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Precipitation kinetics and biological properties of chitosan microparticles produced using supercritical assisted atomization

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ABSTRACT

In this study, the formation of sub-micrometric chitosan (CS) particles via supercritical assisted atomization (SAA) was investigated using supercritical CO_2 spraying medium and aqueous ethanol solution (50%, v/v) as solvent. Results indicated that smaller CS particle were obtained by using low concentrations of the CS solution, high saturator temperatures, and an optimized CO_2-to-CS solution flow ratio. Using the mixed-suspension, mixed-product-removal (MSMPR) population balance model, the precipitation kinetics parameters were determined from CS particle size distributions and the mass-weighted mean sizes of CS particles, which are a function of the precipitation parameters. An efficient tight junction opening effect was induced by the interaction of CS microparticles and Caco-2 cell monolayers as indicated by the marked decrease in the transepithelial electrical resistance (TEER) of Caco-2 cell monolayers incubated with CS particle suspensions. In addition, the reversibility of the TEER changed depending on the viability of the Caco-2 cells, and cell viability was high at low concentrations of CS suspensions.

1. Introduction

Chitosan (CS) is a partially *N*-deacetylated derivative of chitin, which is commonly found in the shells of insect and crustacean and cell walls of some fungi, and is known as the second most abundant natural polysaccharide followed by cellulose. Advantageous characteristics of chitosan have been widely demonstrated, including biocompatibility (Richardson et al., 1999), biodegradability, low toxicity (Kean and Thanou, 2010), cell affinity (Gong et al., 2000), and antimicrobial and antioxidant activities (Rabea et al., 2003; Kim and Thomas, 2007). The protonated form (positively charged) of CS forms electrostatic interactions with negatively charged mucosal surfaces, and thus promotes the transmucosal absorption of drug molecules through the transient widening of the tight junctions (TJs) between epithelial cells (Fernández-Urrusuno et al., 1999). Because of these characteristics, CS has been applied in numerous fields, such as pharmaceuticals (Alpara et al., 2005; Borges et al., 2005), textiles (Hosseini et al., 2013), paper (Kjellgren et al., 2006), agriculture (Grillo et al., 2014), and biotechnology (Rabea et al., 2003; Kim and Thomas, 2007). Molecules cross the intestinal epithelium into the blood by three main pathways, namely the transcellular pathway, paracellular pathway, and carrier-mediated transport. However, hydrophilic molecules cannot diffuse through the cell membrane via the transcellular pathway and instead traverse the epithelial barrier (e.g., TJs) through the paracellular pathway (Ward et al., 2000). The in vitro model of Caco-2 cells is often used to investigate the absorption-promoting property of CS as paracellular permeability enhancers (PPEs), because the Caco-2 cells spontaneously develop highly polarized enterocyte-like characteristics with functional TJs after 3–4 weeks of culture

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Nomenclature

B^0	nucleation rate (no./s m^3)
C	mass concentration of CS in the solution feed stream (mg/mL)
$\overline{d}_{no}$	arithmetic mean particle size (nm)
$\overline{d}_{4,3}$	mass-weighted mean particle size (nm)
F_{CO_2}	volume flow rate of CO_2 at 276 K and 6.5 MPa (cm^3/min)
F_l	volume flow rate of CS solution (cm^3/min)
G	linear particle growth rate (nm/s)
i	order of growth rate in nucleation kinetics equation (Eq. (1))
K_N	constant in nucleation kinetics equation (Eq. (1))
k_V	volumetric shape factor
L	particle size (nm)
$\overline{L}$	arithmetic average size ranging from L_1 to L_2 (m)
n	population density at size L (no./nm m^3)
n^0	population density of nuclei (no./nm m^3)
M_T	suspension density (kg/m^3)
P	pressure (MPa)
R	the volume flow ratio of CO_2 to CS solution
R^2	square of correlation coefficient
T_S	the temperature in saturator (K)
x_i	number of particles within a size range
τ	mean residence time of the mixed suspension (s)
ρ_c	density of CS particle (kg/m^3)
ρ_{CO_2}	density of carbon dioxide (kg/m^3)

superscripts

calc	calculated value
expt	experimental value

on porous membranes under normal culture conditions (Loh et al., 2012). When TJs open in the presence of PPEs, the transepithelial electrical resistance (TEER) is reduced because of an increase in ion flow through TJs. Thus, the absorption-promoting potential of CS can be determined by measuring decreases in the TEER of a cell monolayer incubated with a suspension of CS microparticles (Vllasaliu et al., 2012). In addition, PPEs can also be toxic to cell lines, and Huang et al. (2004) suggested that the in vitro cytotoxicity of CS nanoparticles toward human lung carcinoma cells (A549 cells) was more influenced by degree of deacetylation than molecular weights. They explained that CS particles with a high degree of deacetylation have extended conformation because of high surface charges (zeta potential), and might more readily bind to cell membranes than coiled CSs with a lower degrees of deacetylation. Loh et al. (2012) reported that a higher concentration of CS and smaller CS particles resulted in greater toxicity and proposed a reasonable explanation: larger particles were effectively excluded from cellular uptake into the cytoplasm and a lower concentration of the suspension might mitigate the influence of CS on the TJ integrity.

Conventional methods of CS microparticle production include coacervation (phase separation) (Borges et al., 2005), reverse micelles formation (Liu et al., 2010), spray drying (Cervera et al., 2011), suspension cross linking (Maestrelli et al., 2012), and ionotropic gelation of CS molecular chains with polyanionic tripolyphosphate (Huang et al., 2004; Sadeghi et al., 2008; Loh et al., 2012). Such processes have several limitations, such as the use of organic solvents, the difficulty of separating surfactants, and the requirement of post-processing for reducing solvent residue levels to safe limits. Micrometric particle sizes and low yields are obtained using spray drying (Alpara et al., 2005; Wu et al., 2015). The gelation process requires time-consuming post-treatment steps, such as centrifugation and freeze drying, to obtain CS particle as a dry powder. Many of these common problems could be minimized or eliminated by using supercritical assisted atomization (SAA), which has been suggested as a promising micronization technique (Cai et al., 2008; Wang et al., 2010; Adami et al., 2012; Labuschagne et al., 2014). In SAA, products are obtained in the form of a dry powder through a one-stage process that can be applied to both aqueous and organic solvent systems. SAA has been used to prepare CS microparticles. For example, sub-micrometric particles of CS and CS hydrochloride were prepared using SAA with 1% (v/v) aqueous acetic acid and water, respectively (Reverchon and Antonacci, 2006; Wu et al., 2014). CS-ampicillin coprecipitates and nanostructured CS microparticles loaded with magnetic nanoparticles were also produced using SAA with 1% (v/v) aqueous acetic acid (Reverchon and Antonacci, 2007a; Adami and Reverchon, 2012).

The mechanism of particle formation during SAA process is complicated, involving mass transfer, hydrodynamics, thermodynamics, atomization and precipitation kinetics. SAA fundamentally involves two atomization steps. First, pneumatic atomization produces primary droplets at the exit of the nozzle. These droplets are further disintegrated into secondary droplets through decompressive atomization, which is caused by the rapid release of CO_2 inside the primary droplets (Reverchon and Antonacci, 2006). The droplets are heated in high temperature N_2 in the precipitator to evaporate the solvent and supersaturate the solute in the liquid droplets, thus yielding ultrafine particles. The size and morphology of particles produced using SAA depend on the size of atomized droplets and the solubilized CO_2 in the solution inside the saturator. The CO_2 concentration in the solution must be adequately balanced because a low concentration of CO_2 will not support decompressive atomization and a high concentration of CO_2 in the solution might result in an anti-solvent effect, inducing premature precipitation in the saturator and clogging the nozzle; thus, there is an optimum CO_2 concentration to achieve the required balance. Fortunately, the amount of CO_2 in the solution can be manipulated by the gas-to-liquid flow ratio and is limited by the solubility of supercritical carbon dioxide ($scCO_2$) in solvent. A similar strategy has been reported by Wang et al. (2012) to propose the liquid volume expansion effect as the thermodynamic criterion for choosing the appropriate solvent and optimal operation conditions (including the temperature and pressure of the mixer, and the gas-to-liquid flow ratio) in SAA-HCM process. According to the thermodynamic criterion, a larger liquid volume expansion of the CO_2/acetone mixture (>140%) could markedly decrease the solvation ability, and the solute would precipitate in the mixer before the atomization and fail in the SAA due to the anti-solvent effect. On the contrary, if the liquid volume expansion is less than 80%, SAA could be performed well to afford well-defined spherical cholesterol microparticles. Liparoti et al. (2015) used SAA to produce polyvinylpyrrolidone (PVP) microparticles and demonstrated that the amount of solubilized CO_2 is a crucial factor influencing particle size distribution