



Continuous production of core-shell protein nanoparticles by antisolvent precipitation using dual-channel microfluidization: Caseinate-coated zein nanoparticles



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ABSTRACT

Antisolvent precipitation is commonly used to fabricate protein nanoparticles using a simple batch method that involves injecting a protein-solvent mixture into an antisolvent. In this study, the potential of producing core-shell protein nanoparticles by antisolvent precipitation using a continuous dual-channel microfluidization method was investigated. The solvent phase (zein in ethanol) and antisolvent phase (casein in water) were made to impinge on each other at high velocity, which generates intense shear, turbulent, and cavitation forces that ensure thorough mixing and breakup of the phases. Relatively small core-shell protein nanoparticles ($d < 125$ nm) could be produced using this method when the conditions were optimized. The mean particle diameter decreased with increasing antisolvent-to-solvent ratio, increasing homogenization pressure, increasing ethanol content in the solvent phase, and decreasing zein content in the solvent phase. Depending on the processing conditions employed, zein particles in the range of about 120 nm to over 1000 nm could be produced. The operating conditions were further optimized to increase the final zein concentration and decrease the organic solvent content while still obtaining small particles. The surface potential of the core-shell protein nanoparticles went from positive at low pH to negative at high pH, with a point of zero charge around pH 5. Electron microscopy indicated that the protein particles formed had a roughly spherical shape. The results suggest that the dual-channel microfluidizer could be used to continuously form protein nanoparticles by antisolvent precipitation. Nevertheless, when the microfluidization method was compared with the simple batch method the size of the particles produced under similar conditions were fairly similar.

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1. Introduction

Protein nanoparticles and microparticles may be utilized as natural functional ingredients in numerous applications in the food industry, including as colloidal delivery systems, stabilizers, lightening agents, and texture modifiers (Chen, Remondetto, & Subirade, 2006; Jones & McClements, 2010; Joye & McClements, 2014; Xiao, Li, & Huang, 2016). The ability of protein particles to provide these desirable functional attributes depends on particle properties, such as composition, size, morphology, charge, and polarity (McClements, 2015). Consequently, there is considerable interest in developing processing methods to fabricate protein particles with well-defined physicochemical and structural properties (Matalanis, Jones, & McClements, 2011). A particularly promising method for producing protein particles is antisolvent precipitation (Joye & McClements, 2013; Patel, Hu, Tiwari & Velikov, 2010; Patel, Bouwens & Velikov, 2010; Thorat & Dalvi,

2012). Traditionally, this method is carried out using a simple batch process where a protein/solvent mixture is injected into an antisolvent, which leads to the spontaneous formation of small protein particles. These particles form due to self-association of the protein molecules when the solvent quality changes. For hydrophobic proteins, an organic liquid (such as ethanol) is used as the solvent phase, and water is used as the antisolvent phase (Zhong & Jin, 2009). Conversely, for hydrophilic proteins, water is used as the solvent phase, and an organic liquid is used as the antisolvent phase (Gulseren, Fang, & Corredig, 2012a; Gulseren, Fang, & Corredig, 2012b).

In the current study, a hydrophobic protein (zein) was used to form the core of the protein-nanoparticles, whereas an amphiphilic protein (caseinate) was used to form the shell. This combination was employed because it has previously been shown that relatively small stable core-shell protein nanoparticles can be formed with the antisolvent precipitation method (Davidov-Pardo, Joye, & McClements, 2015; Li et al., 2013; Patel, Bouwens et al., 2010). In this case, a mixture of zein and ethanol are injected into an aqueous buffer solution consisting of caseinate molecules dissolved in water (at a pH away

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from the isoelectric point). After injection, the zein molecules associate with each other when the ethanol diffuses into the surrounding aqueous phase, and the local ethanol concentration surrounding the zein molecules drops below the solubilization limit thereby causing protein precipitation (Zhong et al., 2008). The caseinate is needed in the aqueous solutions to adsorb to the surfaces of the zein particles and cover any exposed non-polar patches. In the absence of this emulsifier, the bare zein nanoparticles have a strong tendency to aggregate due to a strong hydrophobic attraction between the exposed non-polar patches. The adsorption of the caseinate molecules reduces the surface hydrophobicity, and can increase the steric and electrostatic repulsion between the particles, thereby improving their aggregation stability.

As mentioned earlier, protein nanoparticles are normally formed by the antisolvent precipitation using a simple batch approach that involves injecting a protein-solvent mixture into an antisolvent. Recently, it was shown that protein nanoparticles can also be produced by antisolvent precipitation using an impinging jet approach (Li et al., 2014). The authors constructed a mechanical device that consisted of two syringes, one containing the protein-solvent and the other containing the antisolvent. The syringe outlets were connected to a specially designed series of channels that made the two fluid streams impinge upon each other at high velocity. This procedure led to the formation of small protein particles, which was mainly attributed to the intense mixing forces generated when the two liquids collided with each other. In the present study, we investigated the possibility of using a commercially available dual-channel microfluidizer (PureNano®, Microfluidics, Newton, MA) to continuously form protein nanoparticles using the antisolvent precipitation method. The principle behind the microfluidization method is similar to the impinging jet approach, but the former is a continuous method that could be used at an industrial scale (Fig. 1). As part of this study, we examined the impact of various processing parameters on particle properties so that we could form small protein particles with low amounts of organic solvent. We also compared the properties of the protein particles produced using the microfluidization method with those produced using the simple batch injection method to compare their potential advantages and disadvantages.

2. Materials and methods

2.1. Materials

Powdered zein ($\geq 95\%$) was purchased from Sigma Aldrich Corp. (St. Louis MO, USA). Sodium caseinate and ethanol (100% proof) were

purchased from Thermo Fisher Scientific (Waltham, MA, USA). Other chemicals, reagents, and solvents were purchased from Sigma-Aldrich or Thermo Fisher Scientific (Waltham, MA, USA) and were of analytical grade, unless otherwise stated.

2.2. Solution preparation

For the solvent phase, hydrophobic zein from maize was dissolved in a mixture of ethanol and 10 mM citrate phosphate buffer (pH 7). The solution was stirred for 2 h at room temperature to ensure complete hydration of the protein. Solutions were subsequently vacuum filtered with a Whatman Filter (Nr. 42). 1% sodium caseinate was dissolved in 10 mM citrate phosphate buffer (pH 7.0) to prepare the antisolvent phase.

2.3. Nanoparticle preparation

2.3.1. Continuous method

The fabrication of nanoparticles was carried out using a high pressure dual-channel microfluidizer (Microfluidics PureNano, Newton, MA, USA) with Y- and Z-type interaction chambers. The impact of operating pressure (5 to 19 kpsi), solvent-to-antisolvent ratio (1:10 to 1:1), zein concentration (1 to 10% w/v), and ethanol concentration (60–85% v/v) was varied. Based on preliminary experiments, the aqueous phase composition was kept constant, consisting of 1% (w/v) sodium caseinate dissolved in citrate phosphate buffer solution (10 mM, pH 7.0).

The solvent and antisolvent were poured into the two input hoppers of the microfluidizer, and then forced into the mixing chamber under pressure at variable relative flow rates (to alter the solvent-to-antisolvent ratio). Antisolvent precipitation was achieved using a single pass of the samples through the device. After particle formation, ethanol was removed using a vacuum rotary evaporator at 40 °C (Buchi RE 111, Flawil, Switzerland).

2.3.2. Batch method

A simple batch injection method was also used to prepare protein particles by antisolvent precipitation under similar conditions as the dual-channel microfluidizer (with the exception of pressure variation). In this case, the solvent was poured into the antisolvent at a stirring speed of 600 rpm and then the sample was stirred for a further 5 min. Ethanol was removed using the rotary evaporation method mentioned earlier.

Sodium azide (0.02% w/v) was added to all samples after particle formation to prevent any microbial growth during storage.

2.4. Nanoparticle characterization

2.4.1. Particle size and charge

The size and charge of the protein particles was measured using a combined dynamic light scattering/particle electrophoresis instrument (Nano ZS, Malvern Instruments, Malvern, UK). Samples were diluted in 10 mM citrate phosphate buffer solutions at the appropriate pH prior to characterization to prevent multiple scattering effects. The instrument reports the mean particle diameter (Z-average size), polydispersity index (PDI), and ζ -potential of the protein particles. Samples were equilibrated at 25 °C prior to analysis.

2.4.2. pH stability

The ζ -potential versus pH profiles of zein nanoparticles, caseinate/zein-coated nanoparticles, and caseinate solutions (1% w/v) were measured. All samples were prepared at pH 7 and adjusted to pH values ranging from 2 to 9 with phosphoric acid and sodium hydroxide, respectively.

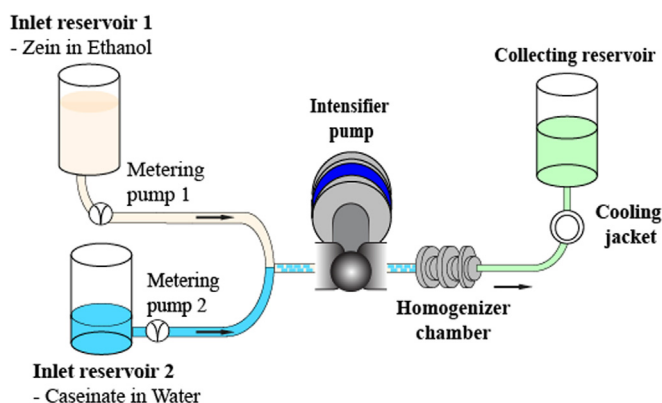


Fig. 1. (a) Schematic illustration of the production of zein nanoparticles using one-step dual-channel microfluidization. A stream of zein in ethanol is made to impinge on a stream of caseinate in water, which results in the formation of zein nanoparticles due to antisolvent precipitation.

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