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Optimization of folic acid nano-emulsification and encapsulation by maltodextrin-whey protein double emulsions

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ABSTRACT

Due to susceptibility of folic acid like many other vitamins to environmental and processing conditions, it is necessary to protect it by highly efficient methods such as micro/nano-encapsulation. Our aim was to prepare and optimize real water in oil nano-emulsions containing folic acid by a low energy (spontaneous) emulsification technique so that the final product could be encapsulated within maltodextrin-whey protein double emulsions. A non ionic surfactant (Span 80) was used for making nano-emulsions at three dispersed phase/surfactant ratios of 0.2, 0.6, and 1.0. Folic acid content was 1.0, 2.0, and 3.0 mg/mL of dispersed phase by a volume fraction of 5.0, 8.5, and 12%. The final optimum nano-emulsion formulation with 12% dispersed phase, a water to surfactant ratio of 0.9 and folic acid content of 3 mg/mL in dispersed phase was encapsulated within maltodextrin-whey protein double emulsions. It was found that the emulsification time for preparing nano-emulsions was between 4 to 16 h based on formulation variables. Droplet size decreased at higher surfactant contents and final nano-emulsions had a droplet size < 100 nm. Shear viscosity was higher for those formulations containing more surfactant. Our results revealed that spontaneous method could be used successfully for preparing stable W/O nano-emulsions containing folic acid.

1. Introduction

Folates are generally thought of as water-soluble vitamins belonging to the B group complex. As folates are synthesized only by microorganisms and plants, humans depend on a variety of dietary sources for the vitamin [1,2]. Folic Acid (FA), also known as pteroylglutamic acid, is the simplest form of folate, which is essential to human health facilitating the single carbon transfer reaction for the synthesis of basic constituents of DNA and RNA which provide the genetic basis of life [3]. FA is composed of three moieties (Fig. 1): a bicyclic 6-methylpterin ring, p-amino benzoic acid and a single molecule of L-glutamic acid, each of which has no vitamin activity when separated [4].

Research over recent decades has shown that low or inadequate folate concentrations may contribute to some malfunctions and disorders [2]. In many countries, particularly the developing populations, people suffer from folate deficiency. Cereal flours have been a primary candidate for fortification as they are consumed by most of the people [3,5,6]. Fortification with FA has some possible limitations since added vitamin may be lost during processing and handling due to its high sensitivity to heat, oxidation, pH, and other environmental conditions. Due to its properties as a vitamin and medicinal compound, FA must be protected against environmental conditions and should have a controlled release. There are several ways to encapsulate and protect hydrophilic bioactive components such as FA including liposomes, multiple emulsions, solid fat particles, biopolymer complexes, niosomes, and biologically derived systems [7,8].

Nano-emulsions are gaining increasing attention in the food and pharmaceutical industry as a novel delivery system for bioactive ingredients [9,10]. Real nano-emulsions are those containing dispersed phased droplet sizes of less than 100 nm, which are called micro-emulsions [11]. The potential benefits of micro-emulsions include optical clarity, high and favorable stability to gravitational separation, flocculation and coalescence, and improved absorption and bioavailability of functional and bioactive components [12,13]. A micro-emulsions is a thermodynamically stable system which forms spontaneously with a totally clear appearance [14].

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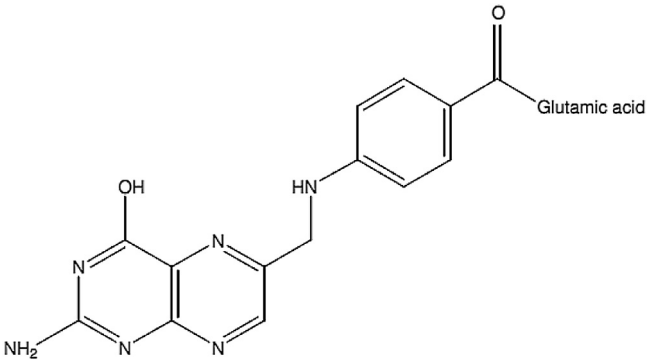

Fig. 1. Chemical structure of folic acid.

The pharmaceutical industry is the dominant field where most applications of nano-emulsions are proposed. Extensive research has been conducted on a variety of drug delivery systems with enhanced solubilization of poorly soluble drugs and improved bioavailability following incorporation into nano-emulsions [15,16]. In the food industry, many food-derived bioactive compounds demonstrate significant health benefits when consumed in relatively high concentrations. Unfortunately, most of these compounds exhibit poor solubility and bioavailability in aqueous-based foods. Recently the development of nano-emulsions loaded with lipophilic and hydrophilic food components has demonstrated the potential of nano-emulsions as a carrier to deliver these bioactive ingredients in food applications [17–19].

Nano-emulsions can be produced by high energy or low energy techniques [20]. High energy methods include homogenizing and sonication devices which are traditionally used in industrial operations because of the flexible control of emulsion droplet size distribution and ability to produce fine emulsions from a wide variety of materials [21,22]. Nano-emulsions produced using low energy methods are called micro-emulsions, which are produced using techniques such as phase inversion composition (PIC), phase inversion temperature (PIT), and spontaneous emulsification [23].

High levels of surfactant and co-surfactant, and long emulsification time limits application of low energy methods for food and pharmaceutical industries. The main goal of this research was optimization of producing micro-emulsions containing folic acid dispersed in canola oil through response surface methodology and its encapsulation within double emulsions of maltodextrin-whey protein in order to protect it from deteriorating environmental and process conditions. Our specific target was to reduce the usage of surfactant and reach the minimum time of emulsification by a low energy nano-emulsion formation technique.

2. Materials and methods

Folic acid (purity > 97%, molecular weight 441.4) and Span 80 (sorbitan mono oleate) was purchased from Sigma-Aldrich Co. (St. Louis, MO), and Merck Chemicals Co. (Germany), respectively. Canola oil was purchased from a local market. Double distilled water was used for preparing W/O micro-emulsions. Whey protein concentrate (80% protein) and maltodextrin (DE = 16–20) were obtained from Arla (Denmark) and Qinhuangdao Starch Co., respectively.

2.1. Micro-emulsion production

W/O micro-emulsions were prepared by spontaneous emulsification according to previously mentioned procedures for making O/W emulsions [24,25] with some modifications. Aqueous phase was prepared by mixing FA solution and Span 80 using a magnetic stirrer (IKA, Germany) at 1000 rpm and then added drop wise to oil phase while magnetically stirring.

2.2. Biopolymer solution preparation

Firstly, a 50% w/w maltodextrin solution was prepared by dissolving maltodextrin into deionized powder. Then, aqueous solution of whey protein concentrate was prepared by dispersing 8 g of WPC powder into deionized water to obtain 100 g solutions containing 0.02% sodium azide as an antimicrobial agent. Solutions were gently stirred for at least 30 min on a magnetic stirrer. Maltodextrin and WPC solutions (in a ratio of 1–1) were mixed together and stored overnight at room temperature for complete hydration of biopolymers and their pH was adjusted to 6.0 using HCL (0.1 M).

2.3. Preparation of W/O/W double emulsions

W/O/W double emulsions were prepared by gradually adding W/O nano-emulsions into the outer aqueous phase of mixed biopolymer solutions (WPC/maltodextrin) while blending by a homogenizer (Heidolph Silentcrusher, Germany) at 12000 rpm for 5 min at 10 °C, and then these coarse emulsions were further emulsified using mentioned homogenizer at 15000 rpm for 8 min at 10 °C [8].

2.4. Droplet size measurement

Droplet size of micro-emulsions was measured firstly through microscopic pictures taken from a Zeisss optical microscope (Germany) and analyzed by ImageJ software [26]. Some samples were analyzed simultaneously using a dynamic light scattering method (Zetasizer Nano Zs, Malvern Instrument, Malvern, UK). To avoid multiple scattering, all samples diluted using 0.1% SDS.

2.5. Shear viscosity

Effect of composition and preparation conditions on viscosity was measured using a Brookfield viscometer (LVDV Pro II, Brookfield Engineering Laboratories, USA) by a spindle S34 [27].

2.6. Color measurement

For evaluating the effect of composition and formulation conditions on color of micro-emulsions, color values ($L^*a^*b^*$) were measured by image analysis using Image J software [26]. We have reported just L value as it corresponds well with the transparent appearance of micro-emulsions.

2.7. Spray drying of double emulsions

The infeed double emulsions were transformed into encapsulated powders in a lab spray drier (Model SP1500, Fanyuan Instrument Co., Shanghai, China) equipped with a pressure air atomizing nozzle at 2.5 bar air pressure, inlet air temperature of 180 ± 5 °C, and outlet air temperature of 90 ± 5 °C with a feed flow rate of 450 mL/h. The dried powder was collected and stored in dark bottle, air tight containers at 4 °C until further analysis.

2.8. Encapsulation efficiency of folic acid

It was necessary to analyze encapsulated folic acid powders in terms of total content and surface content of folic acid in final powders. For surface content, 0.5 g of each sample was dispersed in 20 mL hexane, vortexed for 2 min and filtered using Whatman filter paper no. 41. After adding 10 mL ethanol, the folic acid content was determined spectrophotometrically at 282.5 nm