Title

Straightforward biodegradable nanoparticle generation through megahertz-order ultrasonic atomization

Journal

Applied Physics Letters, 89(6)

ISSN

0003-6951

Authors

Forde, Gareth
Friend, James
Williamson, Tom

Publication Date

2006-08-07

DOI

10.1063/1.2221914

Peer reviewed

Straightforward biodegradable nanoparticle generation through megahertz-order ultrasonic atomization

Gareth Forde,[a] James Friend,[b] and Tom Williamson[c]
Monash University, Wellington Road, Clayton, Victoria 3800, Australia

(Received 23 December 2005; accepted 25 May 2006; published online 9 August 2006)

Simple and reliable formation of biodegradable nanoparticles formed from poly-ε-caprolactone was achieved using 1.645 MHz piston atomization of a source fluid of 0.5% w/v of the polymer dissolved in acetone; the particles were allowed to descend under gravity in air 8 cm into a 1 mM solution of sodium dodecyl sulfate. After centrifugation to remove surface agglomerations, a symmetric monodisperse distribution of particles ϕ 186 nm (SD=5.7, n=6) was obtained with a yield of 65.2%. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2221914]

A simple method for synthesis of unagglomerated biodegradable nanoparticles (<250 nm) in the absence of organic solvents would represent a major processing breakthrough for drug encapsulation and delivery. To this end, a strategy was developed using room-temperature, megahertz-order ultrasound to reliably produce nanosized particles of poly-ε-caprolactone, a biodegradable polymer approved for medical use. While other methods exist to generate small particles, including spray-drying,[1–3] electrostatic atomization,[4] and 20–40 kHz ultrasonic atomization,[5,6] the method shown here can uniquely generate monodisperse polymer nanoparticles with a narrow distribution.

Poor delivery of biopharmaceuticals (e.g., drugs, proteins, and polynucleotides) is a major hurdle in improving their efficacy.[7] One of the current methods to overcome the very low cell transfection efficiencies is to administer large doses of the biopharmaceutical,[8] possibly leading to toxic side effects and drug resistance. Biopharmaceuticals represent the latest generation of therapeutics and are increasing in importance, with 7% of the total pharmaceutical market or US$390 billion in 2002.[9]

Certain biopharmaceuticals (e.g., anticancer therapeutics, DNA, and some proteins and peptides) are intended to migrate to the cell nucleus in order to elicit the required response. The two limiting steps of greatest concern are the cellular uptake of the biopharmaceutical and migration into the cell nucleus across the nuclear pore complex (NPC), difficult for nonsoluble, toxic, or large molecules.[10] New technologies to deliver ϕ10–250 nm particles containing biopharmaceuticals that overcome these barriers have potentially huge applications in the rapidly growing biopharmaceutical market.

The polymeric solution was prepared by dissolving poly-ε-caprolactone (PCL, Sigma-Aldrich), molecular weight 65 000 in acetone (99.5%, LabScan) to create a feedstock solution of 0.5% PCL w/v, with viscosity of 0.402 mPa s at room temperature. The polymer solution feedstock was pumped onto the bottom surface of an inverted 1.0 $\times \phi$30 mm hard lead zirconate titanate (PZT N-61, NEC/Tokin) piezoelectric disk vibrated in either its fundamental (1.645 MHz) or first harmonic (5.345 MHz) thickness mode. The PZT disk was electroded completely on both faces with platinum electrodes, 500 nm thick, and driven with a sinusoidal voltage-controlled input, giving a relatively constant and in-phase output vibration velocity across the entire face of the piezoelectric element, i.e., *piston* vibration, for both modes. The vibration velocity of the surface was measured using a laser Doppler vibrometer (GRAPHTEC AT-3600/0023, Yokohama, Japan) during operation. A thermocouple (455-4371 Type J, RS Components) was used to measure the temperature of the PZT element during the experiment.

Acoustic radiation, transmitted from the PZT element into the droplet, formed surface capillary waves at low power excitation of the PZT element, and atomized the droplet through surface instabilities about the crests of these waves at higher power.[11–13] The feedstock pumped onto the Pt-electroded surface formed a ϕ 10 mm droplet with a wetting angle of approximately 30°, maintained through controlled pumping of the feedstock at 28 ml/h (except as described in Table I) as the fluid surface atomized into 25 C, 45% relative humidity (RH) air, forming fluid polymer particles which descended 8 cm under gravity into a hardening agent, 1 mM sodium dodecyl sulfate (SDS, Sigma-Aldrich) in de-ionized water, as illustrated in Fig. 1.

The nanoparticle-containing hardening agent was then centrifuged at 2500 rpm for 10 min in a swinging bucket rotor to remove any large agglomerates that may have formed, due predominantly to accumulation of the hydrophobic polymer at the air-liquid interface. The particle size (z-average diameter) was determined using Zetasizer (Malvern Instruments Ltd., UK, DT software 4.10b1). The

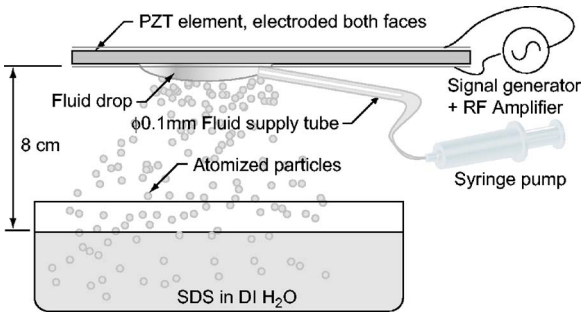

FIG. 1. Illustration of the particle generation method used in the study.

[a]Also at: Department of Chemical Engineering.
[b]Also at: Micro/Nanophysics Laboratory, Department of Mechanical Engineering; electronic mail: james.friend@eng.monash.edu; URL: http://mnrl.monash.edu.au
[c]Also at: Department of Mechanical Engineering.

TABLE I. Results for the ultrasonic atomization of 0.5% poly-ε-caprolactone in acetone under a range of process parameters.

Parameter		D^a	σ^b	No. of runs
Transducer	0.7 m/s	208	5.7	6
Vibration	1.0 m/s	186	5.7	6
Velocity	1.4 m/s	5641	6474.4	6
	1.645 MHz	186	5.7	6
Frequency	5.305 MHz	182	2.3	6
	20 ml/h	187	13.9	6
	28 ml/h	186	5.7	6
Flow rate	36 ml/h	181	6.5	6
	8 cm	186	8.4	3
Atomizer	12 cm	202	3.5	3
height	16 cm	215	4.6	3
	1 mM	186	5.7	6
SDS detergent	10 mM	226	21.4	6
concentration	100 mM	3467	3692.7	6

[a] z-averaged particle diameter (see Refs. 3 and 4).
[b] σ is standard deviation.

z-average diameter is the mean diameter based on the intensity of scattered light that occurs due to Brownian motion of the particles.[14]

The results for the nanoparticle size analysis obtained using the Zetasizer are presented in Table I. Unless otherwise indicated, feedstocks were pumped at a flow rate of 28 ml/h onto the bottom surface of a piezoelectric disk transducer run at an amplitude of 1.0 m/s vibration velocity and a frequency of 1.645 MHz into a hardening agent of 1 mM SDS in water located 8 cm beneath the transducer. The temperature of the PZT element was found to increase rapidly to about 5 °C above ambient during the experiment, with an apparent cooling effect from the constant supply of fluid to the bottom of the element. Under most conditions, as shown in Table I, an average particle diameter of between ϕ 181 and 226 nm was obtained. The particle size frequency distribution under the conditions which resulted in the smallest z-average particle size (181 nm) is shown in Fig. 2, displaying a monodisperse, symmetrical frequency distribution. A small amount of larger agglomerations (ca. 500–700 nm) which were not removed by the centrifuge are visible in the figure. At two certain conditions, submicron z-average diameter particles were not obtained: at 1.4 m/s vibration velocity and for a hardening agent of 100 mM SDS. At 1.4 m/s vibration velocity, observation of the source droplet suggested the atomization process was failing, with either over-

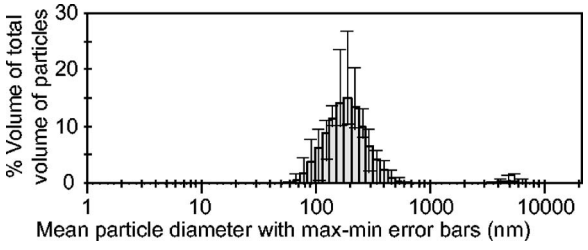

FIG. 2. Histogram of the particle size frequency distribution for atomization of 0.5% w/v PCL in acetone pumped at a flow rate of 36 ml/h onto the bottom surface of a piezoelectric disk vibrated at 1.0 m/s vibration velocity (other test conditions matched standard conditions described in Table I).

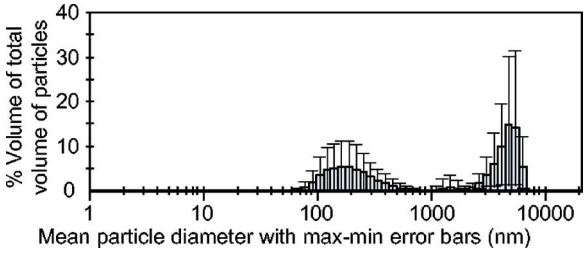

FIG. 3. Histogram of the particle size frequency distribution arising from atomization of 0.5% w/v PCL in acetone pumped at a flow rate of 28 ml/h onto the bottom surface of a piezoelectric disk run at 1.4 m/s vibration velocity (other test conditions matched standard conditions described in Table I).

heating of the liquid causing it to boil away or ejection of far larger droplets from the surface than during typical atomization. Figure 3 displays the bimodal particle size frequency distribution that was obtained at an amplitude of 64%.

For the second case, it is believed that the hydrophobic nature of the polymer prevents the creation of a stable emulsification in water, so a surfactant was employed in the hardening agent to assist in stabilizing the nanoparticle/hardening agent solution. The critical micelle concentration (CMC) of SDS in water is approximately 2.5 g/ℓ or 8.7 mM.[15] In keeping with the findings of Bonard *et al.*,[16] the results from this study show that at concentrations below (1 mM) and slightly above (10 mM) the CMC, no sedimentation of the nanoparticles occurred. At much larger SDS concentrations (100 mM or 11.5 CMC), the particles tended to form larger aggregates that varied greatly in size, indicated by the large z-average particle diameter and large standard deviation (see Table I). Particulate yield reached a maximum of 65.2% as measured by volume of particles in comparison to the volume of PCL polymer introduced into the fluid to be atomized; generally yields with this method were around 50%.

Analysis of variance (ANOVA) studies were performed for each of the process parameters of frequency, amplitude, flow rate, atomizer height, and detergent concentration in the hardening agent. The results of the ANOVA analysis show that, for the conditions listed in Table I, the parameters of amplitude, atomizing surface height, and surfactant concentration in the hardening agent have statistically significant effects on the z-average particle diameter of the PCL nanoparticles ($p=0.05$). Variations in the flow rate and frequency do not have statistically significant effects on the z-average particle diameter ($p=0.05$).

Lang[11] described the acoustic atomization of fluids to form droplets at up to 1 MHz from the breakage of crests of a capillary wave generated upon the fluid surface, and provided an estimate of the droplet size based on the applied acoustic signal frequency along with the source fluid surface tension and density,

$$D = 0.34\sqrt[3]{\frac{8\pi T}{\rho f^2}}, \tag{1}$$

where D, f, T, and ρ are the droplet diameter, applied signal frequency, fluid surface tension, and fluid density, respectively. In his derivation of the equation, Lang noted that the wavelength of the capillary waves generated along the surface of the droplet was actually half the wavelength of the excitation wavelength, and that the droplet size was some

constant fraction of the capillary wavelength [0.34 in Eq. (1)].

The surface tension and density of the 0.5% w/v PCL in acetone were determined to be 26.4 mN/m and 789 kg/m^3, respectively, using a Krüss K9 tensiometer (Krüss GMBH, Hamburg), predicting a droplet size of 2.3 μm using Eq. (1) at 1.645 MHz. Since fully 99.4% w/v of the particle is acetone, which would be expected to quickly evaporate in room-temperature air, and the density of PCL is 1150 kg/m^3, roughly double the density of acetone at 785 kg/m^3, the volume of the particle should become approximately 0.73% of the volume of the atomized droplet. Assuming the droplets and subsequent particles are spherical, the estimated volume of each atomized droplet, 0.697 μm^3, would give a particle of volume 0.005 09 μm^3 after evaporation, a diameter of 223 nm and roughly corresponding to the experimental results. At 5.305 MHz, however, Eq. (1) suggests ϕ 102 nm particles, when, in fact, the particles' diameter remains very nearly constant between the two frequencies as indicated in Table I. This suggests Lang's analysis may be appropriate only for a rough estimation of the particle size if evaporation is taken into account, and that the particle generation mechanism may change between the two frequencies used in this study.

A reliable method to generate biodegradable polymer nanoparticles has been described, and relative to most other generation methods, the method is simple and straightforward. A symmetric monodisperse distribution of nanoparticles averaging ϕ 186 nm was obtained under controlled piston vibration and source fluid delivery conditions of 1.0 m/s vibration velocity and 28 ml/h at 1.645 MHz, with a peak yield of 65.2% of particle volume. Driving the piezoelectric element at higher vibration amplitudes or reducing the source fluid flow rate causes the atomization process to deliver much larger particles and a far wider size distribution, indicating a problem with the atomization process under these conditions.

[1] P. Johansen, H. Merkle, and B. Gander, Eur. J. Pharm. Biopharm. **50**, 413 (2000).
[2] B. Baras, M. Benoit, and J. Gillard, J. Microencapsul. **17**, 485 (2000).
[3] S. Freitas, H. Merkle, and B. Gander, J. Controlled Release **95**, 185 (2004).
[4] H. Watanabe, T. Matsuyama, and H. Yamamoto, J. Electrost. **57**, 183 (2003).
[5] M. Dobre and L. Bolle, Exp. Therm. Fluid Sci. **26**, 205 (2002).
[6] C. Felder, M. J. Blanco-Prieto, J. Heizmann, H. P. Merkle, and B. Gander, J. Microencapsul. **20**, 553 (2003).
[7] C. W. Pouton, Crit Rev. Environ. Control **34**, 51 (1998).
[8] R. B. MacGregor, R. Ginsberg, K. E. Ugen, Y. Baine, C. U. Kang, X. M. Tu, T. Higgins, D. B. Weiner, and J. D. Boyer, AIDS **16**, 2137 (2002).
[9] G. Algra, Genetic Engineering News **22**, 2 (2002).
[10] O. Zelphati, X. Liang, P. Hobart, and P. L. Felgner, Human Gene Therapy **10**, 15 (1999).
[11] R. J. Lang, J. Acoust. Soc. Am. **34**, 6 (1961).
[12] R. R. A. B. Pandit, Ultrasonics **39**, 235 (2001).
[13] F. Barreras, H. Amaveda, and A. Lozano, Exp. Fluids **33**, 405 (2002).
[14] Malvern Instruments Zetasizer Nano, Report No. MRK568–01, 2005.
[15] E. Wanless and W. Drucker, J. Phys. Chem. **100**, 3207 (1996).
[16] J. Bonard, T. Stora, J. Salvetat, F. Maier, T. Stöckli, C. Duschl, L. Forró, W. de Heer, and A. Châtelain, Adv. Mater. (Weinheim, Ger.) **9**, 827 (1997).