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# Physicochemical properties of stable multilayer nanoemulsion prepared via the spontaneously-ordered adsorption of short and long chains

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ABSTRACT

Oil-in-water (o/w) nanoemulsion has great superiority over any other material in the delivery of active substances with low solubility. Whey protein hydrolysate (WPH) is a type of potential emulsifier used to fabricate o/w emulsion because of its rapid adsorption capacity. To further improve the stability of droplets stabilized by WPH, sodium caseinate (SC) and gum Arabic (GA) were combined with WPH to form a short-media-long chain covering layer, which resulted in spontaneously-ordered adsorption on the surface of the droplets. The results showed that the oil droplets were fine and stable, which could be attributed to the rapid adsorption of short chains and the adsorption of SC and GA in sequence. In addition, their stability was enhanced with an increase of the GA ratio, when the GA concentration was 2.0%wt, the emulsion always maintained homogeneity after 30 d of storage.

## 1. Introduction

Nanoemulsions are very fine emulsions made of lipid droplets of nanometric size (d < 200 nm), which have attracted widespread attention since they can act as carriers or delivery systems for bioactive compounds with low solubility (McClements, 2012). A large amount of emulsifiers (20%–30% based on the oil phase wt%) are needed to coat the surface of oil droplets to inhibit droplet aggregation. Interest in replacing synthetic emulsifiers with natural emulsifiers in food and pharmaceutical applications has occurred because of high risk of synthetic emulsifiers (McClements & Gumus, 2016). The natural emulsifiers with the most potential may be proteins and polysaccharides. This is attributed to their wide range of sources and good emulsifying ability (Dickinson, 2015).

To date, several studies have explored the functional properties of protein hydrolysate to broaden the application of proteins with low solubility (Wang et al., 2014, 2016; Yalcin & Celik, 2007). In addition, the enzymatic hydrolysis of proteins can improve their emulsion stability when there is an increase in the exposure of hydrophobic groups to make protein hydrolysate possess amphiphilic characteristics (do Evangelho et al., 2017). Schroder, Berton-Carabin, Venema, and Cornacchia (2017) reported that the adsorption rate of emulsifier on the surface of droplets was closely related to molecular weight and chain length. Therefore, compared with the protein sample, the protein hydrolysate showed advantages in rapid adsorption. However, the covering layer formed by protein hydrolysate was too thin to prevent the coalescence of oil droplets.

Sodium caseinate (SC), a natural protein material, can protect oil droplets from coalescence through electrostatic and steric repulsion because it can form an adequately thick emulsifier layer (Hebishy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017). Therefore, it was used as an emulsifier to stabilize oil droplets (Cho, Yu, & Hwang, 2017; Sabouri, Wright, & Corredig, 2017). In addition, SC is superbly thermostable, which could stabilize the emulsion during sterilization or other heat treatments (Liang et al., 2017).

Polysaccharides are commonly used as stabilizers to formulate o/w emulsions because of their thickening properties and the amount of electrical charges (Nobuhara et al., 2014; Tavernier, Patel, Van der Meeren, & Dewettinck, 2017). Numerous research studies have verified that protein/polysaccharide colloid-stabilized emulsions possess better stability against flocculation and coalescence than emulsion stabilized by pure protein or polysaccharides (Li, Fang, Phillips, & Al-Assaf, 2013; Niu et al., 2015). However, there are very few studies of the protein hydrolysate/polysaccharides complex stabilized in an o/w emulsion.

The objective of this research was to design a hydrolysate/protein/polysaccharide multilayer o/w emulsion. Whey protein hydrolysate (WPH), SC and gum Arabic (GA) were selected as emulsifiers, which could be orderedly adsorbed on the o/w interface according to their

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chain lengths and to fabricate a fine nanoemulsion with high stability. In addition, the effect of GA ratio on the morphological characteristics, stability properties and flow behaviour of multilayer emulsions was investigated.

## 2. Materials and methods

### 2.1. Materials

Corn oil was purchased from the China National Cereals, Oils and Foodstuffs Corporation (Beijing, China). Disodium hydrogen phosphate ($Na_2HPO_4$) and citric acid ($C_6H_8O_7$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). WPH (3–4 kDa) and SC (70–150 kDa) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). GA (280–350 kDa) was purchased from TIC Gums (Belcamp, MD, USA). Sodium azide was purchased from Wuhan Fude Chemical Co., Ltd. (Hubei, China). Fluorescein isothiocyanate (FITC) was purchased from Sigma-Aldrich (Shanghai, China). Nile Red was purchased from Shanghai Guyan Biotechnology Co., Ltd. (Shanghai, China).

### 2.2. Preparation of emulsifier solutions

Six equal amounts of WPH solution were prepared by dissolving 4 g of WPH in 180 mL of buffer solution (10 mM sodium phosphate, pH 7.4) and stirred for 30 min, respectively. Next, 1 g of SC was added to one of the WPH solutions and stirred to obtain a WPH/SC solution. One g of SC and 1, 2, 3 and 4 g of GA were added to WPH solutions and stirred to obtain WPH/SC/0.5% GA, WPH/SC/1.0% GA, WPH/SC/1.5% GA and WPH/SC/2.0% GA solutions, respectively. The solutions were centrifuged to remove insoluble materials.

### 2.3. Preparation of o/w emulsions

The o/w nanoemulsions were prepared by homogenizing 10% (w/w) of the lipid phase with 90% (w/w) of the aqueous phase, and 20 g of corn oil were added to each emulsifier solution, respectively. The o/w mixtures were blended five times using a high-speed blender (IKA T18 basic Ultra-turrax; IKA-Werke, Breisgau, Germany) at 10,000 rpm/min for 2 min each time at room temperature to obtain coarse emulsions, followed by processing with a high-pressure homogenizer (IKA HPH2000/4-SH5; IKA-Werke, Breisgau, Germany) at 100 MPa for five cycles to obtain fine nanoemulsions.

### 2.4. Microstructural analysis

Microstructure of the emulsions was determined using an Axio Vert A1 inverted fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) at 400X magnification. Before analysis, 2 mL emulsions were mixed with a 0.1 mL FITC solution (10 mg/mL dimethyl sulfoxide) to dye the protein and with a 0.1 mL Nile Red solution (1 mg/mL ethanol) to dye the oil phase. Next, 10 μL of each emulsion were placed on a slide under a glass coverslip, which was carefully placed in order to avoid trapping air bubbles, and this was followed by microscopic observation.

### 2.5. Dynamic light scattering (DLS)

The average size of the o/w emulsions was determined by DLS using a Malvern Zetasizer Nano (Malvern Instruments Ltd., Malvern, UK) with a relative refractive index of the dispersed to continuous phases of 1.465.

### 2.6. Determination of zeta potential of nanoemulsions

Nanoemulsions were measured for their electrophoretic mobility by laser Doppler velocimetry using the Malvern Zetasizer Nano. The electrophoretic mobility of each sample was measured three times, and at least 12 runs were performed in each measurement.

### 2.7. Characterization of nanoemulsions

The nanoemulsions were dispersed in deionized water at a concentration of 0.1% at room temperature, and the dispersions were shaken to be uniform. The homogeneous dispersions were left for 4 h, and then were photographed with a Canon IXUS 320 (Canon Co., Ltd, Beijing, China).

### 2.8. Emulsion stability against pH

The freshly prepared emulsion samples were adjusted to pH values ranging from 2 to 10 by adding HCl or NaOH solutions, respectively, and continuously stirring. The samples were then left at room temperature for 24 h before analysis.

### 2.9. Emulsion stability against temperature

The freshly prepared emulsion samples were incubated at fixed temperatures (30–90 °C) for 30 min, while being stirred continuously. Then, the samples were cooled to room temperature and left for 24 h before analysis.

### 2.10. Emulsion stability against ionic strength

For the ionic strength stability test, the emulsions were also prepared with a buffer solution (10 mM). The ionic strength was adjusted to 0, 100, 200, 300, 400 and 500 mM by the addition of the proper amount of NaCl with continuous stirring, respectively. The samples were then left to stand at room temperature for 24 h before analysis.

### 2.11. Emulsion storage stability and creaming index

The measurements of storage stability and creaming index of the emulsion were done according to Piriyaprasarth, Juttulapa, and Sriamornsak (2016). All emulsions were transferred into glass vials, sealed and then stored at ambient temperature for 7, 21 and 30 d. After storage, some emulsions were separated into the 'cream layer' at the top (optically opaque) and the 'serum layer' at the bottom (transparent). The nanoemulsions were photographed at 7, 21 and 30 d intervals with a Canon IXUS 320 (Canon Co., Ltd, Beijing, China). The creaming index was calculated using Eq. (1):

$$\text{Creaming index}\,(\%) = H_S/H_T \quad (1)$$

where $H_S$ is the height of the serum layer measured after a certain time of storage and $H_T$ is the total height of the emulsion.

### 2.12. Interfacial and surface tension measurements

The interfacial and surface tensions of different emulsions were measured using a DCAT21 fully-automatic surface tension meter (Dataphysics Corporation, Regensburg, Germany) with a Wilhelmy plate.

### 2.13. Rheological properties

The continuous shear test of emulsions was carried out using a DHR3 rheometer (TA Instruments, West Sussex, UK), and a cone-plate geometry of 40 mm diameter with a 4° cone angle and 1 mm gap was employed. Apparent viscosities of emulsions were measured on shear rate ramp-up from 0.1 to 100 $s^{-1}$.