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Influence of lysolecithin and Tween 80 on the colloidal stability of branched chain amino acids in a nanosuspension system

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ABSTRACT

This study examined the influence of stabilizers on the solubility and colloidal stability of branched chain amino acids (BCAAs) nanosuspended through high pressure homogenization at 70 °C. Although homogenization increased the initial BCAA solubility, irrespective of pH (pH 3 or 6), homogenization alone was not sufficient to increase their long-term solubility. The incorporation of stabilizers into nanosuspensions increased the saturation concentration of BCAAs but the effect of stabilizers on the increase in the saturation concentration of BCAAs was more pronounced at pH 6.0. At pH 6, Tween 80 dramatically increased the colloidal stability of the BCAA nanosuspensions, independent of the BCAA:stabilizer ratio but not at pH 3. However, the effect of lysolecithin on the colloidal stability of nanosuspended BCAAs varied depending on pH and BCAA:lysolecithin ratio. In lysolecithin-related nanosuspensions, there was no clear relationship between the colloidal stability and nanosuspension conditions including pH and BCAA:lysolecithin ratio. This study could provide a useful information on stabilizer selection for the development of liquid or colloidal products with improved solubility and colloidal stability of nanosuspended BCAAs.

1. Introduction

In the food and pharmaceutical industries, the poor water solubility of materials, such as essential oils, flavors, drugs, and hydrophobic amino acids, greatly hinder their application in product development. This poor water solubility is one of the major obstacles in the development and manufacturing process of these products and is also a reason for their low bioavailability and absorption (Lipinski, 2002). To overcome these limitations of poor water solubility, the food and pharmaceutical industries have employed several techniques, such as solubilization in surfactant solutions, use of cosolvents, micro- and nanoemulsions, liposomes, micelles, incorporation of inclusion complexes, and solid dispersions (Chen, Khemtong, Yang, Chang, & Gao, 2011; McClements, 2010).

Recently, various nanonization strategies, such as nanoemulsion, nanosuspension, nanoencapsulation, and micelles, have emerged as potential delivery systems to increase the solubility of poorly water-soluble functional materials to increase their bioavailability. (Marcato & Durán, 2008; McClements, 2011, 2013; McClements & Rao, 2011). Nanosuspensions are submicron colloidal systems in which hydrophobic particles are generally dispersed into a hydrophilic medium (usually water). High pressure homogenization, a top-down process to create nanosuspensions, does not use harsh solvents. Thus, this approach is widely accepted in the food and pharmaceutical industries. High pressure homogenization is a very useful nanonization technique. High pressure homogenizers generate intense disruptive forces by passing pre-suspended solutions containing poorly soluble materials through a narrow gap in the homogenizer at a high pressure. The solid (or crystalline) poorly soluble materials are mixed into the aqueous phase and are concurrently disaggregated (McClements, 2011). The size of particles can be manipulated by controlling the homogenizer operating conditions such as the intensity and duration of the applied disruptive energy (Jafari, He, & Bhandari, 2007; Qian & McClements, 2011). However, since surface tension due to the large surface area-to-volume ratios of the poorly soluble materials make nanosuspension unstable, nano-sized particles eventually succumb to flocculation and/or coalescence due to Ostwald ripening (Verma, Kumar, Gokhale, &

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Burgess, 2011). A stabilizer is required to maintain the initial size of the nanoparticles and nanodroplets and to give long-term colloidal stability to the nanosuspensions. Stabilizers prevent flocculation of nano-sized particles and droplets through electrostatic repulsion and a steric stabilization effect. The amphiphilic properties of surfactants provide them with several advantages as stabilizers, and their characteristics can dictate the colloidal stability of poorly soluble nanosuspended materials (Mason, Wilking, Meleson, Chang, & Graves, 2006).

Branched chain amino acids (BCAAs), including l-leucine, l-isoleucine, and l-valine, are among the most important essential amino acids and are used in the food and pharmaceutical industries as functional ingredients. BCAAs have variable functions, such as metabolism in muscle cells (Buse & Reid, 1975; Gelfand, Glickman, Jacob, Sherwin, & DeFronzo, 1986; Shinnick & Harper, 1976), as precursors of other amino acids and proteins (Darmaun & Dechelotte, 1991; Ferrando, Williams, Stuart, Lane, & Wolfe, 1995), and as anti-oxidants (Jin et al., 2015). In particular, BCAA supplementation has positive effects on the reduction of exercise-induced muscle damage and the promotion of muscle synthesis (Campbell et al., 2007; Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004), and the decrease in the stress level of the injured rats (Iwasawa et al., 1991). When the BCAAs are supplied in a similar ratio to that they naturally occur in animal protein (2:1:1 = l-leucine:l-isoleucine:l-valine), the beneficial effects of BCAAs could be maximied (Shimomura et al., 2004). However, one obstacle to the utilization of BCAAs in food and pharmaceutical products is their low water solubility. This deficit is the main reason why BCAA-related products are available as capsule, tablet, and powder formations in market (Wu, 2013). In this study, to provide a guideline to select stabilizer for the development of the BCAA-related products having liquid or colloidal form different from the commercially available product forms, this work determined the effect of stabilizers such as Tween 80 and lysolecithin on the solubility and colloidal stability of BCAAs nanosuspended through high pressure homogenization.

2. Materials and methods

2.1. Materials

Branched chain amino acids (BCAA, l-leucine (purity 99.7%), l-isoleucine (purity 99.4%), and l-valine (purity 99.2%)) were a gift from Daesang Inc. (Seoul, Korea). Lysolecithin (Solec™ 8160) and Tween 80 were purchased from DuPont (St. Louis, MO, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. According to the supplier's information on Tween 80, the values for its molecular weight, critical micelle concentration, and hydrophilic-lipophilic balance are 1310 g/mol, 12 μM, and 15, respectively. Additionally, according to the supplier's information on lysolecithin, the values for its molecular weight, critical micelle concentration, and hydrophilic-lipophilic balance are ≈513 g/mol, 20–200 μM, and 9, respectively. All reagents were used as food grade.

2.2. Preparation of the nanosuspensions

The stabilizer solutions were prepared by dissolving lysolecithin or Tween 80 into 10 mM phosphate buffer, and then their values pH were adjusted to 3 or 6. The mixture of BCAAs (l-leucine:l-isoleucine:l-valine = 2:1:1) was directly added into the stabilizer solution to be a final concentration of 5% (w/v). All BCAA suspensions were stirred for 2 h at 25 °C. To prepare the BCAA nanosuspensions, the BCAA suspension preheated at 70 °C was homogenized over 5 cycles at 100 MPa using a high pressure homogenizer (MN400BF, Micronox, Seongnam, Korea). To prevent the crystal creation during homogenization procedure, the homogenization was carried out at 70 °C. The freshly prepared nanosuspension was divided into the storage containers or measurement vials for a Turbiscan LAB optical analyzer (Formulaction, L'Union, France). Nanosuspended BCAAs were stored under inert conditions at 25 °C for up to 20 days.

2.3. Measurement of the solubility of BCAAs in nanosuspensions

Following the method of Starcher (2001) with slight modification, the solubility of the BCAAs was monitored by measuring a ninhydrin reaction in amino acids (l-leucine, l-isoleucine, and l-valine). A 4 N sodium acetate buffer was prepared by dissolving 6.542 g of sodium acetate in 2 mL of glacial acetic acid and distill water up to 10 mL total volume. A stannous chloride solution was prepared by dissolving 1 g of $SnCl_2$ in 10 mL of ethylene glycol. The ninhydrin reagent was prepared by dissolving 0.16 g of ninhydrin in the mixed solution of 6 mL ethylene glycol and 2 mL sodium acetate buffer, followed by the addition of 200 μL of stannous chloride solution under stirring. To measure the BCAA solubility in nanosuspension, sample was collected from the upper layer of storage container and filtered through syringe filter having pore sizes of 0.02 μm to remove the insoluble BCAA crystals. Equal volumes (100 μL) of the ninhydrin reagent and sample solution were added to a glass vial, and the ninhydrin reaction was accelerated by heating at 100 °C for 10 min. The reaction was stopped by adding 1 mL of 50% ethanol followed by cooling at 0 °C for 2 min. The ninhydrin reaction product was measured by using a spectrophotometer (UV1650PC, Shimadzu, Kyoto, Japan) at 575 nm. The content of solubilized BCAAs could be quantified using a calibration curve with BCAA solutions of 0–0.012% (w/v), prepared by dissolving the appropriate amount of BCAA mixture (l-leucine: l-isoleucine:l-valine = 2:1:1) in 10 mM phosphate buffer.

2.4. Measurement of the colloidal stability of BCAA nanosuspensions

The colloidal stability of the BCAA nanosuspensions was assessed by determining the variation in transmission using a Turbiscan LAB optical analyzer (Formulaction, L'Union, France) which is able to analyze physical destabilization of colloidal systems and dispersions. According to the manufacturer, in order to analyze colloidal dispersions including highly concentrated ones, this instrument works on the combination of transmission and backscattering detectors with a vertical scanner. The photons from a near-infrared light source (λ = 880 nm) are sent into the dispersions. These photons, after being scattered many times by the particles or droplets in the dispersion are detected by the 2 detectors located at 180° (for transmitted light) and 45° (for backscattered light) from the light source. Because of transmission intensity depends on particle size and concentration in dispersions (Mengual, Meunier, Cayré, Puech, & Snabre, 1999), the change in particle size is able to be predicted by monitoring the change in the intensity of transmission light.

The prepared nanosuspension was transferred immediately into a measurement vial (70 mm in height, 2 mm in bottom thickness, and 25 mm in internal diameter) to a height of 50 mm (48 mm of sample height). The measurement vial was later placed in the instrument, and then the intensities of transmitted and backscattered lights were periodically recorded by two synchronous optical sensors over the whole sample height (≈55 mm) of the measurement vial with 0.04 mm intervals at 25 °C. The percentage of transmitted light was measured as function of sample height and storage time. Each measurement vial was sectioned into three equally sized layers (bottom layer; 0–16 mm of sample height, middle layer; 16–32 mm of sample height, top layer; 32–48 mm of sample height). The sum of the percentage of transmitted light