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A Comparison of Aerosolization and Homogenization Techniques for Production of Alginate Microparticles for Delivery of Corticosteroids to the Colon

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ABSTRACT

Alginate microparticles incorporating hydrocortisone hemisuccinate were produced by aerosolization and homogenization methods to investigate their potential for colonic drug delivery. Microparticle stabilization was achieved by $CaCl_2$ crosslinking solution (0.5 M and 1 M), and drug loading was accomplished by diffusion into blank microparticles or by direct encapsulation. Homogenization method produced smaller microparticles (45-50 μm), compared to aerosolization (65-90 μm). High drug loadings (40% wt/wt) were obtained for diffusion-loaded aerosolized microparticles. Aerosolized microparticles suppressed drug release in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) prior to drug release in simulated colonic fluid (SCF) to a higher extent than homogenized microparticles. Microparticles prepared using aerosolization or homogenization (1 M $CaCl_2$, diffusion loaded) released 5% and 17% of drug content after 2 h in SGF and 4 h in SIF, respectively, and 75% after 12 h in SCF. Thus, aerosolization and homogenization techniques show potential for producing alginate microparticles for colonic drug delivery in the treatment of inflammatory bowel disease.

Introduction

Corticosteroids are considered one of the most effective therapeutics in the treatment of inflammatory bowel disease (IBD),[1-3] producing consistent and rapid clinical benefit in both ulcerative colitis and Crohn's disease. Hydrocortisone gives a high remission rate in ulcerative colitis of 60%-73% compared to aminosalicylates that are associated with a remission induction of only 20%, thus confining their use to maintenance regimes.[4] However, no other commonly used drug causes as many treatment-related side effects. These include osteoporosis and bone fractures, adrenal insufficiency, and hyperglycaemia which can trigger or worsen diabetes, hypertension, ophthalmologic effects (glaucoma and cataracts), hypokalaemia, myopathy, and psychiatric side effects.[5] These dose-dependent adverse effects could be alleviated by using oral drug-delivery systems that specifically target sites of inflammation in the colon. Moreover, the formulation of microparticulate drug carriers is advantageous in the case of IBD since most patients also suffer from diarrhea, resulting in short gastrointestinal transit times for tablets and pellets (having dimensions ≥200 μm) and therefore the likelihood of a decrease in efficacy. Microparticles smaller than 200 μm have shown more prolonged transit times, thus increasing the opportunity for controlled release of encapsulated drug substances at sites of inflammation.[6,7] Crcarevska et al.[8] produced chitosan-Ca-alginate microparticles loaded with budesonide (BDS), which were effective in treating 2,4,6-trinitro-benzenesulfonic acid—induced colitis in male Wistar rats. Clinical and histological evaluation show that colitis severity (indicated by total score points) was significantly suppressed from 8.8 in the control group that did not receive treatment to 5.1 when rats were treated orally with BDS-loaded microparticles ($n = 5$, $p < 0.05$).

Rodriguez et al.[9] reported enhanced anti-inflammatory activity of BDS, when encapsulated in Eudragit S100 microparticles and administered orally to 2,4,6-trinitro-benzenesulfonic acid—induced colitic rats. Colon/body weight ratio and myeloperoxidase activity decreased significantly compared to the values obtained when the groups were given BDS suspension (5.84 ± 1.73 and 2.79 ± 2.7 vs. 10.04 ± 4.17 and 8.78 ± 3.6, respectively). Total scoring

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of macroscopic and histological damage also show that BDS-loaded microparticles significantly reduced the damage induced in the colonic segments (2.3 ± 1.6) compared to those treated with BDS suspension (4.4 ± 1.1) ($n = 6$, $p < 0.05$).

The primary designs of colon delivery systems are based on transit time,[10,11] enzymatic break down by colon microflora,[12,13] pressure response,[14] and pH-dependence.[15-19]

Colon-specific drug-delivery systems used for the treatment of IBD often rely on pH sensitivity[16] wherein drugs are formulated into solid dosage forms such as tablets, capsules, pellets, microparticles, or nanoparticles and enteric coated using polymers having different pH solubility, principally the methacrylic acid and methylmethacrylate copolymers (Eudragit). However, a serious limitation of this approach relates to the small difference in pH between the lower small intestine or ileum (6.0-7.5) and the colon (6.4-7.6) which can result in premature drug release.

Alginate polysaccharides are derived from kelp and comprise 1→4 linked α-(L)-guluronic (G) and β-(D)-mannuronic (M) acid monomer units. The polymers are well known for their biocompatibility, low toxicity (included in a group of compounds that are generally regarded as safe by the U.S. Food and Drug Administration), relatively low cost, and the ability to form hydrogels under mild, aqueous conditions by the addition of divalent cations such as Ca^{2+}. Alginate has thus been investigated extensively for microencapsulation of diverse entities ranging from conventional drugs to genetically engineered cells designed to secrete biopharmaceuticals.[20,21] The pH sensitivity of alginate has been successfully exploited to control drug release following oral administration.[22-26] Alginate is protonated at pH values lower than its pKa (3.38-3.65),[27] forming a compact alginic acid matrix at gastric pH that acts as a diffusion barrier for encapsulated drug during passage through the stomach.[28,29] In pH media higher than alginate's pKa (correlating with intestinal and colonic fluid), hydration increases over time followed by erosion and solubilization of the alginate hydrogel which facilitate drug release.

A number of techniques have been used to formulate alginate microparticles, ranging from the simple drop technique,[30] to aerosolization,[31] spinning disk atomization,[32] and complex, multistage emulsification processes.[33] The extrusion or drop technique involves droplets of concentrated alginate solution falling from a needle, into calcium-chloride crosslinking solution, resulting in ionotropic gelation of the polymer into a microparticle. Particle size is governed by the size of droplets formed during extrusion and can reach up to a millimeter.[34] Lim and Wan,[30] for example, produced 1-mm spherical beads by extrusion of 2% aqueous sodium alginate solution from a size-14 needle into $CaCl_2$ crosslinking solution. The main disadvantage of this approach is that it is unsuitable for production of microparticles less than 100 μm in size, and deformation of the droplets may occur on impact with the surface of the crosslinking solution, resulting in irregular particle shapes. Spinning-disk atomization produces alginate droplets by centrifugal forces, which are collected and hardened in $CaCl_2$ solution. Aerosolization techniques involve the formation of sprays or aerosols of alginate solution which are deposited into a receiver (crosslinking) phase. Close control over microparticle size in the range 5-200 μm may be achieved by adjusting the spray pressure, liquid flow rate to the spray nozzle, and the distance between the nozzle and surface of the crosslinking solution. Kwok et al.[31] produced Bacillus Calmette Guerin—loaded alginate microparticles using aerosolization with a size range of 5-15 μm. Hariyadi et al.[22] applied the impinging aerosols technique to produce gentamicin sulfate-loaded alginate microparticles with a size range of 10-50 μm. Exposure of droplets of alginate solution to an aerosol of $CaCl_2$ solution in this case, results in efficient contact of the droplet surface with salt solution and avoids droplet deformation due to impact with a liquid surface in a receiver bath. Emulsification techniques involve a complex, multistage approach that is capable of producing particles 1-150 μm in size.[33] Aqueous sodium-alginate solution containing drug is dispersed in an immiscible organic phase to which $CaCl_2$ solution is subsequently added to harden the droplets. However, a major disadvantage of this approach is the use of organic solvents that may lead to toxic residues, and high-burst release of encapsulated drugs is common.[35]

In this study, aerosolization and a homogenization method were compared to assess the potential for production of corticosteroid-loaded alginate microparticles intended for drug delivery to the colon in the treatment of IBD. Alginate's pH sensitivity was exploited to restrict drug release from the microparticles prior to the colon with the aim of providing a high local drug concentration at the site of IBD to increase therapeutic activity and reduce side effects.

Materials and Methods

Protanal® LF 10/60 LS sodium alginate (viscosity of 1% solution at 20°C = 20-70 mPas, molecular weight 180 kDa, percentage guluronic acid: 65%-75%, mannuronic acid: 25%-35%) was supplied by FMC BioPolymer Ltd. (Girvan, UK). Anhydrous calcium chloride (molecular weight: 110.99) (UNILAB) was obtained from Ajax Finechem Pty Ltd. (Sydney, Australia). Hydrocortisone hemisuccinate (HCHS) (water solubility 50 mg/mL) was purchased from Sigma-Aldrich® Pty Ltd. (Castle Hill, Australia). Phosphate-buffered saline (PBS) was obtained from Amresco Inc. (Solon, OH). HCl (0.1 M) and NaOH (0.1 M) were used to adjust the pH of release media to the required level.

Production of HCHS-Loaded Alginate Hydrogel Microparticles

Aerosolization and Diffusion Loading

Alginate microparticles were prepared as previously reported by Hariyadi et al.[36] Aqueous sodium-alginate solution (25 mL, 0.5% and 1% wt/vol) was brought to a spray nozzle (by means of a peristaltic pump [OEM 300; Watson Marlow Pty, Wetherill Park, Australia]) at a flow rate of 10 mL/min (Fig. 1). Aerosolization was achieved using compressed air at 40 or 60 psi, regulated by a control valve (SMC valve AR20-02H, China). Fine alginate droplets were sprayed into 100-mL $CaCl_2$ crosslinking solution of 2 different concentrations (0.5 M and 1 M, respectively) contained in a 500-mL beaker where they were crosslinked by Ca^{2+} ions

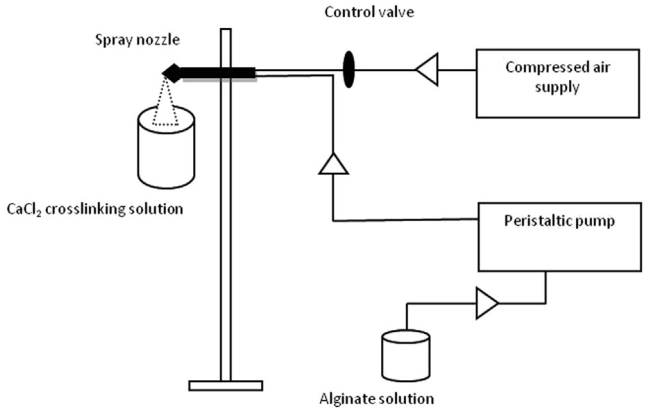

Figure 1. Aerosolization method for production of alginate hydrogel microparticles. Alginate solution is brought to spray nozzle by means of peristaltic pump. Compressed air forces alginate droplets through the nozzle into $CaCl_2$ crosslinking solution.