Food Hydrocolloids 52 (2016) 369–377

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Preparation and optimization of Pickering emulsion stabilized by chitosan-tripolyphosphate nanoparticles for curcumin encapsulation

Bakht Ramin Shah [a, b], Yan Li [a, b], Weiping Jin [a, b], Yaping An [a, b], Lei He [a, b], Zhenshun Li [a, b], Wei Xu [a, b], Bin Li [a, b, *]

[a] College of Food Science and Technology, Huazhong Agriculture University, Wuhan, 430070, China
[b] Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, China

ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form
6 July 2015
Accepted 16 July 2015
Available online 18 July 2015

Keywords:
Chitosan-tripolyphosphate nanoparticles
Pickering emulsion
Curcumin encapsulation

ABSTRACT

In recent decades the preparation of Pickering emulsion has attracted much attention due to a wide range of its useful applicability in cosmetic, food and pharmaceutical industries. The objective of the current study was to optimize the preparation of Chitosan-tripolyphosphate (CS-TPP) nanoparticles stabilized Pickering emulsion as a delivery system for curcumin. The CS-TPP nanoparticles (NPs) were prepared by the ionic gelation techniques between CS and TPP. The effect of preparation conditions, storage time, pH and salt on the stability of Pickering emulsion was evaluated. Then curcumin was encapsulated and its stability and release kinetics were evaluated based on spectrophotometric measurements to quantify the amount of encapsulated curcumin in the Pickering emulsion as a function of time. Overall, these results showed that after optimization, Pickering emulsions could be fabricated with uniform particle size distribution, long-term stability, high stability against pH, salts and can be an effective route for delivery of bioactive compounds.

1. Introduction

Emulsions stabilized by solid particles instead of surfactants are known as “Pickering emulsions”. These emulsions have drawn increasing interest because of their wide range of application in foods, pharmaceuticals and cosmetics, against conventional emulsions using surfactants where toxicity is an issue (Dickinson, 2010; Frelichowska, Bolzinger, Pelletier, Valour, & Chevalier, 2009). In these emulsions unlike surfactants, the particles due to their high energy of attachment irreversibly adsorb at the interface, making the resultant Pickering emulsions more stable for months or even years (Binks, 2002). To prepare Pickering emulsions, a variety of colloidal particles have been successfully employed including inorganic and polymeric colloids such as silica (Binks & Lumsdon, 2000), polymer latex (Binks & Lumsdon, 2001), magnetic particle (Zhang, Su, Ramakrishna, & Lim, 2008), grapheme (Song, Yang, Liu, & Zhao, 2011) and clays and poly methyl methacrylate particles (Yu, Lin, & Li, 2013). Chitosan (CS) the second most abundant biopolymer in nature next to cellulose, derived from the exoskeleton of shrimps and other crustaceans is one of the very few positively charged natural biopolymers existing in the world. Due to its unique characteristics of biodegradability, biocompatibility, bio-adhesion and non-toxicity, CS nanoparticles (NPs) are used as drug delivery systems, nanofibers, biosensors, and edible films (Sogias, Williams, & Khutoryanskiy, 2008). A series of methods are used for producing stable CS NPs for different applications. Preparation of CS NPs by ionic gelation technique has attracted more attention since this process is non-toxic, organic solvent free, convenient and controllable (Agnihotri, Mallikarjuna, & Aminabhavi, 2004). Ionic gelation technique involves the ionic interactions between the positively charged primary amino groups of CS and the negatively charged groups of poly anion, such as TPP, which is the most extensively used ion cross-linking agent due to its non-toxic and multivalent properties (Shu & Zhu, 2002). It is considered that CS–TPP NPs are formed mainly through the electrostatic interaction between positively charged CS and negatively charged TPP molecules. This interaction requires only mild conditions in terms of temperature and pH (Zhang, Oh, Allen, & Kumacheva, 2004) and the NPs size can be controlled by varying the CS: TPP ratio, pH and the molar mass of the CS (Hu et al., 2008; Tsai, Chen, Bai, & Chen, 2011). The physical cross-linking process

* Corresponding author. College of Food Science and Technology, Huazhong Agriculture University, Wuhan, 430070, China.
E-mail address: libinfood@mail.hzau.edu.cn (B. Li).

http://dx.doi.org/10.1016/j.foodhyd.2015.07.015
0268-005X/

between CS and TPP not only avoids the use of chemical cross-linking and emulsifying agents which are often toxic to organisms, but also prevents the possibility of damage to drugs, particularly biological agents (Berger et al., 2004).

Curcumin, a naturally occurring polyphenol derived from *Curcuma longa* has been used in traditional medicine for many Centuries in countries such as India and China (Shishodia, Sethi, & Aggarwal, 2005). Recently, it is reported that curcumin has a wide range of pharmacological applications such as anti-inflammation, anti-human immuno-deficiency virus, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic and anti-cancer with low or no intrinsic toxicity (Yallapu, Jaggi, & Chauhan, 2010). But the clinical advancement of this promising natural compound is hampered by its poor water solubility and short biological half-life resulting in low bioavailability in both plasma and tissues. In this context nanotechnology has been employed in an attempt to increase its retention time and enhance its bioavailability (Cui et al., 2009). However, to the best of authors' knowledge, no such study has been conducted so far that focuses on encapsulation ability, stability and in vitro release of curcumin using tripolyphosphate cross linked CS NPs stabilized Pickering emulsion. Therefore, in the current study, we prepared and optimized CS-TPP NPs stabilized Pickering emulsion in an attempt as a curcumin delivery system.

2. Experimental section

2.1. Materials

Chitosan (CS, Mw 5×10^5–7×10^5 Da) was purchased from Qingdao Yunzhou biochemistry Co., Ltd. (Shandong, China). Medium chain triglyceride (MCT) was purchased from Boxing 145 Chemical Reagent Co., Ltd. (Wuhan, China). Curcumin (95.0% purity), was purchased from National Medicine Group Chemical Reagent Co., Ltd. Sodium Tripolyphosphate (TPP), Glacial acetic Acid, NaCl, $CaCl_2$, NaOH and HCl were purchased from Sinopharm chemical reagent Co., Ltd. (Beijing, China). All the reagents were of analytical grade and used without further purification. Water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus (Millipore, MA, USA) to a resistivity higher than 18.0 MΩ cm.

2.2. Synthesis of CS-TPP NPs

CS-TPP NPs were prepared using the ionic gelling techniques as described by (Konecsni, Low, & Nickerson 2012) with minor modifications. Briefly, different CS solutions (0.2 and 0.5 wt%) were prepared by dissolving CS powder in 0.5% acetic acid solution, which was further diluted with de-ionized water to make a total volume of 200 mL. The mass ratio of CS to glacial acetic acid was kept at 2:3. The resultant mixture was stirred for overnight at room temperature (around 25 °C). A solution of TPP at the concentration of 1.0 mg/mL (0.1 wt%) was prepared by dissolving TPP powder in ultrapure water. The particles were then prepared at different CS:TPP (w/w) ratios of 2.0:1.0, 2.5:1.0, 3.0:1.0, 4.0:1.0, 5.0:1.0, 5:2, 5:3, 5:4 and 5:5, by adding TPP solution drop-wise to CS solution under constant stirring till the formation of an opalescent suspension that is the indication of particles formation.

2.3. Preparation of CS/TPP nanoparticles stabilized Pickering emulsions

A typical preparation procedure of CS-TPP NPs stabilized Pickering emulsions was as follows:

2.3.1. Pickering emulsion with fixed MCT contents

Emulsions with fixed oil contents were prepared by taking 5 wt % MCT and aqueous suspension of different CS-TPP NPs concentrations (95, 75, 55, 45, 35 and 15 v%) in a glass vial. The mixture was then homogenized with an UltraTurrax® T25 device equipped with a S25N-18G shaft (IKA, Germany) rotating at a speed of 10,000 rpm for 3 min. The emulsion so formed was transferred to glass bottles and was stored at room temperature (around 25 °C) for further studies.

2.3.2. Pickering emulsion with different MCT contents

Emulsions with different MCT contents were prepared following the same procedure as described in Section 2.3.1. Briefly, an aqueous suspension (95, 90, 80, 70, and 50 v% respectively) of CS-TPP NPs and different MCT contents (5, 10, 20, 30 and 50 wt%) were taken in a glass vial. The mixture was then homogenized with an UltraTurrax® T25 device equipped with a S25N-18G shaft (IKA, Germany) rotating at a speed of 10,000 rpm for 3 min. The emulsions were transferred to glass bottles and stored at room temperature (around 25 °C) for further studies.

2.4. Evaluation of emulsion stability to different influencing factors

The term “emulsion stability” refers to the ability of an emulsion to keep its properties unchanged over a certain period of time. The more slowly the properties change, the more stable and hence the better the emulsion is. Therefore, the effect of environmental factors, like storage time, pH and ionic strength on the physiochemical characteristics of resultant emulsions were investigated.

a) *Effect of storage time*: In order to study long term stability of the CS-TPP NPs stabilized Pickering emulsion, the emulsions were stored at room temperature (around 25 °C) for one month.
b) *Effect of pH*: To evaluate the effect of pH on the emulsion stability, a series of emulsion samples were prepared and the pH was adjusted to different values in the range of (2–7) by using either HCl or NaOH.
c) *Effect of salts (NaCl and $CaCl_2$)*: The influence of salt on the emulsions stability was examined by adding different amounts of either NaCl or $CaCl_2$ to the emulsions after preparation. Emulsions with different pH (3, 6 and 7) and ionic compositions (0–200 mM NaCl or $CaCl_2$) were then prepared by mixing the initial emulsion with various salt solutions and adjusting the pH using either HCl or NaOH.

2.5. Characterization of the NPs and Pickering emulsion

Particle size distributions of CS-TPP NPs were determined through a dynamic light scattering instrument (Zetasizer NanoZS, Malvern® Instruments, UK). This instrument determines the particle size from intensity-time fluctuations of a laser beam (633 nm), scattered from the sample at a scattering angle of 173° at 25 °C. Each individual measurement was an average of 15 runs. Particle sizes were expressed as the z-average diameter given by the cumulant method. Fourier transform infrared spectroscopy (FTIR) measurements were recorded on NEXUS 470 spectrophotometer using KBr pellets at a resolution of 4 cm^{-1} to evaluate the cross-linking of CS with TPP. The CS and CS-TPP NPs were mixed with KBr in the ratio of 1:150 and ground in a mortar by hand with a pestle. The powder was pressed into pellets under a pressure of 4t. The IR absorbency scans were analyzed in the range of 500–4000 cm^{-1} for changes in the intensity of the sample peaks, with air as the background. Morphology of the particles was investigated with a JEOL transmission electron microscope (TEM)