NOZZLE CONSTRUCTION FOR PARTICLE FORMATION USING SUPER CRITICAL ANTI SOLVENT PRECIPITATION

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ABSTRACT

In the development of drug delivery systems for pulmonal delivery and controlled release systems, small particles (<5 micrometer) with a narrow particle size distribution are desired. Recent techniques using a supercritical fluid as an anti-solvent are considered to be useful alternatives to produce fine powders. Co-precipitation can be realized in one step. In the supercritical fluid antisolvent techniques, carbon dioxide is used as an antisolvent for the solute but as a solvent with respect to the organic solvent. Here one specific version, SAS (Supercritical Anti Solvent), is under investigation. For this process several nozzle configurations are used in order to control particle morphology, particle size and particle size distribution. A nozzle configuration is presented that enables control over process parameters to optimise particle morphology, particle size and particle size distribution. A nozzle construction was built for particle production using the SAS process in a continuous procedure. Experiments were done using dextran and l-PLA to establish the influence of concentrations and dynamics of the process on the product parameters. Process parameters are identified that control particle size between 500 nanometer and 20 micrometer. Particle size distribution of the produced particles is narrow, with a standard deviation of less than 30% of the volumetric median diameter. Furthermore, co-precipitation of model compounds with these polymers of protein and steroid is carried out without influence on particle morphology.

INTRODUCTION

The micronisation based on the use of supercritical antisolvents (SAS) has been suggested during the last decade as an alternative to currently available micronisation techniques for the production of drug delivery particles [1]. The SAS technology has proved to be able to produce particles from low molecular active compounds as well as from polymeric matrix materials. For the production of drug delivery systems it is necessary to control morphology, particle size and particle size distribution to a high standard. The purpose of this study was twofold. First we wanted to improve the control over particle size by modifying the SAS process. We improved the particle formation process by varying the solvent and antisolvent flow rates as well as introducing a delay time after mixing the solvent and the antisolvent. Thus the phase separation could be performed at a relatively low super saturation. Secondly we wanted to find out if this process is able to incorporate model compounds into biodegradable microparticles. The following polymers were used: Dextran and l-poly lactic acid (l-PLA). As model compounds were used cholesterol and Holcus Lanatus extract (protein mixture). We characterised the microparticles by scanning electron microscopy (SEM) and particle size measurements. We determined the degree of cholesterol encapsulation as well as the presence of Holcus Lanatus extract.

EXPERIMENTAL

a. Materials

Dextran (Mw 60.000) and Cholesterol were purchased from Sigma Aldrich. L-PLA (Mw 200000) was kindly provided by Purac Biochem (Gorinchem, The Netherlands). The Holcus Lanatus protein extract was kindly provided by Fornix Biosciences (Lelystad, The Netherlands). Dichloromethane and DMSO were purchased at Sigma Aldrich. Carbon dioxide is provided by Hoek-Loos, The Netherlands. All chemicals were used without further purification.

b. Procedure

A schematic diagram of the SAS apparatus that was employed in the experiments is shown in Fig. 1. A detailed diagram of the nozzle configuration used is shown in Fig. 2. In all experiments a temperature of 40°C and a pressure of 120 bar are used. All SAS experiments start with the delivery of CO2 to the particle collection vessel and the nozzle configuration until the desired pressure is reached. A steady antisolvent flow is then set. Then simultaneously the solution and antisolvent flows through the nozzle configuration are started. Due to the plug flow character in the nozzle construction steady state conditions are obtained. The experiment ends when the delivery of solution to the nozzle is interrupted. However supercritical CO2 continues to flow through the nozzle and the particle collection vessel to wash the apparatus and the product from residual solvent. Washing is stopped after 10 Kg's of CO2 have passed the setup.

Figure 1: Diagram of the used SAS set up

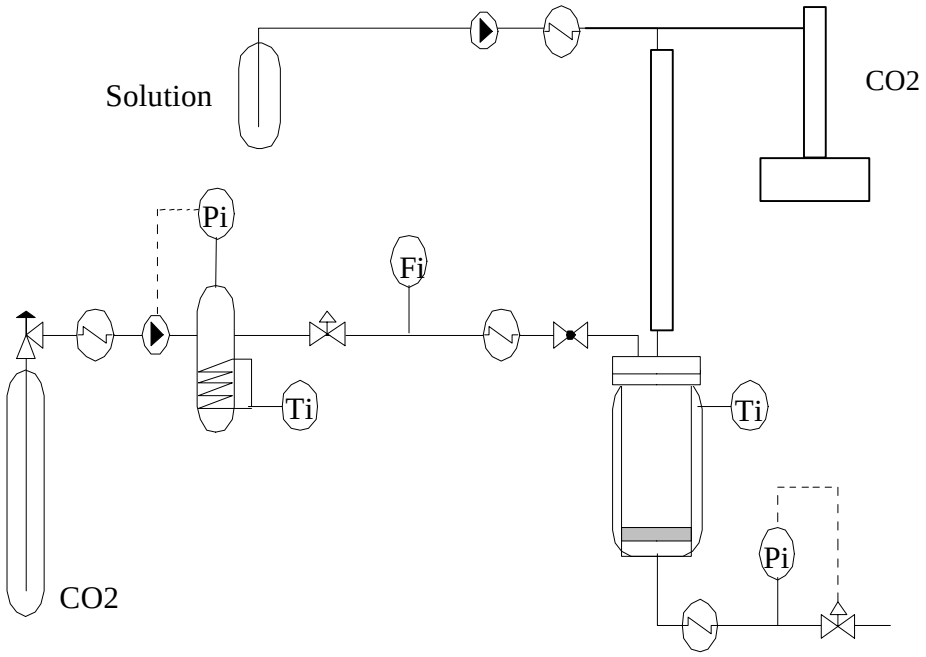
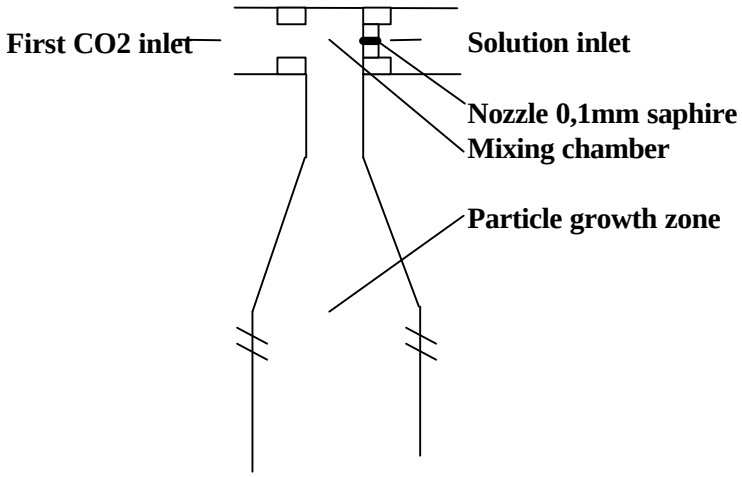


Figure 2: nozzle construction



RESULTS

In table 1 all performed experiments are summarised.

Table 1: Experimental conditions and results

No	X1*	Solution flow rate	CO_2 1 flow rate	Mixing energy (mW)	Mixing time (s)	CO_2 2 flow rate (g/min)	Growth time (s)	Particle size (μm)	S.D. (μm)	Yield
1	0.29	7.2	2.9	16.2	1	220	75	4.5	1.1	75 %
2	0.41	6.0	3.9	9.7	1	220	75	3.5	0.8	79 %
3	0.79	6.0	21.5	9.7	0.4	220	27	0.50	0.09	60 %
4	0.76	10.0	29	34	0.3	190	30	0,72	0.2	75%
5	0.74	2.0	6.0	0.35	1.2	110	292	2.4	0.5	59%

*X: molefraction of solvent / antisolvent in the mixing chamber

Particle formation of dextrane from DMSO yields spherical, unagglomerated particles (figure 3). Also there is a clear relationship between molefraction of solvent and antisolvent at the first mixing step and the resulting particle size. In figure 4 this relationship is graphically depicted.

Figure 3: Dextrane particles

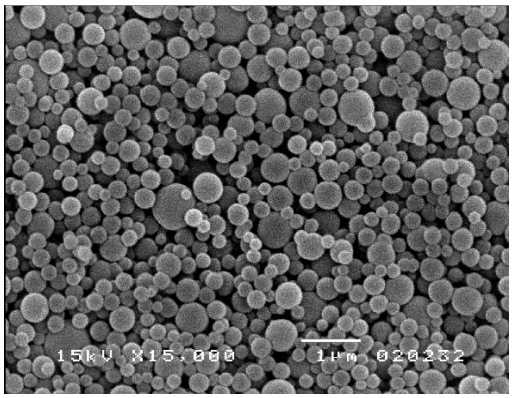
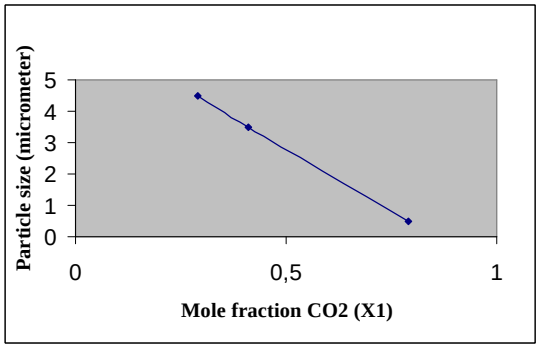


Figure 4: molefraction / particle size



Encapsulation of cholesterol in l-PLA also yields spherical unagglomerated particles (Figure 5). Spectrophotometric analysis shows that l-PLA particles are loaded with 1,2 % cholesterol. Encapsulation of protein in dextrane yields spherical unagglomerated particles containing the protein (Figure 6). The protein is analysed as unaltered by SDS Page.

Figure 5: Cholesterol in l-PLA

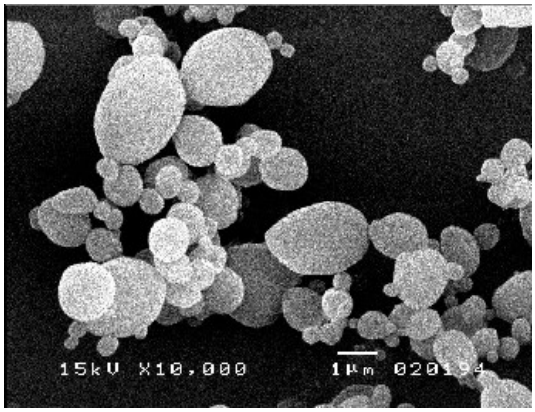
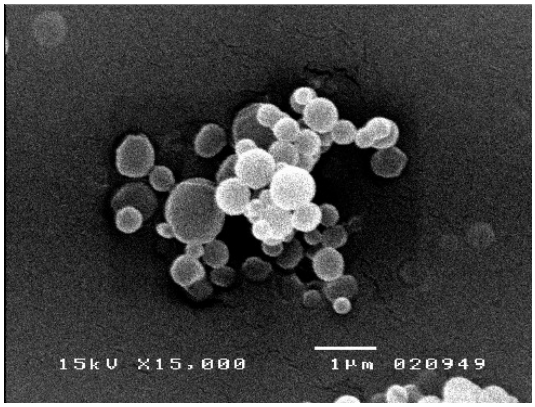


Figure 6: Holcus l. protein in dextran



DISCUSSION

These experiments show that the presented process has a number of possibilities in the production of particles for drug delivery [2]. The particle size can be adjusted, and encapsulation of model compounds is successful. With the formation of dextran particles there is a linear relationship between the mole fraction antisolvent/solvent and the resulting particle size. As a theory that explains this relationship the authors suggest the

following: the phase split that occurs when solvent and antisolvent are first mixed yields both a supercritical gas phase as a viscous droplet still rich in solvent. The mole fraction at first mixing determines the solvent content of these droplets and thus the size of this droplet. Due to the fact that the antisolvent /solvent ratio is relatively low when compared to other authors, the super saturation is low and the phase split only slowly takes place. Therefore a relatively long time is required before phase separation is complete. After this stage the second antisolvent flow is added to extract solvent from the droplets in order to obtain dry and non agglomerating particles. Encapsulation of protein in these experiments was successful at low protein concentrations; higher concentrations lead to agglomeration of particles. For several proteins with a high activity this limitation is not a problem. Future work is addressing the encapsulation of proteins at higher concentrations. Encapsulation of cholesterol in l-PLA yielded particles with a cholesterol content of 1% where an initial loading of 10% was set. Probably the encapsulated cholesterol is extracted from the particles during the washing of the particles at the end of the process. In further experiments this can be avoided by washing the particles with co2 saturated with cholesterol.

CONCLUSION

The presented process is shown to be able to produce particles of polymers with an adjustable size and a narrow particle size distribution. The encapsulation of model compounds was successful.

ACKNOWLEDGEMENTS

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