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Facile continuous production of soy peptide nanogels *via* nanoscale flash desolvation for drug entrapment

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ABSTRACT

A facile continuous production of soy peptide nanogels was demonstrated using a multi-inlet vortex micromixer for the entrapment of active ingredients. The full flow regime in the micromixer was systematically studied to understand the flow impact on nanogel size, drug encapsulation efficiency and drug loading efficiency. Ibuprofen was chosen as a model drug to demonstrate encapsulation capability. The study showed that the nanogel size, drug encapsulation efficiency and drug loading efficiency did not alter significantly as long as the flow rates were in transition and turbulent regimes. The driving force behind the folding of peptides within the microenvironment is kinetic mixing with high flow rates, which dominates in comparison to molecular diffusion, nucleation, and growth. Moreover, the hydrophilic-lipophilic balance of the soy peptides determined the drug encapsulation efficiency and the drug loading efficiency, which did not vary much under different manufacturing parameters. Both characteristics are beneficial to mass production of drug-entrapped peptide. In a 50% water-ethanol mixture, the encapsulation efficiency achieved 70% and the drug loading efficiency was up to 25% in transition and turbulent flow regimes. This work opens the avenues to continuous production of drug-entrapped soy peptide nanogels using a multi-inlet vortex micromixer.

1. Introduction

Protein-based biomaterials, especially food-grade protein, have been considered as the next-generation nanocarrier to synthetic polymers because natural biomaterials are metabolizable, non-antigenic, nontoxic, non-immunogenic, more absorbable, biocompatible, and biodegradable (Wang and Hasan, 2008; Gagner et al., 2014; Hawkins et al., 2008). For various therapeutics and imaging agents with diverse physico-chemical properties among different forms of protein-based nanoparticles, protein nanogels shows superior advantages of much larger surface area, swelling capacity, higher stimuli sensitivity, and higher functionalization capacity to encapsulate, store and release active ingredients (Kabanov and Vinogradov, 2009; Oh et al., 2009; Jahanshahi and Babaei, 2008). Among various food-grade proteins, soy proteins have been the most attractive biomaterials for use as potential amphiphilic entrapment matrix materials mainly due to their naturally balanced composition of hydrophobic and hydrophilic functional groups. The two major components of soy protein are approximately 40% of β-conglycinin, (7S globulin, molecular weight 140–170 kDa) and 60% of glycinin (11S globulin, molecular weight 300–380 kDa) (Teng et al., 2009; Zhang et al., 2012; Liu et al., 2007). β-Conglycinin (7S globulin) is a trimer (α, α′ and β subunits) sharing a large degree of amino acid homologies (Mujoo et al., 2003; Thanh and Shibasaki, 1978). Glycinin (11S globulin) is a hexametric globulin consisting of six subunits of acidic and basic polypeptides, and its quaternary structure is stabilized via disulfide, electrostatic and hydrophobic interactions (Staswick et al., 1984). Both globulins are the precursor of the bioactive soy peptides with plenty of active groups (amino, carboxyl and hydroxyl groups), that offer many functional sites for encapsulation and releasing active gradients (Singh et al., 2014). Because soy peptides share the same composition of amino acids as soy protein but without the globulin structures (food allergens) to cause the allergic response, soy peptide nanoparticles have recently been drawing positive attention. One of the intriguing questions by utilizing bioactive soy peptide chain to entrap small molecules is whether the soy protein is fully unfolded to the primary structure.

Both water-soluble (e.g. soy, albumin) (Teng et al., 2012; Kratz, 2008) and insoluble (e.g. zein, gliadin) (Xu et al., 2011; Ezpeleta et al., 1996) protein biomaterial candidates have been reported as drug, imaging agents, and nutraceutical delivery carriers in a small batch production (Liu and Tang, 2014) The most common method to prepare protein-based nanoparticles (e.g. albumin, whey, zein, soy protein, etc.)

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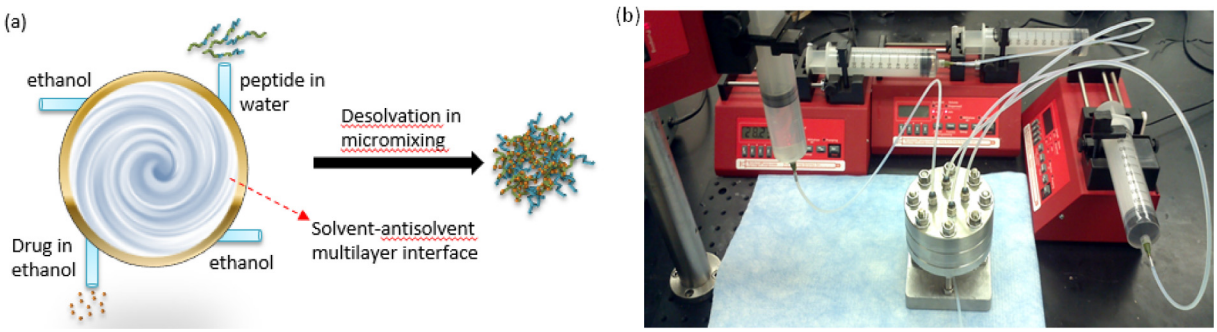

Fig. 1. (a) Illustration and (b) actual experimental setup to prepare soy peptide nanogels using a multi-inlet vortex micromixer (MIVM).

is the desolvation method, a thermodynamically driven self-assembly process (Lohcharoenkal et al., 2014; Weber et al., 2000; Wang et al., 2015; Bi et al., 2017). Self-assembly of protein nanoparticles is triggered by adding the anti-solvent agent to the protein solution dropwise. Denatured protein experiences the differential solubility and subsequently hydrophobic effect as the driving force folds polypeptide chains and exposes the hydrophilic groups in the aqueous phase. Meanwhile, hydrophobic groups on the peptide chains rapidly entrap hydrophobic small molecules to lower the free energy of dissolution. After self-assembly at the solvent-antisolvent interface, the protein nanoparticles are crosslinked. The dropwise desolvation method is simple but time-consuming in adding drop by drop at a time. It is difficult and unfeasible to attempt massive production of encapsulated products by dropwise desolvation method. Furthermore, the dropwise method is limited to precise control of protein nanoparticle formation and encapsulation at limited solvent-antisolvent interface in bulk solution.

In this work, nanoscale flash desolvation in a multi-inlet vortex micromixer (MIVM) is utilized to continuously produce soy peptide nanogels, illustrated in Fig. 1(a). Prud'homme group first presented the design of the MIVM (Liu et al., 2008). After that, many polymeric and lipid nanoparticles were reported by the MIVM production (Shen et al., 2011; Fang et al., 2012; Liu et al., 2008). Recently MIVM has also been combined with spray drying techniques to increase bioavailability of nanoparticle (Suzuki et al., 2017). Inspired by mass production of nanoparticles in the MIVM, we explore the formation of peptides nanogels in the MIVM. The four inlets are connected either with a solvent or with an antisolvent stream. Each stream of the four inlets of the MIVM independently contributes momentum to driving rapid micromixing in the microchamber at different flow rates. Thus, it creates a high supersaturation rate over a shorter period than the molecular nucleation and growth rate. Under this supersaturation environment, nucleus formation starts once the critical nucleation concentration is reached. Consequently, the bulk solute concentration decreases, and nucleation stops. Subsequently, growth of nuclei by solute aggregation continues until the bulk solute concentration reaches the saturation concentration. Concurrently, the macromolecule (e.g. polymer, lipid, protein, etc.) faces differential solubility at the solvent-antisolvent interface under supersaturation which drives the self-assembly. Rapid micromixing with flash solvent replacement leads to the self-assembly of the hydrophobic groups on the hydrophobic solute surface. The growth of nuclei is inhibited because the hydrophilic groups surround by the hydrophobic groups, blocking growth. This mechanism results in formation of uniform nanoparticles with a high production rate. As a comparison, formation and growth of nuclei by desolvation in a batch vessel by the dropwise method cannot reach high local supersaturations immediately, and thus results in wide particle size distributions and uncontrolled aggregation in bulk solution (Johnson and Prud'homme, 2003; Saad and Prud'homme, 2016).

Herein, soy protein was first denatured using urea and then subjected to nanoscale flash desolvation in the MIVM to form peptide nanogels. Peptide nanogels were characterized in terms of the particle size, zeta potential and morphology by varying flow rate, solvent composition, and peptide concentrations. To understand the contribution of the nanoscale differential solubility at the interface to nanogel formation, we compared nano flash desolvation with conventional dropwise desolvation methods as well. Finally, to demonstrate the feasibility of entrapping hydrophobic drugs, Ibuprofen was chosen as the drug model.

2. Material and methods

2.1. Materials

Soy protein Isolate (SPI) (PRO-FAMFAM®981), containing 96.0% protein on a dry basis, was provided by Archer Daniels Midland Company (ADM, Decatur, IL, U.S.A.). Urea (CH4N2O), as a biology-grade denaturing reagent for proteins, was purchased from Thermo Fisher Scientific Inc. (Springfield, NJ, USA). D, L-glyceraldehydes (≥90%) were purchased from Sigma-Aldrich (Saint Louis, MO, USA) as the crosslinker. Ethanol (200 Proof, > 99.2%, Decon Labs, Inc.) and HPLC-grade deionized (DI) water (J.T. Baker®) were used as solvent and anti-solvent. Ibuprofen (≥98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A homebuilt MIVM with a central mixing chamber of 5.92 mm diameter and 1.2 mm depth was fabricated by using stainless steel.

2.2. Preparation of soy peptide by urea denaturation

The denaturation of SPI by urea was utilized to unravel polar/nonpolar and charged amino acids of soy peptides and keep those functional groups bioactive by Van der Waals attraction. SPI powder dissolved in DI water. The SPI suspension was prepared with concentrations from 1 mg/mL to 15 mg/mL. Then each SPI suspension was dissolved with 8 M urea under mild stirring at 23 °C for 20 min and incubated at 60 °C for 60 min. The heated suspension gently cooled down to room temperature for 30 min. After cooling down, the solution was transferred to dialysis tubes (cut-off molecular weight of 12–14 kD, Spectrum Laboratories, Fort Meade, FL, USA). The urea was removed during 24-hour dialysis with three-time replacements of fresh DI water every 8 h to avoid aggregation while the urea concentration decreased. Afterwards, the dispersion was filtered through a polyethylene filter (pore size 2.7 μm) to remove aggregates.

2.3. Preparation of soy peptide nanogels in a multi-inlet vortex micromixer

The fully unfolded soy peptide suspension was used as the raw material to form peptide nanogels. In Fig. 1, four inlets were connected to plastic syringes (MonojectTM 60 mL with Luer-Lock Tip, Covidien) with four Polytetrafluoroethylene (PTFE) tubes (1.5875 mm ID), respectively. Three of the syringes contained pure ethanol as the anti-solvent. One of syringes contained the soy peptide suspensions. We used four syringe pumps (New Era Pump System, Inc., Farmingdale, NY,