Sustainable Formation of Curcumin Nanoparticle: Stirred Tank and Confined Impinging Jet Reactor

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***Abstract*—As natural extract from tumeric, curcumin has driven many people interests. However, the wide application for curcumin is limited by its poorly water solubility. This study explored the possibility to prepare curcumin nanoparticles with a sustainable precipitation by applying pH-shift and avoiding organic solvents. With Pluronic F68 as the surfacatant, this study used two modes of operation, stirred tanks and confined impinging jet reactors, for precipitating curcumin. The effects of energy dissipation, and drug-surfactant mass ratio on particle size discussed. As a result, curcumin nanoparticles with size ranging from 180 to 350 nm and drug loading as high as 87.5% were obtained. We noticed that nanoparticle size decreases with stirring speeds for stirred tanks, but plays a less important role in confined impinging jet reactor. The influence of drug/surfactant mass ratio was more significant in controlling particle size.**

***Index Terms*—pH-shift driven precipitation, curcumin, drug nanoparticles, sustainable, poorly water soluble drug Index**

I. Introduction

As the extract from natural South Asian spice turmeric, curcumin is known with various interesting properties such as anticancer, antioxidant, anti-inflammatory, anti-HIV and anti-microbial [1]. However, the use of curcumin is limited due to its low solubility in water, which is about 0.021 mg/mL [2]. One approach to enhance the bioavailability of curcumin is to increase local drug concentration through increased particle surface area by reducing powder size to nano-scale. Nanoparticles are usually prepared in top-down or bottom-up methods. Although top-down approaches are widely used in industry, bottom-up methods are favored in lab-scale for lower energy and time consumption, low contamination and flexibility in controlling particle morphology [3].

Preparing nanoparticles formed by precipitating drug out from the solution is attractive due to the convenience in controlling temperatures, pressures and ease of scale-up. [1] The preparation contains generation of supersaturation, nucleation, growth by coagulation and condensation, and usually uncontrolled agglomeration [4]. Based on studies of LaMer VK and Dinegar RH, nucleation followed by diffusional growth happens once molecular concentration reaches a critical point, which is higher than its saturated solubility, to reduce the free energy of the system [5]. Therefore, properties of formed particles such as size and morphology are highly influenced by supersaturation rate and uniformity [1]. In order to form monodisperse nanoparticle suspension, nucleation and growth need to take place within a short period of time. Besides, high degree of supersaturation, uniform spatial concentration distribution in solutions and minimal growth are necessary to obtain monodisperse nanoparticles [6].

The supersaturation can be generated by temperature quenching [7], chemical reaction, addition of antisolvent and shift in pH value [8]. The degree of supersaturation from temperature quenching is low [7] and addition of miscible antisolvent or adjusting pH to the isoelectric point to reduce solubility are widely used to obtain high degree of supersaturation. The effect of mixing on spatial concentration distribution as well as particle size, size distribution and particle morphology in preparing nanoparticle with antisolvent precipitation were discussed in Shad W.'s study [9]. Moreover, modes of operations with different energy dissipation were found to be related to particle size, morphology and even the crystallinity [10].

Besides, M. E. Matteucci, M. A. Hotze, K. P. Johnston, and R. O. Williams III had observed that the percentage of surfactant upon precipitation plays an important role in particle size [6]. Similar phenomenon was noticed by C.D. Hou et al, who found particle size decreases while surfactant concentration increases in preparing azithromycin nanoparticle via reactive precipitation method [8].

Nanoparticles by precipitating drug out from the solution were applied on many drugs including curcumin [1], [3]. However, the precipitation of curcumin via shift in pH value has not been systematically studied, which drive our interests as a sustainable method avoiding any organic solvent. In this study, we first examined the formation of curcumin nanoparticles by pH-shift driven precipitation with stirred tanks, where the effect of stirring speeds, sequence of addition and drug/surfactant ratio on size were analyzed. Second, we studied the potential of using confined impinging jet reactor, a mode of operation with higher energy dissipation, for preparing smaller curcumin nanoparticles. By comparing the size of curcumin nanoparticles, we aimed to discuss the

Manuscript received March 1st, 2014; revised May 12th, 2014.

doi: 10.12720/jomb.4.2.154-158

influences of mode of operation. Last but not least, the storage stability of nanoparticle suspension was studied.

II. Materials and Methodology

A. Materials

Poorly water soluble drug curcumin (95% curucuminozoid from Alfa Aesar.co) was used as purchased. Potassium hydroxide from Sigma-Aldrich in solid form was used to dissolve curcumin, while hydrochloric acid (25%, from Riedel-de Haen) in liquid form was applied for pH adjustment. The surfactant Pluronic F68 from Sigma-Aldrich was used as stabilizer for controlling the growth in nanoparticle size.

B. Methodology

pH-shift precipitation via magnetic stirring: Curcumin was dissolved in 1M KOH as the stock before further dilution with DI water to 5 mg/mL in 0.1M KOH. Meanwhile, the stabilizer Pluronic F68 was dissolved in HCl at designed concentration. The concentration of acid and base was altered so that the pH value of final mixture equals to 7. In each batch, two millilitre of 0.1M KOH containing drug was added by one-shot pipetting to equal volume of 0.1M HCl containing the surfactant with continuously stirred in 15 mL glass vials at ambient temperature (22.0 ± 1.0 ℃). The suspensions of nanoparticles were collected after one minute to allow sufficient mixing, and then washed with three cycles of centrifugation at 14,000 rpm for 10 minutes to remove excess drug and surfactant. Afterwards, the sediments were further suspended and stored in -20^{o}C freezer prior to 24-hour freeze-drying. The stirring speed values examined were 100 rpm, 250 rpm, 500 rpm, 750 rpm, and 1000 rpm. The sequence of addition was either base to acid or acid to base. Different drug/surfactant ratios (0.1-4) were achieved by changing surfactant concentration or volumes, where the concentration of acid and base was altered accordingly.

pH-shift precipitation via confined impinging jet reactor: The confined impinging jet reactors were designed as with two coaxial jets impinge head-on to generate intense turbulent energy dissipation by impinging two jets of high speed in a small reactor volume and were set as shown in Fig. 1. Curcumin and Pluronic F68 were dissolved at the same concentration of 5 mg/mL in 0.1M KOH and in 0.1M HCl, respectively. Both solutions were placed in 50 mL stainless syringes assembled with tubing of 0.02-inch inner diameter. The velocity of impinging jet and energy dissipation was adjusted with syringe pumps by tuning the volumetric flow rate. Different drug/surfactant ratios were achieved by changing volumes only. As the maximum working flow rate was 68 mL/min, the experiment was designed as the flow rates of solution with larger volume fixed at 10, 17, 34, 51 and 68 mL/min at various drug/surfactant ratios. After impinging, the suspensions of curcumin nanoparticles were washed with three cycles of centrifugation at 14,000 for 10 minutes to remove excess drug and surfactant. Afterwards, the sediments were further suspended and stored at -20^{o}C freezer prior to 24-hour freeze-drying.

C. Methodology

Morphology: The curcumin nanoparticle suspension upon preparation was immediately observed under optical microscope (Olympus) to check the morphology at wet state. A JEOL JSM6390LA analytical scanning electron microscope was used to analyze the morphology of freeze-dried curcumin nanoparticle after 80s of sputter coating by JEOL JFC-1600 coater.

Particle size: The size distribution and zeta-potential of curcumin nanoparticles were analysed by dynamic laser scattering with Brookhaven 90PLUS Particle Size Analyzer. In order to prevent significant particle growth, the fresh nanoparticle suspensions were stored in a 4℃ fridge and analyzed within 24 hours. For studying stability of nanoparticle suspension, the size distribution were analyzed after 10 days of storage at 4℃.

Drug loading: The drug loading defined as mass ratio of drug in nanoparticles was obtained by dissolving known mass of nanoparticles in 0.1M KOH and subsequently analysis with UV-vis Spectrophotometer (Shimadzu UVmini-1240) at a wavelength of 459 nm.

Statistical analysis: Two-way ANOVA was applied to study the effect of two independent factors on the dependent variable with Origin85.

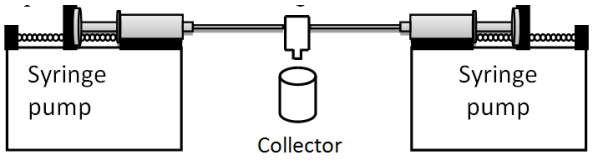

Figure 1. Schematic of confined impinging jet reactor

III. Results and Discussions

A. Ph-Shift Precipitation via Stirred Tank

Fig. 2 shows the optic morphology of fresh curcumin nanoparticle suspension prepared at different stirring speeds when 0.1M KOH containing 5 mg/mL curcumin is added to equal volume of 0.1M HCl containing 5mg/mL F68. A wide size distribution containing large particles and small particles was observed when the stirring speed is as low as 100 rpm, indicating the stirring speed was not efficient for uniform spatial concentration distribution. As the stirring speed increases, large particles gradually disappear and the size distribution becomes narrower. The surface morphology of freeze-dried curcumin nanoparticles from the run by adding base to acid at 1000 rpm is shown in Fig. 3, where curcumin nanoparticles around 200 nm were observed.

Fig. 4 shows particle size obtained with DLS of curcumin nanoparticles prepared at different addition sequences and different stirring speeds. The addition sequence has apparent effect on particle size especially when stirring speed is low. This can be explained as failure for generating an outburst supersaturation for monodisperse nanoparticle suspension. In the condition of adding base to acid, the solubility in the suspension at

the moment of base contacts acid is low, giving a high supersaturation ratio for nucleation while following supply of drug contributes more to growth than nucleation. Therefore, the stirring speed plays a more significant role in this condition as the supersaturation ratio at different area in the bottle is highly dependent by mixing. At low stirring speed, the mixing is not enough to allow same drug concentration through the suspension and leads to a wide size distribution. The trend of decreasing particle size agrees with the optic images shown in Fig. 2.

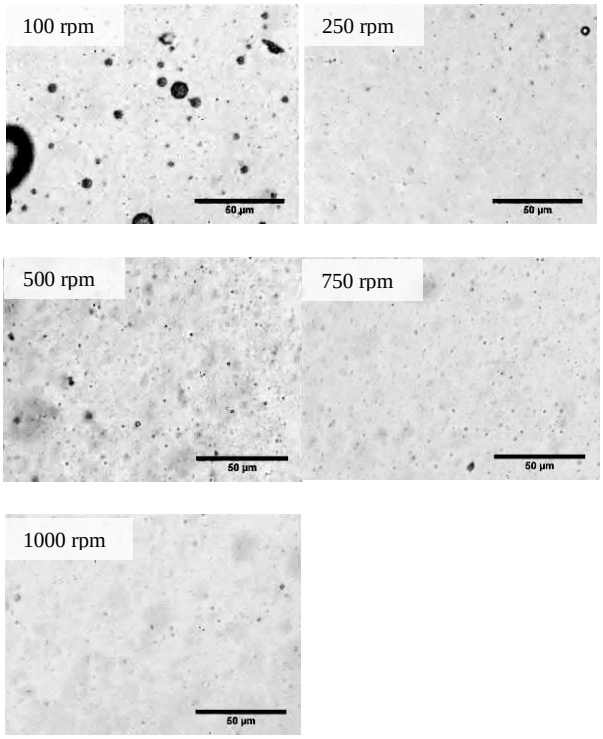

Figure 2. Note how the caption is centered in the column.

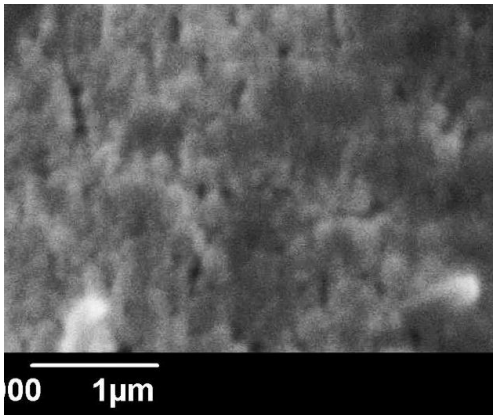

Figure 3. SEM images of curcumin nanoparticles prepared at 1000 rpm, base added to acid.

When we change the addition sequence as acid to base, the drug solubility keeps decreasing as acid is added to the mixture. Once the pH value decreases to a value (usually at the end of addition) that generates supersaturation, nucleus are formed within one short period to deplete drug, which is more suitable for preparing small and monodisperse nanoparticle suspension. The particle size decreases with increasing stirring speed but the effect is less obvious due to supersaturation is achieved with the last droplet of acid, before which the drug concentration is already uniform during the addition.

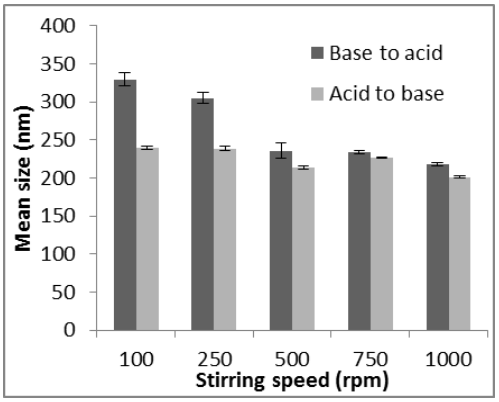

Figure 4. Particle size of curcumin nanoparticles at different addition sequences and different stirring speed.

As shown in Fig. 5, we studied the influence of drug-surfactant mass ratio by changing volume ratios or concentrations while fixing the preparation at a condition with 1000 rpm as stirring speed and addition sequence as acid to base. The particle size grows with increase in drug/surfactant ratio, indicating that when drug/surfactant ratio is high, the amount of surfactant is insufficient to cover the nucleus, leading significant particle growth by condensation and coagulation. [5].

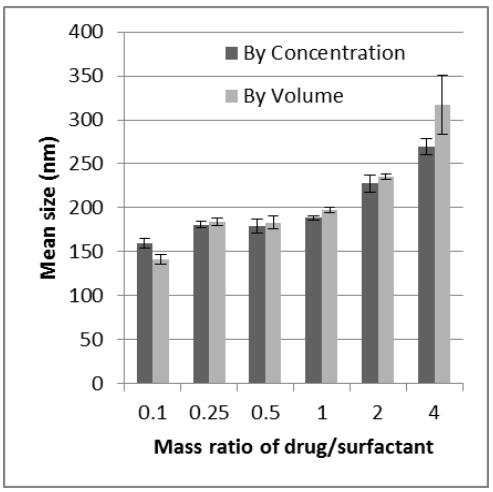

Figure 5. Particle size of curcumin nanoparticles at different addition sequences and different stirring speed.

The difference between changing drug/surfactant ratios by changing the concentration or changing volume is small but is not negligible, especially at drug/surfactant ratio of 0.1 and 4, where 20 mL or 0.5 mL acid is added to 2 mL base. In these conditions, the concentration of drug is different in the final mixture. When the drug/surfactant ratio is larger than 1, the volume of acid is smaller than base. Therefore, both degree of supersaturation and drug concentration are higher than the run with same drug/surfactant ratio but achieved with

equal volume. Higher drug concentration leads to more particle growth in this condition, which eventually leads to smaller particles. Similarly, when the drug/surfactant ratio is less than 1, the run with adjusted volume has a lower drug concentration and smaller particles as a result.

B. pH-shift Precipitation via Confined Impinging Jet Reactor

The effect of mixing energy and drug/surfactant ratios were studied in preparing monodisperse nanoparticle suspension. The mixing energy and drug-surfactant mass ratio were tuned by flow rate and volume of both solutions, respectively. In confined impinging jet reactor, the effect of addition sequence is not studied as acid and base are designed as impinging at the same time and the supersaturation keeps constant through the impinging process. To be specific the experiments were designed as shown in following figure.

We further analyzed the significance of each independent factor on the dependent variable, with energy dissipation and drug-surfactant mass ratio set as two independent factors and particle size as the dependent variable. To simplify the study of mixing energy on particle size, we use the sum of kinetic energy of both jets as in x1 assuming the kinetic energy of flow exiting mixer ignorable. From the study in [11] of B.K. Johnson and R. K. Prud'homme, the energy dissipation rate is proportional to energy input in this system P, which can be further expressed as in Eq. (1) where m stands for mass flow rate (kg/s) and u stands for jet velocity (m/s) when each jet are redirected to perpendicular direction in the system.

$$P \propto (\frac{1}{2} m_1 u_1^2 + \frac{1}{2} m_2 u_2^2) \quad (1)$$

From the two-way ANOVA analysis at a significance level of α=0.05, it is found that the population means of mixing energy are not significantly different (p value = 0.91997>0.05) while population means of drug/surfactant ratio are significantly different (p value = 0.00118<0.05). Based on the p values, it is concluded that in preparing curcumin nanoparticles with confined impinging jet reactor via pH-shifting, Therefore, it is fair for us to conclude the energy dissipation in confined impinging jet reactor has less influence than drug/surfactant on particle size at the experimental conditions.

Sizes of curcumin nanoparticles obtained at varied drug/surfactant ratios and from confined impinging jet reactor are shown in Fig. 6. It is noticed that generally larger drug/surfactant ratios yield bigger particles except for one outlier at drug/surfactant = 0.2, flow rate equals to 34 mL/min. However, contradictory to our expectations, the difference in size by change in flow rate is minimal. At given conditions, the energy dissipation rate of run at highest flow rate (68 mL/min) is at least several hundred times of the run at lowest flow rate (10 mL/min). Hence, the change in size maynot be proportional to that of energy dissipation. Or the difference in flow rate is not high enough cogenerate obvious difference in size.

In Fig. 7 we show mean size of curcumin nanoparticles prepared at different drug/surfactant ratios while the flow rate of larger volume is fixed at 51 mL/min. The mean size increases with drug/surfactant ratios, which is as the same as the trend obtained with stirred tanks.

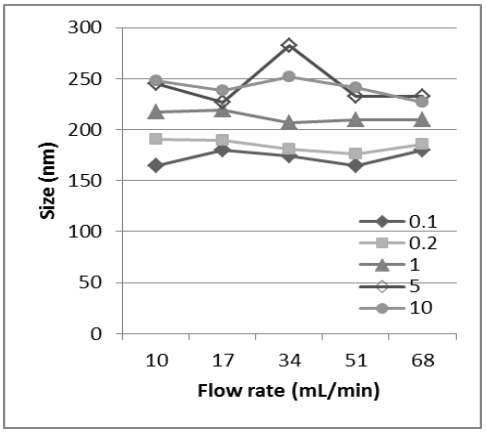

Figure 6. Paricle size of curcumin nanoparticles at different drug/surfactant mass ratios (0.1, 0.2, 1, 5, 10) and at different flow rates.

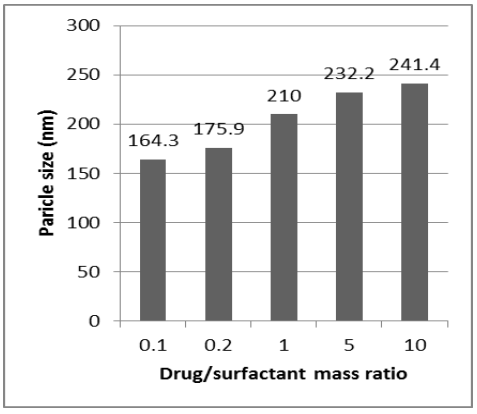

Figure 7. Mean size of curcumin nanoparticle at different drug/surfactant ratios with flow rate of larger volume fixed at 51 mL/min.

C. Summary of Curcumin Nanoparticle with pH-Shift Driven Precipitation

Although the confined impinging jet reactor was expected to generate rate smaller particles than the stirred tanks, it is observed that for the optimal runs, not only the size but also the size distribution from both runs were very close. However, it is not fair enough to conclude that stirred tanks are better as the design of experiments with confined impinging jet reactor may not being perfect. There is no obvious difference in each run for drug loading and the drug loading of all nanoparticles with efficient washing was about the same, which is about 87.5 ±9.7%.

D. Stability

The storage stability of nanoparticles suspension under 4℃ was shown in Fig. 8. Particle size increase around 20 nm were found in the run drug/surfactant = 0.1while a particle size increase around 60 nm was found in the run drug/surfactant = 4. The presence of large amount of

surfactant not only reduces particle size upon preparation but also helps in keeping nanoparticles from further growth.

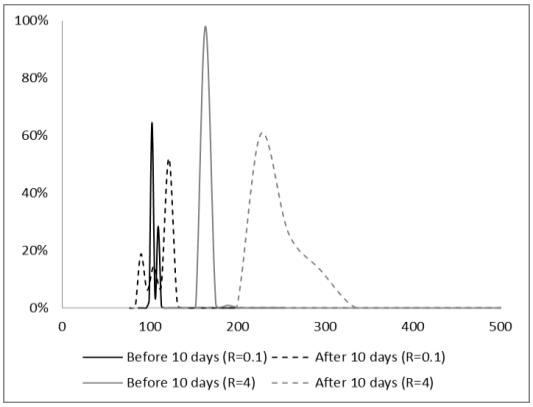

Figure 8. Storage stability of curcumin nanoparticles in 10 days.

IV. Conclusion

In this study, we prepared curcumin nanoparticles in the range of 180nm to 300nm with 87.5% drug loading by pH-shift driven precipitation with two modes of operation: stirred tank and confined impinging jet reactors. The sequence of addition and method for adjusting drug/surfactant ratio are found to have obvious effects on particle size. In our preparation of curcumin nanoparticles with stirred tank and confined impinging jet reactors, we found that the influence of drug-surfactant mass ratio was more significant compare to energy dissipation when we keep the mode of operation unchanged. For further preparation of nanoparticles with poorly water soluble drugs, pH-shift driven precipitation might be an interesting alternative as a safe and low cost way without using organic solvents.

Acknowledgment

The authors wish to thank Dr Kunn Hadinoto for his support andsupervision. We would also thank Hou Zheng and Tanuwidjaja Kristie for their efforts in preliminary studies. This work was supported by GlaxoSmithKline Singapore under their Green and Sustainable Manufacturing Research Initiative.

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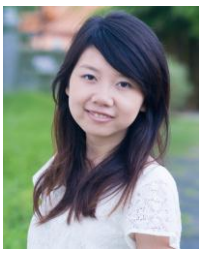

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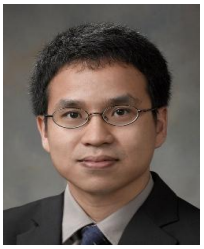

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