Colloids and Surfaces A: Physicochem. Eng. Aspects 520 (2017) 62–71

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Research Paper

A new solubility enhancement strategy of capsaicin in the form of high-payload submicron capsaicin-chitosan colloidal complex

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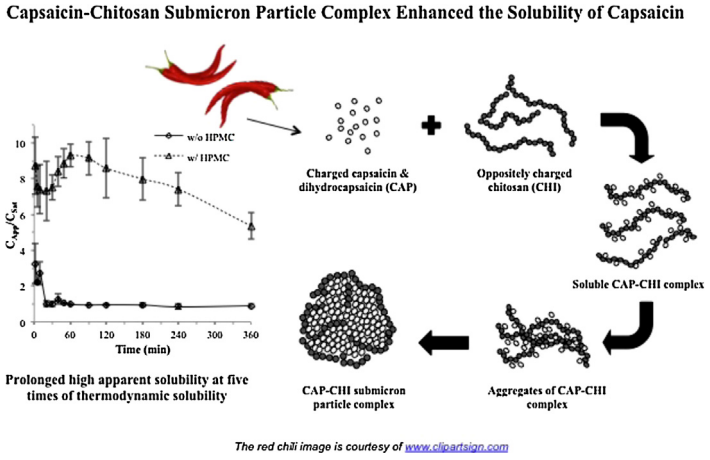
HIGHLIGHTS

- CAP submicroplex is easily prepared by ambient mixing of CAP and CHI solutions.
- Structural integrity of CAP submicroplex is governed by concentration, pH, $R_{CHI/CAP}$.
- CAP submicroplex is efficiently prepared at ≈85% CAP usage rate with ≈75% payload.
- Partially amorphous CAP submicroplex produces prolonged high apparent solubility.
- CAP submicroplex has similar antimicrobial activity as the native CAP.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form 19 January 2017
Accepted 23 January 2017
Available online 24 January 2017

Keywords:
Capsaicin bioavailability
Capsaicin nanoparticles
Drug-polysaccharide complexation
Self-assembly
Macromolecule complexation

ABSTRACT

Clinical application of capsaicin – a major component of chili peppers known for its numerous therapeutic activities – faces the hurdle of poor oral bioavailability due to its low aqueous solubility. While capsaicin nanocapsules have been extensively investigated as a bioavailability enhancement strategy, their low payload limits their effectiveness. Herein we developed a new bioavailability enhancement strategy of capsaicin in the form of high-payload submicron capsaicin-chitosan colloidal particle complex (or submicroplex in short) prepared by electrostatically driven self-assembly complexation between capsaicin and chitosan. The effects of preparation conditions (i.e. capsaicin concentration, chitosan/capsaicin ratio, and pH) on the (a) structural integrity of the capsaicin submicroplex upon centrifugation and freeze drying, (b) physical characteristics (i.e. size, zeta potential, payload, colloidal stability), and (c) preparation efficiency were investigated, from which the optimal preparation conditions were

Abbreviations: AA, acetic acid; AUC, area under the curve; C_{App}, apparent solubility; C_{sat}, thermodynamic saturation solubility; CAP, natural capsaicin; CE, complexation efficiency; CFU, colony forming unit; CHI, chitosan; DMSO, dimethyl sulfoxide; DSC, differential scanning calorimetry; FESEM, field emission scanning electron microscope; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; HPMC, hydroxypropylmethylcellulose; MHB, Mueller Hinton broth; MIC, minimum inhibitory concentration; MW, molecular weight; OD_{600}, optical density at 600 nm; PBS, phosphate buffered saline; PCS, photon correlation spectroscopy; $R_{CHI/CAP}$, charge ratio of CHI to CAP prior to complexation; TGA, thermogravimetric analysis.

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http://dx.doi.org/10.1016/j.colsurfa.2017.01.069
0927-7757/

determined. The optimal formulation exhibited (1) high payload (≈75%), (2) high colloidal stability, and (3) good solubility enhancement capability attributed to its partially amorphous form, resulting in high apparent solubility that was maintained for 6 h at 5 × of the thermodynamic solubility. Lastly, the complexation with chitosan did not have any adverse effect on the antimicrobial activity of capsaicin, hence signifying the preservation of capsaicin's bioactivities in the submicroplex.

1. Introduction

Capsaicinoids – a group of alkaloid compounds responsible for the pungent taste of chili peppers – is made up of approximately 90% capsaicin and dihydrocapsaicin. Capsaicin is well known for its various therapeutic activities ranging from the well-established analgesic, antioxidant, and antimicrobial activities to the more recently discovered antidiabetic, cancer chemopreventive, and cardioprotective activities [1,2]. Despite its rapid absorption in the intestine, oral delivery of capsaicin results in low bioavailability due to its first-pass metabolism in the liver and poor aqueous solubility (<0.1 mg/mL) [3]. Consequently, the clinical use of capsaicin thus far has been limited to its topical application to the skin, mostly as pain relief aids, attributed to the slower metabolism of capsaicin in the skin [4,5].

Current research efforts to enhance the oral bioavailability of capsaicin have been focused predominantly on the nanoencapsulation strategy motivated by the prolonged capsaicin retention time afforded by nanocapsules. The prolonged retention time was attributed to the nanoscale size, sustained release functionality, and stabilization provided by the capsule matrix against metabolism [3]. Various formulations of capsaicin nanocapsules ranging from polymeric nanoparticles [6–8] to polymeric micelles [9] and liposomes [10] have been developed, where the nanoencapsulation was shown to improve the in vivo bioavailability of capsaicin when compared to its native form.

The nanocapsules, however, exhibit a major drawback in their low capsaicin payload (<20%) irrespective of their formulation approach. The low payload leads to a large amount of doses required to achieve the desired therapeutic effects, hence increasing the risk of adverse side effects due to the excessive intake of carrier materials. The high dose requirement also makes clinical application of capsaicin less attractive from the economics and patient compliance' perspectives. Importantly, the nanocapsules possess a limitation in the maximum amount of capsaicin in the systemic circulation that they can generate due to the aforementioned poor aqueous solubility of capsaicin. Consequently, improvements in the bioavailability of capsaicin afforded by the nanocapsules have been fairly limited [3].

Herein we developed an alternative oral bioavailability enhancement strategy of capsaicin in the form of submicron capsaicin-chitosan particle complex (or submicroplex in short) exhibiting high payload and high apparent aqueous solubility. The submicroplex was prepared by electrostatically driven self-assembly complexation between anionic capsaicin (CAP) and oppositely charged chitosan (CHI) based on the well-established principle of drug-polyelectrolyte complexation [11]. The CAP-CHI submicroplex preparation was simple, rapid, and cost effective involving only mixing of the CAP and CHI solutions under ambient condition.

CHI was used as the polyelectrolyte because of the following reasons: (1) CHI was readily ionized in acid to produce charges opposite to the anionic CAP 12, (2) CHI was naturally abundant with well-established biocompatibility and biodegradability [13], and (3) the inclusion of CHI had been shown to enhance the intestinal absorption of various bioactive molecules attributed to the epithelial tight junction opening mediated by CHI [14,15]. More importantly, CHI has been widely used in various biomedical applications, including drug delivery systems, attributed to its biocompatibility and versatility [16–19].

First, the present work aimed to determine the feasible range of preparation condition to produce physically robust CAP-CHI submicroplex that could withstand the harsh external stresses exerted by the two widely used subsequent processing steps for submicron particles after their preparation, i.e. (1) centrifugation for purification and (2) freeze drying for solid dosage form production. For this purpose, we investigated the effects of (a) CAP concentration, (b) charge ratio of CHI to CAP, and (c) preparation pH on the structural integrity of the resultant CAP-CHI submicroplex upon centrifugation and freeze drying.

Second, the present work aimed to evaluate the effects of varying the feasible preparation condition on the (i) physical characteristics (i.e. size, zeta potential, and payload) and (ii) preparation efficiency (i.e. CAP utilization rate, overall yield) of the CAP-CHI submicroplex produced, from which the optimal preparation condition was determined. Third, the CAP-CHI submicroplex prepared at the optimal condition was examined in terms of its (1) colloidal stability during 24-h-storage in ambient condition, (2) in vitro solubility enhancement capability and dissolution rate, and lastly (3) in vitro antimicrobial activity against clinically derived bacterial pathogen, where the aim was to provide a preliminary assessment on the effect of complexation with CHI on the bioactivity of CAP.

2. Materials and methods

2.1. Materials

Low molecular weight (MW) chitosan (50–190 kDa), natural capsaicin composed of roughly 65% capsaicin and 35% dihydrocapsaicin (MW = 305.4 g/mol), potassium hydroxide (KOH), glacial acetic acid (AA), hydroxypropyl methylcellulose (HPMC), potassium bromide (KBr), trehalose, and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (Singapore). Absolute ethanol and acetonitrile (>99.8%) were purchased from Merck Millipore (Singapore). Phosphate buffered saline (PBS, pH 7.4) and Mueller Hinton broth (MHB) for bacterial cell culture were purchased from BD Diagnostics (Singapore) and 1st Base (Singapore), respectively. Clinically derived strain of *Pseudomonas aeruginosa* bacteria was provided by laboratory of clinical microbiology at National University Hospital (Singapore).

2.2. Methods

2.2.1. Preparation of CAP-CHI submicroplex

CAP having pKa of 9.76 [20] was fully deprotonated upon dissolving in 0.1 M KOH (pH 13) to form the negatively charged CAP with a charge density of 3.27×10^{-6} mol-charge/mg. CHI having pKa of 6.5 [12] was protonated upon its dissolution in aqueous AA solution to form the positively charged CHI with a charge den-