PRODUCTION OF DRUG NANOPARTICLES OF CONTROLLABLE SIZE USING SUPERCRITICAL FLUID ANTISOLVENT TECHNIQUE WITH ENHANCED MASS TRANSFER

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ABSTRACT
The use of supercritical fluids in the area of material processing and for particle formation has been known for several years now. The advantages of supercritical fluid processing include mild operating temperatures, production of solvent free particles and easy micro encapsulation of particles. One of the attractive methods of particle processing using supercritical fluid is the Supercritical Antisolvent (SAS) technique. Although this technique has numerous advantages, it still cannot produce fine particles in the sub-micron range (<300 nm) for soft materials. A significant improvement in the SAS process has been proposed in this work, to obtain particles of controllable size that are up to ten-fold smaller and have narrower size distributions. Like the conventional SAS technique, the new technique Supercritical Antisolvent Precipitation with Enhanced Mass Transfer (SAS-EM) also utilizes supercritical carbon dioxide as the antisolvent, but in this case the solution jet is deflected by a surface vibrating at an ultrasonic frequency, that atomizes the jet into much smaller droplets. Furthermore, the ultrasound field generated by the vibrating surface enhances mass transfer and prevents agglomeration through increased mixing. The particle size is easily controlled by varying the vibration intensity of the deflecting surface, which can be adjusted by changing the power supplied to the attached ultrasound transducer. This new technique is demonstrated by the formation of nanoparticles of different pharmaceuticals such as lysozyme, tetracycline and griseofulvin.

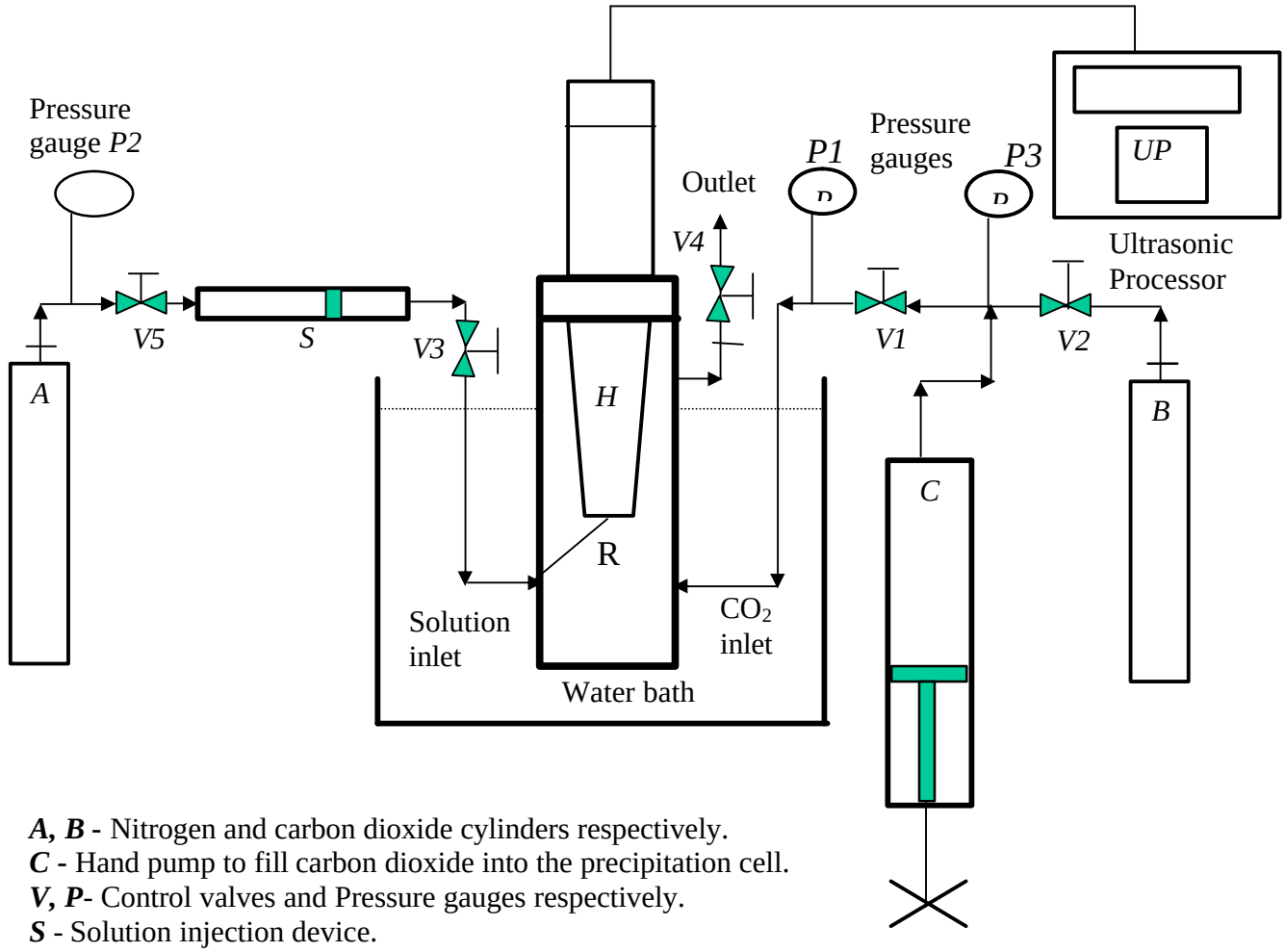
INTRODUCTION
Nanoparticles are important in developing delivery systems for controlled release of drugs. These systems can improve the therapeutic efficacy of drugs, *in-vitro* and *in-vivo* stability, bioavailability, targetability, and bio-distribution to reduce toxicity [1]. The delivery systems involving nanoparticles studied so far include, polymer nanoparticles with the drug dispersed within the polymer matrix, drug nanoparticles coated with a biodegradable polymer, polymer nanoparticles with the drug adsorbed on the surface, and nanoparticle suspensions for poorly soluble drugs [2].

There have been several methods in the past for the manufacture of drug nanoparticles. Some of the conventional techniques include spray drying and ultra fine milling [3, 4]. The major disadvantage of these techniques is that they produce particle having a broad size distribution (0.5 - 25 μm) and only a small fraction of the particles produced are in

the nanometer range [5]. Drug nanoparticles can also be prepared by precipitation process leading to the formation of hydrosols, but these methods have limitations due to difficulties in containing and controlling particle growth. In recent years however, supercritical fluid technologies such as Rapid Expansion of Supercritical Solutions (RESS) [6-8] and Supercritical Antisolvent (SAS) [9-11] precipitation have emerged as attractive methods for drug and biological particle formation. Some of the advantages of these techniques include mild operating temperatures and absence of residual solvent. The particles obtained by these techniques are 0.7 - 5.0 μm in size and have a narrow size distribution. Although these techniques are getting increasingly popular, in most cases they still do not produce particles predominantly in the nanometer range (< 300 nm) necessary for drug targeting and controlled release. In the SAS process the operating temperature, pressure and concentration of the injecting solution have so far been investigated as size control parameters, but none of these parameters have been found to produce a significant decrease in the particle size over a wide range. Again the extensive application of the RESS technique is limited by solubility limit of the solid being precipitated in supercritical fluid.

Keeping this in mind, we have proposed a technique that can be used to manufacture particles in the nanometer range having a very narrow size distribution. This new technique, supercritical antisolvent with enhanced mass transfer (SAS-EM) [12], is a modification of the conventional SAS process and overcomes the currently existing limitations of the SAS process. Like the SAS process, the new technique also uses supercritical CO_2 as an antisolvent. The modification in the new technique is that it utilizes a surface, vibrating at an ultrasonic frequency to atomize the solution jet into micro-droplets. Moreover, the ultrasound field generated by the horn surface provides a velocity component in the y-direction (direction normal to the vibrating surface) that greatly enhances turbulence and mixing within the supercritical phase resulting in high mass transfer between the solution and the antisolvent. The combined effect of fast rate of mixing between the antisolvent and the solution, and reduction of solution droplet size due to atomization, provides particles approximately ten-fold smaller than those obtained from the conventional SAS process. The use of high energy sonic waves for particle precipitation using supercritical fluids has also been suggested Subramaniam et al. (1997) [13]. In their case a specialized nozzle, of the type commercialized by Sonomist, Model 600-1 was used to generate and focus the high frequency sonic waves for atomization. Randolph et al. (1993) [14] also employed a specialized nozzle during the precipitation of poly (L-lactic acid) particles using the SAS technique. The nozzle in their case was a Sonotek atomizer having a capillary tube, which was vibrated at 120 kHz to produce a narrow cylindrical spray. The proposed technique also uses high frequency sound for atomization but the atomization process is brought about by introducing the solution on a vibrating surface in the form of a thin liquid film. No specialized nozzle has been used in this technique.

In this paper we demonstrate the application of SAS-EM technique for the formation of tetracycline, griseofulvin, and lysozyme micro and nanoparticles required for drug delivery and other pharmaceutical purposes.



A, B **-** Nitrogen and carbon dioxide cylinders respectively.
C **-** Hand pump to fill carbon dioxide into the precipitation cell.
V, P- Control valves and Pressure gauges respectively.
S - Solution injection device.
R - Precipitation cell maintained at high pressure.
UP - Ultrasonic processor.
H - Titanium Horn

Figure 1. Schematic diagram of the SAS-EM apparatus

METHOD

A schematic representation of the apparatus is shown in Figure 1. The main component of the apparatus consists of a high-pressure ultrasound precipitation cell *(R)* approximately 80 cm^3 in volume. A titanium horn (Sonics and Materials, Inc.) having a tip 1.25 cm in diameter is attached to the precipitation cell to provide the ultrasonic field and the vibrating surface necessary for atomization. The vibrations of the horn surface are generated by a 600 W (maximum power), 20 kHz ultrasonic processor (Ace Glass, Inc.). The ultrasonic processor is designed to deliver constant amplitude. The amplitude of vibration of the ultrasonic horn is directly proportional to the total input power and can be directly controlled by adjusting the power supplied to the ultrasound transducer. A collection plate is placed inside the precipitation chamber for collecting the particles. High pressure inside the chamber is generated using a HIP hand pump (*C*). Valves *V1*

and *V2* are used to fill up the HIP hand pump with fresh CO_2. Temperature inside the precipitation cell is maintained by placing it in a constant temperature water bath. The solution containing the solid to be precipitated is injected inside the precipitation cell using a "solution injection device" (*S*), which consists of a stainless steel cylinder containing a piston. The piston divides the cylinder into two chambers. The antibiotic solution is placed inside one of these chambers and is delivered into the cell by using pressurized nitrogen in the other chamber. The device *S* is connected to the vessel by means of a 75-µm i.d. fused quartz capillary tube. A pressure drop of 28 bar is maintained across the capillary tube and the device *S* in order to spray the solution inside the precipitation chamber. The capillary tube is placed at an angle of 40^o with respect to the horn surface in a manner such that the capillary opening touches the horn surface. Supercritical CO_2 is fed inside the precipitation chamber through the inlet port located at the bottom of the vessel. Valve *V1* is used to control the flow of Supercritical CO_2 into the high-pressure chamber. Pressure inside the chamber is measured using a pressure gauge *P1*. The outlet port is located on top of the precipitation chamber and valve *V4* is used to control the depressurization process. The pressure difference across the capillary and the solution injection device is measured using the pressure gauge *P2* and *P1*.

All the precipitation experiments were carried out in the batch mode and in an identical manner. First ultrasonic precipitation cell was filled with carbon dioxide up to desired operating pressure. The temperature inside the chamber was maintained using a water bath. Approximately 1.5-2.0 g of solvent containing the drug (5 mg/ml) was then loaded into the "solution injection device" (*S*). The ultrasonic horn inside the cell was then switched on at the desired vibration amplitude by adjusting the input power, and the solution was introduced inside the precipitation chamber through the 75 µm capillary tube, placed against the horn surface at an angle of 40^o. As soon as the solution jet was introduced into the precipitation chamber particles were precipitated in the nanometer range due to enhanced mass transfer between the solvent and the supercritical fluid. Motion between the particles inside the chamber was increased due to the ultrasonic field generated by the horn surface, which prevented agglomeration. The injection process was typically completed in 2 - 3 minutes and the power supply to the ultrasonic horn was turned off thereafter.

Next was the washing step in which residual solvent, left dissolved in supercritical CO_2 was removed by continuously purging the precipitation chamber with fresh CO_2. The complete cleaning process required approximately 7-8 times the vessel volume of fresh CO_2. The precipitation cell was then allowed to slowly depressurize till it reached ambient pressure. The chamber was then opened and the collection plate was removed and taken for particle analysis.

RESULTS

Results of the precipitation experiments conducted using the SASEM technique at 96.5 bar, 37 ^oC and at different values of ultrasound power supply, for various pharmaceutical compounds, have been shown below. The experiments clearly illustrate that with increasing power supply to the transducer there is a decrease in the size of the precipitated particles.

Lysozyme particles:

Power supplied (W)	Size Num. avg. (nm)	Size Vol. avg. (nm)	Standard deviation (nm)
0	1200	2000	970
12	730	1040	350
30	650	860	340
60	240	260	140
90	190	190	220
120	230	360	350

Tetracycline particles:

Power supplied (W)	Size Num. avg. (nm)	Size Vol. avg. (nm)	Standard deviation (nm)
0	800	1100	970
30	270	400	480
60	200	230	172
90	184	200	133
120	110	125	75

Griseofulvin particles:

Solvent in Use	Total power supply (W)	Morphology of GF Particles obtained.	Volume avg. (Spherical. GF particles)	Mean size of needle shaped GF crystals
DCM	0	Long needle shaped GF crystals.	-	Several mm long.
DCM	150	Larger yield of spherical shaped GF nanoparticles. Very few Shorter needle shaped GF nanoparticles.	0.5 µm	3.8 µm long 1.4µm wide
DCM	180	Predominantly spherical shaped GF particles. Very little yield of short needle shaped GF particles.	0.3 µm	2.0µm long 1.6 µm wide
THF	0	Long fibers of GF	-	-
THF	150	Predominantly spherical GF nanoparticles. Few short needles shaped GF nanoparticles.	0.3 µm	3.8 µm long 4.6µm wide
THF	180	Larger yield of spherical shaped GF nanoparticles. Very few Shorter needle shaped GF nanoparticles.	0.2 µm	2.1 µm long 1.7 µm wide

CONCLUSIONS

We have demonstrated the precipitation of tetracycline and lysozyme nanoparticles having sizes as low as 125 nm and190 nm using the SAS-EM technique. The particles obtained by this process are 5-8 times smaller than those obtained from the conventional SAS process. We have also been successful in reducing the size of griseofulvin particles from several millimeter long needles obtained in the conventional SAS process to 0.2-2.0 μm size particles. Particle sizes in the SAS-EM technique are easily controlled by adjusting the power supplied to the transducer. At higher ultrasound power, apart from a decrease in the particle size, the standard deviation in particle size in most cases is also much lower. Thus, particles having a narrower size distribution are obtained using the SASEM technique.

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