

Production of Polymer Nanosuspensions Using Microfluidizer® Processor Based Technologies

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ABSTRACT

Polymer nanoparticles are often used for controlled drug delivery of active pharmaceutical ingredients (APIs). Microfluidizer® Processor based technologies offer two options for production of polymer nanoparticles. The first is an emulsion method, which involves dissolving the polymer and API in the oil phase of an emulsion and then subsequent removal of the oil. The second is a precipitation method, in which the polymer and API are dissolved in a solvent and then forced to precipitate inside the high shear mixing zone when mixed with an antisolvent. These methods are compatible with a wide variety of polymer/API systems. The focus of this work is to identify the effects of varying key parameters such as process pressure, relative flow rates of the streams, and formulation on the particle size distribution.

This article showcases polymer nanosuspensions in the range of 50-500 nm that were prepared with two different polymers, using both techniques. Furthermore, these tests indicate that an API was successfully encapsulated within the nanoparticles.

Keywords: polymer, nanoparticles, nanoemulsion, drug encapsulation, Microfluidizer®

1 INTRODUCTION

The creation and use of chaperone systems in drug delivery and diagnostic imaging has greatly broadened the applications, and thus needs, for polymer nanosuspensions.[2,3] The enhancement of surface to volume ratios obtained when these nanosuspensions are created provides unique capabilities for functionalization of the surface required for specificity. Encapsulation of APIs and contrast agents within these biocompatible polymers is readily accomplished using versatile Microfluidizer based technologies for processes that are reproducible and scalable. Furthermore, the probability of physical property changes due to processing is reduced. Especially when compared to sonication, the most commonly used laboratory scale technique, where cavitation and sonochemistry issues may arise.

Two techniques are reported here that can create nanosuspensions of many different polymer types with varying particle sizes by controlling the formulation and process variables. Microfluidics Reaction Technology

(MRT) was used with the solvent/anti-solvent precipitation method. Particle size distribution can be controlled by varying parameters such as processing pressure, degree of supersaturation and the ratio of solvent and anti-solvent streams.[4] This process has the advantage of producing nanosuspensions in a single step which is ideal for process intensification. The emulsion evaporation method was implemented using a Microfluidizer Processor; i.e., dissolving a polymer in a solvent, creating a nanoemulsion with an immiscible continuous phase, then removal of the solvent to produce the nanosuspension. Particle size distributions can be controlled by varying process parameters and/or formulation.[5]

Both systems control the amount and form of energy dissipation that occurs at specific locations in the system, i.e., directed toward maximizing the useful work in forming surfaces and interfaces. Narrow flow channels convert the energy input to high fluid velocities. These jet streams impinge upon each other in precision fabricated micro-liter sized interaction chambers (Figure 1). Various degrees of mixing intensity (i.e., macro-, meso-, or micro-mixing) and associated level of turbulence intensity (i.e., eddy sizes) are obtained depending upon the energy dissipation rate. The size of the smallest eddies formed, and thus the Kolmogorov scale for the desired diffusion and reaction coordinates, are in the 50-200 nanometer range. This platform can achieve processing pressures of up to 276 MPa (40,000 psi), generate fluid velocities of over 400 m/s and achieve energy dissipation values exceeding 10^7 W/kg.[6].

2 EXPERIMENTAL APPARATUS

The core of this technology is a continuous microreactor (reaction chamber) based on impinging jet design, described earlier, see Figure 1. Two opposing jets form as fluids flow through two microchannels within the chamber. The jets collide inside a microliter volume where the fluids mix at the nanometer scale. Average fluid velocities inside the channels may exceed 400 m/s, which is orders of magnitude higher than existing impinging jet reactors.[7] A planar array of opposed pairs of such channels ensures effective scaling up of the technology.

High velocities through the channels are achieved by applying high pressures to the fluid upstream of the channels. Pressures up to 207 MPa (30,000 psi) are required for such velocities generated using a hydraulically or

pneumatically driven pressure multiplier referred to as an intensifier.

Depending on the application, a variety of feed systems can be used. For simple “top-down” processing, which includes particle size reduction, a single feed system is used. For “bottom-up” processes which involve particle generation as a result of chemical or physical processes, a multiple stream feed system is used.[7] This system delivers multiple, separate streams to the processor at controlled rates. Mixing of the streams is minimized prior to the chamber, and maximized inside the chamber. Therefore particle formation is suppressed prior to the reaction chamber. Uniform mixing at the nanometer scale inside the reaction chamber ensures uniform particle production conditions in addition to nanoparticle formation.

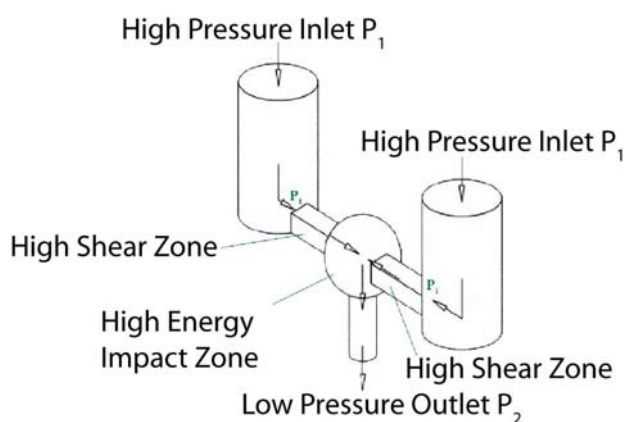


Figure 1. Schematic of the flow path of the reaction chamber. Copyright by Microfluidics, 2007.

3 PROCEDURE

Nanosized polymer particles were generated using two methods: (a) emulsion-evaporation, and (b) precipitation.

3.1 Emulsion Method

This method is a “top down” approach that involves the formation of a stable emulsion of a polymer/solvent solution with an immiscible non-solvent liquid and the subsequent removal of the solvent. For some of these tests, an API, carbamazepine, was added to the solvent phase to be incorporated inside the polymer particles.

Microfluidizer® Processors are the industry standard in forming nanoemulsions. For this method, a nanoemulsion was formed by first preparing a coarse emulsion with the solvent and aqueous streams using the IKA T-25 high shear mixer, then processing with a Microfluidizer® processor. The nanoemulsion size was controlled by varying the processing pressure, the number of passes and the concentration of the oil phase.

The solvent was then removed from the emulsion leaving only the polymer particles suspended in the water phase. There are many different ways of removing the solvent such as evaporation and co-solvent extraction. When the API was involved, the goal was to incorporate it within the polymer particle.

This method can be used for any polymer/API/solvent/non-solvent system. For illustration, this method was used to make poly(lactic-co-glycolic acid) (PLGA) particles. The polymer was dissolved in dichloromethane (DCM) at concentrations between 10 and 80 mg/ml. It was then mixed at concentrations of 1-10% dichloromethane with water that contained poly(vinyl alcohol) (PVA) to form a coarse emulsion. The processing pressure was varied between 70 and 140 Mpa and multiple passes were performed with some of the material. All these tests were performed on the M-110EH Microfluidizer® Processor with the F20Y (75 µm)–H30Z (200 µm) chamber configuration.

The solvent was then removed using several different methods that have different driving forces to obtain particles with different sizes. The evaporation method was performed in a rotovap at 25 kPa absolute for 10-20 minutes depending on the concentration of the dichloromethane. The temperature of the sample was maintained at room temperature using a water bath. The co-solvent extraction process was performed by mixing the emulsion with a co-solvent immediately after processing. The co-solvent does not dissolve the polymer, but is miscible with both the water and organic phases.

3.2 Precipitation Method

This method is a “bottom up” process that involves the precipitation of the polymer from a solution, by adding a polymer/solvent/API solution to a miscible anti-solvent. The addition of the anti-solvent results in a supersaturated condition with subsequent polymer dissolution. These streams were mixed inside the interaction chamber at various shear rates by controlling the orifice size and processing pressure.

A surfactant was added to the anti-solvent (water) in order to: (a) to stabilize the nanoparticles and limit their growth, and (b) to minimize agglomeration of the particles and thereby to create a stable suspension. A non-ionic surfactant was used, Solutol® HS 15 (polyoxyethylene esters of 12-hydroxystearic acid) from Bayer.

Nanosuspensions of two different polymers, Poly(epsilon-caprolactone) (PCL) and poly(D,L-lactide-co-glycolide) (PLGA) were produced using the precipitation method. These polymers were dissolved in acetone at concentrations ranging from 10mg/ml to 40 mg/ml. These solutions were mixed with water that contains a surfactant with flow ratios in the range of 1:2 – 1:10. Process pressures were varied between 35 – 140 mPa.

3.3 Drug Encapsulation

To date, there have only been qualitative measurements of the amount of drug that was encapsulated within the polymer nanoparticles during these tests. These were obtained by performing two replicate tests, both with the same concentrations of API; one with the and one without polymer. These samples were analyzed using optical microscopy to identify any large drug particulates.

4 ANALYSIS

4.1 Particle Size Analysis

The particle size distribution of these samples was measured using the Malvern Zetasizer® which uses dynamic light scattering. The samples were measured at 25°C with water as the continuous phase and PLGA as the particle phase. The results given are the Z-Average, which is a volume weighted average.

4.2 Electron Microscopy

Two different electron microscopy techniques were used for this process. The emulsion evaporation samples were analyzed using a transmission electron microscope (TEM); model JEOL, JEM 1010 TEM, operated at 60 kV. A staining material was used to increase the contrast of the particles. The samples that were prepared using the precipitation technique were analyzed using a scanning electron microscope (SEM); Hitachi S-4800 FESEM.

4.3 Light Microscopy

To determine if the API was encapsulated within the polymer particles, the samples were analyzed using a light microscope. Although it is unable to achieve resolution at the nanoparticle scale, it is powerful enough to see preliminarily whether or not the API has been encapsulated.

5 RESULTS

5.1 Emulsion method

Table 1. Results from the processing of the polymer nanoparticles using the emulsion method.

#	C PLGA (mg/ml)	% DCM	Pres. (MPa)	# of Passes	Evap. (nm)	Co-Solv. (nm)
1	10	5	70	1	223	129
2	10	5	70	2	100	76
3	10	5	70	3	114	99
4	10	1	70	1	3254	124
5	10	10	70	1	168	116
6	10	5	105	1	127	84
7	10	5	140	1	140	149
8	40	5	70	1	193	146
9	80	5	70	1	168	119

The results from the emulsion tests are shown in Table 1. All of these tests were performed with 1% PVA dissolved in the water phase to stabilize the emulsion. The concentration of the PLGA in the DCM is given as “C PLGA”; the amount of oil phase that is mixed with the water phase as “% DCM”; the process pressure as “Pres” and the Z-average particle size for the two different solvent removal techniques as “Evap.” for the solvent evaporation technique and “Co-Solv.” for the co-solvent extraction technique.

Figure 3 is a TEM image of particles formed using the emulsion method; sample #9. The black specks that are present in the picture are identified as the contrast agent, phosphotungstic acid, which was used to enhance imaging.

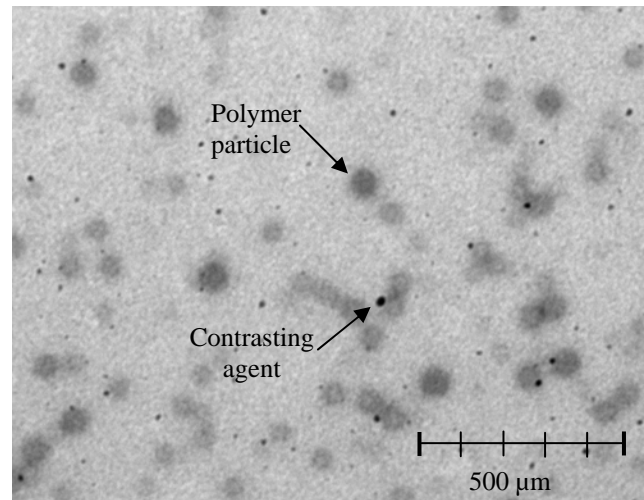


Figure 3. TEM image of the polymer nanoparticles generated after test #9 of the emulsion method.

5.2 Precipitation Method

Table 2. Results from the processing of the polymer nanoparticles using the precipitation method.

#	Polymer	% Acetone	C Poly. (mg/ml)	Shear ($s^{-1} \times 10^6$)	Z-ave. (nm)
1	PCL	10	20	1.2	445
2	PCL	10	20	1.2	517
3	PCL	10	20	6.0	281
4	PCL	10	40	6.0	341
5	PCL	10	10	6.0	258
6	PCL	10	20	6.8	280
7	PLGA	10	20	6.0	230
8	PLGA	9	10	6.8	184
9	PLGA	17	10	6.8	173
10	PLGA	25	10	6.8	177
11	PLGA	33	10	6.8	212

The results from the precipitation tests are presented in Table 2. All of these tests were performed with 1% Solutol dissolved in the water phase to stabilize the dispersion. The

type of polymer used is given as “Polymer”; the concentration of the polymer in the acetone as “C Poly”; the amount of acetone that is mixed with the water phase as “% acetone”; the shear rate, which is a function of pressure and orifice size, as “Shear”; and the Z-average particle size as “Z-ave.” Figure 4 is a SEM image of particles formed via the precipitation method; sample #3.

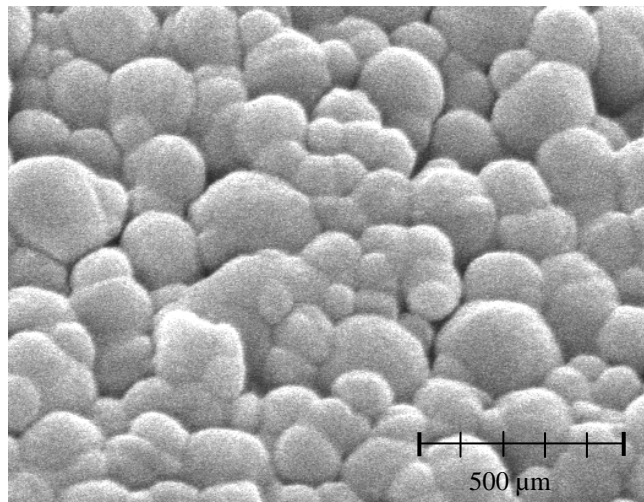


Figure 4. SEM image of the polymer nanoparticles generated after test #3 of the precipitation method.

5.3 Drug Encapsulation

Pictures taken with the optical microscope from the drug encapsulation tests can be found in Figure 5. These samples were prepared with the conditions from test #10 of the precipitation method. The absence of API particles in 5a indicate that the drug is encapsulated within the polymer as opposed to the free crystalline form seen in 5b.

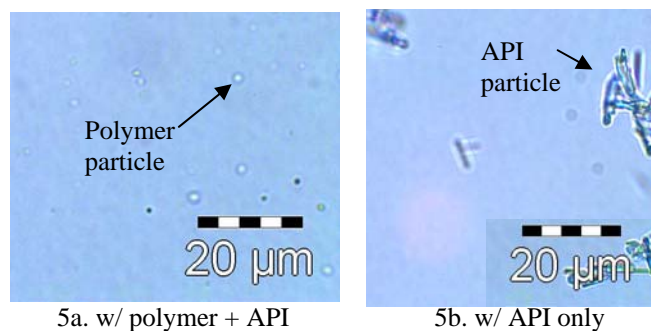


Figure 5. Optical microscope images from test # 10 of the precipitation tests with polymer and API (5a) and with API only (5b).

6 CONCLUSIONS

Polymer Nanosuspensions in the range 50-500 nm, with two different polymers, have been created successfully

using both the emulsion and the precipitation methods. By controlling the processing parameters, nanosuspensions with various polymer sizes and densities were created.

For the emulsion method, the dispersions that were prepared by co-solvent extraction were, in general, smaller than those prepared by the evaporation method. This may be due to the stability of the emulsion after processing or the agglomeration of the particles either during or after drying process. The co-solvent extraction step was performed immediately after processing. Some time (5-30 min.) elapsed before the evaporation technique was performed which may have enabled the emulsions to ripen.

By varying the process pressure (70-140 MPa) and number of passes (1-3, the size of the polymer particles varied in the range of 75-250 nm. Given a desired formulation, it is likely that the particle size of the dispersion can be controlled by selecting the appropriate processing conditions.

It appears that the API was encapsulated within the polymer nanoparticles during these tests. Future work will involve quantifying both the amount of API encapsulated and the release rate as a function of process parameters.

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