Spray Freeze-Drying as an Alternative to the Ionic Gelation Method to Produce Chitosan and Alginate Nano-Particles Targeted to the Colon

ALEXANDER GAMBOA,[1] VALERIA ARAUJO,[1] NELSON CARO,[1] MARTIN GOTTELAND,[2] LILIAN ABUGOCH,[1] CRISTIAN TAPIA[1]

[1]Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Independencia, Santiago de Chile, Chile
[2]Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago de Chile, Chile

Received 30 January 2015; revised 26 June 2015; accepted 4 August 2015

Published online 25 August 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24617

ABSTRACT: Chitosan and alginate nano-composite (NP) carriers intended for colonic delivery containing prednisolone and inulin were obtained by two processes. Spray freeze-drying using chitosan (SFDC) or alginate (SFDA) was proposed as an alternative to the traditional chitosan–tripolyphosphate platform (CTPP). NPs were fully characterised and assessed for their yield of particles; level of prednisolone and inulin release in phosphate and Krebs buffers; and sensitivity to degradation by lysozyme, bacteria and faecal slurry. NPs based on chitosan showed similar properties (size, structure, viscoelastic behaviour), but those based on SFDC showed a higher mean release of both active ingredients, with similar efficiency of encapsulation and loading capacity for prednisolone but lower for inulin. SFDC was less degraded in the presence of lysozyme and *E. coli* and was degraded by *B. thetaiotaomicron* but not by faecal slurry. The results obtained with SFDA were promising because this NP showed good encapsulation parameters for both active ingredients and biological degradability by *E. coli* and faecal slurry. However, it will be necessary to use alginate derivatives to reduce its solubility and improve its mechanical behaviour.
 J Pharm Sci 104:4373–4385, 2015
Keywords: nanoparticles; spray freeze-drying; alginate; chitosan; colonic drug delivery; biodegradable polymers

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease that occurs in the middle or lower part of the gastrointestinal tract. IBD includes ulcerative colitis and Crohn's disease. The drugs commonly used are anti-inflammatory agents, 5-aminosalicylates and corticosteroids for the treatment of moderate and severe IBD and immunosuppressive agents for the treatment of the disease in severe stages. A major challenge in the therapy of IBD is the prevention and reduction of drug-related side effects.[1] Chitosan and alginate have been widely studied as drug delivery platforms targeted to the colon because the drug release from these systems is triggered by the enzymatic activity of the colonic resident micro-biota. Additionally, chitosan shows high mucoadhesive properties that have been used to prolong chitosan residence in the gastrointestinal tract.[2–6]

Patients with ulcerative colitis have exhibited both diminished mucus-layer barrier properties because of overall depletion of goblet cells and higher permeability of the intestinal epithelium, allowing nano-composites (NPs) to be more easily transported into the mucosa. Therefore, mucoadhesive NPs would be particularly appropriate for site-specific drug delivery in IBD patients. In addition, the efficient diffusion of NPs through the mucus is critical for drug effectiveness. The advantages of NPs include a tendency to be phagocytosed by macrophages and neutrophils at sites of inflammation and during interactions with the mucosal layer, which is thinner in inflamed tissues.[7–9] Size and charge are major determinants of the particles ability to passively target the inflamed intestinal mucosa and reach maximum retention times in the tissue. A size-dependent accumulation pattern of micro- and nanoparticles has been reported in rats with induced ulcerative colitis, specifically in inflamed intestinal regions. Within the small intestine and the colon, 15% of the 0.1-μm particles were found to adhere to inflamed areas, whereas approximately 5% of particles were found in non-inflamed areas. The same distribution was present but less pronounced for 1-μm beads, and 10-μm particle distribution showed only a minor resemblance to this pattern.[10] NPs based on polivinyl alcohol-polylactide encapsulated in a mixture of chitosan alginate loaded with Lys-Pro-Val (KPV), an anti-inflammatory tripeptide, were produced and evaluated in a mouse model colitis. NPs of 400 nm can overcome physiologic barriers and target KPV to inflamed areas.[11] However, recent studies in human IBD patients who received a rectal enema consisting of either 10^{10} micro-particles or 10^{13} nano-particles demonstrated practically no accumulation of NPs (250 nm) in the inflamed mucosa, though deposition of micro-particles (3.0 μm) was noted.[12] Similar findings were obtained with PLGA-based nano- (300 nm) and microparticles (3.0 μm) where the targeting efficiencies in terms of particle translocation and deposition were investigated in Ussing chamber experiments using intestinal tissue from human IBD patients.[13] The reason for the discrepancy between animal and human studies is unclear.[12] Another relevant aspect to be considered is the surface charges of the NPs, which affect their mobility through the mucus. The diffusion rate of anionic NPs is shown to be 20–30 times faster than that of cationic particles. In contrast, positive chitosan charges support adhesion to the mucins, as described above. Thus, a balance between

Correspondence to: Cristian Tapia (Telephone: +56-2-2-9781640; Fax: +56-2-2-2227900; E-mail: ctapia@uchile.cl)

Journal of Pharmaceutical Sciences, Vol. 104, 4373–4385 (2015)

mucoadhesive and mucus-penetrating properties of the particles is important for efficient delivery of the drug to the inflamed mucosa.[7,8]

The most common procedure for the preparation of chitosan NPs is ionic gelation with tripolyphosphate (TPP) because only mild temperature and pH conditions are used and because the NP size can be controlled by varying the ratio of chitosan to TPP, the pH or the molar mass of chitosan. NPs prepared by dropping a TPP solution in CS showed a *Z*-average of 350–400 nm with a mass ratio of 3:1 to 4:1 (CS:TPP), a CS MW of 350 kDa and DD > 75%.[14] Using CS of MW 150 kDa and a mass ratio of 5:1–6:1 (CS:TPP) yielded NPs with a *Z*-average of 200 nm and a zeta potential of 25 mV.[15] In another procedure, the CS was dropped into the TPP solution. NPs of 120–150 nm measured by transmission electron microscopy (TEM) were obtained with LMW CS and a mass ratio of 5:1 (CS:TPP).[16] The effect of different initial sizes of CS–TPP nano-particles and storage conditions using a phosphate buffer at different pH values (5.5, 7.5 and 9.9) and on storage stability in a phosphate buffer of pH 7.5 at 25°C was evaluated. The size of the nano-particles decreased with the increase of the pH in the solution because CS–TPP NPs have a metastable structure that changes easily with the pH and ionic strength of the solutions.[17]

Spray freeze-drying (SFD) is a method developed to enhance the wetting and dissolution properties of water-insoluble active pharmaceutical ingredients (APIs). SFD is a simple process based on the atomisation of a feed solution containing APIs and/or excipients above the cryogenic liquid surface to produce frozen NPs, which are subsequently lyophilised. The ultra-rapid freezing rate prevents the phase separation of solutes within the feed solution and induces the formation of amorphous structures with high surface areas.[18] This method has not been used in the manufacture of chitosan and alginate NPs and in the present study we propose SFD as an alternative to ionic gelation.

The aim of this work was to compare NPs obtained by the SFD procedure using chitosan (SFDC) and alginate (SFDA) as encapsulating agents with chitosan–TPP (CTPP) NPs obtained by ionic gelation by examining: (1) the encapsulation parameters of prednisolone and inulin; (2) the physicochemical properties of NPs; (3) the release of the active ingredients in compendial and bio-relevant buffers; and (4) the degradation of NPs by lysozyme, bacteria (*E. coli* and Bacteroides) and faecal slurry.

MATERIALS AND METHODS

Materials

Low-molecular-weight chitosan [reduced viscosity ($\eta_{sp/c}$) = 203 (mL/g), viscosity average molar mass (Mv) = 269 kDa and degree of acetylation (DA) = 21.7% determined using conditions described elsewhere[19]], alginic acid sodium salt of low viscosity from *Macrocystis pyrifera* (viscosity of the 2% solution at 25°C 100–300 mPa) and inulin–fluorescein isothiocyanate (FITC) were purchased from Sigma–Aldrich Inc. (St. Louis, Missouri). Prednisolone was obtained from Indukern (Mainland, China). Lysozyme (81,989 units/mg) from chicken egg white was purchased from Fluka (Bornem, Belgium). *E. coli* ATCC25022 (Biomedical Laboratory Reference National Public Health Institute, Santiago, Chile) and *Bacteroides thetaiotaomicron* ATCC 29741 (Medica Tec, S.A., Santiago, Chile) were used in bacterial experiments. All other chemicals used were analytical grade.

NP Preparation

Preparation of CTPP NPs by Ionic Gelation

Twenty-five milligrams of the non-ionic, surfactant polyol Pluronic F-127, 25 mg of FITC-labelled inulin, and 2.5 mL of prednisolone, previously dissolved in ethanol (10 mg/mL), were added to 10 mL of a 1% (w/v) chitosan–acetic acid solution and diluted with distilled water to reach 100 mL. The mixture was left stirring overnight and then filtered through a 0.45-μm membrane. The solution was loaded into two 50-mL syringes mounted on an infusion pump (Model KDS200; KD Scientific©, Holliston, Massachusetts). The solution was pumped at a rate of 1.8 mL/min over 50 mL of an aqueous solution of sodium TPP at 0.3% (w/v). The resulting suspension was centrifuged at 24,000*xg* for 30 min. The pellet was separated from the supernatant and both fractions were separately frozen (−30°C for 1 day) and subsequently freeze dried (−55°C and 6.7 Pa) for 2 days before further characterisation.

Preparation of Chitosan NPs by SFD (SFDC)

The same chitosan solution used in preparation of CTPP was applied to an HPLC pump (Model 510; Waters, Milford, Massachusetts) using the tip of a flattened PEEK tube (polyether ether ketone, length 7 cm, internal diameter 50 μm) to produce the spray solution. One hundred millilitres of the chitosan solution was sprayed onto 200 mL of liquid nitrogen contained in a 400-mL beaker immersed in bucket containing 300 mL of liquid nitrogen. Chitosan solution was sprayed on the surface of liquid nitrogen at a distance of approximately 4 cm at a flow rate of 6.0 mL/min and a fixed pressure of 2.07×10^7 Pa. Liquid N_2 agitations was adjusted to produce a vortex of approximately 4 cm in height. Once all the solution was sprayed, the vessel containing the product was removed and left to evaporate excess N_2. The solid product obtained was extracted from the vessel using a spatula, placed in a plastic Petri dish of 13.5-cm diameter, covered with a layer of aluminium foil with holes to facilitate lyophilisation (−55°C and 6.7 Pa) for a period of 2 days. Subsequently, the sample was ground in a porcelain mortar and then stored at 4°C.

Preparation of Alginate NPs by SFD (SFDA)

Twenty-five milligrams of Pluronic F-127, 25 mg of inulin–FITC, and 2.5 mL of prednisolone, previously dissolved in ethanol (10 mg/mL), were added to 10 mL of an alginate solution in water 1% (w/v), and diluted with water to reach 100 mL. The mixture was left overnight with stirring and then filtered through a 0.45-μm membrane. One hundred millilitres of the alginate solution was applied to an HPLC pump using the same procedure described for SFDC preparation.

Characterisation of NPs

Zeta Potential, Polydispersity Index and Particle Hydrodynamic Diameter

Nano-composites were measured at 25°C using a Zetasizer Nano ZS-20 (Malvern Instruments, UK) operating at 4.0 mW and 633 nm with a fixed scattering angle of 173°. Thirty milligrams of NPs were dispersed in 50 mL of distilled water, left in an orbital shaker at 25 ± 0.1°C and 50 ± 5 rpm for 1–3–6