Production of Stable Drug Nanospensions Using Microfluidics Reaction Technology

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ABSTRACT

Many hydrophobic drugs are difficult to formulate in ways that ensure high bioavailability. The formation of stable drug nanosuspensions is an attractive formulation strategy that does not limit the dosage amount. Microfluidics Reaction Technology (MRT) was used to produce drug nanosuspensions via solvent and anti-solvent crystallization. The core of this technology is a continuous and scalable microreactor based on impinging jets. Inside the reactor, liquid reactants encounter highly turbulent conditions and interact at the nanometer level. Several Active Pharmaceutical Ingredients (APIs) were used to demonstrate the technology. The particle sizes of the suspensions depended on the particular API, surfactant(s), supersaturation, and energy dissipation levels expressed as process pressure. The median particle sizes of suspensions produced by MRT varied in the range of 50-767 nm. The suspensions were stable with a single exception. For certain APIs the process efficiency exceeded 80%.

Keywords: crystallization, nanosuspension, microfluidizer, microreactor, turbulence

1 INTRODUCTION

A large number of compounds with potentially high pharmacological value fail to pass initial screening tests because they are too hydrophobic to be effectively formulated. An attractive formulation strategy for such drugs is the formation of stable drug nanosuspensions, which increase the bioavalability of the drugs and do not limit the dosage amount [1-3]. Current methods for manufacturing nanosuspensions rely mainly on reducing the particle size of drug powders in dry or wet formulations [4-5]. Such "top-down" processes may be slow and energy demanding. In contrast, "bottom-up" processes allow the formation and stabilization of nanosuspensions without the need for size reduction.

A "bottom-up" process was developed that uses solvent and anti-solvent crystallization in conjunction with Microfluidics Reaction Technology (MRT) to produce drug nanosuspensions. Crystallization is used routinely to purify compounds or to promote the formation of certain crystalline structures [6]. Changes in temperature, pH or composition of the solvent are often used to induce crystallization. In this work, an API solution interacted with a miscible liquid which was a poor API solvent.

At the core of MRT is a continuous, impinging jet microreactor scalable to at least 50 lit/min [4]. In the reactor, high-velocity liquid reactants are forced to interact inside a microliter scale volume. The reactants mix at the nanometer level as they are exposed to high shear stresses and turbulence. MRT provides precise control of the feed rate and the mixing location of the reactants. This ensures control of the nucleation and growth processes, resulting in uniform crystal growth and stabilization rates.

Several APIs were used to demonstrate the technology. The effects of process pressure and supersaturation were examined previously for a single API [4]. That work was expanded to include a variety of APIs, solvents and surfactants, as reported here.

2 EXPERIMENTAL APPARATUS

2.1 Reaction Chamber

Figure 1 shows a schematic of the fluid path inside the reaction chamber [4]. Two opposing jets form as fluids flow through two microchannels within the chamber. The jets collide inside a microliter volume where the fluids mix in the nanometer scale. Typical dimensions of channel depth and width are in the range of 75 to 150 microns. Average fluid velocities inside the channels may exceed 400 m/s. A planar array of opposed pairs of such channels ensures effective scaling up of the technology.

Impinging jet reactors have been used in the past for a variety of reactions [7, 8]. The flow inside Microfluidics chamber is intensely turbulent, unlike the flow in existing reactors. The average channel velocities, jet Reynolds numbers and energy dissipation levels are orders of magnitude higher in Microfluidics chambers than in these other reactors. Typical values for jet Reynolds numbers of Microfluidics chambers are over 20,000, as compared to only 100-2000 in existing technologies [7,8].

2.2 Microfluidizer® Processor

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. In Microfluidizer® processors such pressures are generated using a hydraulically or pneumatically driven pressure multiplier called an intensifier [4].

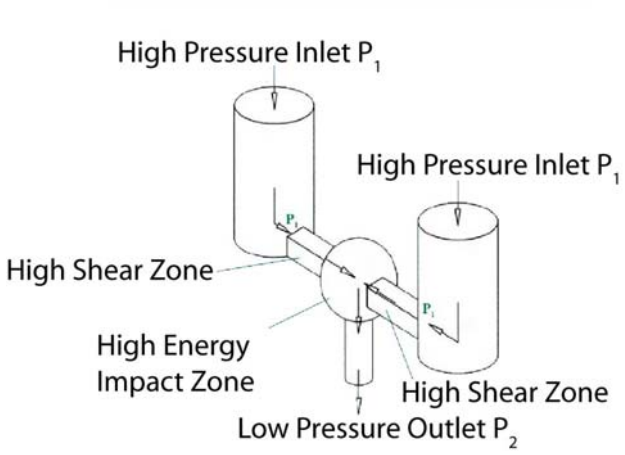

Figure 1. Schematic of the flow path of the reaction chamber. Copyright by Microfluidics, 2007.

Two reactant streams, a solvent and an anti-solvent, are combined at the appropriate proportions just upstream of the intensifier so that mixing of the streams is kept to a minimum prior to entering the reaction chamber, see Figure 2. The contact time of the two reactant streams prior to entering the reaction chamber is less than 200 ms. The flow path and the velocities of the reactant mix are such that intense mixing is avoided prior to entering the reaction chamber.

The total flowrate of the machine is determined by the operating pressure and the geometrically fixed dimensions of the reaction chamber. The flowrate of one of the reactant streams is controlled using a peristaltic pump. Therefore, the desired flowrate ratio of the reactant streams is obtained by controlling the total flowrate of the reactants and that of one of the reactants.

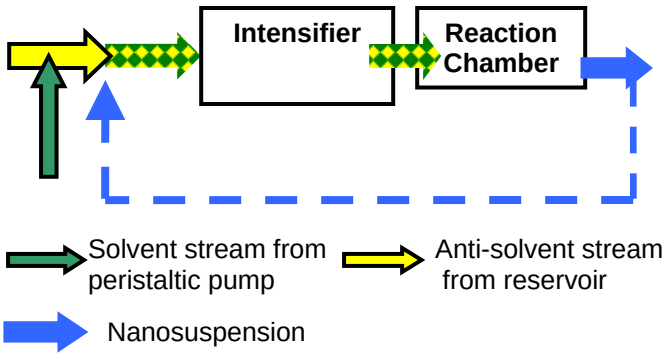

Figure 2. The reactant streams mix just upstream of the reaction chamber. Copyright by Microfluidics, 2007.

3 PROCEDURE

3.1 Determination of Solubilities

The approximate solubilities of the APIs in the solvent and various solvent/anti-solvent solutions were determined whenever possible. This information was used to calculate the supersaturation, which is the driving force of crystallization [6] and is defined as the ratio of the actual concentration to the saturation concentration of the solute. Solubilities are also used to calculate the theoretical efficiency of the process.

It was not possible to obtain solubility data for all the APIs and at all conditions of interest. Lack of adequate quantities of APIs was the main reason. In such cases simple tests were conducted by mixing solutions of APIs with the anti-solvent to ensure adequate precipitation, as described below.

3.2 Crystallization Experiments

Crystallization experiments using the MRT were conducted at 138 and 83 MPa (20,000 and 12,000 psi) process pressures. The reaction chamber that was used had a minimum channel dimension of 75 microns.

The solvents were either dimethyl sulfoxide (DMSO) or N-Methyl-2-Pyrrolidone (NMP) and the antisolvent was water, see Table 1. The water volume flowrate was typically 3-10 times the solvent volume flowrate, resulting in superaturation between 1.4 and 9.

Whenever the solubilities were known, the solvent to anti-solvent ratio was determined by the supersaturation. When the solubilities were not known, a simple test was conducted by adding water to an API solution to determine conditions under which a substantial amount of the API had crystallized and precipitated. This condition was then modified as seen fit, based on the results of the crystallization experiments.

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. Two non-ionic surfactants were used, Solutol® HS 15 (polyoxyethylene esters of 12-hydroxystearic acid) from Bayer and a hydrophobically modified inulin polymeric surfactant, INUTEK SP1 from Orafti.

The "bottom up" process to produce nanosuspensions was compared to a typical "top down" process. As a typical "top down" process the standard Microfluidizer® technology was selected. This technology is routinely used for particle size reduction of dispersions and emulsions in a variety of industries, including the pharmaceutical industry.

API	Function	Solvent (Sol.)	Antisolvent (AS)	Ratio (AS)/(Sol.)	API conc. in solvent (mg/ml)	Surfactant	MRT Process Pressure (MPa)
Azithromycin	Antibiotic	DMSO	Water	4	75	Solutol	138
Oxycarbazepine	Anticonvulsant	DMSO	Water	4	40	Solutol	138
API-1	NSAIS	NMP	Water	5	50	Solutol/None	83
API-2	Antibiotic	DMSO	Water	3-10	5-20	Solutol/None	138
Loratadine	Antihistamine	NMP/DMSO	Water	4 & 10	40 &100	Solutol/INUTEK	138

Table 1. Crystallization conditions for various APIs.

For the "top down" experiments, suspensions of micron size crystals were processed with a regular Microfluizider® high shear processor. These crystals had been grown under no shear conditions by pouring the solvent and anti-solvent streams in a beaker and then allowing the crystals to grow without stirring the fluids.

Five different model APIs were used for testing, as listed in Table 1. The APIs were selected so they belong to different chemical families and had different pharmacological activities. There were two antibiotics (*azithromycin* and *API-2*), an antihistamine (*loratadine*), an anticonvulsant (*oxycarbazepine*) and a Non-Steroidal Anti-Inflammatory (NSAIS, *API-1*). The molecular weights of the APIs were in the range of 228-749.

3.3 Material Characterization

The particle size was determined using particle size analyzers based on either Static or Dynamic Light Scattering (SLS or DLS). In addition, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and optical microscopy were used to obtain information with respect to size and shape of the particles.

Several steps were required to prepare the samples for SEM and TEM analysis. The dispersions were initially centrifuged to separate the solids from the solvent/anti-solvent mixture and the majority of the surfactant. The solids were then rinsed with water and subsequently were filtered, and either air dried or dried in a vacuum oven.

4 RESULTS

Table 2 describes the results from crystallization experiments for different APIs. Particle size and shape, the measuring/visualization methods and details of the crystallization process are included.

Crystallization of *azithromycin* using MRT resulted in a nanosuspension with median particle size of 50-100 nm. The particle size was measured using DLS and was confirmed by TEM, see Figure 3. The nanosuspension was stable both in the solvent and anti-solvent mixture, and in water after the solvent was removed.

Crystallization of *oxycarbazepine* using MRT resulted in stable nanosuspensions with median particle size of 767 nm, as determined by SLS. The particle size distribution was bimodal, with a peak at about 340 nm and another at about 1 micron. It is possible that the smaller particle population is a result of crystallization taking place inside the reaction chamber. The larger particle population may be a result of crystallization starting prior to the interaction chamber. Finally, about 5% of the particles by volume were in the range of 2.5-6 microns.

In contrast, crystallization of *oxycarbazepine* under control conditions (beaker) resulted in needles having lengths of about 20 microns and widths of about 5 microns. The particle size of the control *oxycarbazepine* sample was reduced using a standard Microfluidizer® processor ("top-down" process). The median particle size of the sample was reduced to 1.2 microns after 25 passes through the processor. The processed sample contained a population of about 5% of particles with sizes in the range of 4-6 microns. Therefore, smaller particles were produced using MRT in a single step than by reducing the particle size in multiple (25) steps.

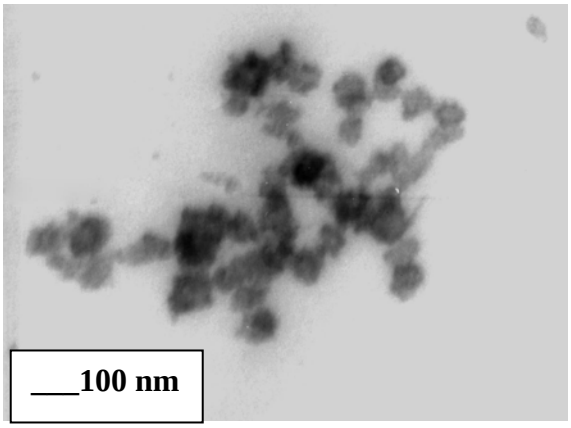

Figure 3. TEM picture of azythromycin particles produced with MRT.

Crystallization of *API-1* using MRT resulted in stable nanosuspensions with median particle size of 400 nm, as

API	Median Particle Size (nm)	Measurement Method	Shape	Stability	Surfactant (Solvent)	Processing method
Azithromycin	50 -100	TEM/DLS	Spheroidal	Stable	Solutol	MRT
Oxycarbazepine	767	SLS	Unknown	Stable	Solutol	MRT
"	5000×20,000	SLS	Needle	Stable	Solutol	Control
"	1200	SLS		Stable	Solutol size reduction	Control with after 25 passes
API-1	397	SLS	Unknown	Stable	Solutol/None	MRT
API-2	166-312	SEM/LS	Needle	Stable	Solutol/None	MRT
Loratadine	183	DLS	Unknown	Very unstable	Solutol (NMP)	MRT
"	379	DLS	Needle	Rather unstable	INUTEK (NMP)	MRT
"	90	DLS	Unknown	Very unstable	Solutol (DMSO)	MRT
"	332	DLS	Needle	Rather unstable	INUTEK (DMSO)	MRT

Table 2. Results from crystallization experiments.

determined by SLS. The presence of surfactant, Solutol, did not affect the primary particle size of the suspension, but it reduced the propensity of the particles to agglomerate.

Crystallization of *API-2* using MRT resulted in stable nanosuspensions with median particle sizes of 166-312 nm, as determined by SEM and SLS. Similarly to API-1, the presence of surfactant (Solutol) did not affect the primary particle size of the API-1 suspension. The median particle size of *API-2* and the process efficiency decreased as supersaturation decreased from about 8 to 1.4. The efficiency varied in the range of 32% and 83%.

Loratadine exhibited very different behavior than all other APIs. Submicron particles were formed immediately after using MRT or mixing the solvent and anti-solvent streams in a beaker. However, the suspension was unstable since the particles grew, sometimes during particle size measurements.

Different surfactants and solvents were investigated in an effort to increase the stability of *loratadine* suspension. When Solutol was used as a surfactant, the initial particle size was about 90 nm, but a reproducible particle size measurement was difficult or impossible to achieve. This was true with both solvents that were tried, DMSO and NMP. When INUTEK was used as a surfactant, the resulting particle size was larger, in the order of 332-379 nm. However, the stability of the particles was much higher.

Overnight, the *loratadine* particles that were produced with MRT formed uniform needles 10-20 microns long and 0.5-2 microns wide. The particles that were produced with the control method formed needles of hundreds of microns in length and tens of microns in width.

5 SUMMARY

Microfluidics developed a continuous and scalable reaction technology (MRT) based on an impinging jet microreactor. High pressures force liquid reactants to form turbulent, opposing jets, with velocities up to 400 m/s. Such jets collide in a microliter scale volume forcing the reactants to mix in the nanometer scale, minimizing diffusion limitations.

MRT was successfully used to produce nanosuspensions of model APIs using solvent and anti-solvent crystallization. The median particle size varied in the range of 50-767 nm. The suspensions were stable after production with a single exception. The stability of the less stable suspension was influence by the type of surfactant present. Finally, MRT was demonstrated to be more effective in producing nanosuspensions than standard, particle size reducing methods.

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