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Amorphous ternary nanoparticle complex of curcumin-chitosan-hypromellose exhibiting built-in solubility enhancement and physical stability of curcumin

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ABSTRACT

The low aqueous solubility of curcumin (CUR) had greatly limited the clinical efficacy of CUR therapy despite its well-known potent therapeutic activities. Previously, we developed amorphous nanoparticle complex (nanoplex) of CUR and chitosan (CHI) as a solubility enhancement strategy of CUR by electrostatically-driven drug-polyelectrolyte complexation. The CUR-CHI nanoplex, however, (1) lacked a built-in ability to produce prolonged high apparent solubility of CUR in the absence of crystallization-inhibiting agents, and (2) exhibited poor physical stability during long-term storage. For this reason, herein we developed amorphous ternary nanoplex of CUR, CHI, and hypromellose (HPMC) where HPMC functioned as the crystallization inhibitor. The effects of incorporating HPMC on the (1) physical characteristics and (2) preparation efficiency of the CUR-CHI-HPMC nanoplex produced were investigated. Compared to the CUR-CHI nanoplex, the HPMC inclusion led to larger nanoplex (≈300–500 nm) having lower zeta potential (≈1–15 mV) and lower CUR payload (≈40–80%), albeit with higher CUR utilization rates (≈100%) attributed to the CUR interactions with both CHI and HPMC. The CUR-CHI-HPMC nanoplex's physical characteristics could be controlled by varying the HPMC to CHI ratio in the feed. Subsequently, the CUR-CHI-HPMC and CUR-CHI nanoplexes were examined in terms of their (1) storage stability, (2) dissolution characteristics in simulated gastrointestinal fluids, and (3) *in vitro* solubility enhancement. The results showed that the CUR-CHI-HPMC nanoplex exhibited superior (i) amorphous state stability after twelve-month storage, (ii) dissolution characteristics, and (iii) solubility enhancement in simulated gastrointestinal fluids, with minimal cytotoxicity towards human gastric epithelial cells.

1. Introduction

The potent therapeutic activities of curcumin (CUR) – a natural polyphenol isolated from turmeric – as anti-inflammatory, antioxidant, antimicrobial, and anticancer agents have been well established, resulting in the vast application of CUR as oral dietary supplements [1,2]. The low aqueous solubility of CUR («1 mg/mL) and the consequential poor oral bioavailability, however, have greatly limited its true therapeutic potential clinically [3]. Numerous solubility enhancement strategies of CUR have therefore been proposed, for example, via amorphization [4,5], chemical conjugation [6,7], encapsulation [8,9], cyclodextrin inclusion complex [10,11], and nanonization [12,13]. Among these strategies, amorphization and nanonization represent the more feasible strategies for large-scale implementation attributed to (i) the high CUR payload of their products, (ii) their organic solvent-free preparation, and (iii) less intricate preparation techniques [3,14].

To take advantage of the solubility enhancement afforded by amorphization and nanonization, our group previously developed a solubility enhancement strategy of CUR that combined amorphization and nanonization principles in the form of amorphous

Abbreviations: ASD, amorphous solid dispersion; AUC, area under the curve; C, supersaturated concentration of CUR; C_{sat}, saturation solubility of CUR; CE, complexation efficiency; CHI, chitosan; CUR, curcumin; DLS, dynamic light scattering; DSC, differential scanning calorimetry; FESEM, field emission scanning electron microscope; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; HPMC, hypromellose; $M_{HPMC/CHI}$, mass ratio of HPMC to CHI; PXRD, powder x-ray diffraction; $R_{CHI/CUR}$, charge ratio of CHI to CUR; SGJ, simulated gastric juice; SIJ, simulated intestinal juice; TGA, thermal gravimetric analysis; UV–vis, ultraviolet visible; USP, United States Pharmacopeia.

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nanoparticle complex of CUR and chitosan (CHI) [15]. First, the metastable amorphous state of the CUR-CHI nanoparticle complex (or nanoplex in short) enabled it to generate highly supersaturated CUR concentration upon dissolution, resulting in high apparent solubility of CUR. The high apparent solubility would lead to enhanced CUR bioavailability if the high supersaturation level could be maintained over a time period sufficient for CUR absorption across the gastrointestinal lumen [16,17].

Second, owing to its nanoscale size, the amorphous CUR-CHI nanoplex exhibited superior supersaturation generation *in vitro* to microscale amorphous solid dispersion (ASD) of CUR, which represented a well-established amorphization strategy of CUR [18]. The superior supersaturation generation of the CUR-CHI nanoplex was attributed to the faster dissolution rate afforded by its nanoscale size [18]. Furthermore, the CUR-CHI nanoplex also exhibited superior characteristics to the ASD of CUR and other CUR solubility enhancement strategies due to its (1) simple, fast, and cost-effective preparation involving only mixing of CUR and CHI solutions under ambient condition, and (2) high CUR utilization rate (>90%) resulting in minimal CUR wastage [15].

Nevertheless, the current CUR-CHI nanoplex formulation exhibits two major drawbacks. First, while the CUR-CHI nanoplex could generate a high supersaturation level, the supersaturation level rapidly decreased in the absence of crystallization-inhibiting agents in the dissolution medium [15]. The CUR-CHI nanoplex thus did not possess a built-in capability to generate a prolonged high supersaturation level necessary for bioavailability enhancement. From the product formulation perspectives, the crystallization-inhibiting agents must thus be added in the subsequent formulation step, namely during the oral solid dosage form preparation of the nanoplex. This approach is less than ideal because the supersaturation generation of the CUR-CHI nanoplex becomes dependent on the dissolution rate of the crystallization inhibitors from the solid dosage form, which itself is influenced by a myriad of factors [19,20].

Second, the CUR-CHI nanoplex exhibited poor physical stability during long-term storage in its dry-powder form, where parts of its amorphous form underwent crystallization, resulting in its diminished solubility enhancement capability [18]. Moreover, the amorphous state stability of the CUR-CHI nanoplex remained lacking even when it was stored as intimate mixtures with crystallization inhibitors [18]. The amorphous state stability of the nanoplex could only be maintained when it was stored in the presence of a large amount of crystallization inhibitors and other adjuvants, resulting in undesirably low nanoplex contents (<30%) [21].

To address these drawbacks, the present work aimed to incorporate hypromellose – a well-established polymeric crystallization inhibitor [22] – into the CUR-CHI nanoplex early at the nanoplex formation step, rather than at the solid dosage formulation step as previously done. Herein we hypothesized that the presence of hypromellose at the nanoscale would provide the CUR-CHI nanoplex with a built-in capability (1) to prolong its high supersaturation level upon dissolution and (2) to enhance its long-term physical stability.

The first objective of the present work was to investigate the feasibility of forming amorphous ternary nanoplex of CUR-CHI-hypromellose via the same preparation principle (i.e. drug-polyelectrolyte complexation) used in the CUR-CHI nanoplex preparation. In this technique, the electrostatic interaction between ionized drug molecules (i.e. CUR) and oppositely charged polyelectrolytes (CHI) resulted in the formation of soluble CUR-CHI complexes that subsequently aggregated due to inter-CUR hydrophobic interactions. The complex aggregates then precipitated out of the solution to form the CUR-CHI nanoplex upon reaching a critical aggregate mass [15].

In this regard, hypromellose had been known to readily form aggregates with non-ionic molecules via hydrophobic and hydrogen bond interactions [23,24]. In fact, hydrogen bond interactions between hypromellose and CUR had been reported when they were formulated as ASD of CUR [25,26]. We postulated that a similar kind of interactions between hypromellose and CUR occurred during the nanoplex formation, resulting in the formation of amorphous ternary nanoplex.

The second objective of the present work was to examine the optimal formulation of the CUR-CHI-hypromellose nanoplex and compare it against the CUR-CHI nanoplex in terms of their (1) physical stability during long-term storage, (2) dissolution characteristics in simulated gastrointestinal fluids, and (3) *in vitro* supersaturation generation. The optimal formulation of the CUR-CHI-hypromellose nanoplex was determined by investigating the effects of (i) mass ratios of hypromellose to CHI and (ii) charge ratios of CHI to CUR on the physical characteristics and preparation efficiency of the CUR-CHI-hypromellose nanoplex produced. In addition, we also examined the cytotoxicity of the CUR-CHI-hypromellose nanoplex towards human gastric epithelial cells NCI-N87 to evaluate its potential *in vivo* applications as oral dietary supplement.

2. Materials and methods

2.1. Materials

Curcumin (CUR) (95% curcuminoid) was purchased from Alfa Aesar (Singapore). Chitosan (CHI) (50–190 kDa, 75–85% deacetylation), hypromellose (hydroxypropylmethylcellulose, HPMC) (26 kDa), potassium and sodium hydroxides (KOH, NaOH), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), hydrogen chloride (HCl), glacial acetic acid (AA), and ethanol were purchased from Sigma-Aldrich (Singapore). The materials and methods for the cytotoxicity test were provided in the Supplementary Materials.

2.2. Methods

2.2.1. Preparation of CUR-CHI-HPMC nanoplex

The CUR-CHI-hypromellose nanoplex (hereinafter referred as CUR-CHI-HPMC nanoplex for brevity) was prepared at two different charge ratios of CHI to CUR ($R_{CHI/CUR}$) (i.e. 0.7 and 1.0) at a fixed CUR concentration of 5 mg/mL. The effects of the mass ratios of HPMC to CHI ($M_{HPMC/CHI}$) in the feed solution were investigated in the range of 0.33–2.0 and 0.23–1.4, respectively, for $R_{CHI/CUR}$ = 0.7 and 1.0. The sample calculation for $R_{CHI/CUR}$ was provided in the Supplementary Materials. Briefly, CHI was dissolved in 1.2% (v/v) aqueous AA solution (pH 2.7) at either 6.4 mg/mL ($R_{CHI/CUR}$ = 0.7) or 9.1 mg/mL ($R_{CHI/CUR}$ = 1.0). CHI having pK_a of 6.5 [27] was protonated upon dissolution in AA to form cationic CHI molecules. Separately, HPMC was dissolved in 0.1 M KOH (pH 13) at different concentrations (i.e. 2–12 mg/mL) depending on the desired $M_{HPMC/}$CHI. Afterwards, CUR powder was added to the HPMC solution in 0.1 M KOH at 5 mg/mL, where CUR subsequently dissolved as CUR having pK_a of 8.4, 9.9, and 10.5 [28] was fully deprotonated at pH 13 to form anionic CUR molecules.

Next, the (CUR + HPMC) solution was mixed immediately upon its preparation with equal volume of the CHI solution to minimize alkaline degradation of CUR, resulting in mixed solution exhibiting pH 4.4. On this note, the CUR degradation rate at pH 13 expressed as the% CUR loss was reported in two separate studies to be equal to approximately 50% after 48 h and 67% after 20 h with negligible losses after 1 h [28]. Hence, CUR degradation in the (CUR + HPMC) solution prior to its mixing with the CHI solution was minimal.