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1 **A Trade-off between Solubility Enhancement and Physical Stability upon Simultaneous**
2 **Amorphization and Nanonization of Curcumin in Comparison to Amorphization Alone**

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Abstract

The numerous health benefits of curcumin (CUR) have not been fully realized due to its low aqueous solubility, resulting in poor bioavailability. While amorphization of CUR via amorphous solid dispersion (ASD) represents a well-established CUR solubility enhancement strategy, simultaneous amorphization and nanonization of CUR via amorphous CUR nanoparticles (or nano-CUR in short) have emerged only recently as the plausibly superior alternative to ASD. Herein we examined for the first time the amorphous nano-CUR versus the ASD of CUR in terms of their (1) *in vitro* solubility enhancement capability and (2) long-term physical stability. The ASD of CUR was prepared by spray drying with hydroxypropylmethylcellulose (HPMC) acting as crystallization inhibitor. The amorphous nano-CUR was investigated in both its (i) aqueous suspension and (ii) dry-powder forms in which the latter was prepared by spray drying with adjuvants (i.e. HPMC, trehalose, and soy lecithin). The results showed that the amorphous nano-CUR (in both its aqueous suspension and dry-powder forms) exhibited superior solubility enhancement to the ASD of CUR attributed to its faster dissolution rates. This was despite the ASD formulation contained a larger amount of HPMC. The superior solubility enhancement, however, came at the expense of low physical stability, where the amorphous nano-CUR showed signs of transformation to crystalline after three-month accelerated storage, which was not observed with the ASD. Thus, despite its inferior solubility enhancement, the conventional ASD of CUR was found to represent the more feasible CUR solubility enhancement strategy.

Keywords: curcumin; nanoparticles; amorphous solid dispersion; spray drying; poorly soluble drugs

45 **List of abbreviations**

46	AUC	area under the curve
47	ASD	amorphous solid dispersion
48	C _{Sat}	thermodynamic saturation solubility of CUR
49	C _{Supersat}	supersaturated concentration of CUR
50	CHI	chitosan
51	CUR	curcumin
52	DLS	dynamic light scattering
53	DTA	differential thermal analyzer
54	FESEM	field emission scanning electron microscope
55	HPLC	high performance liquid chromatography
56	HPMC	hydroxypropylmethylcellulose
57	nano-CUR	curcumin nanoparticles
58	PBS	phosphate buffered saline
59	PXRD	powder x-ray diffraction
60	PVP	poly (vinylpyrrolidone)
61	SEM	scanning electron microscope
62	TGA	thermogravimetric analysis
63	UV-Vis	ultraviolet visible spectroscopy

64

1. Introduction

Curcumin (CUR) - a natural polyphenol isolated from turmeric plants - has been widely consumed as oral dietary supplements attributed to its well-established vast therapeutic activities (e.g. anti-inflammatory, antioxidant, anticancer) (Prasad et al., 2014b). However, the poor bioavailability of CUR caused primarily by its low aqueous solubility ($<< 1$ mg/mL) has greatly limited the therapeutic effectiveness of CUR (Berginc et al., 2012; Siviero et al., 2015). Numerous strategies to enhance the CUR solubility (e.g. amorphization, nanonization, complexation, concomitant delivery) have been pursued (Anand et al., 2007). Among these strategies, amorphization (i.e. the process of formulating CUR into amorphous solids) represents one of the most effective strategies because of its ability to produce high apparent solubility of CUR (Serrano et al., 2015).

The high apparent solubility of amorphous CUR is attributed to the highly supersaturated concentration generated by the metastable state of the amorphous form (Brough and Williams, 2013). The amorphous CUR was typically prepared in the form of microscale amorphous solid dispersion (ASD) in which CUR was molecularly dispersed in the amorphous matrix of polymers exhibiting high glass transition temperature, such as hydropropylmethylcellulose (HPMC) and poly (vinylpyrrolidone) (PVP) (Chuah et al., 2014; Liu et al., 2016; Paradkar et al., 2004; Teixeira et al., 2016). Spray drying and hot melt extrusion represent the two most widely used ASD preparation methods (Brough and Williams, 2013). In ASDs, the metastable state of the amorphous form is stabilized by molecular mobility restrictions of the active compound by the polymer matrix (Baghel et al., 2016). Upon dissolution, the polymer stabilizer in the ASD functions to suppress (1) the nucleation of the supersaturated solution and (2) the solution-mediated nucleation of the remaining solid phase by Ostwald ripening, resulting in high and prolonged apparent solubility (Sun and Lee, 2013).

Recently, nanonization (i.e. the process of formulating CUR into nanoparticles) has emerged as an attractive CUR solubility enhancement strategy attributed to the fast dissolution afforded by the large specific surface areas of nanoparticles (Borin et al., 2016; Naksuriya et al., 2014; Sun et al., 2012). Not surprisingly, a synergistic CUR solubility enhancement strategy that combines the amorphization and nanonization principles has been developed in the form of amorphous CUR nanoparticles (or nano-CUR in short) that exhibit both high apparent solubility and fast dissolution (Nguyen et al., 2015; Shin et al., 2016).

91 In theory, the fast dissolution of amorphous nanoparticles should make them less vulnerable to the
92 aforementioned solution-mediated nucleation of the amorphous solid phase, resulting in their superior
93 supersaturation generation compared to the microscale ASDs (Jog and Burgess, 2017). In practice, the superior
94 supersaturation generation of amorphous nanoparticles compared to their ASD counterparts has been demonstrated
95 in several studies for several small-molecule drugs (Dhumal et al., 2008; Matteucci et al., 2007; Miller et al., 2012;
96 Mou et al., 2011). This type of study, however, has not been carried out for the ASD of CUR and amorphous nano-
97 CUR. Therefore, the first objective of the present work was to evaluate the amorphous nano-CUR against the ASD
98 of CUR prepared by spray drying in terms of their *in vitro* solubility enhancement capability.

99 Besides solubility enhancement, the physical stability of the amorphous state during its shelf life represents
100 another important characteristics of amorphous CUR formulations, where the inclusion of crystallization inhibitors,
101 such as HPMC and PVP, is typically needed to stabilize the amorphous form (Chavan et al., 2016). In this regard,
102 nanoparticle formulations of active compounds are typically stored in their dry powder forms due to the poor storage
103 stability of the aqueous suspension forms (Abdelwahed et al., 2006; Fonte et al., 2014). Therefore, the second
104 objective of the present work was to develop dry powder formulation of the amorphous nano-CUR by spray drying,
105 where the spray-dried nano-CUR was designed to exhibit good physical stability during storage, while maintained
106 the solubility enhancement characteristics possessed by the raw amorphous nano-CUR.

107 For this purpose, the effects of (1) the mass ratio of the nano-CUR to the total drying adjuvant used, and (2) the
108 adjuvant compositions (i.e. HPMC: trehalose: soy lecithin) were investigated. Herein the roles of HPMC were
109 twofold, namely (1) to stabilize the amorphous nano-CUR powders during storage and (2) to inhibit crystallization
110 of the supersaturated solution generated upon the nano-CUR dissolution (Tajarobi et al., 2011). **HPMC was used as**
111 **the crystallization inhibitor because it had been shown to be superior to PVP in inhibiting CUR crystallization**
112 **(Gosangari and Dyakonov, 2013; Meng et al., 2015; Wegiel et al., 2014). Furthermore, ASD of CUR prepared with**
113 **HPMC had been successfully demonstrated *in vivo* to enhance the bioavailability of CUR (Chuah et al., 2014). On**
114 **this note, the roles of trehalose and soy lecithin were to prevent irreversible coalescences of the nano-CUR upon**
115 **drying and to improve surface wettability of the nano-CUR powders upon dissolution, respectively.**

116 In short, the present work aimed to answer the following two research questions pertaining to the solubility
117 enhancement of CUR, i.e. (1) was the solubility enhancement of the amorphous nano-CUR superior to that of the

ASD of CUR? If yes, the next question was (2) could the dry-powder form of the amorphous nano-CUR prepared by spray drying maintain the superior solubility enhancement of its aqueous suspension counterpart, while at the same time exhibited good long-term physical stability? The results showed that the amorphous nano-CUR did exhibit superior solubility enhancement to the ASD of CUR, but a trade-off existed between the solubility enhancement and the physical stability, thereby identifying the optimal amorphous nano-CUR formulation remained a challenge.

2. Materials and methods

2.1. Materials

CUR from turmeric rhizome (95% total curcuminoid content), ethanol, and phosphate buffered saline (PBS) (pH 7.4) were purchased from Alfa Aesar (Singapore), Merck Millipore (Singapore), and 1st Base (Singapore), respectively. Chitosan (CHI) (50-190 kDa, 75-85% deacetylation), D-trehalose, HPMC (26 kDa), potassium hydroxide (KOH), sodium chloride (NaCl), and glacial acetic acid were purchased from Sigma-Aldrich (Singapore). Lecithin (L- α -phosphatidylcholine) from soy (95% purity) was purchased from Avanti Polar Lipids (USA).

2.2. Methods

2.2.1. Preparation and characterization of the amorphous nano-CUR

The nano-CUR was prepared by a bottom-up electrostatically-driven drug-polyelectrolyte complexation method presented earlier in Nguyen et al. (2015). Briefly, 5 mg/mL CUR was dissolved in 0.1 M KOH to form anionic CUR molecules, while 6 mg/mL CHI was dissolved separately in 1.2% (v/v) acetic acid solution to form cationic CHI molecules. Subsequently, equal volumes of the CUR and CHI solutions (25 mL each) were mixed under gentle stirring and the resultant nano-CUR suspension was ultrasonicated for 15 s at 20 kHz (VC 505, Sonics, USA). Next, the nano-CUR suspension was washed by two cycles of centrifugation (14,000 $\times$ g) to remove excess CUR and CHI after which the nano-CUR was re-suspended in deionized water for characterizations.

The size and zeta potential of the nano-CUR were characterized by dynamic light scattering (DLS) after 100 $\times$ dilution, using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The nano-CUR morphology was examined by field emission scanning electron microscope (FESEM) (JSM 6700F, JEOL, USA). The CUR payload defined as the amount of CUR per unit mass of the nano-CUR was determined by dissolving a known mass of the nano-CUR in 80% (v/v) ethanol. Afterwards, the amount of CUR in the ethanol

solution was determined by UV-Vis spectrophotometer (UV Mini 1240, Shimadzu, Japan) at the optimal CUR absorbance wavelength of 423 nm (Leung et al., 2008).

2.2.2. Preparation of the ASD of CUR

The ASD of CUR was prepared by spray drying using B-290 mini spray-dryer (Büchi, Switzerland) at feed solid concentration of 1.0% (w/v) with CUR: HPMC ratio equal to 40: 60 (w/w). The drying temperature, feed flow rate, and atomizing flow rate were fixed at 120°C, 5 mL/min, and 300 L/h, respectively. The feed solution was prepared by dissolving the native CUR together with soy lecithin at 10:1 mass ratio in 80% (v/v) ethanol solution after which the ethanol solution was mixed with 1% (w/v) aqueous HPMC solution. The CUR payload in the ASD was determined by the same method described in the above.

2.2.3. Preparation of the dry-powder amorphous nano-CUR

The dry-powder form of the amorphous nano-CUR was prepared by spray drying at the same feed solid concentration, drying temperature, feed flow rate, and atomizing flow rate as the ones used for the ASD of CUR. As summarized in Table 1, the CUR content in the feed was varied from 40% (w/w) to 60% (w/w) at different adjuvant compositions (i.e. formulations *a* to *l*), where the soy lecithin composition was fixed at 1/10 of the CUR composition. Aqueous solutions of HPMC and trehalose and ethanol solution of soy lecithin (all at 1% w/w) were prepared separately after which they were mixed with the aqueous suspension of the nano-CUR at the predetermined compositions shown in Table 1.

2.2.4. Physical characterization of the dry-powder CUR

The amorphous state of the spray-dried products (i.e. ASD of CUR and amorphous nano-CUR) and their stability during accelerated storage (i.e. three months at 40°C and 75% relative humidity) were examined by powder x-ray diffraction (PXRD) using D8 Advance X-ray Diffractometer (Bruker, Germany). The PXRD was performed between 10° and 70° (2θ) with a step size of 0.02°/s. The PXRD of the native CUR was also performed for comparison. The abovementioned accelerated storage condition was roughly equivalent to twelve-month storage under the normal condition of 25°C and 60% relative humidity (Grimm, 1998). The 75% relative humidity condition was generated by placing an open container of saturated NaCl solution in a desiccator maintained at 40°C.

The morphologies (i.e. size, shape) of the spray-dried products were examined by scanning electron microscope (SEM) (JSM 6390LA, JEOL, USA). The thermal stability of the spray-dried products was examined by

thermogravimetric (TGA)/differential thermal analyzer (DTA) using Pyris Diamond TG/DTA (Perkin-Elmer, USA) at heating rate of 10°C/min in the range of 25°C to 400°C. The TGA/DTA analysis was performed on the spray-dried products before and after the accelerated storage. The TGA/DTA analysis was also carried out for the native CUR, native CHI, HPMC, trehalose, and soy lecithin.

For the spray-dried amorphous nano-CUR, the aqueous re-dispersibility was characterized in terms of the size of the nano-CUR recovered from the spray-dried particles upon their immersion in an aqueous medium. Briefly, in triplicates, 2 mg of the spray-dried particles were suspended in 400 mL deionized water under gentle stirring for 30 min. Next, 2 mL aliquot of the resultant particulate suspension was withdrawn after which the size of the nano-CUR in the suspension was characterized by DLS as previously described. A good aqueous re-dispersibility was characterized by nearly complete reconstitution of the spray-dried particles to individual nano-CUR particles as reflected by a minimal change in the nano-CUR size before and after spray drying.

2.2.5. Dissolution rate under sink condition

The dissolution rates under a sink condition (i.e. at CUR concentration $\ll$ its thermodynamic saturation solubility $C_{\text{Sat}} = 4.15 \mu\text{g/mL}$ (Nguyen et al., 2015)) were characterized in triplicates for the amorphous nano-CUR (both its aqueous suspension and spray-dried forms) and the ASD of CUR. Briefly, the CUR particles were dispersed at $\frac{1}{4} C_{\text{Sat}}$ in 230 mL PBS placed in a shaking incubator maintained at 37°C. Next, 2 mL aliquot was withdrawn at specific time points over a 2-h period and 2 mL fresh PBS was added back to the dissolution medium. The aliquot was then syringe filtered (0.2- μm pore size) and the CUR concentration in the aliquot was determined by UV-Vis spectrophotometer at 423 nm as previously described.

2.2.6. Supersaturation generation

Next, the dissolution rates of the three CUR particles under a non-sink condition (i.e. CUR concentration $\gg C_{\text{Sat}}$) were carried out in triplicates in order to characterize the supersaturation generation of the amorphous forms. The supersaturation generation was characterized with and without the presence of pre-dissolved crystallization-inhibiting HPMC (2 mg/mL) in the dissolution medium. Briefly, the CUR particles were dispersed in 100 mL PBS at CUR concentration equal to $15 \times C_{\text{Sat}}$ in a shaking incubator maintained at 37°C. Next, 400 μL aliquot was withdrawn at specific time points over a 24-h period. The aliquot was then syringe filtered and immediately diluted ten times with fresh PBS to prevent CUR precipitation from the supersaturated solution.

The supersaturated CUR concentration in the aliquot (C_{Supersat}) was then determined by high performance liquid chromatography (HPLC) using Agilent 1100 (Agilent Technologies, USA) at detection wavelength of 423 nm (Leung et al., 2008). Aqueous ethanol solution 80% (v/v) at 1.0 mL/min was used as the mobile phase, resulting in CUR's retention time of ≈ 2.5 min, in ZORBAX Eclipse Plus C18 column (250 x 4.6 mm, 5 μm particle size). The supersaturation generation of the CUR particles was reported as the ratio of C_{Supersat} to C_{Sat} .

3. Results and discussion

3.1. Comparing the solubility enhancement of amorphous nano-CUR versus ASD of CUR

3.1.1. Physical characteristics

We first compared the physical characteristics (i.e. size, shape, CUR payload) of the amorphous nano-CUR versus that of the ASD of CUR as the dissolution rate was widely known to be greatly influenced by the particle characteristics. The amorphous nano-CUR exhibited a roughly elongated shape as shown in the FESEM image in Fig. 1A with an average size of 167 ± 12 nm and zeta potential of 16 ± 2 mV as measured by DLS, where the positive zeta potential was attributed to the presence of cationic CHI on the nano-CUR surface. The CUR payload of the nano-CUR was equal to $81 \pm 9\%$ (w/w) with the remaining mass was made up of CHI acting as colloidal stabilizer of the nano-CUR.

The ASD of CUR, on the other hand, was roughly spherical in the size range of 5-35 μm as shown in the SEM image in Fig. 1B and with CUR payload of $38 \pm 3\%$ (w/w) with the remaining mass was made up of mostly the crystallization-inhibiting HPMC. In this regard, the ASD of CUR was prepared by spray drying at 40% (w/w) CUR content to facilitate its direct comparison with the optimal dry-powder formulation of the amorphous nano-CUR to be presented later in Section 3.2, where a higher CUR content above 40% (w/w) was reported to result in poorer solubility enhancement for the spray-dried nano-CUR.

3.1.2. Dissolution rate under sink condition

Not unexpectedly, the amorphous nano-CUR was found to exhibit a faster dissolution rate under sink condition than the ASD of CUR attributed to its much smaller size. Specifically, $80 \pm 5\%$ (w/w) of the initial CUR payload was dissolved from the nano-CUR after 5 min versus only $54 \pm 1\%$ (w/w) was dissolved from the ASD of CUR over the same period (Fig. 2A). On this note, the gradual decrease in the % CUR recovered upon reaching the peak concentration in Fig. 2 was due to the well-known hydrolytic degradation of CUR in a physiological pH

environment (Sun et al., 2010), particularly at low CUR concentrations under the sink condition investigated here. As a result, the % CUR recovered never reached 100% for both the amorphous nano-CUR and the ASD of CUR.

3.1.3. Supersaturation generation

Next, the solubility enhancement capability of the amorphous nano-CUR was evaluated against the ASD of CUR in terms of the supersaturation generation. Both the amorphous nano-CUR and the ASD of CUR exhibited the “spring-and-parachute” supersaturation profiles characteristic of an amorphous form (Sun and Lee, 2013), where the supersaturation level rapidly reached a peak (“spring”) owed to the fast dissolution of the amorphous solids, followed by a gradual decrease in the supersaturation level due to crystallization of the supersaturated solution (“parachute”) (Fig. 2B). In the absence of pre-dissolved HPMC as a crystallization inhibiting agent in the dissolution medium, the maximum achievable supersaturation levels for the amorphous nano-CUR and the ASD of CUR were $(9.9 \pm 0.9) \times C_{\text{Sat}}$ and $(4.2 \pm 0.6) \times C_{\text{Sat}}$, respectively, which were both achieved approximately after 10 min (Fig. 2B). The higher maximum achievable supersaturation level for the amorphous nano-CUR was attributed to its aforementioned faster dissolution rate, which in turn minimized the time window for the Ostwald ripening crystallization of the amorphous particles (Matteucci et al., 2007).

The supersaturation level of the amorphous nano-CUR, however, quickly decreased to $(4.8 \pm 0.5) \times C_{\text{Sat}}$ after just 0.5 h and continued to decrease to $(1.8 \pm 0.1) \times C_{\text{Sat}}$ after 1 h due to quick crystallization of the highly supersaturated CUR solution caused by the lack of crystallization-inhibiting agent in the nano-CUR formulation. In contrast, the supersaturation level of the ASD of CUR slowly decreased from its peak value and settled at $(2.1 \pm 0.1) \times C_{\text{Sat}}$ after 2.5 h owed to the presence of HPMC in the ASD formulation (Fig. 2B). The supersaturation levels of the amorphous nano-CUR and ASD of CUR remained at the abovementioned values over the next 8 h before they eventually settled down back to $1 \times C_{\text{Sat}}$ after 24 h (data not plotted).

These supersaturation time-profiles over the 8-h-period resulted in the area-under-the-curve (AUC) equal to 67 ± 3 and 74 ± 4 $\mu\text{g/mL}\cdot\text{h}$ for the amorphous nano-CUR and ASD of CUR, respectively. The comparable AUC achieved by the amorphous nano-CUR, without the need of incorporating HPMC in its formulation, signified its superior inherent solubility enhancement capability. Furthermore, the significantly higher CUR payload of the amorphous nano-CUR at $\approx 80\%$ versus $\approx 38\%$ for the ASD of CUR should translate to a lower dosage requirement for the amorphous nano-CUR while achieving the same AUC. These results hence answered our first research

question by demonstrating that simultaneous amorphization and nanonization of CUR was the superior solubility enhancement strategy compared to amorphization alone.

3.2. Spray drying formulation of the amorphous nano-CUR

3.2.1. Preliminary screening

We proceeded to determine the spray-drying formulation of the amorphous nano-CUR that could preserve its superior solubility enhancement characteristics in the dry-powder form. The spray-drying formulation that exhibited the optimal dissolution characteristics was determined systematically by first (i) investigating the aqueous re-dispersibility of the spray-dried particles, followed by (ii) examining the dissolution rate under sink condition, and finally (iii) evaluating the supersaturation generation. In terms of the physical characteristics, the spray-dried nano-CUR shared a similar morphology with the ASD of CUR, where the particles were roughly spherical as seen in the SEM image (Fig. S1 of the Supplementary Materials) in the size range of 10 to 30 μm . The same morphology (i.e. size, shape) was observed for all the spray-dried nano-CUR independent of the adjuvant compositions.

The spray-dried nano-CUR was found to exhibit relatively good aqueous re-dispersibility for the range of CUR contents (i.e. 40-60% w/w) and adjuvant compositions investigated (i.e. formulations *a* to *l* in Table 1). The spray-dried nano-CUR (10-30 μm) readily re-dispersed in an aqueous medium to smaller particles in the nanosize range of 350 to 550 nm as shown in Fig. 3A. The larger re-dispersed nano-CUR size compared to its original size before spray drying (≈ 170 nm) was postulated to be caused by coalescence of a small number of nano-CUR particles upon exposure to the high temperature in spray drying. The good aqueous re-dispersibility of the spray-dried nano-CUR boded well for their ability to maintain the fast dissolution rate exhibited by the nano-CUR suspension.

In this regard, spray drying of the nano-CUR at higher CUR contents (i.e. > 60%) resulted in particles with poor aqueous re-dispersibility and low production yield (data not shown), hence it was not pursued further. Moreover, the nano-CUR content investigated was capped at 60% (w/w) because there ought to be sufficient amount of HPMC to stabilize the amorphous state of the spray-dried nano-CUR. On this note, while the spray-drying formulation of the amorphous nano-CUR contained more than one polymer (i.e. CHI and HPMC), it was distinct from amorphous ternary solid dispersions made up of drug-polymer-polymer interactions reported in Prasad et al. (2016) and Prasad et al. (2014a). The reason was because the spray-dried nano-CUR was made up of physical dispersion of the nano-

CUR in the polymer matrix, instead of being molecularly dispersed in a completely miscible drug-polymer-polymer phase, as was the case in the amorphous ternary solid dispersion.

3.2.2. Dissolution rate under sink condition

The spray-drying formulations were subsequently narrowed down by examining their dissolution rates under sink condition and compared them with that of the amorphous nano-CUR suspension. Starting with the spray-dried nano-CUR prepared at CUR = 40% (w/w) (i.e. formulations *a*, *b*, and *c* in Table 1), the spray-dried nano-CUR exhibited slower dissolution rates than the nano-CUR suspension (Fig. 3B). This was not unexpected considering that the dissolution of the spray-dried nano-CUR could only proceed fully upon dissolution of the water-soluble excipients. Specifically, $78 \pm 6\%$ of CUR was recovered in the dissolution medium from the nano-CUR suspension after 10 min, whereas only $35 \pm 2\%$, $68 \pm 3\%$, and $21 \pm 2\%$ of CUR were recovered from the formulations *a*, *b*, and *c*, respectively, over the same period. In this regard, the significant variations in the % CUR recovered among the *a*, *b*, and *c* formulations indicated the importance of the adjuvant compositions, where the dissolution rates were found to increase with increasing HPMC content in the formulation.

For the spray-dried nano-CUR prepared at CUR = 50% (w/w) (i.e. formulations *d*, *e*, and *f*), the % CUR recovered after 10 min were $24 \pm 3\%$, $52 \pm 5\%$, and $47 \pm 4\%$, respectively (Fig. 4A). Again, a strong dependence of the dissolution rates on the adjuvant compositions was demonstrated. For the spray-dried nano-CUR prepared at CUR = 60% (w/w) (i.e. formulations *g*, *h*, and *i*), however, the dissolution rates worsened greatly. The % CUR recovered even after 30 min for these formulations was only $\approx 30\%$ independent of the excipient compositions (Fig. S2 of the Supplementary Materials). These results led us to conclude that the dissolution rates of the spray-dried nano-CUR deteriorated with increasing nano-CUR content.

Subsequently, we investigated the effect of adding soy lecithin to improve the dissolution rates by virtue of the enhanced wettability of the spray-dried particles in the presence of surface-active lecithin. The study was performed at CUR = 40% (w/w) (i.e. formulations *j*, *k*, and *l*). The results showed that the addition of soy lecithin was only effective in improving the dissolution rates for those formulations with HPMC contents lower or equal relative to the trehalose (i.e. formulations *j* and *l*). Specifically, the % CUR recovered after 10 min increased from $\approx 35\%$ and 21% in the formulations *a* and *c* without lecithin, respectively, to $70 \pm 1\%$ and $36 \pm 4\%$ in the formulations *j* and *l* with lecithin, respectively (Fig. 4B). In contrast, compared to the formulation *b* without lecithin, the presence of soy

lecithin in the formulation *k* had a minimal effect on the % CUR recovered ($68 \pm 2\%$). These results suggested that the addition of soy lecithin essentially had the same effect as increasing the HPMC content.

The spray-dried nano-CUR formulations with the optimal dissolution characteristics had thus been narrowed down to the formulations *j* and *k* with lecithin (i.e. CUR: HPMC: trehalose = 40:30:30 and 40:40:20, respectively). Both formulations *j* and *k* exhibited the maximum % CUR recovered $\approx 70\%$ that was achieved after approximately 10 min. Their maximum % CUR recovered was only slightly lower than that of the nano-CUR suspension ($\approx 80\%$) achieved over the same period. In the next section, the supersaturation generations of the formulations *j* and *k* were evaluated in order to determine the optimal formulation between the two.

3.2.3. Supersaturation generation

In the absence of pre-dissolved HPMC in the dissolution medium, the supersaturation generation by the spray-dried nano-CUR from the formulations *j* and *k* were found to be superior to that of the nano-CUR suspension, despite the lower supersaturation peak exhibited by the spray-dried nano-CUR (Fig. 5A). Owing to their slower dissolution rates, the formulations *j* and *k* exhibited lower maximum achievable supersaturation levels at $(7.1 \pm 1.1) \times C_{\text{Sat}}$ and $(8.1 \pm 0.8) \times C_{\text{Sat}}$ after 10 min, respectively, compared to the nano-CUR suspension whose supersaturation peak was at $(9.9 \pm 0.9) \times C_{\text{Sat}}$. However, unlike the nano-CUR suspension, which exhibited a rapid decrease in its supersaturation level upon reaching the peak, the supersaturation levels of the spray-dried nano-CUR decreased at a slower rate to $(3.6 \pm 0.3) \times C_{\text{Sat}}$ and $(3.9 \pm 0.5) \times C_{\text{Sat}}$ for the formulations *j* and *k*, respectively, after 1 h. The supersaturation levels then remained approximately at these values for a prolonged period of at least 8 h.

The prolonged supersaturation levels at nearly $4 \times C_{\text{Sat}}$ were attributed to the presence of HPMC in the spray-dried nano-CUR formulations. Between the formulations *j* and *k*, the latter was found to exhibit slightly higher supersaturation levels over 8 h attributed to its higher HPMC content (40% versus 30%). As a result of the prolonged supersaturation level, the spray-dried nano-CURs were determined to exhibit larger AUCs in their supersaturation time-profiles (AUCs = 94 ± 3 and 120 ± 7 $\mu\text{g/mL}\cdot\text{h}$ for the formulations *j* and *k*, respectively) compared to the nano-CUR suspension (AUC = 67 ± 3 $\mu\text{g/mL}\cdot\text{h}$). The same trends, where (1) the spray-dried nano-CURs exhibited larger AUCs in their supersaturation time-profiles than the nano-CUR suspension, and (2) the formulation *k* had larger AUC than the formulation *j*, were observed in the presence of pre-dissolved HPMC in the dissolution medium (Fig. 5B).

Based on these results, the formulation *k* was concluded to be the spray-dried nano-CUR formulation with the optimal solubility enhancement capability. Significantly, the supersaturation generation of the spray-dried nano-CUR from the formulation *k* was also superior to that of the ASD of CUR, which had the same nano-CUR content as the formulation *k* (i.e. 40% w/w). The plot comparing the supersaturation generation of the formulation *k* versus the ASD of CUR was provided in Fig. S3 of the Supplementary Materials. Specifically, as reported earlier in Section 3.1.2, the AUC of the supersaturation time-profile of the ASD of CUR was equal to $74 \pm 4 \mu\text{g/mL}\cdot\text{h}$, hence it was significantly lower than the AUC of the formulation *k* at $120 \pm 7 \mu\text{g/mL}\cdot\text{h}$. The lower AUC exhibited by the ASD of CUR was despite its significantly higher HPMC content (i.e. 60%) compared to only 40% for formulation *k*.

These results answered the first part of our second research question in which we determined that the amorphous nano-CUR exhibited superior solubility enhancement to the ASD of CUR in both their aqueous suspension and dry-powder forms. In the next section, we evaluated whether the superior solubility enhancement of the spray-dried nano-CUR from the formulation *k* was accompanied by its good physical stability.

3.2.4. Physical stability

The amorphous state of the spray-dried nano-CUR from the formulation *k* was verified by PXRD immediately after their preparation (i.e. 0 month), where the PXRD pattern showed the absence of sharp crystalline peaks observed in the native CUR that were replaced by the appearance of broad amorphous halos (Fig. 6A). After the accelerated three-month storage (i.e. 3 months), however, the spray-dried nano-CUR began to show small peaks at $2\theta \approx 15$ and 25° in its PXRD patterns indicating the appearance of small crystalline forms. Moreover, the small peaks were accompanied by the disappearance of the broad amorphous halos observed earlier. The PXRD results hence indicated that parts of the amorphous form in the spray-dried nano-CUR had re-crystallized during the accelerated storage, signifying a loss of physical stability.

The loss of physical stability of the spray-dried nano-CUR from the formulation *k* after the accelerated storage was reaffirmed by the thermal analysis using DTA. Immediately after its preparation, the DTA thermograph of the spray-dried nano-CUR did not show the endothermic melting point peak at $\approx 174^\circ\text{C}$ that was characteristic of the crystalline CUR (Fig. 6B), hence the amorphous state of the spray-dried nano-CUR was again verified. After the accelerated storage, however, a small downward peak in the DTA thermograph was observed in the temperature range of $160\text{--}175^\circ\text{C}$ signifying the presence of small crystalline forms. On this note, the TGA results showed that the

spray-dried nano-CUR began to decompose at $\approx 270^{\circ}\text{C}$ due to the decomposition of CUR and the adjuvants (Fig. S4 of the Supplementary Materials). The DTA thermographs above 270°C were thus excluded from the analysis.

For comparison, the PXRD and DTA results of the ASD of CUR, which had the same HPMC content in its formulation as the spray-dried nano-CUR ($\approx 40\%$ w/w), showed that the ASD particles exhibited good physical stability after the accelerated storage (Fig. 6). Specifically, the PXRD pattern and the DTA thermograph of the ASD of CUR showed the absence of sharp crystalline peaks and a small downward peak at around $160\text{--}170^{\circ}\text{C}$, respectively. These results suggested that the molecular mobility restriction of CUR provided by the HPMC matrix was lacking in the spray-dried nano-CUR because CUR was physically dispersed in the form of nano-CUR in the HPMC matrix. In contrast, CUR in the ASD was molecularly dispersed in the HPMC matrix, resulting in its higher physical stability as CUR and HPMC were known to exhibit good drug-polymer miscibility and strong molecular interactions via hydrogen bonds (Meng et al., 2015). Therefore, the superior solubility enhancement of the amorphous nano-CUR compared to the ASD of CUR was obtained at the expense of its lower physical stability after long-term storage.

4. Conclusions

The present work investigated two types of solubility enhancement strategy of CUR, i.e. (1) simultaneous amorphization and nanonization of CUR (i.e. amorphous nano-CUR) and (2) amorphous solid dispersion (i.e. ASD of CUR) in terms of their (i) *in vitro* solubility enhancement capability and (ii) long-term physical stability. The amorphous nano-CUR was investigated in two forms, i.e. its aqueous suspension and dry-powder forms prepared by spray drying with adjuvants (i.e. HPMC, trehalose, and soy lecithin). First, the results showed that the amorphous nano-CUR, in both its aqueous suspension and dry-powder forms, exhibited superior solubility enhancement to the ASD of CUR. The superior solubility enhancement of the amorphous nano-CUR, which was observed despite the higher HPMC content of the ASD acting as crystallization inhibitors, was attributed to its faster dissolution rates.

Second, a trade-off, however, existed between solubility enhancement and long-term physical stability upon simultaneous amorphization and nanonization of CUR. The amorphous nano-CUR was found to experience fractional loss of its amorphous form after the accelerated storage that was not evident in the ASD of CUR. The lack of physical stability of the amorphous nano-CUR was caused by the lack of molecular mobility restriction by the HPMC matrix. Significantly, the present work showed that while simultaneous amorphization and nanonization

might yield superior solubility enhancement for most small-molecule drugs owed to the resultant faster dissolution, it might come at the expense of the physical stability. Therefore, the future research direction will be to develop amorphous nano-CUR with inherently built-in physical stability, for example, by incorporating crystallization-inhibiting agents during the nanoparticle formation step, instead at the later formulation step.

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475

Figure captions

- Fig. 1 (A) FESEM image of the nano-CUR and (B) SEM image of the ASD of CUR prepared by spray drying with HPMC
- Fig. 2 (A) Dissolution rate under sink condition and (B) supersaturation generation of the amorphous nano-CUR suspension versus the ASD of CUR
- Fig. 3 (A) Aqueous re-dispersibility of the spray-dried nano-CUR as a function of the adjuvant compositions; (B) dissolution rate under sink condition for the spray-dried nano-CUR formulations *a*, *b*, and *c*
- Fig. 4 Dissolution rates under sink condition for the spray-dried nano-CUR (A) formulations *d*, *e*, and *f* and (B) formulations *j*, *k*, and *l* with soy lecithin
- Fig. 5 Supersaturation generation of the spray-dried nano-CUR formulations *j* and *k* (A) without and (B) with the presence of pre-dissolved HPMC in the dissolution medium
- Fig. 6 (A) PXRD patterns and (B) DTA thermographs of the spray-dried particles before and after the accelerated three-month storage

Table caption

- Table 1 A summary of the spray-dried nano-CUR formulations investigated (Note: + with the addition of lecithin, o without the addition of lecithin)