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Evaluation on the Use of Confined Liquid Impinging Jets for the Synthesis of Nanodrug Particles

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Most pharmaceutical compounds can benefit from being produced with a small particle size to enhance processing or therapeutic performance. Confined liquid impinging jets (CLIJ) were employed in this study to evaluate the feasibility and limitations in the production of nanodrugs (i.e., particle size in the nanorange). Four drugs from different pharmaceutical classes and water solubilities—salbutamol sulfate, mannitol, ibuprofen, and cyclosporine—were examined. Particles of salbutamol sulfate and cyclosporine with diameters of approximately 300 nm were successfully achieved. The use of CLIJ thus shows potential in the production of nanopharmaceuticals for certain compounds.

Keywords confined liquid impinging jet; nanodrug; nanoparticle; crystallization; drug delivery

INTRODUCTION

In delivering drugs into the body, one needs to consider the path and the rate of delivery. With typical drug administration methods such as oral, topical, or aerosol inhalation, the reduction in the primary particle size of the drugs may improve product performance. For drugs targeting respiratory diseases, particles of below 5 μm in size are commonly used. However, due to faster absorption, drugs for systemic delivery via the lungs may be improved by using particles in the nanosize region (Sham, Zhang, Finlay, Roa, & Löbenberg, 2004). Currently most pharmaceutical drugs are micronized by milling to enhance delivery, but the high energy involved in the process could induce amorphous regions which can affect the stability of the product (Buckton, Butler, Thielmann, & Williams, 2001; Kawakami, Numa, & Ida, 2002; Newell, Buckton, Butler, Thielmann, & Williams, 2001; Ward & Schultz, 1995).

An alternative method of reducing particle size is recrystallization of the drug in a well-mixed system. Previous mixing technologies require mechanical agitation to homogenize two or more reactants. Confined liquid impinging jets (CLIJ) mix two liquid streams based on the velocities of the solutions and require no external mechanical mixing (Johnson & Prud'homme, 2003a). This technology is a single-pass process where rapid mixing occurs within the confined mixing chamber, compared with traditional mixing processes where mixing usually occurs within a longer timescale.

To control the particle size, the micromixing time (τ_{mix}) is required to be shorter than the formation time of the product ($\tau_{reaction}$; Chen et al., 2004), and for an antisolvent crystallization reaction this depends on various factors such as reactant concentration, solvent to antisolvent ratios, nucleation and growth times, and temperature. In a previous study, it was shown that flash nanoprecipitation using CLIJ was feasible to synthesize stabilized nano-organic active particles, such as β-carotene and polystyrene-*block*-poly(ethylene oxide) with a medium size of 100 nm (Johnson & Prud'homme, 2003b). While this is potentially useful for controlled-release drug delivery systems, the product limits the possible drug delivery pathways as copolymers is not expected to be suitable for respiratory drug delivery. In two recent publications, CLIJ was used for quasi-emulsion precipitation of pharmaceuticals, mainly to propose and test the mechanism of quasi-emulsion precipitation (Wang, Gillian, & Kirwan, 2006) as well as control of polymorph formation during antisolvent precipitation (Wang & Kirwan, 2006). It is more useful to produce nanodrugs with no additives or those generally regarded as safe (GRAS) in

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minimum amounts for a wider range of applications. For example, only a very limited number of additives (e.g., lactose, lecithin, and oleic acid) is available for inhalation pharmaceutical products. Furthermore, the presence of additives may have an undesirable effect on changing the drug particle physicochemical properties. However, additives could be introduced in the CLIJ process if it is found to be safe (and acceptable by the regulatory authorities) and useful in the final formulation.

The drugs used in this work were salbutamol sulfate, mannitol, ibuprofen, and cyclosporine. Salbutamol sulfate is a bronchodilator widely used in asthma management. Mannitol can be used for mucociliary clearance (Daviskas et al., 1997) as well as for the diagnosis of asthma in subjects (Brannan, Koskela, Anderson, & Chew, 1998). Ibuprofen is a nonsteroidal anti-inflammatory drug, commonly used for pain relief, and cyclosporine is an immunosuppressive agent for the prevention of transplant rejections. Salbutamol sulfate (SS) and mannitol are water soluble while ibuprofen and cyclosporine are poorly water soluble.

The aim of this study was to evaluate the feasibility and limitations of CLIJ for the production of nanodrugs.

METHODS AND MATERIALS

Materials

SS was purchased from Inter-Chemical Ltd., China; mannitol was purchased from Rocquett Frères, France; ibuprofen was purchase from Shasun Chemicals and Drugs Ltd., India; and cyclosporine was purchase from Fujian Kerui Pharmaceutical Co. Ltd., China. Sodium dodecyl sulfate (SDS) was supplied by Sigma-Aldrich, Australia; isopropanol (IPA), chloroform, and acetone were from Biolab, Australia; lecithin (Emultop) from was from Degussa, USA; dextrose monohydrate was from MyoPure, Australia; and plastic syringes (50 mL, 29.1 mm ID) were supplied by Livingstone, Australia.

CLIJ Mixer

The CLIJ experimental setup (Figure 1) was built similarly to the original design by Johnson and Prud'homme (2003a) with a jet diameter of 0.5 mm and a conical outlet diameter of 1 mm. Two syringe pumps (model PHD 2000, Havard Apparatus, USA) were used to maintain continuous flow and volume of the two liquid streams from the syringes into the mixing chamber.

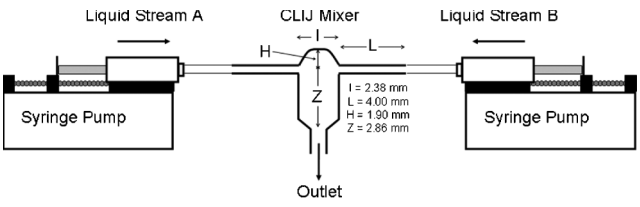

FIGURE 1. A schematic of the confined liquid impinging jet configuration used.

CLIJ Mixing of SS

SS, with and without surfactant, was examined. SS (10.5 mg) was dissolved with SDS (11.5 mg) in distilled water (4 mL). The solution volume was increased to 50 mL with IPA, and loaded into a plastic syringe while pure IPA (50 mL) was loaded into a separate syringe. Each syringe was connected to the CLIJ mixer with both syringe pumps set to dispense simultaneously at 120 mL/min for 50 mL. Mixing of the two liquids resulted in precipitation of SS, and the suspension was collected from the mixer outlet for particle characterization. For the surfactant-free sample, only SDS was omitted while other conditions were maintained.

CLIJ Mixing of Mannitol

Saturated mannitol solution (18% w/v in distilled water) was loaded into a plastic syringe, while clean acetone (50 mL) was loaded into a separate syringe. Both syringes were connected to the CLIJ mixer and set to dispense 10 mL and 50 mL at 120 mL/min for mannitol and acetone, respectively. In a separate set of impinging, SDS (10% w/v), was added to the saturated mannitol solution while maintaining the other conditions.

CLIJ Mixing of Ibuprofen

Ibuprofen (1g) was dissolved in ethanol (10 mL) and loaded into a 50 mL syringe while distilled water (10 mL) was loaded into a separate syringe. Both syringes were connected to the CLIJ mixer and set to simultaneously dispense at 120 mL/min for 10 mL with the suspension collected from the outlet for particle characterization. A separate set was carried out with SDS (11.5 mg) dissolved in the distilled water (10 mL) component. The mixing condition was kept the same.

CLIJ Mixing of Cyclosporine

Cyclosporine (0.7 g) was dissolved in ethanol (10 mL) and loaded into a 50 mL syringe, while distilled water (30 mL) was loaded into a separate syringe. Both syringes were connected to the CLIJ mixer with the cyclosporine-containing syringe set to dispense at 40 mL/min for 10 mL and the water-containing syringe at 120 mL/min for 30 mL. The product was collected from the mixer outlet in a stirred beaker containing distilled water (50 mL) for quenching prior to particle characterization. In a separate set, lecithin (0.3 g) and dextrose monohydrate (1.5 g) were dissolved in the distilled water component (30 mL) while other conditions were kept constant.

Particle Size Distribution

The volume weighted particle size distribution (PSD) of the resulting suspension of each drug collected from the mixer outlet was examined by laser diffraction (Mastersizer S or 2000, Malvern, Worcs, UK) based on the refractive index (RI) of the drug and dispersant for each system, as shown in Table 1.

TABLE 1
Refractive Index (RI) Values for Drugs and Dispersants Used for Particle Size Distribution Measurements

Drug	RI Drug	Dispersant	RI Dispersant
Salbutamol sulfate	1.553	IPA	1.378
Mannitol	1.520	Chloroform	1.444
Ibuprofen	1.436	Water	1.333
Cyclosporine	1.578	Water	1.333

The imaginary RI used for the drugs was 0.100.

The spread of the size distribution is expressed by the span (defined as the difference between the particle diameters at the 10% and 90% cumulative volume, divided by the median particle diameter) or the geometric standard deviation (GSD) if the particles follow log-normal size distribution.

Dynamic light scattering (Zetasizer Nano ZS, Malvern, Worcs, UK) was used to examine the presence of small particles or nucleation in solution.

Microscopy

Particle morphology of the drugs collected was examined by optical microscopy (CH40, Olympus, Japan) or high-resolution scanning electron microscopy (SEM; JSM 6000F, Joel, Japan) operating at 5 kV.

RESULTS AND DISCUSSION

Precipitation of Nanoparticles

Precipitation occurs in several stages: chemical reaction and/or supersaturation, nucleation, solute diffusion, and crystal growth (Dirksen & Ring, 1991). In this work, each system reaches supersaturation by the introduction of an antisolvent, which then leads to nucleation and subsequent crystal growth. Particles in the nanosize range can be obtained by rapid micromixing of reactants to enhance nucleation while suppressing crystal growth. Both nucleation and crystal growth depend on the supersaturation level which, in turn, is closely related to the level of micromixing. Thorough micromixing leads to the same supersaturation for all the nuclei in the liquid, resulting in uniform growth and particle size. On the other hand, insufficient mixing will lead to growth disparity among different nuclei, resulting in a wide particle size distribution. When the micromixing time is significantly less than the induction time to establish a steady-state nucleation rate, the latter will be nearly uniform spatially, and the particle size distribution can be controlled at a uniform level. This is achieved in the present study by the CLIJ mixer which utilizes confined liquid impinging jets to intensify mass and heat transfer in multiphase systems (Johnson & Prud'homme, 2002, 2003a). During the process, the fluids going through the jets are spread and split into thin films, threads, and very thin filaments under the high shear created by the high speed. This results in intense micromixing between the liquid elements, with the micromixing time in this process estimated to be < 1 ms (Johnson & Prud'homme, 2002, 2003a).

Common GRAS surfactants such as SDS and lecithin can be added to maintain the stability of the crystallized particles and prevent aggregation throughout the crystallization process. Small molecules, such as dextrose, can also be added into the system to act as a stabilizer. The surfactant molecules are able to cover the surface of the crystallized particles and reduce interaction between particles. The effect of the added surfactant is discussed in this work.

Precipitation of Water-Soluble Compounds

The CLIJ-processed, surfactant-free SS particles show a bimodal size distribution, while particles with added surfactant show two distinct distributions (Figure 2). Surfactant-free SS has peaks at 20 μm and 80 μm with a volume median diameter (VMD) of 34 μm. When SDS was added, the sample showed a major peak between 0.1 μm and 1 μm, with a VMD of the 318 nm (span 0.710, GSD 1.33), and a minor peak between 1 μm and 10 μm, where the overall VMD was 390 nm. The SS particles are needlelike with a width of approximately 100 nm, as shown in the SEM photo (Figure 3).

Without surfactant, it is clear that the precipitation of SS was not well controlled within a broad size distribution. The use of surfactant assists the production of particles in the nanorange as it stabilizes the particles so further growth does not continue. The small population of large particles is not caused by an unstable system, but most probably by the lower dispensing rate of the liquid streams at the beginning and end of the process when the syringe pumps were started or stopped, leading to poorer mixing and consequent formation of larger particles.

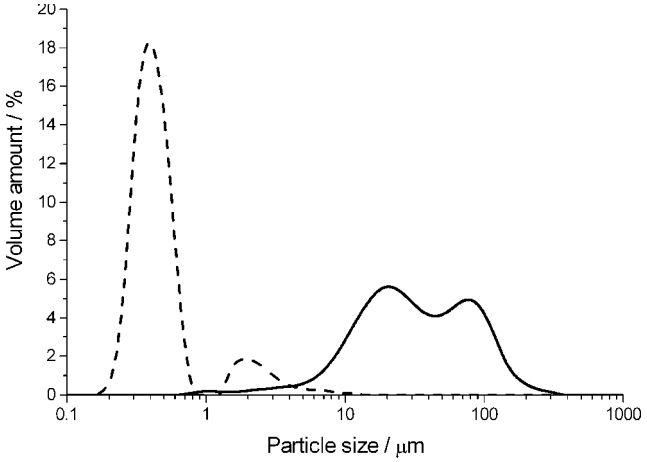

FIGURE 2. Particle size distribution of processed salbutamol sulfate, without SDS (—) and with SDS (- - -).

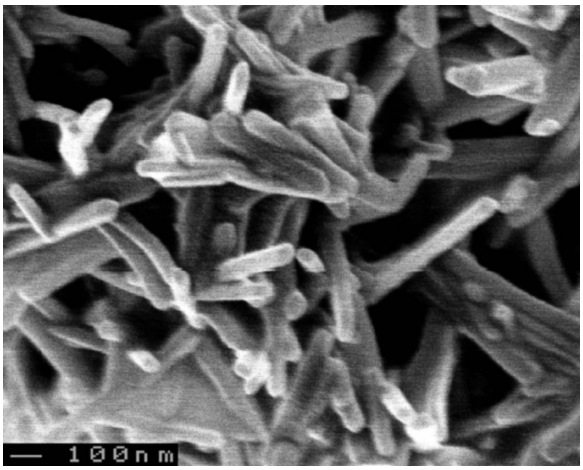

FIGURE 3. SEM image of precipitated salbutamol sulfate with SDS.

FIGURE 5. SEM image of precipitated mannitol with SDS.

Like SS, surfactant-free CLIJ precipitation of mannitol also shows a broad size distribution with a VMD of 13 μm, but with added SDS, the VMD increased to 22 μm (Figure 4). Under SEM, the particles were elongated and platelike (up to 5 μm long with a width as small as 0.2 μm; Figure 5). With the addition of SDS, the larger particle size distribution may be due to an increase in solubility of mannitol in the final water-acetone mixture as the initial mannitol solution is already at saturation. It should be pointed out that light scattering for sizing these samples was only for qualitative comparisons as the particles were elongated rather than spherical. Solubility of mannitol in 1:5 water:acetone solution was determined to be 5.8 mg/mL, while the addition of 10% SDS increased the mannitol solubility to 9.6 mg/mL. This was not observed in the SS precipitation as the solvent to antisolvent ratio was 1:24, compared with 1:5 for mannitol. Increase in solubility increases the formation time of the particles for the same precipitation driving force applied (antisolvent). While the increase in formation time should, in theory, produce smaller particles since the mixing time is relatively faster, it is only applicable if the time scale is within the residence time of mixing in the confined chamber. If the formation has not occurred before leaving the mixing zone, it loses the mixing advantage of the CLIJ as the collected mixture may separate during sitting due to effects such as diffusion and form larger particles.

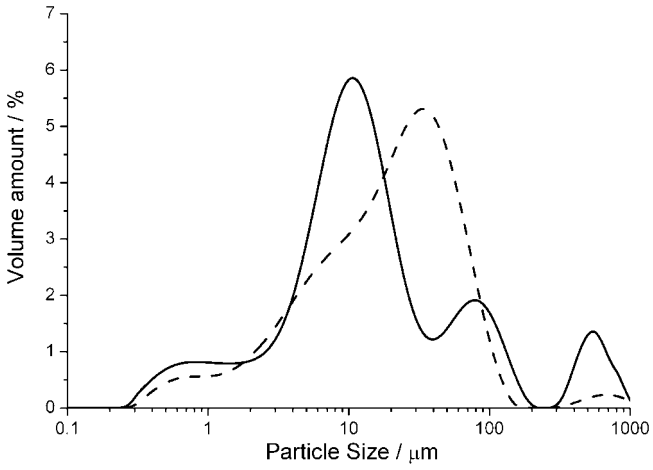

FIGURE 4. Particle size distribution of precipitated mannitol, without SDS (—) and with SDS (- - -).

It is also possible to decrease the formation time by increasing the solvent to antisolvent ratio. One method to increase the solvent to antisolvent ratio while maintaining high mixing velocities of the two liquid streams is to have a lower ratio of one liquid stream while impinging against pure antisolvent, as used in the SS precipitation (1:11.5 then 1:1). This only works if the mixing of the lower ratio does not form precipitate prior to CLIJ mixing. To test for nucleation or precipitation, the initial solutions (SS solution, pure IPA) and the mixture was examined by dynamic light scattering. It was found that the degree of nucleation or precipitation, if any, was insignificant and could not be detected. An alternative method is to have multiple liquid streams impinging simultaneously.

As both SS and mannitol products are needlelike in nature, it must be noted that laser diffraction report the distribution based on the volume of an equivalent sphere. Elongated particles would scatter light differently to spherical particles. As they are measured under the same assumptions within the light-scattering instrument, the particle size distribution is used as a tool to compare the effect of added SDS relative to SDS-free precipitation.

Precipitation of Poorly Water-Soluble Compounds

In the case of ibuprofen, without SDS the VMD was 190 μm with the main peak at 240 μm (Figure 6). With SDS, the suspension obtained directly after mixing showed a broad particle size distribution ranging from about 0.1 to > 100 μm. However, the suspensions were unstable as shown by the shift in PSD when remeasured two minutes later. The VMD of the lower

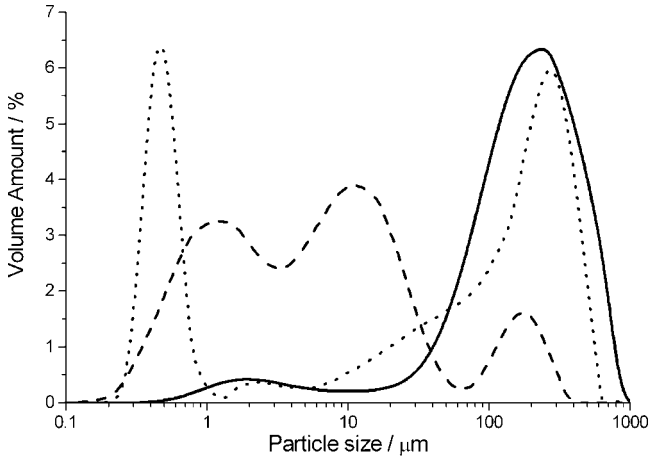

FIGURE 6. Particle size distribution of ibuprofen without additives (—), directly processed with additives (- - -), and two minutes later with additives (···).

size fraction (after two minutes) was 439 nm (span 0.709, GSD 1.36), while the VMD of the larger size fraction was > 100 μm. The ibuprofen crystals did not wet properly and floated (with trapped air bubbles) to the liquid surface, making further size measurement by laser diffraction difficult even with further addition of surfactant. Optical microscopy was then employed to observe the final size and morphology, where the particle length was approximately 30 μm to 50 μm (Figure 7).

As CLIJ is a single-pass process, mixing can only occur once, so precipitation of the compound must complete soon after mixing. If depletion of solute during nucleation and crystal growth is incomplete, secondary crystallization and further growth may occur, as seen in the case of ibuprofen. The increase in size could be due to aggregation of small particles; however, optical microscopy (Figure 7) showed that they were large individual crystals. The driving force of crystallization was greater than the stabilization force of the surfactant.

In some systems, different solvent:antisolvent ratios are required for quick precipitation, and to maintain the ratio, both the volume and flow rates need to be adjusted. For cyclosporine, the solvent:antisolvent ratio used was 1:3, which produced a broad distribution with a VMD of 541 nm and the main peak of 275 nm. By adding lecithin and dextrose monohydrate, the distribution narrowed and had a VMD of 294 nm (span 1.017, GSD 1.46; Figure 8). After drying, the particles were found to be spherical as shown in the SEM photo (Figure 9) with a mean diameter of approximately 260 nm. The apparent fusing of the particles is likely due to the additives on the particle surface after drying rather than agglomeration.

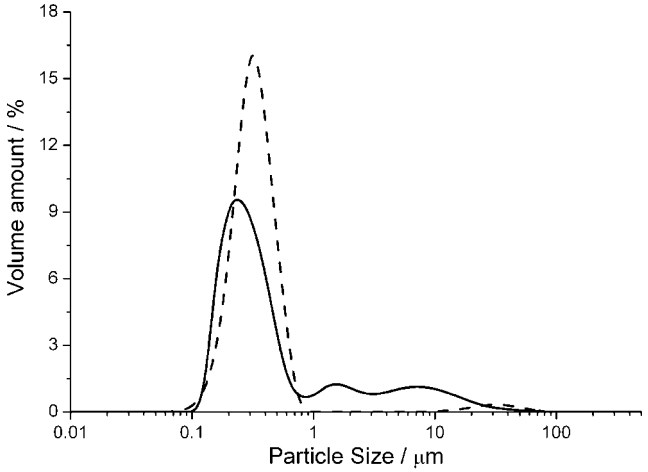

FIGURE 8. Particle size distribution of processed cyclosporine, without SDS (—) and with SDS (- - -).

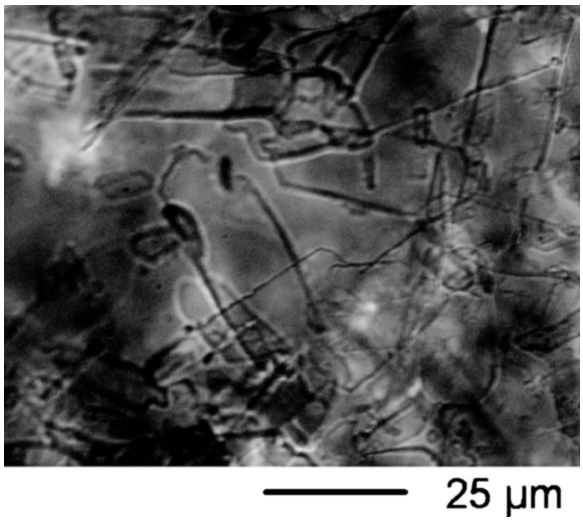

FIGURE 7. Optical microscope image of precipitated ibuprofen with additives.

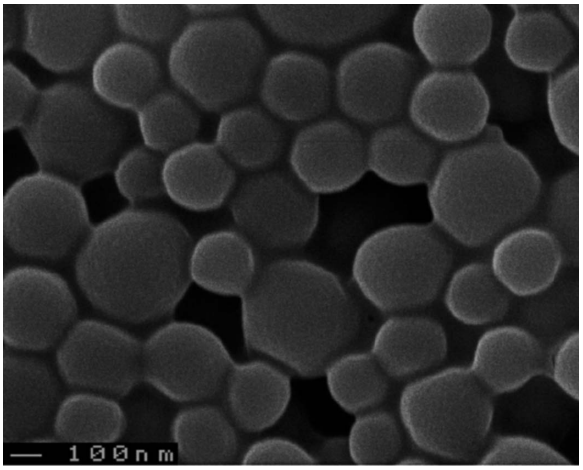

FIGURE 9. SEM image of precipitated cyclosporine with SDS.

To prevent further growth of crystals, a quenching process could be employed, and in the case of cyclosporine, the crystallized product was quenched in a large volume of antisolvent (water). This is to ensure that any drugs remaining in solution are forced to crystallize and prevent growth to existing crystals. The small population of large particles in the cyclosporine PSD (Figure 8) may be a result of the uncontrolled crystallization from quenching in addition to poor mixing at the start and end of the CLIJ process, though the number of large particles is low considering the distribution is volume weighted.

CONCLUSIONS

This work has shown that nanodrugs can be produced for water-soluble and poorly water-soluble compounds, SS and cyclosporine, by the CLIJ technique. The CLIJ process is not suitable for all compounds as shown by the results from the mannitol and ibuprofen. It strongly reinforces that CLIJ is a single-pass process designed for flash nanoprecipitation, where precipitation should form during impinging. Suitability does not depend on water solubility, but rather the formation time of the compound in the environment to which it is exposed. The environment would need to be optimized so that the formation time is longer than the mixing time but less than total residence time. Once it is achieved, narrower distributions can be obtained by stabilising the compound through the addition of surfactants. Quenching of the system may be used to arrest further growth of particles. The CLIJ precipitation process can be refined or tailor-made using other GRAS additives to enhance the success of nanoparticle production.

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