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Research paper

Facile fabrication of microparticles with pH-responsive macropores for small intestine targeted drug formulation

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ABSTRACT

Oral drugs present the most convenient, economical, and painless route for self-administration. Despite commercialization of multiple technologies relying on micro- and nanocrystalline drugs, research on microparticles (MPs) based oral biopharmaceuticals delivery systems has still not culminated well enough in commercial products. This is largely due to the drugs being exposed to the destabilizing environment during MP synthesis process, and partly because of complicated process conditions. Hence, we developed a solvent swelling-evaporation method of producing pH-responsive MPs with micron-sized macropores using poly(methacrylic acid-co-ethyl acrylate) in 1:1 ratio (commercial name: Eudragit® L100-55 polymer). We investigated the effects of temperature and evaporation time on pore formation, freeze-drying induced pore closure, and the release profile of model drugs (fluorescent beads, lactase, and pravastatin sodium) encapsulated MPs in simulated gastrointestinal tract conditions. Encapsulated lactase/pravastatin maintained > 60% of their activity due to the preservation of pore closure, which proved the potential of this proof-of-concept microencapsulation system. Importantly, the presence of macropores on MPs can be beneficial for easy drug loading, and solve the problem of bioactivity loss during the conventional MP fabrication-drug encapsulation steps. Therefore, pH-sensing MPs with macropores can contribute to the development of oral drug formulations for a wide variety of drugs and bio-macromolecules, having a various size ranging from genes to micron-sized ingredients with high therapeutic efficacy.

1. Introduction

Microparticles (MPs) are widely utilized as delivery vehicles in food, pharmaceuticals, and cosmetics industries due to their capability of encapsulating substances, preserving them in unfavorable conditions, and delivering them at the desired target sites [1–5]. These carriers are broadly classified into two main categories: solid MPs that have drug molecules dispersed in their polymer matrix and hollow MPs with a hollow interior to encapsulate the pharmaceutical ingredients [6,7]. Application-specific functionalization of microencapsulation systems allowed for the production of smart delivery carriers, which respond to the surrounding stimuli (e.g., temperature and pH) [6,8–11]. For oral drug delivery applications, pH-responding carriers are ideal candidates. This is due to their pH-dependent ionization/deionization behavior, which can be utilized for the protection of the drugs/biopharmaceuticals in the unfavorable pH conditions and subsequent delivery to the target sites [12,13].

A number of MP fabrication methods, such as phase separation, spray-drying, and emulsion-solvent evaporation/extraction have been employed to realize the desired features and characteristics in drug delivery carriers [14–18]. The key idea of phase separation method is adding a solvent, which cannot dissolve the drug or the polymer, but is miscible with the solvent containing the drug and polymer. Adding the non-solvent causes the phase separation of the polymer, which leads to the formation of coacervate droplets [19]. However, the use of organic solvent will inevitably raise concerns about both the safety and destabilization of therapeutic biomolecules [20,21]. In the case of the spray-drying method, the fast evaporation of the organic solvent in the drying chamber can be advantageous for the formation of fine MPs with reduced exposure of drugs to the organic solvent [22]. However, this method may involve complicated optimization of the fabrication parameters such as feed rate, temperature, and concentration of different types of excipients and drugs [22–24]. Emulsion-solvent evaporation/extraction method has been most widely employed to fabricate MPs. In this method, the organic solvent can be removed from the emulsion by either leaching the volatile organic solvent in the dispersed

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phase (solvent evaporation) or by transferring the emulsion to a quenching medium (solvent extraction) for the solidification of MPs [25–27]. However, the main drawbacks of these systems are associated with the low throughput, insufficient encapsulation ability, inadequate protection of the drugs in the harsh environment, and inefficient release profiles at the target sites [28–32]. Particularly, the main problem is related to the efficacy of encapsulated drugs. During the encapsulation process, biopharmaceuticals are inherently exposed to the destabilizing elements, such as organic solvents, O-W interface, and hydrophobic interaction with the polymer matrix, because of the process limitations [33–38].

To resolve these problems, Kumar et al. in our group developed MPs with a pH-responsive macropore using a new O/W emulsion/solvent extraction protocol. In pored MPs made of an anionic copolymer (Eudragit® S100, abbreviated as S100), the presence of surface pores is responsible for the direct and solvent-free encapsulation, thus avoiding destabilization of the drugs during the loading process [29]. MPs with macropore exhibited a wide range of advantages originating from their unique architecture, such as the easy loading of drugs and pH-modulated pore opening-closing behavior. Although the few micron-sized MPs developed by Kumar et al. had the capability for the encapsulation of large-size ingredients (i.e. 100 nm drug), bigger MPs with micron-scale pores promise for the encapsulation and delivery of even larger-sized biopharmaceuticals, such as viruses and bacteria and offer an excellent platform for the administration of large doses of drugs. Nevertheless, increasing the size of the droplets in the emulsion drastically decreases the Laplace pressure inside the particles prepared through the emulsion solvent evaporation/extraction techniques, leading to the destabilization of the emulsion droplets [39]. Therefore, the development of a new process to fabricate large MPs with pH-responsive macropores is recognized as another critical challenge for the successful implementation of the oral drug delivery systems.

This research aims to develop an innovative solvent evaporation method for fabricating MPs with pH-responsive macropores that can be used for the small intestine-targeted delivery of oral drugs to the small intestine. Here, we report a facile technique to manufacture MPs with macropores by utilizing the Eudragit® L100-55 polymer (hereafter referred to as L100). This is based on our observation that the L100 polymer powder consists of MPs with a few micron-sized surface pores. The size of these MPs ranges from a few to tens of micrometers. Hence, our approach was to devise a facile method that can modify the original powder to have macroscopic pores, and use them as an oral drug delivery system. As a biocompatible FDA approved enteric formulation, L100 is an anionic environmental-sensitive copolymer with pKa around 5.5, which is not metabolized in the body, remains stable at the gastric environment, and responds to intestinal pH. The original polymer powder, which consists of anionic MPs, was incubated in a single organic phase, dichloromethane (DCM). The reasons for selecting DCM include: 1) the insolubility of the polymer in DCM, 2) the small size of the solvent molecule, which facilitates its diffusion through the polymer matrix, and 3) its low boiling point (39.6 °C), which is much lower than the glass transition temperature (T_g) of the polymer. The suspension went through the solvent evaporation step to form and enlarge the interior void space and the surface pores present in the original polymer particles. This approach incorporates the critical parameters previously investigated in the solvent evaporation method for the creation of surface pores in the MPs [29,40]. As such, the effects of solvent evaporation temperature and the incubation time were evaluated to test the pore size controllability in the MP system. The encapsulation capability of the MPs with macropores was tested by using three different sizes of fluorescent particles (i.e. 100 nm, 1 μm, and 4 μm), and their pH-dependent release behavior was monitored in the simulated GI conditions. The protective efficacy of the MPs was assessed by measuring the remaining activity of β-galactosidase, an enzyme used as a supplement for the lactose intolerance, using the *ortho*-nitrophenyl-β-galactoside (ONPG) assay. Lastly, the viability of the pored MPs as a delivery system was further confirmed by measuring the pH-dependent release profiles through pore closure/opening and the capability of preserving the structural stability of an encapsulated drug, pravastatin sodium [HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor]. For this purpose, the high-performance liquid chromatography-mass spectroscopy (HPLC-MS) and HPLC- UV/Vis methods were utilized.

2. Methods

2.1. Materials

Poly(methacrylic acid-co-ethyl acrylate) in 1:1 ratio, commercially known as Eudragit® L100-55 (hereafter referred to as L100), was obtained from Evonik Canada Inc. (Burlington, Ontario, Canada). 2-Nitrophenyl β-D-galactopyranoside, β-galactosidase from Aspergillus Oryzae, galactose and lactose assay kit, sodium dodecyl sulfate, acetonitrile, and disodium hydrogen phosphate were acquired from Sigma-Aldrich (St Louis, Missouri, USA). Yellow-green fluorescent beads with different sizes (100 nm, 1 μm, and 4 μm) were purchased from Life Technologies (Carlsbad, CA, USA).

2.2. Fabrication of pored MPs and pore closure by freeze-drying

To test the effects of temperature on MPs, 5 g of L100 polymer (abbreviated as $MP_{Original}$) was added to 100 mL DCM in a beaker and then vortexed for 4–5 s. The aliquots of the polymer-DCM suspension were stir-dried overnight at the room temperature (RT). Also, aliquots of the polymer-DCM suspension (10 mL) were put into 250 mL Petri-dishes for solvent removal by incubation in an oven under two different conditions: (1) incubation at 65 °C for 30 min, followed by overnight incubation at 37 °C, and (2) overnight incubation at 37 °C.

To investigate the effect of incubation time of L100 polymer-DCM mixture on the morphology of MPs, the suspension in the sealed container was further stir-incubated at 39 °C (50–60 rpm) in a water bath. This is based on the prediction that the temperature just below the boiling point of the DCM would maximize the diffusion of the organic phase into the polymer matrix. Aliquots (10 mL) were taken over the course of several incubation time intervals (0, 30, and 120 min; abbreviated as $MP_{0\ min}$, $MP_{30\ min}$, and $MP_{120\ min}$, respectively). These samples were dried in a glass petri-dish in an incubator (Isotemp Incubator, Thermo Fisher Scientific). After the initial drying at 65 °C for 30 min, the temperature was brought down to 37 °C for overnight incubation. Subsequently, MPs were collected for further analysis.

Pores on MPs were closed by utilizing a freeze-drying method, following the protocol developed by Kumar et al [29]. Briefly, 20 mg of each sample was suspended in 1 mL DI water, which was then frozen in the liquid nitrogen and freeze-dried using the protocol reported in the above-mentioned study. (AdVantage Pro Freeze Dryer, SP Scientific; Warminster, PA). The presence of residual DCM of MPs was investigated using Fourier transform infrared (FTIR) spectroscopy (Thermo Nicolet NEXUS 870 FTIR ESP, Thermo Fisher Scientific).

2.3. Preparation and pH-dependent release behavior of fluorescent bead-encapsulated MPs

Encapsulation ability of MPs based on the size of the ingredients was tested using three different types of fluorescent beads: 100 nm, 1 μm, and 4 μm. In a typical procedure, 50 mg of MP sample from each condition, i.e. $MP_{Original}$, $MP_{0\ min}$, $MP_{30\ min}$, and $MP_{120\ min}$, was added to 1 mL carboxylate-modified yellow-green fluorescent bead solution. The bead solution had been 10-fold diluted in DI water from the original product. Vacuum on/off cycle was applied four to five times to lower the surrounding pressure, replace the air pockets inside the MPs with the ingredients solution and fluorescent beads through the surface pore [29]. Fluorescent bead-encapsulated MPs were subsequently