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Millifluidic Synthesis of Amorphous Drug-Polysaccharide Nanoparticle Complex with Tunable Size Intended for Supersaturating Drug Delivery Applications

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Abstract

The conventional bulk mixing method to prepare amorphous drug-polysaccharide nanoparticle complex (or drug nanoplex in short) has a major drawback in the lack of size control for the nanoplex produced, hence limiting its potential applications as a supersaturating drug delivery system for bioavailability enhancement of poorly soluble drugs. For this reason, we developed a continuous millifluidic synthesis platform of the drug nanoplex exhibiting high size tunability using curcumin (CUR) and chitosan (CHI) as the models for drug and polysaccharides, respectively. The nanoplex size tunability was achieved by controlling the residence time of the CUR and CHI solutions in the millifluidic reactor, where their slow diffusive mixing at the liquid-liquid interface resulted in a well-regulated nanoplex growth as a function of the residence time. The effects of the preparation pH, molecular weight of CHI, millifluidic tube diameter, and flowrate on the nanoplex size tunability were investigated from which the optimal preparation condition was determined. At the optimal condition, the CUR nanoplex was roughly ≈115 nm in size with zeta potential of ≈15 mV and ≈72% (w/w) CUR payload. The millifluidic synthesis also maintained the high CUR utilization rate (≈80%) exhibited by the bulk mixing method. Most importantly, the ability to produce significantly smaller nanoplex (six-fold smaller) via millifluidics led to the generation of higher (≈8.5× of CUR saturation solubility) and prolonged (≈8 h) supersaturation level. These results bode well for the bioavailability enhancement potential of the drug nanoplex.

Keywords: millifluidics; microfluidics; drug-polymer complexation; curcumin nanoparticles; continuous pharmaceutical manufacturing

List of abbreviations

AA	acetic acid
AUC	area under the curve
CFD	computational fluid dynamics
CHI	chitosan
CUR	curcumin
FESEM	field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
HMW	high molecular weight
HPLC	high performance liquid chromatography
HPMC	hydroxypropylmethylcellulose
ID	internal diameter
LMW	low molecular weight
MMW	medium molecular weight
OD	outer diameter
PBS	phosphate buffered saline
PCS	photon correlation spectroscopy
PDI	polydispersity index

Nomenclatures

$C_{Supersat}$	supersaturated concentration (mg/mL)
C_{Sat}	saturation solubility (mg/mL)
d	inner diameter of the millifluidic tube (mm)
D	molecular diffusion coefficient (cm^2/s)
F	flow rate (mL/min)
h	mixing length scale in the radial direction (mm)
L	axial distance from the Y-junction (cm)
$R_{CHI/CUR}$	charge ratio of CHI to CUR

78	Re	Reynolds number
79	t_{Mix}	diffusive mixing time for diffusing half of the tube diameter (s)
80	t_{Res}	residence time (s)
81	μ	dynamic viscosity (cp)
82	ν	kinematic viscosity (cm^2/s)
83	U	fluid velocity (cm/s)

1. Introduction

Nanoscale amorphous drugs have been demonstrated in several studies to be a superior supersaturating drug delivery system for poorly soluble drugs, in comparison to the conventional approach of microscale amorphous solid dispersions [1-4]. Their superior supersaturation generation is attributed to the faster dissolution rate afforded by the nanoscale that shortened the time window for solution-mediated crystallization of the remaining solid phase by Ostwald ripening [5]. Amorphous drug-polysaccharide nanoparticle complex (or drug nanoplex in short) represents a new class of nanoscale amorphous drugs that possess a number of attractive characteristics, i.e. (a) high drug payload [6, 7], (b) simple yet highly efficient preparation [8], and (c) prolonged supersaturation generation and better amorphous state stability compared to the conventional formulation of nanoscale amorphous drugs [9].

The drug nanoplex is formed by electrostatically driven self-assembly complexation between the drug and oppositely charged polysaccharides, where their strong attractive interactions prevent the drug molecules from assembling themselves into ordered crystalline structures upon precipitation of the complex, resulting in the formation of an amorphous state [9]. Thus far, the amorphous drug-polysaccharide nanoplex has been prepared in a batch process in which the drug solution is added in bulk to the polysaccharide solution at room temperature under constant stirring. While the bulk mixing method is simple, control over the resultant nanoplex size is lacking. Varying the batch mixing time was found to be ineffective due to the rapid nature of the nanoplex formation [8]. Furthermore, varying the key variables in drug-polysaccharide complexation (i.e. pH and charge ratio of polysaccharide to drug) was found not to have statistically significant influence on the nanoplex size [6, 7].

Not unlike any other particulate drug products, the size of the drug nanoplex can have significant influences not only its physicochemical characteristics (e.g. dissolution rate, colloidal stability), but also on the subsequent process of preparing its solid dosage forms (e.g. drying, granulation). Therefore, the ability to control the nanoplex size is crucial at its stage of development. In this regard, a continuous microfluidic platform has been found effective in producing highly monodisperse nanoparticles with a tunable size for a wide range of materials. The size monodispersity is resulted from the homogeneous reaction environment created by the rapid mixing in the microchannel [10-12]. For nanoparticle formation by electrostatically driven complexation with polysaccharides, a precise control of the nanoparticle size has been successfully demonstrated in microfluidics by means of controlling the residence time of the fluids undergoing complexation [13, 14].

A millifluidic synthesis platform has recently emerged as an alternative to microfluidics for production of nanoparticles, particularly for inorganic nanoparticles (e.g. gold, silver, copper) [15-17]. This was attributed to (1) the less susceptibility of millifluidics to fouling as a result of its larger channel dimension, (2) its cost effectiveness attributed to the ease of fabrication and simple operation, and (3) its higher throughput that makes nanoparticle production in the manufacturing scale closer to realization [17]. Importantly, the millifluidic platform exhibits these attractive features while also possesses the ability of microfluidics to produce nanoparticles having precisely tunable sizes [15-17]. Recently, the application of millifluidics in the production of organic nanoparticles (i.e. cellulase) has been demonstrated [18] denoting the increased interests in the millifluidic synthesis platform beyond production of inorganic metal nanoparticles.

Herein we demonstrated the application of millifluidics in the synthesis of amorphous drug nanoplex with tunable size using curcumin as the model drug. Curcumin (CUR) is a natural polyphenolic compound isolated from turmeric well known for its wide-ranging therapeutic activities [19]. Oral bioavailability of CUR, however, is poor due to its rapid metabolism and low aqueous solubility [20]. Previously, we synthesized CUR nanoplex as a new bioavailability enhancement strategy of CUR by means of complexation with chitosan (CHI) using the bulk mixing method [6]. The sizes of the CUR nanoplex prepared at different pH and charge ratios of CHI to CUR (twelve runs in total) were fairly constant at around 250-300 nm denoting the lack of tunable size in the bulk mixing method [6].

The objective of the present work was to investigate the feasibility of employing a simple millifluidic reactor to produce CUR nanoplex with a tunable size by means of residence time variations. The residence time was varied by collecting the nanoplex at different axial distance from the inlet, hence essentially varying the reactor length. The present work also examined the impacts of the preparation pH, CHI's molecular weight, tube diameter, and flowrate on the tunability of the nanoplex size by the residence time variations. The CUR nanoplex prepared at the optimal condition was characterized and compared with the nanoplex prepared by the bulk mixing method in terms of their physical characteristics (i.e. size, polydispersity index, zeta potential, payload, supersaturation generation, and amorphous state) and preparation efficiency (i.e. CUR utilization rate, overall yield).

2. Materials and Methods

2.1. Materials

Materials for millifluidics setup*:** High-density polyethylene Y-junction connector (orifice ID 0.794 mm) and Tygon® microbore tubing of two different sizes, i.e. ID 0.762 mm (OD 2.286 mm) and 1.588 mm (OD 3.175 mm) were purchased from Cole-Parmer (USA). ***Materials for nanoplex preparation: CUR (98%) was purchased from Alfa Aesar (Singapore) and CHI of three different molecular weights (MW), i.e. low (LMW, μ = 20-300 cp), medium (MMW, 200-800 cp), and high (HMW, 800-2000 cp), with 75-85% deacetylation were purchased from Sigma-Aldrich (Singapore). Ethanol (absolute) and phosphate buffered saline (PBS, pH 7.4) were purchased from Merck Millipore (Singapore) and 1st Base (Singapore), respectively. Potassium hydroxide (KOH), glacial acetic acid (AA), and hydroxypropyl methylcellulose (HPMC) were purchased from Sigma-Aldrich (Singapore).

2.2. Methods

2.2.1. Millifluidic preparation of CUR nanoplex

The schematic of the millifluidic synthesis platform consisting of two syringe pumps and horizontal milliscale tubes as the reactor (i.e. ID 0.762 or 1.588 mm) was shown in Fig. 1. Y-junction connector was used to promote the mixing between the CUR and CHI solutions required for the nanoplex formation. As illustrated in Fig. S1 of the Supplementary Materials, slight bends downstream of the Y-junction were present aimed at promoting further mixing between the two solutions. The CUR nanoplex was prepared at a constant charge ratio of CHI to CUR ($R_{CHI/CUR}$ = 0.8), which was determined to be optimal in our earlier work [6]. This $R_{CHI/CUR}$ value translated to CUR and CHI concentrations of 5 and 6 mg/mL, respectively.

Briefly, aqueous solution of anionic CUR in 0.1 M KOH (pH 13) and aqueous solution of cationic CHI in AA were injected by the syringe pumps (Legato 200, KD Scientific, USA) at equal flow rates (i.e. 0.10, 0.25, 0.50, and 0.75 mL/min) to the Y-junction. Their electrostatic interactions led to the formation of soluble CUR-CHI complex that subsequently formed clusters owed to the adsorption of more ions onto the growing CUR-CHI complex. Afterwards, the clusters precipitated to form the CUR nanoplex upon reaching a critical mass (Fig. 1). The CUR nanoplex suspension produced was collected in a microcentrifuge tube and centrifuged for 25 min at 14,000×g, followed by its washing and redispersion in deionized water for characterizations. The CUR nanoplex was collected from three stages (i.e. Stages 1, 2, and 3) to study the effect of residence time (t_{Res}) on its size (Fig. 1).

In this regard, Stage 2 was defined as the axial distance from the Y-junction at which particulate products began to be visible to the naked eyes suggesting significant nanoplex formation had taken place, where the visible

products were likely in the form of agglomerates of the nanoplex. Stage 3 was a distance downstream of Stage 2 at which diffusive mixing of the CUR and CHI solutions across the width of the millifluidic tube was visually observed to have taken place. The advanced mixing at Stage 3, which was also marked by an increased presence of visible products in the tube, was reflected by the gradual color change of the CUR solution from dark red in 0.1 M KOH (alkaline pH) near the Y-junction to yellow as it slowly mixed with the CHI solution in AA (acidic pH). Stage 1 was defined as a distance upstream of Stage 2 at which the gradual color change of the CUR solution had begun, but particulate products were not yet visible.

The exact axial distances of Stages 1, 2, and 3 varied depending on the pH, flowrate, tube diameter, and CHI's MW used, hence so did t_{Res}. Sample calculations of t_{Res} at the different stage were provided in the Supplementary Materials. In addition, Computational Fluid Dynamics (CFD) simulation using COMSOL Multiphysics® version 5.0 software was carried out to help in understanding the effects of different millifluidic operating conditions on the diffusive mixing of the CUR and CHI solutions inside the millifluidic channel, which in turn affected the resultant nanoplex size. For the bulk mixing method used as the control experiment, the details were presented in our earlier work [6], hence they were not repeated here for brevity.

2.2.2. Physical characterizations of CUR nanoplex

The size, polydispersity index (PDI), and zeta potential of the CUR nanoplex were determined by photon correlation spectroscopy (PCS) using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The size of the CUR nanoplex prepared at the optimal millifluidic condition was verified by image analysis of its Field Emission Scanning Electron Microscope (FESEM) image (JSM-6700F, JEOL, USA) using ImageJ software (NIH, USA) with minimum particle counts of 200. Freeze-dried nanoplex powders sputter coated with platinum were used for the FESEM.

The CUR payload, which was defined as the amount of CUR present per unit mass of the nanoplex, was determined by dissolving a known mass of freeze-dried nanoplex powders in 80% (v/v) aqueous ethanol solution after which the amount of CUR was quantified by UV-Vis spectrophotometer (UV Mini 1240, Shimadzu, Japan) at absorbance wavelength of 423 nm. The reported size, PDI, zeta potential, and CUR payload were based on a minimum of three replicates.

The presence of CUR in the nanoplex prepared by millifluidics was verified by Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum One, Perkin–Elmer, USA) performed between 450 and 4000 cm^{-1} at 1 cm^{-1} spectral resolution. The FTIR was also performed for the native CUR, native CHI, and CUR nanoplex prepared by the bulk mixing method. The amorphous state of the CUR nanoplex prepared by millifluidics was examined by Powder X-ray Diffraction (PXRD) analysis between 10° and 70° (2θ) with a step size of 0.02°/s using D8 Advance X-ray Diffractometer (Bruker, Germany). The PXRD analysis was also performed for the native CUR and CUR nanoplex prepared by the bulk mixing method.

2.2.3. Preparation efficiency

The efficiencies of the nanoplex preparation by millifluidics and the bulk mixing method were characterized in triplicates by the CUR utilization rate and the overall yield defined in Eq. 1 and 2, respectively. For the CUR utilization rate, the mass of CUR that formed the nanoplex was determined from the difference between the initial mass of CUR added and the mass of CUR recovered in the supernatant after centrifugation of the nanoplex suspension. The amount of CUR in the supernatant was quantified by UV-Vis spectroscopy at 423 nm after 100× dilution in 80% (v/v) aqueous ethanol solution. For the overall yield, the mass of CUR nanoplex produced was determined by 24-h freeze drying of the CUR nanoplex suspension after washing.

$$\text{CUR utilization rate (\%)} = \frac{\text{Mass of CUR that formed nanoplex}}{\text{Initial mass of CUR added}} \times 100 \quad (1)$$

$$\text{Overall yield (\%)} = \frac{\text{Mass of CUR nanoplex produced}}{\text{Initial mass of CUR and CHI added}} \times 100 \quad (2)$$

2.2.4. Supersaturation generation

The supersaturation generations of the CUR nanoplex prepared by millifluidics and the bulk mixing method were characterized in triplicates by adding excess nanoplex at 15× of CUR's saturation solubility (C_{Sat} = 4.15 µg/mL) to 8.5 mL PBS in a shaking incubator maintained at 37°C. At specific time points over an 8-h period, 400 µL aliquot was withdrawn and syringe filtered (0.2-µm pores). The filtered aliquot was immediately diluted five times with fresh PBS to prevent CUR precipitation from the supersaturated solution. Subsequently, the amount of CUR in the aliquot was determined by High Performance Liquid Chromatography (HPLC) (Agilent 1100, Agilent Technologies, USA) at detection wavelength of 423 nm using ZORBAX Eclipse Plus C18 column (250 x 4.6 mm, 5 µm particle size). Aqueous ethanol solution 80% (v/v) was used as the mobile phase at 1.0 mL/min, resulting in

CUR retention time of 2.5 min. The supersaturation generation was characterized with or without the presence of a crystallization inhibitor (i.e. hydroxypropylmethylcellulose, HPMC at 2 mg/mL) in the dissolution medium.

3. Results and discussion

3.1. Mixing of CUR and CHI solutions in the millifluidic reactor

For the range of operating conditions investigated, the flow in the millifluidic reactor fell into the laminar flow regime with Reynolds number (*Re*) smaller than 30 denoting the significant role of molecular diffusion over convective motion on the mixing of CUR and CHI solutions [21]. For the basic condition (i.e. tube ID = 0.762 mm, flowrate = 0.25 mL/min), the diffusive mixing time (t_{Mix}) for diffusion over half of the tube diameter was calculated to be equal to 145 s, which translated to a reactor length (L) of approximately 1.3 m. Sample calculations for *Re*, t_{Mix}, and L were provided in the Supplementary Materials.

These calculations showed that the mixing of the CUR and CHI solutions in the millifluidic reactor occurred slowly by design, where the millifluidic reactor was purposely not equipped with passive mixers or hydrodynamic flow focusing tools that were widely employed in microfluidics, because we aimed to maintain a simple, cost-effective, hence highly scalable, millifluidic reactor design. The slow mixing was verified by the CFD simulation that showed two segregated streams flowing side by side near the Y-junction outlet, where mixing only occurred at the interface, and the streams remained segregated at 30 cm downstream of the Y-junction (Fig. S2 of the Supplementary Materials).

More importantly, the slow mixing of the CUR and CHI solutions was favored in the nanoplex formation because a rapid mixing would cause the CUR anions in the KOH solution to quickly lose their charge due to the rapid pH drop upon mixing with the CHI solution in AA. The loss of charge would lead to fewer charged CUR molecules available for complexation with CHI, resulting in lower CUR utilization rates. The slow mixing, on the other hand, enabled both the CUR and CHI solutions to maintain their opposite charges as they flowed side by side forming a liquid-liquid interface between them. The attractive interactions between the CUR anions and CHI cations at the interface resulted in the formation of CUR-CHI complex, where consecutive adsorptions of the CUR anions and CHI cations to the CUR-CHI complex led to its size growth as the residence time was increased. This well-controlled nanoplex formation mechanism at the liquid-liquid interface enabled us to tune the size of the nanoplex produced by simply controlling the residence time of the CUR and CHI solutions.

3.2. Size control of the CUR nanoplex via residence time variations

3.2.1. Effect of preparation pH

The effects of the preparation pH on the CUR nanoplex size tunability were investigated for the basic condition by varying the AA concentration used to dissolve CHI, while keeping a constant pH for the CUR solution at pH 13 as the solubility of CUR (pKa 8.4, 9.9, and 10.5) was found to greatly diminish at a lower pH [22]. LMW CHI was used in this study. The AA concentration was investigated in the range of 0.4% and 1.2% (v/v) (i.e. $2.95 \leq pH \leq 2.71$) in which CHI (pKa 6.5) was fully protonated [23]. Under this condition, the preparation pH investigated was in the range of 12.30 and 4.62 achieved upon a complete mixing of the CHI and CUR solutions.

For all the preparation pH, the results in Fig. 2A showed that the nanoplex experienced an increase in size with increasing residence time (t_{Res}) as the collection stage of the millifluidics was moved downstream from Stage 1 to Stage 3. Hence, the results were in agreement with our hypothesis that the nanoplex size could be controlled by t_{Res} variations. The values of t_{Res} corresponding to Stages 1-3 of the different runs and sample calculations for t_{Res} were presented in Table 1 and Supplementary Materials, respectively. The size dependence on pH was evident in terms of the absolute size of the nanoplex produced at each stage. Specifically, at AA = 0.4% (pH 12.30), large nanoplex (646 ± 188 nm) was produced in Stage 1 where the size increased further to 1321 ± 30 nm in Stage 3. This result was in agreement with our previous finding from the bulk mixing method [6], where large particulate products were produced at alkaline preparation pH, which was caused by agglomeration of individual nanoplex due to the lack of CHI charge available to stabilize the nanoplex at pH high above its pKa.

The pH range in which there was a clear demarcation in the nanoplex sizes obtained from Stages 1-3 was observed at AA = 0.6% and 0.8% corresponding to the mixed solution pH of 5.43 and 4.98, respectively (Fig. 2A). Specifically, at AA = 0.6%, the nanoplex size gradually increased from 115 ± 7 nm in Stage 1 to 209 ± 35 nm in Stage 2 and 311 ± 12 nm in Stage 3. Likewise, at AA = 0.8%, the nanoplex size gradually increased from 145 ± 20 nm in Stage 1 to 277 ± 18 nm in Stage 2 and 433 ± 63 nm in Stage 3. The clear demarcation in the nanoplex sizes obtained from Stages 1-3 signified the high tunability of the nanoplex size in this pH range. For this reason, the CUR nanoplex was prepared in the subsequent studies at AA = 0.6% at which the smallest nanoplex size was produced.

The pH dependence of the nanoplex size tunability could be explained from the effect of pH on the mixing between the CUR and CHI solutions. As shown in Table 1, the values of t_{Res} corresponding to Stages 1-3 were found to decrease with increasing AA concentration (lower pH) from ≈33-71 s at AA = 0.6-0.8% to ≈10-39 s at AA = 1.0-1.2%. On this note, the significantly longer t_{Res} at AA = 0.4% (≈38-133 s), where large particles (> 1 um) were produced, was visually observed to be caused by fouling of the millifluidic tube due to the large particle formation, resulting in the severely diminished mixing. The shorter t_{Res} at lower pH signified faster mixing that in turn led to smaller variations in the nanoplex sizes obtained from Stages 1-3 as the nanoplex formation was likely completed sooner. The faster mixing at lower pH was postulated to be caused by the higher enthalpy of neutralization generated when increasingly acidic CHI solution was mixed with the basic CUR solution. This led to a higher solution temperature, which in turn enhanced mass diffusivity of the solutes resulting in the faster mixing.

3.2.2. Effect of MW of CHI

The high tunability of the CUR nanoplex size observed when LMW CHI was used at AA = 0.6%, however, was no longer evident for CHI of larger MWs (Fig. 2B). Specifically, a clear demarcation in the nanoplex size did not exist between Stages 2 and 3 for MMW CHI and between Stages 1 and 2 for HMW CHI. The values of t_{Res} corresponding to Stages 1-3 were found to increase with increasing MW of CHI (Table 1), hence denoting slower mixing between the CUR and CHI solutions, which was not unexpected as solutes of larger molecular sizes were known to possess smaller mass diffusion coefficients [21]. This trend was in contrast to the earlier trend in the AA variation, where slower mixing led to better size demarcation between stages.

However, unlike the effect of AA variation in which the slower diffusion occurred in both liquid phases, the slower mixing here was dictated by the smaller mass diffusivity of the longer CHI chains. It was postulated that the slower diffusive motion of CHI disrupted the consecutive adsorption pattern of the oppositely charged ions at the interface, resulting in less controlled nanoplex formation. The results also indicated that a shorter t_{Res} was needed to produce a given nanoplex size when CHI of higher MW was used. For example, to produce ≈300 nm nanoplex, the values of t_{Res} required were equal to 71, 50, and 44 s for the LMW, MMW, and HMW CHI, respectively. This was not unexpected as the longer CHI chains meant more sites were available for CUR adsorption, resulting in the formation of larger nanoplex for a given t_{Res}. Nevertheless, LMW CHI was used in the subsequent studies owing to the better size tunability of the nanoplex produced.

3.2.3. Effect of tube diameter

The effect of tube diameter on the size of the nanoplex produced and its tunability were investigated by increasing the tube diameter more than two fold to 1.588 mm at a constant flowrate of 0.25 mL/min. The use of a larger tube diameter increased t_{Mix} to 630 s, hence an even longer reactor length (> 5.7 m) was needed to achieve a complete radial mixing of the CUR and CHI solutions. As a result, the values of t_{Res} corresponding to Stages 1-3 were shown in Table 1 to increase significantly from 33-71 s for the 0.762-mm tube to 150-400 s for the 1.588-mm tube. The slower diffusive mixing in the larger tube was verified by the CFD simulation as presented in Fig. S3 of the Supplementary Materials.

In comparison to the results of the 0.762-mm tube, the results in Fig. 3A showed that slightly larger nanoplex was produced at each stage in the 1.588-mm tube (i.e. 115 ± 7, 209 ± 35, and 311 ± 12 nm for the 0.762-mm tube versus 244 ± 31, 335 ± 22, and 339 ± 17 nm for the 1.588-mm tube in Stages 1, 2, and 3, respectively). The demarcation in the nanoplex size between stages, however, diminished in the larger tube, particularly between Stages 2 and 3. These results reaffirmed the previous finding that slower diffusive mixing did not necessarily lead to better nanoplex size tunability.

3.2.4. Effect of flow rate

Next, the effects of increasing flowrate on the nanoplex size tunability were investigated with the aim of increasing the nanoplex production throughput of the millifluidic reactor. For the basic condition, the production throughput was currently at approximately 0.52 mg/min or 0.03 g/h. The effects of t_{Res} variations on the nanoplex size tunability were re-examined at different flowrates (i.e. 0.10, 0.25, 0.50, and 0.75 mL/min) using the 0.762-mm tube. Increasing the flowrate at a constant tube diameter resulted in faster axial convective motions of the CUR and CHI solutions, which for a given reactor length essentially meant that a shorter time window for the diffusive mixing to take place. As a result, the values of t_{Res} corresponding to Stages 1-3 were shown in Table 1 to increase with increasing flowrate because longer axial distances were needed for the diffusive mixing, and consequently the nanoplex formation, to take place. The delayed diffusive mixing observed at higher flowrates was confirmed by the CFD simulation as presented in Fig. S4 of the Supplementary Materials.

The results showed that the demarcation in the nanoplex size between Stages 1-3 diminished at flowrates above 0.25 mL/min (Fig. 3B), hence denoting the adverse effect of the faster convective motion on the nanoplex size

tunability. In addition, the nanoplex size prepared at the highest flowrate (i.e. 0.75 mL/min) exhibited greater experimental uncertainties compared to the lower flowrates, further denoting the diminished size control at higher flowrates. The clear size demarcation observed at 0.10 mL/min reaffirmed the importance of operating at low flowrates in achieving size tunability of the nanoplex produced.

These results suggested that increased production throughput could not be achieved by simply increasing the flowrate, as it would lead to poorer control of the nanoplex size. Hence, a trade-off existed between the nanoplex size tunability and production throughput. The future research direction will be to increase the nanoplex production throughput to gram-scale production by simultaneously increasing the tube diameter and flowrate in order to maintain the same t_{Res}, followed by scaling out the nanoplex production by running multiple reactors in parallel. Our back-of-the-envelope calculation showed that if the production throughput from a single reactor could be doubled, the gram-scale production could be achieved by running a reasonable number of sixteen reactors in parallel.

The size range of the nanoplex produced, nevertheless, did not vary significantly with the change in the flowrate. For the flowrates at which the nanoplex size tunability was evident (i.e. 0.10 and 0.25 mL/min), smaller nanoplex was consistently produced at 0.25 mL/min compared to at 0.10 mL/min (Fig. 3B). This was despite the fact that they exhibited the same t_{Res} values for Stages 1-3, hence denoting similar diffusive mixing at 0.10 and 0.25 mL/min (Table 1). Thus, the variations in the nanoplex size were not caused by the difference in t_{Res}. The slower convective motion at 0.10 mL/min was postulated to cause the CUR-CHI complex at the interface to flocculate with each other due to the lower shear force generated by the fluid motion in the vicinity of the complex, resulting in the formation of larger nanoplex.

3.3. Physical characteristics and preparation efficiency

The optimal preparation condition for the CUR nanoplex with tunable size was determined to be at AA = 0.6% and 0.25 mL/min using LMW CHI in the 0.762-mm tube. Herein the physical characteristics of the CUR nanoplex having the smallest size (i.e. 115 ± 12 nm obtained from Stage 1 of the optimal preparation condition) were presented. The size measurement by PCS was first verified by size analysis of the FESEM image of the CUR nanoplex. The CUR nanoplex collected from Stage 2 was used as the representative sample for FESEM owed to its abundant production compared to Stage 1. The FESEM image in Fig. 4A showed non-spherical nanoplex with an

average size of 185 ± 44 nm, which was only slightly lower than 209 ± 35 nm measured by PCS. Thus, the tunability of the CUR nanoplex size derived from the PCS measurements was successfully verified.

The nanoplex size exhibited PDI of 0.352 ± 0.034 suggesting a degree of polydispersity that was not unexpected in a slow mixing process due to the generation of inhomogeneous reactive environment. Note: PDI > 0.7 characterized a broad particle size distribution [24]. Not unexpectedly, similar values of PDIs at around 0.35-0.45 were observed for the nanoplexes prepared at various conditions (Figs. S5 and S6 of the Supplementary Materials). The nanoplex possessed a positive zeta potential of 15.7 ± 1.5 mV denoting the presence of CHI on the nanoplex surface and a high CUR payload of 72.3 ± 2.7% (w/w).

For comparison, the CUR nanoplex prepared by the bulk mixing method at the same condition was significantly larger at 603 ± 141 nm, where the larger standard deviation between batches was not unexpected from a bulk mixing process (Table 2). In this regard, the smaller CUR nanoplex (≈250-300 nm) reported in our earlier study using the bulk mixing method [6] was attributed to the sonication step that was not carried out here. Other than the size, both methods resulted in the production of nanoplexes having highly comparable physical characteristics in terms of their PDI, zeta potential, and payload.

The presence of CUR in the nanoplex prepared by millifluidics was verified by FTIR analysis, which showed the characteristics bands of CUR at 1626, 1508, and 1272 cm^{-1} in the FTIR spectrum of the CUR nanoplex (Fig. 4B). These bands also appeared in the FTIR spectra of the native CUR and the nanoplex prepared by the bulk mixing method, but not in the spectrum of the native CHI. These band corresponded to the stretching vibrations of the C=C-C_{ring}, C=O, and enol C-O bonds of CUR, respectively [25].

The millifluidic synthesis platform exhibited a high CUR utilization rate of 80.6 ± 1.6% (w/w) even in Stage 1, which was only slightly lower than 88.9 ± 4.0% observed for the bulk mixing method at the same condition (Table 2). A higher CUR utilization rate at nearly 100% was observed in Stage 3 (data not shown). Despite the high CUR utilization rate, the overall yield of the millifluidic synthesis platform was relatively low at 35.4 ± 1.3% (w/w) suggesting that a significant amount of CHI in the feed did not transform into nanoplex. A similarly low yield of 42.4 ± 1.7% was observed for the bulk mixing method at the same condition. Reducing the amount of CHI in the feed (i.e. lower $R_{CHI/CUR}$), however, was shown in our earlier study to adversely affect the colloidal stability of the resultant nanoplex, hence it was not pursued here [6].

3.4. Supersaturation generation

In the absence of HPMC, the CUR nanoplex prepared at the optimal condition was capable of generating a high degree of supersaturation that reached 9.0 ± 0.7 ×C_{Sat} at the maximum, followed by its rapid drop to 3.7 ± 0.9 ×C_{Sat} after 30 min before gradually settling back to C_{Sat} after 3 h (Fig. 5A). Hence, the CUR nanoplex exhibited the characteristic "spring and parachute" supersaturation profile of amorphous drug formulations [26]. In this regard, the amorphous state of the CUR nanoplex prepared by millifluidics was verified by the PXRD analysis, which showed broad amorphous halos in contrast to the sharp crystalline peaks observed for the native CUR (Fig. 5B).

Despite their significant difference in size, the supersaturation generation of the nanoplexes prepared by the millifluidic (≈100 nm) and bulk mixing (≈600 nm) methods could hardly be distinguished in the absence of HPMC due to rapid crystallization of the dissolved CUR from the highly supersaturated solution. For this reason, the supersaturation generation was re-evaluated in the presence of HPMC, where the roles of HPMC were twofold, i.e. to prevent the solution-mediated crystallization of CUR and to suppress the Ostwald-ripening crystallization of the amorphous solid phase undergoing dissolution [5].

In the presence HPMC, the nanoplex prepared by millifluidics was capable of generating a maximum supersaturation level at 11.3 ± 1.0×C_{Sat} that was maintained for 1.5 h, followed by its gradual decrease to 8.5 ± 0.1×C_{Sat} after 8 h (Fig. 5A). For comparison, the nanoplex prepared by bulk mixing exhibited a lower maximum achievable supersaturation level at 9.4 ± 0.4×C_{Sat} and a lower supersaturation level after 8 h at 6.2 ± 0.7×C_{Sat} due to its significantly larger size. As a result, the nanoplex prepared by millifluidics exhibited a larger area-under-the-curve (AUC) in the time versus concentration plot, which could translate to a higher bioavailability *in vivo*. These results signified the importance of the nanoplex size in determining its bioavailability enhancement capability.

4. Conclusions

The present work successfully demonstrated size control of amorphous CUR nanoplex in a continuous millifluidic platform, which was not achievable in the conventional bulk mixing method. The nanoplex size tunability was achieved by controlling the residence time of the CUR and CHI solutions undergoing electrostatically driven complexation in a millifluidic reactor operated under diffusion-dominated flow regime. The size tunability was found to be influenced by the preparation pH, MW of CHI used, tube diameter, and flowrate through their impacts on the rate of diffusive mixing in the millifluidics, which was verified by CFD simulation. Not unlike the

bulk mixing method, the millifluidic synthesis was found to favor acidic preparation pH, while the use of higher MW CHI and increasing tube diameter and flowrate from the basic condition (i.e. 0.762 mm tube ID and 0.25 ml/min) had adverse effects on the CUR nanoplex size tunability.

The optimal millifluidic preparation condition at which there was a clear demarcation in the nanoplex size as a function of the residence time was determined to be at pH 5.43 (AA = 0.6%) and 0.25 mL/min using LMW CHI in 0.762-mm tube. The nanoplex size collected from Stage 1 of the optimal condition was roughly 115 nm with ≈72% CUR payload. Other than the size, in comparison to the bulk mixing method, the CUR nanoplex prepared by the millifluidics exhibited similar physical characteristics (i.e. zeta potential, PDI, CUR payload) and preparation efficiency (i.e. CUR utilization rate and yield). Importantly, the ability to control the nanoplex size in the millifluidics enabled us to produce a significantly smaller nanoplex compared to the bulk mixing method (i.e. six fold smaller) without sonication. The smaller nanoplex led to the generation of a higher and more prolonged supersaturation level at ≈8.5× of the saturation solubility maintained for 8 h. The improved supersaturation generation afforded by the nanoplex prepared by millifluidics boded well for its potential bioavailability enhancement capability.

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Figure captions

Fig. 1 Schematic of the millifluidic reactor setup to prepare amorphous CUR-CHI nanoplex with tunable sizes

Fig. 2 Effects of (A) preparation pH represented by the AA concentrations used and (B) MW of CHI on the size tunability of the CUR nanoplex produced

Fig. 3 Effects of (A) tube diameter and (B) flowrate on the size tunability of the CUR nanoplex produced

Fig. 4 (A) FESEM image of the CUR nanoplex prepared at the optimal condition and (B) FTIR spectra confirming the presence of CUR in the nanoplex

Fig. 5 (A) Supersaturation generation profiles of the CUR nanoplexes prepared by the millifluidic and bulk mixing methods, (B) PXRD patterns confirming the amorphous state of the CUR nanoplex prepared by millifluidics

Table captions

Table 1 The residence time as a function of preparation pH, MW of CHI, tube diameter, and flowrate

Table 2 A summary of comparison between the CUR nanoplexes prepared by the millifluidic and bulk mixing method in terms of their physical characteristics and preparation efficiency