Examining practical feasibility of amorphous curcumin-chitosan nanoparticle complex as solubility enhancement strategy of curcumin : scaled-up production, dry powder transformation, and long-term physical stability

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1 **Examining Practical Feasibility of Amorphous Curcumin-Chitosan Nanoparticle Complex**
2 **as Solubility Enhancement Strategy of Curcumin: Scaled-Up Production, Dry Powder**
3 **Transformation, and Long-Term Physical Stability**

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Abstract

Amorphous curcumin-chitosan nanoparticle complex (or nanoplex in short) was recently developed as a new solubility enhancement strategy of curcumin (CUR) - a natural herb well known for its vast therapeutic activities. For its subsequent clinical application and commercialization, the present work aimed to address the three remaining research questions pertaining to the CUR nanoplex, i.e. (1) was the nanoplex preparation scalable? (2) could the nanoplex maintain its solubility enhancement capability in the powder form? (3) could the nanoplex resist crystallization, which would jeopardize its solubility enhancement capability, during long-term storage? First, the results showed that gram-scale production of the CUR nanoplex was readily achieved at high CUR utilization rate without significant adverse effects on the physical characteristics. Stable CUR nanoplex with size, zeta potential, and CUR payload of $\approx 100\text{-}300$ nm, 18 mV, and 80%, respectively, was produced. The scaled-up production, nevertheless, resulted in lower yield due to lower nanoplex recovery in the purification step. Second, the CUR nanoplex powders, when formulated correctly with drying adjuvants, maintained the solubility enhancement capability of the suspension form, despite their slower dissolution velocity. High apparent solubility at approximately twice of CUR's thermodynamic solubility was demonstrated for 8 h. Third, the CUR nanoplex powders maintained its amorphous state after twelve-month storage when stored as physical mixture with crystallization-inhibiting agents. In short, the present results successfully established the CUR nanoplex as a practical and effective solubility enhancement strategy of CUR.

Keywords: nanoparticles; dissolution; amorphization; curcumin; freeze drying

48 **Abbreviations**

49	AUC	area under the curve
50	C _{Sat}	thermodynamic saturation solubility
51	C _{Supersat}	supersaturated concentration
52	CE	complexation efficiency
53	CHI	chitosan
54	CUR	curcumin
55	DLS	dynamic light scattering
56	DTA	differential thermal analyzer
57	FESEM	field emission scanning electron microscope
58	FTIR	Fourier transform infrared spectroscopy
59	HPLC	high performance liquid chromatography
60	HPMC	hydroxypropyl methylcellulose
61	PBS	phosphate buffered saline
62	PXRD	powder x-ray diffraction
63	RH	relative humidity
64	TG	thermogravimetric
65	UV-Vis	ultraviolet visible spectroscopy

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1. Introduction

Curcumin - the main bioactive compound of turmeric plants - is well known for its plethora of therapeutic activities ranging from anti-inflammatory and antibacterial to anticancer and antioxidant activities [1]. The presumably immense therapeutic benefits of curcumin, however, have not been fully realized due to its low aqueous solubility (< 1 mg/mL), which in turn causes its poor bioavailability upon oral administration [2]. Recently, nanoparticulate formulations of curcumin (nano-curcumin) have emerged as an effective solubility enhancement strategy of curcumin by virtue of the fast dissolution velocity afforded by the nanoparticles attributed to their large specific surface areas [3-5].

Previously, we developed a new class of nano-curcumin formulation in the form of amorphous curcumin-chitosan nanoparticle complex (or curcumin nanoplex in short), which were superior to the existing nano-curcumin formulations in terms of (1) the high curcumin payload ($> 80\%$) and (2) simple, yet highly efficient, preparation involving only the mixing of curcumin and chitosan solutions under an ambient condition [6]. The solubility enhancement capability of the curcumin nanoplex was successfully demonstrated in its aqueous suspension form, where the nanoplex, upon dissolution, generated a high and prolonged apparent solubility at roughly four to five times of the thermodynamic saturation solubility of curcumin [6]. The high apparent solubility that was maintained for several hours was attributed primarily to the amorphous state of the curcumin nanoplex that was capable of producing highly supersaturated curcumin concentration upon dissolution [6].

The curcumin nanoplex was prepared by drug-polysaccharide complexation technique that relied on electrostatic binding between curcumin and the oppositely charged chitosan to form the nanoplex. As illustrated earlier in Nguyen, Yu, Kiew and Hadinoto [6], the electrostatically driven complexation between curcumin and chitosan resulted in the formation of soluble curcumin-chitosan complex, which subsequently formed aggregates among themselves due to hydrophobic interactions between the bound curcumin molecules. The complex aggregates then precipitated out of the solution upon reaching a critical mass to form the curcumin nanoplex.

Thus far, the drug-polysaccharide complexation technique had only been carried out in a very small production volume (i.e. 2 mL) in which good mixing between curcumin and chitosan, which was required for their electrostatic binding to take place, was readily achieved. For the curcumin nanoplex to gain traction as the preferred formulation platform for nano-curcumin, the scalability of the curcumin-chitosan complexation technique ought to be

demonstrated. Thus, the first objective of the present work was to investigate the scalability of the curcumin nanoplex preparation by assessing (1) the physical characteristics (i.e. size, zeta potential, curcumin payload, solid concentration, and colloidal stability), and (2) preparation efficiency (i.e. complexation efficiency of CUR, overall yield) of nanoplex prepared at larger production volumes.

Next, as solid dosage forms (e.g. tablets, capsules) remain the formulation of choice for oral drug delivery [7], the curcumin nanoplex, which currently is prepared as aqueous suspension, must undergo transformation to dry powders for it to be readily processed into solid dosage forms. Previous studies on other drug nanoplexes, however, showed that the dry powder transformation in the absence of drying adjuvants had adverse effects on the drug nanoplex's dissolution characteristics, which was caused by irreversible aggregation of the nanoplex upon drying [8, 9]. Therefore, the second objective of the present work was to examine the solubility enhancement capability of the dry powder form of the curcumin nanoplex prepared in the presence of drying adjuvants.

Lastly, while the amorphous state of the nanoplex had been established as the primary reason for its solubility enhancement capability, the amorphous state stability of drug nanoplex powders during long-term storage had not been investigated before. In this regard, amorphous solid dispersions of drugs, if they are not formulated correctly, are notoriously prone to recrystallization during storage due to its metastable state, resulting in the loss of their solubility enhancement capability [10, 11]. Thus, the third objective of the present work was to evaluate the amorphous state stability of the curcumin nanoplex powders during long-term storage.

In a nutshell, the outcomes of the present work provided answers to three of the most important remaining research questions regarding the curcumin nanoplex, i.e. (1) could the curcumin nanoplex be prepared at a larger scale and how did the production scale affect its physical characteristics and preparation efficiency? (2) could we maintain the solubility enhancement capability of the curcumin nanoplex after its dry powder transformation when drying adjuvants were used? and lastly (3) did the curcumin nanoplex powders remain in their amorphous state during long-term storage and whether the inclusion of crystallization-inhibiting agent was needed? The results of these three studies demonstrated the definite potential of the curcumin nanoplex as the solubility enhancement strategy of curcumin.

2. Materials and Methods

2.1. Materials

Curcumin (CUR) (98%) and low molecular weight chitosan (CHI) (50-190 kDa with 75-85% deacetylation) were purchased from Alfa Aesar (Singapore) and Sigma-Aldrich (Singapore), respectively. Ethanol (absolute) and phosphate buffered saline (PBS) (pH 7.4) were purchased from Merck Millipore (Singapore) and 1st Base (Singapore), respectively. D-Trehalose, hydroxypropyl methylcellulose (HPMC), potassium hydroxide (KOH), sodium chloride (NaCl), and glacial acetic acid (AA) were purchased from Sigma-Aldrich (Singapore).

2.2. Methods

2.2.1. Scaled-up preparation of CUR-CHI nanoplex

CUR with pK_a of 8.4, 9.9, and 10.5 [12] was dissolved at 5 mg/mL in 0.1 M KOH (pH 13) resulting in the formation of anionic CUR molecules. CHI with pK_a of 6.5 [13] was dissolved separately at 6 mg/mL in 1.2% (v/v) acetic acid solution (pH 4.4) resulting in the formation of cationic CHI molecules. Next, equal volumes of the CUR and CHI solutions were mixed under gentle stirring using magnetic stirrer, immediately after their preparation to minimize alkaline degradation of CUR. The resultant CUR-CHI nanoplex suspension was ultrasonicated for 15 s at 20 kHz (VC 505, Sonics, USA) to disperse the nanoplex aggregates that might form. Subsequently, the nanoplex suspension was washed by two cycles of centrifugation (14,000×g) to remove excess CUR and CHI after which the washed nanoplex suspension was re-suspended in deionized water for characterizations.

In the scaled-up production, the production volume (i.e. CUR + CHI volumes) was gradually increased from 2 mL to 800 mL (i.e. 2, 10, 50, 200, 400, and 800 mL). The upper limit for the production volume was dictated by the maximum capacity of the high-speed centrifuge (Sorvall ST 16, Thermo Fisher Scientific, USA) available in our laboratory. The centrifugation time was gradually increased from 25 min at 2-mL production volume to 2.5 h at 400-mL production volume at a fixed centrifugation speed (14,000×g). The centrifugation for the 800-mL volume was carried out in two rounds at 400-mL each time for a total of 5 h centrifugation time. Likewise, the ultrasonication was gradually increased from one cycle at 2-mL production volume to nine cycles at 800-mL production volume (15 s for each cycle) at a fixed ultrasonication frequency (20 kHz).

2.2.2. Physical characterizations of CUR-CHI nanoplex

The size and zeta potential of the CUR-CHI nanoplex in its aqueous suspension form were characterized by dynamic light scattering (DLS) after 100× dilution, using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The nanoplex size was confirmed by field emission scanning electron

microscope (FESEM) (JSM-6700F, JEOL, USA) using a sample from the aqueous nanoplex suspension after overnight drying at room temperature.

The CUR payload, which was defined as the amount of CUR contained per unit mass of the nanoplex, was determined by dissolving a known mass of the CUR-CHI nanoplex in 80% (v/v) aqueous ethanol solution. The amount of CUR in the ethanol solution was then quantified by UV-Vis spectrophotometer (UV Mini 1240, Shimadzu, Japan) at the optimal absorbance wavelength of 423 nm [12]. In addition to the CUR payload, the presence of CUR in the nanoplexes was verified by Fourier transform infrared spectroscopy (FTIR) using Spectrum One FT-IR Spectrometer (Perkin-Elmer, USA) performed between 500 and 4000 cm⁻¹ at 1 cm⁻¹ spectral resolution. The FTIR analysis was also performed for the native CUR and native CHI for comparison.

The solid concentration of the CUR-CHI nanoplex suspension was determined by freeze drying a known volume of the nanoplex suspension after which the solid concentration was calculated by dividing the freeze-dried solid mass to the suspension volume used. The colloidal stability of the nanoplex suspension after its preparation was examined by DLS monitoring of the size and zeta potential at specific time points (i.e. 0, 2, 4, 6, and 24 h) during storage at room temperature. Minimal fluctuations in the size and zeta potential indicated good colloidal stability. The reported size, zeta potential, CUR payload, and colloidal stability were based on a minimum of three independent replicates.

2.2.3. Preparation efficiency of CUR-CHI nanoplex

The efficiency of the CUR-CHI nanoplex preparation was characterized in triplicates in terms of (1) the complexation efficiency (CE) and (2) the overall production yield, as defined in Equations 1 and 2, respectively. In the CE determination, the mass of CUR that formed the nanoplex in Eq. (1) was determined from the difference between the initial mass of CUR added and the mass of CUR recovered in the supernatant after the first centrifugation of the nanoplex suspension. The amount of CUR in the supernatant was determined by UV-Vis spectrophotometer at 423 nm after 10× dilutions. In the yield determination, the mass of the nanoplex produced in Eq. 2 was determined from the freeze-dried mass of the washed nanoplex suspension.

$$\text{CE (wt. \%)} = \frac{\text{Mass of CUR that formed CUR-CHI nanoplex}}{\text{Initial mass of CUR added}} \times 100 \quad (1)$$

$$\text{Yield (wt. \%)} = \frac{\text{Mass of CUR-CHI nanoplex produced}}{\text{Initial masses of CUR and CHI added}} \times 100 \quad (2)$$

2.2.4. Preparation of CUR-CHI nanoplex powders

The CUR-CHI nanoplex powders were prepared by freeze drying of the nanoplex suspension in the presence of drying adjuvants for 24 h in Alpha 1-2 LD Plus freeze dryer (Martin Christ, Germany) at -52°C and a vacuum level of 0.05 mbar. Two drying adjuvants (i.e. trehalose and HPMC) at different compositions were used at two different nanoplex solid contents (i.e. 50% and 60% w/w) as summarized in Table 1. Trehalose - a widely used cryoprotective agent for nanoparticles [14] - was added to prevent irreversible coalescence of the nanoplex upon freeze drying as this might adversely affect the nanoplex's dissolution characteristics. Whereas HPMC - a well-established crystallization inhibiting agent [15] - was added to suppress the crystallization of the amorphous nanoplex during their dissolution and storage. Owing to their high aqueous solubility, both trehalose and HPMC dissolved upon immersion of the nanoplex powders in the dissolution medium, resulting in the liberation of the nanoplex from the adjuvant matrix.

2.2.5. Dissolution tests under sink and non-sink conditions

Prior to the dissolution tests, aqueous re-dispersibility of the CUR-CHI nanoplex powders prepared by the different adjuvant formulations was visually examined in duplicates by dispersing a small amount of the nanoplex powders in deionized water. Good aqueous re-dispersibility was characterized by full reconstitution of the nanoplex powders back to the nanoplex suspension upon immersion in water. Subsequently, the dissolution tests were carried out in triplicates for the adjuvant formulations exhibiting good aqueous re-dispersibility.

First, the dissolution rate of the CUR-CHI nanoplex powders was carried out under a sink condition at a concentration below the thermodynamic saturation solubility of CUR (i.e. $C_{\text{Sat}} = 4.15 \mu\text{g/mL}$). Briefly, the CUR-CHI nanoplex powders were dispersed at $1.5 \mu\text{g/mL}$ in 230 mL PBS maintained at 37°C in a shaking incubator. Afterwards, 2 mL aliquot was withdrawn at specific time points over a 2-h period, while 2 mL fresh PBS was simultaneously added back to the dissolution vessel. Subsequently, the aliquot was syringe filtered (0.2- μm pore size) after which the CUR concentration in the aliquot was determined by UV-Vis spectrophotometry at 423 nm.

Second, the dissolution rate of the CUR-CHI nanoplex powders was carried out under a non-sink condition to characterize the supersaturation generation capability of the amorphous nanoplex powders. Briefly, the nanoplex powders were added at $15 \times C_{\text{Sat}}$ to 100 mL PBS maintained at 37°C in a shaking incubator. Afterwards, 400 μL aliquot was withdrawn and syringe filtered at specific time points over a 24-h period. The aliquot was then immediately diluted five times with fresh PBS to prevent CUR precipitation from the supersaturated solution.

Next, the CUR concentration in the aliquot was determined by High Performance Liquid Chromatography (HPLC) using Agilent 1100 (Agilent Technologies, USA) at detection wavelength of 423 nm [16]. Aqueous ethanol solution 80% (v/v) was used as the mobile phase at 1.0 mL/min in ZORBAX Eclipse Plus C18 column (250 x 4.6 mm, 5 μ m particle size), resulting in the CUR's retention time of approximately 2.5 min. The supersaturation generation of the nanoplex powders was characterized as the ratio of the supersaturated concentration generated (C_{Supersat}) to C_{Sat} . The effect of having pre-dissolved HPMC in the dissolution medium on the supersaturation generation was also investigated at 2 mg/mL. The dissolution tests of the CUR-CHI nanoplex suspension were carried out following the same protocols for comparison.

2.2.6. Long-term storage stability testing

The CUR-CHI nanoplex powders were stored for three months in an open container inside a desiccator (3 L) under an accelerated storage condition of 40°C and 75% relative humidity (RH), which was equivalent to approximately **twelve-month** storage under normal condition (i.e. 25°C and 60% relative humidity) [17]. The high RH condition was generated by placing NaCl saturated solution in the desiccator while keeping its temperature at 40°C. Two types of powders were stored, i.e. (1) the CUR-CHI nanoplex itself and (2) the physical mixture of the CUR-CHI nanoplex and HPMC at 1:1 ratio. **The physical mixture was prepared by blending equal masses of the freeze-dried CUR-CHI nanoplex powders and the native HPMC (i.e. 50 mg each), followed by gentle shaking to minimize powder segregations.**

The amorphous state stability of the nanoplex powders was examined by Powder X-ray Diffraction (PXRD) operated between 10° and 70° (2 θ) with a step size of 0.02°/s using D8 Advance X-ray Diffractometer (Bruker, Germany). The amorphous state stability was also examined by thermogravimetric (TG)/differential thermal analyzer (DTA) using Pyris Diamond TG/DTA (Perkin-Elmer, USA). Briefly, the TG/DTA was performed by filling 5 mg powders in a sealed alumina crucible followed by heating from 25°C to 400°C at 10°C/min. The PXRD and TG/DTA analysis were carried out for the native CUR, native CHI, CUR-CHI nanoplex powders, **and their physical mixture with HPMC** immediately after their preparation and after the three-month accelerated storage.

3. Results and discussion

3.1. Scaled-up production of CUR-CHI nanoplex

The results showed that the product of the complexation between CUR and CHI remained in the nanoscale size upon the increase in the production volume. Specifically, the CUR-CHI nanoplex size decreased slightly with increasing production volume from 227 ± 19 nm at 2 mL to 139 ± 4 nm at 400 mL and 168 ± 18 nm at 800 mL (Fig. 1A), as a result of the increased ultrasonication cycle at larger production volumes. Not unexpectedly, the smaller sizes observed at larger production volumes were accompanied by their slightly higher zeta potentials, which increased from 13 ± 2 mV at 2 mL to 18 ± 3 mV at 400 mL and 16 ± 2 mV at 800 mL (Fig. 1A). Furthermore, the CUR payload remained constant at ≈ 80 -85% (w/w) upon increase in the production volume (Fig. 1B). These results hence signified the scalability of the CUR-CHI complexation in terms of the physical characteristics of the CUR-CHI nanoplex produced.

The larger production volume, however, resulted in the decrease in the solid concentration of the CUR-CHI nanoplex suspension from 2.1 ± 0.2 mg/mL (or 0.21% w/v) at 2 mL to 1.6 ± 0.2 mg/mL (or 0.16% w/v) at 400 mL and 800 mL (Fig. 1B). The lower solid concentration at larger production volumes was caused by the inevitably lower effectiveness of the centrifugation step in recovering the nanoplex from larger suspension volumes. This issue could be resolved by using a centrifuge of larger capacity, which unfortunately was not available in our laboratory. At the current solid concentration, roughly 1.28 g of CUR-CHI nanoplex were produced in a single 800-mL batch. Therefore, gram-scale production of the nanoplex could be readily achieved from several 800-mL batches.

The nanoscale size of the CUR-CHI complex prepared at 400 mL production volume was verified by its FESEM image, which showed rectangle-like shaped particles in the size range of 100-300 nm (Fig. 2A). The size from FESEM hence was in agreement with the size from DLS reported earlier. The colloidal stability of the CUR-CHI nanoplex suspension prepared in the 400-mL batch was demonstrated by the minimal variations in its size and zeta potential during the 24-h storage, where they remained in the narrow range of 140-180 nm and 13-18 mV, respectively (Fig. 2B).

The presence of CUR in the nanoplex prepared at the largest production volume was verified by FTIR, which showed the appearance of the characteristics bands of the native CUR at 1626, 1508, and 1272 cm^{-1} in the FTIR spectra of the CUR-CHI nanoplex prepared in the 400-mL batch (Fig. 3A). These bands corresponded to the stretching vibrations of the C=C-C_{ring}, C=O, and enol C-O bonds of CUR, respectively [18]. As expected, these bands were also present in the FTIR spectrum of the CUR-CHI nanoplex prepared in the 2-mL batch, but not in the native CHI's

spectrum. As discussed earlier in Nguyen, Tran and Hadinoto [19], the complexation between CUR and CHI in the CUR-CHI nanoplex was evident from the disappearance of the stretching NH_2 band of the native CHI at 3350 cm^{-1} and the reduced intensity of the phenolic OH band of the native CUR at 3500 cm^{-1} in the CUR-CHI nanoplex spectrum (Fig. S1 of the Supplementary Materials), which were resulted from the electrostatic interaction between the charged amine group of CHI and the phenol group of CUR.

The scalability of the CUR-CHI nanoplex preparation was also evident in the preparation efficiency, where the CE remained high at approximately 95% (w/w) up to the 400-mL production volume before it dropped to $84 \pm 2\%$ at the largest production volume investigated (i.e. 800 mL) (Fig. 3B). The trend in the CE indicated that the same level of mixing between the CUR and CHI solutions was maintained using the magnetic stirring method up to the 400-mL production volume and the mixing deteriorated at larger volume. The yield, however, gradually decreased from $43 \pm 2\%$ (w/w) at 2 mL to $29 \pm 3\%$ (w/w) at 400 mL and $32 \pm 2\%$ at 800 mL (Fig. 3B) caused by the aforementioned lower nanoplex recovery by the centrifugation step at larger production volumes.

3.2. Aqueous re-dispersibility of CUR-CHI nanoplex powders

The aqueous re-dispersibility tests of the CUR-CHI nanoplex powders showed that the adjuvant formulations *b* and *e* resulted in nanoplex powders that re-dispersed poorly in an aqueous environment. Their poor aqueous re-dispersibility was evident from the photographs of these powders after their re-dispersion in water, which showed the appearance of mostly clear solution with the powder sediments at the bottom (Fig. S2 of the Supplementary Materials). In contrast, photographs of the re-dispersed nanoplex powders prepared by the other adjuvant formulations (i.e. *a*, *c*, *d*, and *f*) showed the appearance of highly turbid yellowish suspensions, which were similar to the appearance of the original CUR-CHI nanoplex suspension.

As indicated in Table 1, the similarity between the *b* and *e* formulations lay in the lower trehalose presence relative to that of HPMC (i.e. 10% trehalose versus 40% and 30% HPMC, respectively). In the other formulations, trehalose was present at least at the same concentration as that of HPMC, if not higher. These results signified the importance of the adequate presence of cryoprotective trehalose in minimizing the irreversible coalescence of the CUR-CHI nanoplex during freeze drying. The poor aqueous re-dispersibility of the *b* and *e* powders did not bode well for their dissolution characteristics as the irreversible coalescence greatly reduced the nanoplex surface areas available for

dissolution. Therefore, the subsequent dissolution tests were carried out only for the nanoplex powders exhibiting good aqueous re-dispersibility (i.e. *a*, *c*, *d*, and *f* formulations).

3.3. Dissolution of CUR-CHI nanoplex: powders versus aqueous suspension

Owing to its amorphous state and nanoscale size, the CUR-CHI nanoplex exhibited a fast dissolution velocity under sink condition in its aqueous suspension form. Nearly 80% of the CUR payload was recovered in the dissolution medium in less than 5 min (or 0.083 h) (Fig. 4A). The decrease in the amount of CUR recovered afterwards was due to the hydrolytic degradation of CUR in a physiological pH environment, such as PBS [20]. This degradation was particularly significant under the sink condition (i.e. low CUR concentration) investigated here. As a result, 100% recovery of the CUR payload in the dissolution medium was not demonstrated.

In comparison, the CUR-CHI nanoplex powders from the *a* and *c* formulations, which both had 50% (w/w) nanoplex contents, exhibited significantly slower dissolution velocities. Specifically, for the *a* and *c* formulations, only approximately 10% and 40% of the CUR payload were recovered in the dissolution medium after 0.5 h, respectively (Fig. 4A). A similar trend was observed for the powders from the *d* and *f* formulations, which had 60% (w/w) nanoplex contents. For the *d* and *f* formulations, the amounts of CUR recovered after 0.5 h were only approximately 20% and 40% of the CUR payload, respectively (Fig. 4B).

The slower dissolution velocities of the nanoplex powders were not unexpected considering that the adjuvants had to first dissolve, followed by liberation of the nanoplex before the CUR dissolution could essentially take place. Among the four formulations, the powders from the *c* and *f* formulations, which had more trehalose than HPMC, exhibited the two fastest dissolution rates. These results again signified the importance of having enough cryoprotectant in the dry powder formulation to achieve better dissolution characteristics. In this regard, while the *c* and *f* formulations exhibited similar dissolution rates, the latter was determined to be the optimal formulation attributed to its higher nanoplex content. Therefore, the supersaturation generation capability of the CUR-CHI nanoplex powders prepared by the *f* formulation was examined next.

3.4. Supersaturation generation of CUR-CHI nanoplex: powders versus aqueous suspension

In the absence of pre-dissolved HPMC in the dissolution medium, the aqueous suspension of the CUR-CHI nanoplex exhibited the typical “spring and parachute” supersaturation profile of amorphous drug formulations [21]. Specifically, the supersaturation level peaked at $(9.2 \pm 1.0) \times C_{\text{Sat}}$ after 10 min (“spring”) followed by its decrease to

(4.8 ± 0.5) $\times C_{\text{Sat}}$ after 30 min (“parachute”) (Fig. 5A). Subsequently, the supersaturation level decreased to $\approx 2 \times C_{\text{Sat}}$ after 1 h and remained at that value for the next 7 h (Fig. 5A), before it eventually settled at $1 \times C_{\text{Sat}}$ after 24 h (data not shown). This supersaturation profile hence reaffirmed the solubility enhancement capability of the CUR-CHI nanoplex over a prolonged period.

In comparison, the CUR-CHI nanoplex powders also exhibited the “spring and parachute” supersaturation profile, however, at a considerably lower peak level of (3.3 ± 0.5) $\times C_{\text{Sat}}$, which was reached immediately upon dissolution (< 5 min) (Fig. 5A). The supersaturation level then decreased to (2.1 ± 0.1) $\times C_{\text{Sat}}$ after 30 min and remained at that value for 8 h (Fig. 5A), before it eventually settled at $1 \times C_{\text{Sat}}$ after 24 h (data not shown). The diminished supersaturation level generated by the powder form was not unexpected considering its abovementioned slower dissolution velocity. The slower dissolution velocity was known to increase the solution-mediated crystallization propensity of the remaining amorphous solid phase undergoing dissolution, resulting in the lower supersaturation generation [22].

In the presence of pre-dissolved HPMC in the dissolution medium, a similar supersaturation profile was observed for the CUR-CHI nanoplex suspension, albeit at a higher peak supersaturation level of (14.4 ± 1.2) $\times C_{\text{Sat}}$ that was achieved immediately upon dissolution (Fig. 5B). The supersaturation level then rapidly dropped to $\approx 4 \times C_{\text{Sat}}$ after 20 min (Fig. 5B) and remained at that value over 24 h (data not shown). Hence, the supersaturation generation of the nanoplex suspension was improved in the presence of pre-dissolved HPMC, which was not unexpected considering HPMC inhibited the crystallization propensity of the supersaturated solution.

In contrast, the CUR-CHI nanoplex powders exhibited a diminished supersaturation generation in the presence of pre-dissolved HPMC. Specifically, the highest supersaturation level was only (2.4 ± 0.3) $\times C_{\text{Sat}}$ that was achieved slowly after 6 h (Fig. 5B). This was followed by its gradual decrease before it eventually settled at $1 \times C_{\text{Sat}}$ after 24 h (data not shown). The diminished supersaturation generation was caused by the slower dissolution velocity of HPMC in the nanoplex powders due to the lower concentration gradient driving force of HPMC between the powders and the dissolution medium. This resulted in slower liberation of the nanoplex from the adjuvant matrix, causing its diminished supersaturation generation.

In short, the results presented in Section 3.3 and 3.4 demonstrated the adverse effect of the dry powder transformation by freeze drying on the CUR-CHI nanoplex’s dissolution characteristics, even with the use of drying

adjuvants. Nevertheless, while the dissolution velocity of the nanoplex powders under the sink condition was lower by roughly 50% compared to the aqueous suspension form (Fig. 4), the area-under-the-curve (AUC) of the supersaturated concentration versus time plot of the nanoplex powders (Fig. 5) was only slightly lower at approximately 11% over the 8-h-period (i.e. from 72 $\mu\text{g/mL}\cdot\text{h}$ to 64 $\mu\text{g/mL}\cdot\text{h}$).

In this regard, the highly comparable AUC of the supersaturation versus time plot between the nanoplex suspension and nanoplex powders was not unexpected considering the difference in their supersaturation profiles was only significant in the first 1 h of dissolution (Fig. 5). Significantly, these results suggested that the nanoplex powders remained effective in enhancing the CUR solubility, despite their slower dissolution velocity. This was attributed to their ability to produce prolonged supersaturation level, albeit at a lower maximum achievable supersaturation level. These results boded well for the practical application of the CUR-CHI nanoplex as a solubility enhancement strategy of CUR.

3.5. Long-term stability of CUR-CHI nanoplex powders

The amorphous state of the CUR-CHI nanoplex powders from the *f* formulation after its preparation (i.e. 0 month) was demonstrated by their PXRD pattern in which broad amorphous halos were present, which were in contrast to the sharp crystalline peaks exhibited by the native CUR (Fig. 6A). Their amorphous state was reaffirmed by the DTA thermograph that showed the absence of an endothermic peak at around 174°C corresponding to the crystalline melting point, which was again in contrast to the thermograph of the native CUR (Fig. 6B). On this note, the TG thermograph showed that the CUR-CHI nanoplex started to decompose at around 240°C (Fig. S2 of the Supplementary Materials), hence the DTA thermograph above 240°C were excluded in the analysis.

After the accelerated storage for three months, which was equivalent to twelve-month storage under normal condition, the CUR-CHI nanoplex powders, however, began to exhibit characteristics of a crystalline form. This was as evidenced by the appearances of (i) sharp crystalline peaks at $2\theta \approx 8$ and 17 in their PXRD pattern (Fig. 6A) and (ii) a small endothermic peak at around 174°C in their DTA thermograph (Fig. 6B). Hence, the PXRD and DTA results after the twelve-month storage indicated that the CUR-CHI nanoplex powders had begun to recrystallize during storage. The amorphous state stability of the CUR-CHI nanoplex powders, nevertheless, could be improved by storing them as physical mixtures with HPMC powders. After the twelve-month storage, the PXRD pattern and DTA thermograph of the physical mixture showed the absence of sharp crystalline and melting point peaks, respectively,

hence denoting its good amorphous state stability (Fig. 6). In the physical mixture, the physical separation of the amorphous CUR afforded by the HPMC inclusion coupled with the high glass transition temperature of HPMC resulted in the improved physical stability of the amorphous CUR-CHI nanoplex powders during storage [23].

4. Conclusions

First, the present work successfully demonstrated the scaled-up production of amorphous CUR-CHI nanoplex in which nanoplex having similar physical characteristics (i.e. size $\approx$ 100-300 nm, zeta potential $\approx$ 18 mV, CUR payload $\approx$ 85%, and colloidal stability over 24 h) was produced at high complexation efficiency at the largest production volume investigated (i.e. 800 mL). The scaled-up production, however, resulted in a lower solid concentration in the nanoplex suspension ($\approx$ 0.16% w/v), hence lower production yield ($\approx$ 30%), due to the reduced nanoplex recovery in the centrifugation step at larger production volumes. Second, the present work showed that the CUR-CHI nanoplex powders, when formulated correctly with drying adjuvants, could maintain the prolonged supersaturation generation capability of the nanoplex suspension, albeit at a slower dissolution velocity. A high apparent solubility at approximately twice of the saturation solubility of CUR was demonstrated by the optimal formulation for at least 8 h. The optimal formulation required a predominant presence of trehalose as the cryoprotectant to prevent irreversible coalescence of the nanoplex during the dry powder transformation by freeze drying. Third, the amorphous state stability of the CUR-CHI nanoplex powders during long-term storage was achieved by storing them as physical mixture with HPMC as the crystallization-inhibiting agent. In summary, the present results successfully established the CUR-CHI nanoplex as a highly feasible solubility enhancement strategy of CUR attributed to its straightforward production scalability, supersaturation generation capability, and long-term stability in the dry powder form in the presence of crystallization-inhibiting agent.

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Figure captions

Fig. 1 Effects of production volume on (A) size and zeta potential; (B) CUR payload and solid suspension concentration

Fig. 2 (A) FESEM image and (B) colloidal stability of the CUR-CHI nanoplex prepared in 400-mL batch

Fig. 3 (A) FTIR spectra of the native CUR, native CHI, CUR-CHI nanoplexes prepared in 2-mL and 400-mL batches; (B) Effects of production volume on CE and yield

Fig. 4 Dissolution rate under sink condition for CUR-CHI nanoplex powders at (A) 50% nanoplex content (i.e. *a* and *c* formulations) and (B) 60% nanoplex content (i.e. *d* and *f* formulations)

Fig. 5 Supersaturation generation of CUR-CHI nanoplex powders from formulation *f* (A) with and (B) without the presence of pre-dissolved HPMC in the dissolution medium

Fig. 6 (A) PXRD patterns and (B) DTA thermographs of the native CUR, native CHI, CUR-CHI nanoplex, and its physical mixture with HPMC at 0 month and after twelve-month storage

Table captions

Table 1 A summary of the different CUR-CHI nanoplex powder formulations investigated

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6