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A Simple Confined Impingement Jets Mixer for Flash NanoPrecipitation

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Abstract

Johnson and Prud'homme¹⁴ introduced the confined impingement jets (CIJ) mixer to prepare nanoparticles loaded with hydrophobic compounds (e.g., drugs, inks, fragrances, or pheromones) via flash nanoprecipitation (FNP). We have modified the original CIJ design to allow hand operation, eliminating the need for a syringe pump, and we added a second antisolvent dilution stage. Impingement mixing requires equal flow momentum from two opposing jets one containing the drug in organic solvent the other an antisolvent, typically water. The subsequent dilution step in the new design allows rapid quenching with high antisolvent concentration that enhances nanoparticle stability. This new CIJ-D mixer (confined impingement jets with dilution) is a simple, cheap and efficient device to produce nanoparticles. We have made 55 nm diameter β -carotene nanoparticles using the CIJ-D mixer. They are stable and reproducible in terms of particle size and distribution. We have also compared the performance of our CIJ-D mixer with the vortex mixer, which can operate at unequal flow rates²⁰, to make β -carotene-containing particles over a series of turbulent conditions. Based upon dynamic light scattering (DLS) measurements, the new CIJ-D mixer produces stable particles of a size similar to the vortex mixer. Our CIJ-D design requires less volume and provides an easily operated and inexpensive tool to produce nanoparticles via FNP and to evaluate new nanoparticle formulation.

Keywords

CIJ mixer; nanoparticles; flash nanoprecipitation; drug delivery; β -carotene

Introduction

Nanoparticles have recently received enormous attention as a drug delivery tool.^{5–8,29} Flash nanoprecipitation (FNP) is a simple technique that is used to prepare polymeric nanoparticles with a high loading of hydrophobic compounds, including drugs.^{14,15,17} As

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shown in Fig. 1,³³ a hydrophobic drug and an amphiphilic block copolymer (e.g. PEG-*b*-PLA, polyethylene glycol-*b*-polylactic acid) are co-dissolved in a water miscible organic solvent (e.g. THF), which is then impinged at high velocity against an antisolvent (water) to create turbulent mixing and high supersaturation. The supersaturation promotes co-precipitation of the hydrophobic drug and the hydrophobic block of the copolymer to form nanoparticles.^{2,10,19,20} Mixing and precipitation occur within milliseconds inside the small internal mixing chamber.

Johnson and Prud'homme¹⁴ first described FNP using a confined impingement jets mixer (CIJ). In this design, a syringe pump was used to drive two opposing liquid streams (A and B in Fig. 1) at high velocity into the mixing chamber. Prud'homme and others have used this device to successfully make a variety of nanoparticles.^{1,14–16,21,23} The CIJ design was inspired by the simple T mixer that is commonly used to mix liquids or act as a chemical reactor.^{9,27} T mixers have also been used to mix monomers and oligomers for reaction injection molding.^{18,22,25,28}

To avoid reducing the mixing efficiency (via one stream backing up the other), the two streams in the CIJ mixer must operate at near equal momentum. In practice, this requires approximately a 1:1 volume flow rate, limiting the highest achievable supersaturation level. To operate at unequal flow ratios, Prud'homme and coworkers developed a multi-inlet vortex mixer.^{11,20} In this design, the mixing chamber is connected to four inlets and the liquid streams meet at an angle as opposed to the head-on impingement that is characteristic of the CIJ mixer. The vortex mixer can be applied to a wide range of solvent ratios and materials. However, the device is time consuming to clean and digitally programmed syringe pumps are usually required to control the inlet flow rates.⁴ In terms of operation, cleaning and cost, the CIJ mixer is preferred. Nevertheless, the vortex mixer is able to achieve higher levels of supersaturation.

Here we report a modified CIJ design, the CIJ-D mixer, that is simpler than the original Johnson and Prud'homme design, yet overcomes the limitation of 1:1 solvent ratio. To emphasize its simpler design and easier handling with equivalent function, we have compared the average size of β -carotene nanoparticles made using our CIJ-D mixer vs. the vortex mixer.

Design

Fig. 2 shows the confined impingement jets with dilution (CIJ-D) design. Two features distinguish it from the original CIJ mixer: hand operation and an antisolvent dilution stage. By using relatively small, low friction syringes, turbulent flow can be achieved with simple, rapid hand motion, eliminating the need for syringe pumps. The small sample size and easy operation made the CIJ-D ideal for screening candidate formulations. A metal plate connects the two syringes to insure simultaneous actuation. To increase the supersaturation, the outlet stream from the CIJ-D chamber immediately flows directly into a large volume of water.

The design dimensions for the CIJ mixing chamber was recommended by Johnson and Prud'homme.¹⁴ Two pathways lead to a small chamber with a confined volume, where the

organic solvent and water impinge to create turbulence. A ratio of entrance channel length to diameter $L/d = 6.1$ was used to insure stable jets. The ratio of chamber height above the inlet nozzle to diameter is $H = 0.8D$, and the length to diameter ratio $H+Z = 2.0D$ was held constant to maintain geometric similarity upon scale-up.¹⁴ The chamber volume is 25 μL . The outlet tube runner length should be at least ten times the outlet diameter $K/\delta > 10$, in order to create a pressure drop and ensure that the chamber is filled with liquid during impingement.

Fig. 3 shows the dimensions of our CIJ-D chamber. The main body of the CIJ mixer was made of high density polyethylene (HDPE), with two inlets and adapters (IDEX Health & Science, P604) fitted with threaded syringes, and one outlet adapter (IDEX Health & Science, P205-X). Two additional side openings resulted when horizontal jet pathways were drilled during manufacturing. These ports are sealed with threaded plugs (IDEX Health & Science, P203-X) during FNP experiments, but can be opened for thorough cleaning.

A typical procedure for making nanoparticles is as follows. β -carotene was used as the generic hydrophobic molecule ($\log P = 15.5$, ACD model from www.emolecules.com). PEG-*b*-PLA (polyethylene glycol-*b*-polylactic acid, molecular weight: 5,000-*b*-10,000 Da²⁶) was used as the amphiphilic block copolymer. 25 mg β -carotene and 25 mg PEG-*b*-PLA were dissolved in 2.5 mL of THF and transferred to a 3 mL plastic syringe (Kendall, Tyco Healthcare, Mansfield, MA). 2.5 mL of deionized water was loaded in a second 3 mL plastic syringe. A metal plate was placed on the top of the syringes to ensure simultaneous actuation which occurred over a period of approximately 5 s. The co-mixed stream was immediately diluted into a bottom reservoir pre-loaded with 45 mL of deionized water. The final composition of the 50 mL dispersion was THF:H₂O = 5:95, containing 0.1 wt% of nanoparticles. Mass average particle size and size distribution was determined by dynamic light scattering (DLS) (ZetaPALS, Brookhaven Instruments, diode laser BI-DPSS wavelength of 659 nm, round cuvette) using REPES method,^{12,13} immediately after nanoparticles were prepared following the procedure of Zhu.³³ The light intensity correlation function was collected at 25 °C and a scattering angle of 90°.

Performance

To test the CIJ-D mixer, nanoparticles were prepared following the typical procedure given above. Dynamic light scattering gave mass average diameter of 38 nm with polydispersity of 1.6 and standard deviation of 6 nm for three measurements on three separately mixed samples. The particles were stable for several weeks. In other block copolymer and model drug studies, we have also used PEG-*b*-PLA and PEG-*b*-PLGA (polyethylene glycol-*b*-poly(lactic-co-glycolic acid)) with a series of molecular weight (5,000-*b*-10,000 Da, 5,000-*b*-15000 Da et al.) to make nanoparticles loaded with β -carotene. Zhu³³ used hydrocortisone, paclitaxel, betulin and their derivatives to test the capability of the CIJ-D mixer for preparing polymeric nanoparticles. Chow and his researchers³ assessed the CIJ-D mixer by making curcumin nanoparticles. Prud'homme and coworkers³¹ have used the CIJ-D mixer making polystyrene nanoparticles. They were successfully produced, reproducible in terms of size.

As mentioned above, the dilution stage allows high supersaturation while maintaining the 1:1 flow ratio of the impinging streams. In order to demonstrate the importance of the dilution stage, four groups of particles were made following the procedure described in Section 2 but with differing dilution. For this study, we used pure β -carotene (no block copolymer) to make particles. These particles show good short-term (~4 hr) stability due to slightly negative surface charge²⁴ as judged by zeta potential (ζ) measurements.³²

In group 1, nanoparticles were made following the recommended procedure, immediate dilution into 45 mL water, THF/H₂O = 0.05. The resulting mass average size was 55 nm. In group 2, nanoparticles were made without dilution. They were unstable, and aggregated to micron size in seconds. In group 3, dilution was delayed from ~10 ms (residence time in the mixer and outlet tube) to 5 s. This also resulted in unstable microparticles (> 1 μ m). In group 4, nanoparticles were made with immediate (10 ms) water dilution but less than 45 mL (e.g., 5 mL, 10 mL, and 25 mL or THF/H₂O = 0.33, 0.20 and 0.091). The particle sizes from group 4 are shown in Fig. 4. None of them produced 55 nm nanoparticles, in contrast to the usual dilution, THF/H₂O = 0.05. Instead, all were much bigger, approximately 1400 nm, 200 nm and 167 nm, respectively and eventually unstable. These experiments show that immediate dilution with a significant amount of water is indispensable to produce small and stable nanoparticles.

We also compared the sizes of these pure β -carotene nanoparticles made by the CIJ-D mixer to those made by a vortex mixer. Particle size was varied by varying the flow rate, Q . This was accomplished simply by changing hand velocity during mixing. Mixer efficiency is typically correlated with Reynolds number, the ratio of inertial force to viscous force. The Reynolds number was calculated by:

$$Re = \frac{\text{Inertial force}(= \rho V^2)}{\text{Viscous force}(= \mu V/d)} = \frac{\rho V d}{\mu} = \frac{\rho Q c}{\mu A} \quad (1)$$

In our case, Reynolds number was calculated by accumulating multiple streams when using the CIJ-D or vortex mixer.

$$Re = \sum_{i=1}^n Re_i = \frac{d}{A} \sum_{i=1}^n \frac{\rho_i Q_i}{\mu_i} = \frac{4}{\pi d} \sum_{i=1}^n \frac{\rho_i Q_i}{\mu_i} \quad (2)$$

where ρ is the density of the fluid (kg/m³), V is the mean fluid velocity (s), d is the stream inlet diameter, μ is the viscosity of the fluid (kg/m·s), A is the pipe cross-sectional area (m²) which in our case was the same for all inlets of a given mixer, and Q_i is the volumetric flow rate (m³/s) of the i th inlet stream.

For example, in CIJ mixing, $n=2$ for two streams, ρ is 1.0×10^3 kg·m⁻³ for H₂O, or 8.89×10^2 kg·m⁻³ for THF at room temperature, μ is 1.0×10^{-3} Pa·s at room temperature for H₂O, or 4.8×10^{-4} Pa·s for THF, and d is 5×10^{-4} m for the CIJ-D mixer.

The vortex mixer has four inlets, each with diameter $d=1.45\times10^{-3}$ m. For all four streams, we assumed $\mu=1.0\times10^{-3}$ Pa·s and $\rho=1.0\times10^{-3}$ kg·m⁻³ at room temperature, since 90% H₂O/10% THF has a similar kinematic viscosity to that of water.

The mass average diameter shown in Fig. 5 are averages of three measurements on three separately mixed samples prepared under the same conditions. The vortex mixing data were taken from Zhu.³³ The error in the calculated Reynolds number comes from variation of injection time. In vortex mixing, errors are minor since the injection time is mechanically controlled by syringe pumps, but it is relatively large in CIJ-D mixing due to uncertainty in timing the hand motion. At the highest Reynolds number, the injection time of the CIJ-D mixer was 4.2 ± 0.3 s. 2.5 mL of solvents in two 3 mL syringes were used thus the flow rate was 1.2×10^{-6} m³/s. Considering this flow rate and the tubing dimensions (1 mm inside diameter and 15 mm long), the residence time in the chamber and outlet tube before dilution was approximately 10 ms. For $Re < 1000$, a syringe pump was used with the CIJ-D mixer since hand operation cannot be controlled well enough to create steady mixing over minutes.

The results in Fig. 5 show good agreement between the new CIJ-D design and the vortex mixer. Due to rapid injection the CIJ-D can reach $Re > 4000$, twice the limit for the vortex mixer, and particle size of 30 nm, half the smallest size from the vortex mixer. At the same Re particle size for the CIJ-D is slightly smaller.

A potential problem with hand operation of the CIJ-D mixer is the start-up of flow. The Re at the beginning of impingement will be lower due to the time required to accelerate the syringes. The same problem occurs with syringe pumps, but because the impingement time is longer, it is possible to discard the first part of the product. This start-up transient could lead to broader particle size distribution, however, we observed broad size distribution in both the CIJ-D mixer and the vortex mixer. For example, at $Re\approx1750$ (shown in Fig. 5), DLS gave averaged size polydispersity indices³³ of 0.5–0.8 for both samples.

Conclusion

A simple modification of Johnson and Prud'homme's confined impingement jets (CIJ) mixer has been used to make stable and reproducible nanoparticles. The addition of a dilution stage after mixing results in higher levels of supersaturation, overcoming the limitation of equal volume ratios required in the original CIJ design. For our β -carotene particles we found that the dilution needed to be rapid, < 5 s after mixing, and extensive, >5 fold. Hand-operated impingement with small syringes creates sufficient turbulent mixing, ideal for flash nanoprecipitation (FNP) of hydrophobic compounds with diblock copolymers to form kinetically trapped, sterically stabilized nanoparticles. With these modifications, the new mixer, called CIJ-D to emphasize the addition of a dilution stage, uses very small volumes and is easy to operate and inexpensive, making it more effective for rapid screening of small quantities of new materials, via FNP, compared to alternative mixers and other methods. It is especially attractive for evaluation of new drug formulations for their ability to produce nanoparticles.

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References

1. Akbulut M, Ginart P, Gindy ME, Theriault C, Chin KH, Soboyejo W, Prud'homme RK, 2009 Generic method of preparing multifunctional fluorescent nanoparticles using Flash NanoPrecipitation. *Adv Funct Mater* 19:718–725.
2. Cheng CY, Vigil RD, Fox RO, 2010 A competitive aggregation model for Flash NanoPrecipitation. *J Colloid Interface Sci* 351:330–342. [PubMed: 20800847]
3. Chow S, Sun C, Macosko CW, Chow A, 2011 Comparative assessment of the performance between confined impingement jet and multi-inlet vortex mixers for controlled production of curcumin nanoparticles. American Association of Pharmaceutical Scientists Meeting.
4. D'addio SM, 2011 Flash nanoprecipitation video: <http://www.princeton.edu/research/news/princetoninvention/video/index.xml>
5. Feng SS, Chien S, 2003 Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. *Chem Eng Sci* 58:4087–4114.
6. Ferrari M, 2005 Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer* 5: 161–171. [PubMed: 15738981]
7. Faraji AH, Wipf P, 2009 Nanoparticles in cellular drug delivery. *Bioorg Med Chem* 17: 2950–2962. [PubMed: 19299149]
8. Horn D, Rieger J, 2001 Organic nanoparticles in the aqueous phase-theory, experiment and use. *Angew Chem Int Ed* 40:4330–4361.
9. Hunter TG, Nash AW, 1935 Liquid-liquid extraction systems. *Ind Eng Chem*. 27:836–845.
10. Gavi E, Marchisio DL, Barresi AA, 2007 CFD modeling and scale-up of confined impinging jet reactors, *Chem Eng Sci*. 62:2228–2241.
11. Gindy ME, Panagiotopoulos AZ, Prud'homme RK, 2008 Composite block copolymer stabilized nanoparticle: simultaneous encapsulation of organic actives and inorganic nanostructure. *Langmuir* 24:83–90. [PubMed: 18044945]
12. Jakes J, 1988 Testing of the constrained regularization method of inverting laplace transform on simulated very wide quasilastic light-scattering auto-correlation functions. *Czech J Phys*. 38 (12): 1305–1316
13. Jakes J, 1995 Regularized positive exponential sum (REPES) program-a way of inverting laplace transform data obtained by dynamic light scattering. *Collect Czech Chem Commun*. 60 (11):1781–1797.
14. Johnson BK, Prud'homme RK, 2003a Chemical processing and micromixing in confined impinging jets. *AIChE J* 49:2264–2282.
15. Johnson BK, Prud'homme RK, 2003b Flash NanoPrecipitation of organic actives and block copolymers using a confined impinging jets mixer. *Aust J Chem*. 56:1021–1024.
16. Johnson BK, Prud'homme RK, 2003c Mechanism for rapid self-assembly of block copolymer nanoparticles. *Phys Rev Lett* 91:1183021–1183024.
17. Johnson BK, Prud'homme RK, 2004 Process and apparatuses for preparing nanoparticle compositions with amphiphilic copolymers and their use. U.S.P.A.P 0091546 A1.
18. Lee LJ, Ottino JM, Ranz WE, Macosko CW, 1980 Impingement mixing in reaction injection molding. *Poly Eng Sci* 20:868–874.
19. Liu Y, Fox RO, 2006 CFD predictions for chemical processing in a confined impinging-jets reactor. *AIChE J*. 52:731–744.
20. Liu Y, Cheng CY, Liu Y, Prud'homme RK, Fox RO, 2008 Mixing in a multi-inlet vortex mixer (MIVM) for flash nano-precipitation. *Chem Eng Sci* 63:2829–2842.

21. Lince F, Marchisio DL, Barresi AA, 2009 Smart mixers and reactors for the production of pharmaceutical nanoparticles: proof of concept. *Chem Eng Res Des* 87:543–549.
22. Macosko CW, 1989 RIM, fundamentals of reaction injection molding. Hanser, New York.
23. Marchisio DL, Rivautella L, Barresi AA, 2006 Design and scale-up of chemical reactors for nanoparticles precipitation. *AIChE J* 52:1877–1887.
24. Mordt RC, Walton JC, Burton GW, Hughes L, Ingold KU, Lindsay DA, Moffatt DJ, 1993 Oxidative-degradation of β -Carotene and β -8'-carotenal. *Tetrahedron* 49:911–928.
25. Nguyen LT, Suh NP, 1986 Processing of polyurethane/polyester interpenetrating polymer networks by reaction injection molding: Part 2. mixing at high Reynolds numbers and impingement pressures. *Poly Eng Sci.* 26:799–842.
26. Qian HT, Wohl AR, Crow JT, Macosko CW, Hoyer TR, 2011 A Strategy for control of “random” copolymerization of lactide and glycolide: application to synthesis of PEG-b-PLGA block polymers having narrow dispersity. *Macromolecules*
27. Treybal RE, 1963 Liquid extraction, 2nd ed. McGraw-Hill, New York.
28. Tucker CL, Suh NP, 1980 Mixing for reaction injection molding. I. impingement mixing of liquids. *Poly Eng Sci* 20:875–886.
29. Wang X, Yang L, Chen Z, Shin DM, 2008 Application of nanotechnology in cancer therapy and imaging. *CA Cancer J Clin.* 58:97–110. [PubMed: 18227410]
30. Wohl AR, Kalscheuer S, Lee HS, Han J, McCormick A, Macosko CW, Panyam J, and Hoyer TR, 2012 A silicate ester prodrug strategy for improving the therapeutic index of paclitaxel. *JACS*,
31. Zhang C, Pansare VJ, Prud'homme RK, Priestley RD, 2012 Flash nanoprecipitation of polystyrene nanoparticles. *Soft Matter*, 8: 86–93.
32. Zhu ZX, Margulis-Goshen K, Magdassi S, Talmon Y, Macosko CW, 2010 Polyelectrolyte stabilized drug nanoparticles via flash nanoprecipitation: a model study with β -carotene. *J Pharm Sci* 99:4295–4306. [PubMed: 20143406]
33. Zhu ZX, 2010 Polymer stabilized nanosuspension via flash nanoprecipitation: nanoparticle formation, formulation and stability. PhD thesis.

Fig. 1.
Schematic of flash nanoprecipitation process.³³

Fig. 2.
The CIJ-D mixer, hand operated with subsequent dilution.

Fig. 3.
Dimensions of the CIJ-D mixer made of HDPE.

Fig. 4.
Mass average diameter of β -carotene particles vs. THF/water ratio in group 4 ($Re \approx 1750$).

Fig. 5. Mass average size of β -carotene particles (d_m) vs. Reynolds number (Re). Particles were made using both the vortex mixer and CIJ-D mixer. The Reynolds number was calculated using equation (2).