Release of Prednisolone and Inulin from a New Calcium-Alginate Chitosan-Coated Matrix System for Colonic Delivery

VALERIA ARAUJO,[1] ALEXANDER GAMBOA,[1] NELSON CARO,[1] LILIAN ABUGOCH,[1] MARTIN GOTTELAND,[2] FERNANDO VALENZUELA,[1] HAMID A. MERCHANT,[3] ABDUL W. BASIT,[3] CRISTIÁN TAPIA[1]

[1]Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Av. Vicuña Mackenna 20, Providencia, Santiago de Chile, Chile

[2]Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago de Chile, Chile

[3]Department of Pharmaceutics, UCL School of Pharmacy, University College London, London WC1N 1AX, UK

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ABSTRACT: Putative colonic release formulations of calcium (Ca)-alginate coated with chitosan containing two different actives, prednisolone and inulin, were prepared in three different sizes, beads ($D_{50} = 2104\ \mu m$) and microparticles ($D_{50} = 354$ and $136\ \mu m$). The formulations were tested in standard phosphate buffer and biorelevant Krebs bicarbonate buffer at pH 7.4, and were further evaluated in the presence of the bacterium *E. coli*. Product yield and encapsulation were higher with prednisolone than with inulin. In Krebs bicarbonate buffer, a clear relationship between particle size and prednisolone release was observed. In contrast, release of inulin was independent of the particle size. In phosphate buffer, the particles eroded quickly, whereas in Krebs buffer, the particles swelled slowly. The difference in behavior can be attributed to the formation of calcium phosphate in the phosphate buffer medium, which in turn weakens the Ca-alginate matrix core. In the presence of *E. coli*, the formulations were fermented and the release of prednisolone was accelerated. In conclusion, the buffer media affects formulation behavior and drug release, with the bicarbonate media providing a better simulation of *in vivo* behavior. Moreover, the susceptibility of the formulations to bacterial action indicates their suitability as carriers for colonic drug delivery. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2748–2759, 2013

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INTRODUCTION

It has been shown that the use of bacteria as a trigger mechanism for colonic drug release improves specificity compared with a pH-responsive approach. These bacteria produce enzymes that are capable of breaking down undigested polysaccharides in the colonic contents.[1] Polysaccharides such as alginate and chitosan are preferentially degraded by bacteria in the colon[2–4]; these findings have led to the development of chitosan–alginate drug delivery systems for site-specific drug delivery in the colon.[2–7]

Procedures for the manufacture of particles of calcium (Ca)-alginate core coated with chitosan have been extensively studied. The main formulation factors studied have been the effect of composition of the alginates and the cross-linking ions,[8] the molecular weight and degree of acetylation of chitosan on the formation of the polyelectrolyte complex,[9,10] and coagulation time and the pH of the coagulation medium.[11] One of the most effective procedures is the two-step method, where Ca-alginate microparticles are recovered and subsequently coated with chitosan.[12] The reaction occurs mainly on the surface of Ca-alginate core to form a membrane. It has been reported that a thicker membrane with better antiswelling ability is obtained with low molecular weight chitosan compared with high molecular weight chitosan.[10] Thus, in this work, it has been used to optimize the conditions described in the literature for the two-step method. In this system, we have encapsulated active ingredients (AIs) with high

Correspondence to: Cristián Tapia (Telephone: +562-29781640; Fax: +562-22227900; E-mail: ctapia@uchile.cl)

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molecular weight as proteins and conventional low molecular weight drugs. According to our knowledge, to date, there have been no reports on encapsulation and drug delivery behavior of a combination of high-/low-molecular-weight AIs in this system. In particular, it has been reported in the literature that alginate/chitosan microparticles containing prednisolone[13] and Ca-alginate beads containing inulin[14] but not the combination of both in chitosan–alginate system intended for colon-specific delivery. Some recent studies suggest that prebiotics that produce quite selective changes in the composition of the microbiota may have benefits in irritable bowel syndrome. It has been suggested that inulin should receive more attention for colon-specific delivery of bioactive food components as it is cheap, has many health benefits by itself, and can be applied in combination with almost all encapsulation techniques.[15–17]

The aim of this work was to develop novel formulations of Ca alginate coated with chitosan containing prednisolone and inulin as the AIs with the intention of evaluating drug release in simulated intestinal and colonic conditions in the presence of the bacterium *E. Coli*.

In particular, in this work, we prepare three different sizes of particles from beads to microparticles by dropping or spraying alginate solution into $CaCl_2$ solution, which was then coated by chitosan. The effect of the particle size on the load of the drug was studied, and the drug-dissolution behavior was evaluated based on the swelling/erosion behavior in both phosphate and Krebs buffers. The swelling/erosion data were analyzed according to the obstruction-scaling model proposed by Amsden[18] to estimate the intragel diffusion of prednisolone and inulin in the core of the Ca alginate. Additionally, the formulations were tested against bacterial-strains-cultured *E. coli* ATCC 25922.

MATERIALS AND METHODS

Chitosan (low molecular weight) was obtained from Sigma–Aldrich Inc. (St. Louis, Missouri), which had the following properties: intrinsic viscosity ($\eta_{sp/c}$) = 203 mL/g, viscometric molecular weight (M_v) = 269 kDa, and degree of acetylation (DA) (%) = 21.7. These properties were determined using conditions described elsewhere.[19]

Alginic acid sodium salt of medium viscosity from *Macrocystis pyrifera* (AS) was obtained from Sigma–Aldrich Inc. . The viscosity of the 2% solution at 25°C was 3500 mPas.

1. Prednisolone was obtained from Ferring Pharmaceuticals (København S, Denmark).
2. Inulin–FITC was obtained from Sigma–Aldrich Inc., St. Louis, MO
3. Calcium chloride was supplied from Scharlau Chemie (Barcelona, Spain).
4. All other chemicals used were of analytical grade.
5. *E. coli* ATCC25022 (Biomedical Laboratory Reference National Public Health Institute, Santiago, Chile).

Preparation of Beads and Microparticles Loaded with Prednisolone and Inulin–FITC

The beads and microparticles were made by dispersing 100 mg of prednisolone, inulin–FITC, and Pluronic F-127 (block copolymer of ethylene oxide and propylene oxide, Sigma–Aldrich Inc., St. Louis, MO) in 20 mL of an aqueous solution of sodium alginate (medium viscosity 3500 mPas) at 1% (w/v) with a magnetic stirrer. This dispersion was pumped using a peristaltic pump (Masterflex 7523-35; L/S tubing 14; Masterflex, Barrington, Illinois) at different rates (1–3–5 mL/min) into an automatic spray gun (Walther Pilot mod WA-XV, Wupertal, Germany) fitted with a 0.5- or 1.5-mm diameter nozzle. The dispersion was dropped (beads) or sprayed (microparticles, varying air flow and pressure) into 500 mL of a solution of calcium chloride at 0.5% (w/v) in water. The mixture was stirred with a magnetic stirrer until all of the suspension was added. Then, the particles obtained were separated by using a sieve and were washed three times with water. Next, the particles were immersed in 10 mL of chitosan solution at 1% (w/v) in acetic acid (Aldrich; low molecular weight) for 30 min. Then, the particles were separated by using a sieve and dried in a tray dryer (Labtech mod LDO-080F, Namyangu, Korea) for 24 h at 30°C and sieved through 16 mesh for the beads, 20 mesh for micro-M, and 100 mesh for micro-S. Particles with a moisture content of 10% were obtained.

Determination of the Size of the Beads and Microparticles

Dried bead or microparticles (40 mg) were dispersed in 25 mL of distilled water and observed under a microscope (ZEISS model AXIOSTAR PLUS; magnification 5×; Oberkochen, Germany) equipped with a digital camera (NIKON mod E450; Tokyo, Japan). For each experiment, at least 50 particles were measured. The diameter of the particles was measured using Axiovision 4.8 software (ZEISS, Oberkochen, Germany). The particle size distribution and span were characterized by the ratio of $(D_{90}–D_{10})/D_{50}$, in which D_{90}, D_{10}, and D_{50} represent the diameter below which 90%, 10%, and 50% by diameter of the particles are found, respectively.