosan particulate by freeze drying

The nanoparticle suspensions were centrifuged (10,000 rpm, 30 min, $T = 25$ °C) in the presence of different cryoprotectants. Specifically, 200 μL of glycerol, mannitol (1% w/v) and sucrose (1% w/v) solutions, respectively, was used in order to investigate the best excipients with respect to the best re-suspension properties. Supernatants were removed and the nanoparticles were re-suspended in deionized water. These suspensions were frozen

Table 1
Preparative characteristics of the different formulations.

Concentration of CH/TPP	CH/TPP molar ratio	Formulation
0.20% w/v CH–0.15% w/v TPP	10:1.5	A
0.16% w/v CH–0.15% w/v TPP	8:1.5	B

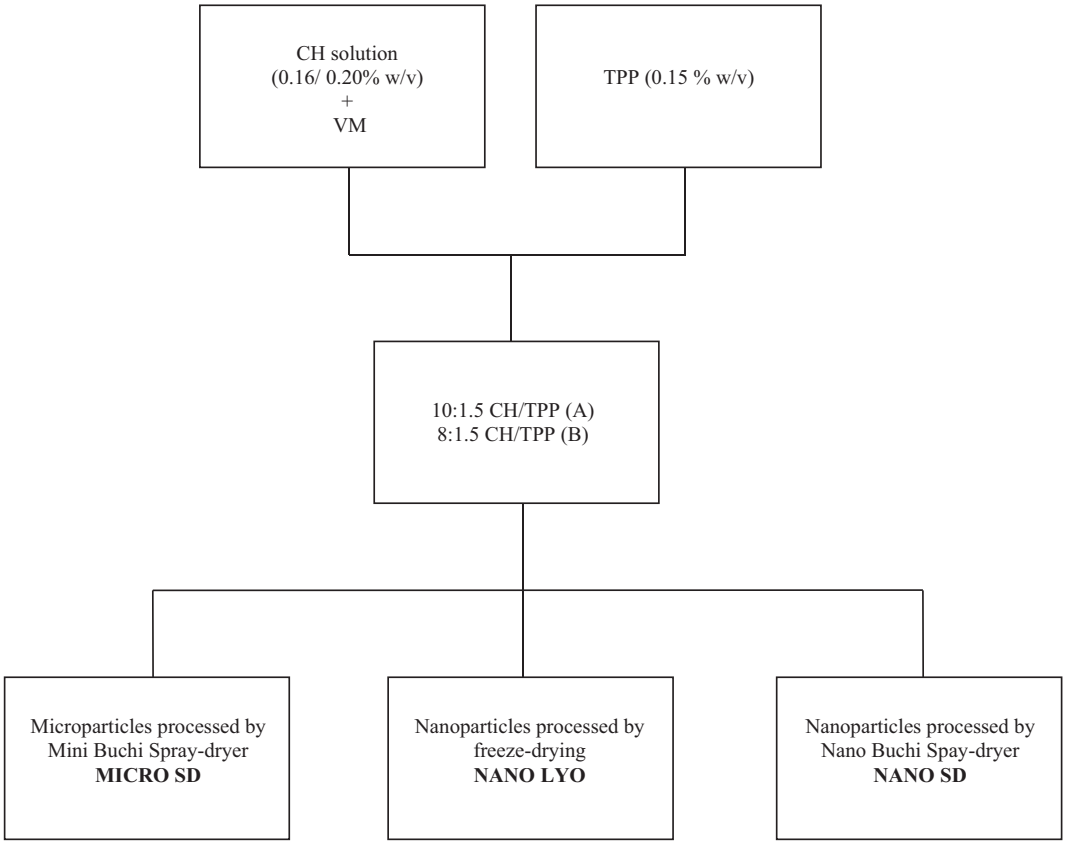

Fig. 1. Flow sheet of the experimental procedure for the micro/nanoparticle preparation.