WATER AS MODIFIER OF THE DMSO-CO_2 PHASE BEHAVIOUR: NEW APPROACH FOR POLYMER MICRONIZATION USING THE PCA PROCESS

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To avoid the existing limitations of the PCA process for the precipitation of proteins and polar polymers a new approach based on the use of water to modify the phase behaviour of the system DMSO-CO_2 has been carried out. The addition of water to the DMSO shifts the mixture critical pressure of the solution-CO_2 system to higher pressures and opens a new operating window in which the PCA process can be carried out. Following this approach it was possible to develop a process to produce 1-10 µm particles of N-trimethyl chitosan chloride (TMC) suitable for inhalation.

The influence of operating variables such as pressure, nozzle size or solution flow rate on particle morphology and size was studied. At the optimal operating conditions FITC-ovalbumine was encapsulated.

INTRODUCTION

Chitosan and its N-trimethyl derivatives are non toxic, biocompatible polymers with important applications in pulmonary drug delivery. N-trimethylchitosan chloride (TMC) overcomes chitosan limited solubility at the neutral physiological pH values (7.4) and has been shown to be an effective and safe enhancer of the absorption of peptides and proteins through mucosal epithelia. Moreover, cationic polymers as TMC have the potential for DNA complexation and can be used as non-viral vectors for gene delivery.

The use of TMC microparticles to administer proteins or gene is receiving a lot of attention in the last years [1]. Established preparation techniques for TMC microparticles as spray-drying and coacervation produce irregular microparticles of wide size distribution. These microparticles are stabilised by cross-linking with e.g. sodium sulphate. Although these stabilised microparticles may serve for drug carrier purposes they lack absorption enhancement properties. The purpose of this research project is to study alternative micronisation techniques that avoid the use of stabilisers for the polymer.

The main objective of this work is to study the feasibility of the Precipitation with a Compressed Antisolvent process (PCA) to produce TMC microparticles suitable for pulmonary administration.

The use of the PCA process to precipitate proteins and polar polymers from DMSO using CO_2 as antisolvent has some difficulties. When operating below the mixture critical pressure of the mixture DMSO-CO_2, solution droplets formed by atomisation in the nozzle dry slowly due to the low solubility of DMSO in the surrounding CO_2 gas phase. A wet product or a film over the filter plate are often obtained operating at these conditions [2]. On the other hand, proteins and polar polymers have an extremely low solubility in CO_2. When working above the critical pressure of the mixture high supersaturations are induced as soon as the proteins or polymers get in contact with the CO_2 and agglomerated nanoparticles are formed

To avoid the existing limitations of the PCA process for the precipitation of proteins and polar polymers, water was used to modify the phase behaviour of the system DMSO-CO_2. Following this approach it was possible to develop a process to produce TMC particles suitable for inhalation.

MATERIALS AND METHODS

Materials

N-trimethyl chitosan with a 48% substitution degree was synthesised by methylation of chitosan (ChitoClear, Primex Ingredients ASA, Ireland) following the procedure described by Sieval et al. [3]. Substitution degree of the synthesised TMC was determined by ^{1}H- NMR. In this article, TMC 48 refers to trimethyl chitosan with a 48% trimethylation in the molecule.

Carbon dioxide (99.97%) was supplied by Hoek Loos. DMSO (99.7%) was purchased from Acros Organics. Water had an ultrapure grade. FITC-ovalbumine was supplied by Sigma Aldrich. The water content of each solution was analysed by Karl –Fisher titration.

Particle characterisation methods

Processed particles were analysed by a JEOL JSM-5400 Electron Scanning Microscope.
Confocal laser scanning microscopy visualisation was used to study the dispersion of FITC ovalbumine in the TMC.

Apparatus

The polymer solution is sprayed directly into a 4 liter vessel using sapphire nozzles of variable diameter (80-120 μm). A detailed description of the setup and the procedure has been given elsewhere [2]. Carbon dioxide flow rate was 20kg/h in all the experiments. At

least 8 kg of CO_2 were used to rinse the reactor and avoid the recondensation of water and DMSO on the particles during depressurisation.

RESULTS AND DISCUSSION

Phase behaviour DMSO-CO_2 and water-DMSO-CO2.

From the data reported by Vega et al [4] the critical pressure for the mixture DMSO-CO_2 was estimated to be around 85 bar at 41ºC. It was expected that the presence of water in the system would displace the bubble point of the mixture DMSO-CO_2 to higher pressures. The phase behaviour of the system water-DMSO-CO_2 was measured at 40 ^{o}C to support this hypothesis. The influence of the presence of small amounts of TMC in the solution was also studied.

The phase behaviour measurements were performed in a solubility cell described in [5].A mixture of a known composition was placed in the view cell. Keeping the temperature constant, the volume of the cell was decreased (so, pressure in the cell increased) until the first vapour bubble appears (bubble point) or until the first droplets of solution were seen (dew point). The experimental error in the determination of the pressure was around 2.5 bar. Results of these measurements are shown in figure 1.

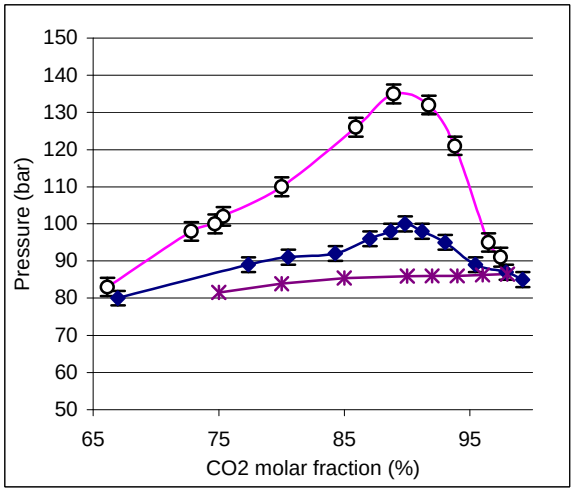

Figure 1. *LV equilibrium measurements*

- *x Vega et al.[4], no water in the DMSO, 41^{o}C;*
- *2%wt water in the DMSO solution, 40^{o}C ;*
- *3.5%wt water in the DMSO solution, 40^{o}C;*

These results show that when the water concentration content of the solution is a few percent increased, the LV equilibrium line of the mixture DMSO-CO_2 considerably shifts to higher pressures. The presence of TMC is expected not to influence the LV behaviour of the water-DMSO-CO_2 system.

PCA experiments

Experiments are classified as below the mixture critical pressure and above the mixture critical pressure in order to distinguish between the way of contact between the solution

and the CO_2 [6]. Below the mixture critical pressure there is an interface between the solution and the CO_2 and solution droplets are formed. Above the critical pressure of the solvent-CO_2 mixture, mixing of the fluids is expected to be faster than droplet formation.

Experiments above Pc, mixture

All these experiments where performed at 40^{o}C and 120 bar. Concentration of TMC in the DMSO solution was in the range 0.1-0.5%wt.

Changes in flow rate in the range 5-20ml/min and nozzle size (80, 100 or 120 microns) did not have any influence on the morphology and size of the TMC particles obtained by spraying a DMSO solution containing 2%wt water. Due to the high supersaturation levels reached in the polymer solution as it gets in contact with the CO_2, TMC nanoparticles are formed under these conditions.

As the water content of the solution increases, the morphology of the precipitated polymer changes from nanoparticles to microspheres (see Figure 2). These results can be explained using the phase diagram shown by figure 1. When spraying a 1.5 %wt water solution at 120 bar and 40^{o}C, the operating region is always in the area of completely miscibility between the solution and the CO_2. Polymer nanoparticles are produced as a result of a fast precipitation in a mixture of solution and CO_2. As the water content of the solution increases, the mixture critical pressure of the mixture moves to above the operating conditions and precipitation of polymer takes place at the interface between the solution droplets and the CO_2. That is the reason why at higher water content (3.75%wt water) microparticles are obtained. At water contents between 2-3.5 %wt a mixture of nanoparticles and microspheres is observed. Water concentrations in the DMSO solution above 4 %wt produced a wet product.

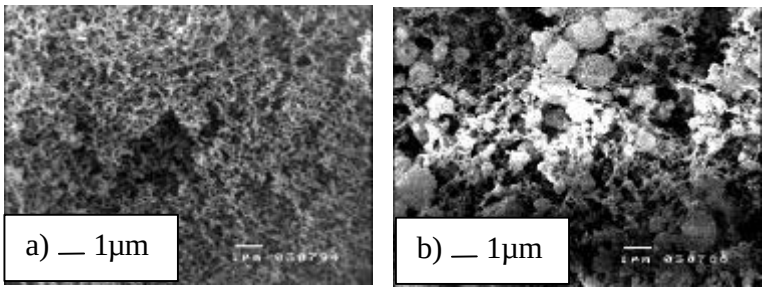

Figure 2. *TMC 48 particles produced by spraying at 120 bar and 40^{o}C.*
a) 2 %wt water,
b) 2.7 %wt water

Experiments below $P_{c,mixture}$

Working at pressures below 80 bar a wet product was always obtained over the filter plate. As pressure increases to 90 bar, the extraction of the liquid solvent to the supercritical phase is enhanced due to the higher solubility of the DMSO-water mixture in the vapour phase. Also as pressure increases atomisation of solution produces smaller droplets and mass transfer is favoured. All experiments described in this section were performed at 90 bar and 40 ^{o}C.

Keeping the water concentration in the DMSO solution as 2.7%wt, changes of solution flow rate (10-20ml/min) and nozzle diameter (80-100 micron) didn't seem to influence particle size of the precipitated TMC.

Water content of the solution has to be kept above 1.5%wt to be at conditions below the mixture critical pressure of the mixture when working at 90 bar. The stability of the droplets' surface increases when water content of the DMSO solution increases from 2%wt to 2.7%wt. If water content increases above 4%wt in the DMSO solution, a wet product is obtained over the filter plate.

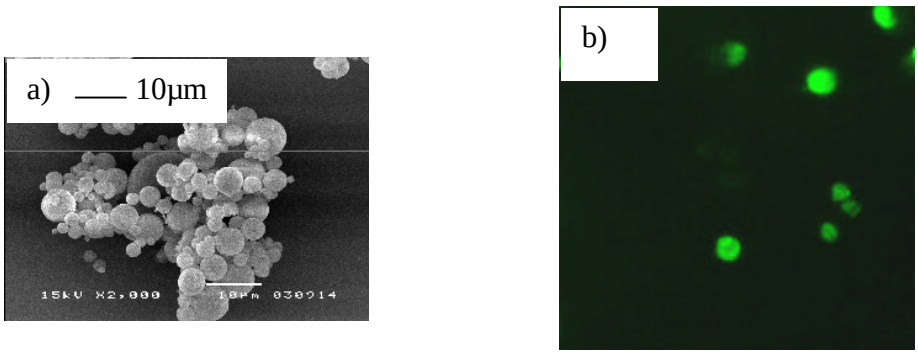

Figure 3. *Encapsulation of FITC-ovalbumine in TMC 48 at the optimised conditions (90 bar, 40^{o}C, 2.7%wt water in DMSO, 10ml/min solution flow rate, 100 µm nozzle)*

a) SEM picture of particles
b) *Confocal laser scanning microscopy visualisation*

Encapsulation of ovalbumine was performed at the more favourable conditions to carry out the process. Figure 3.a shows the SEM picture of the produced particles. Their size is comparable to the ones obtained in absence of protein. Analysis of the fluorescence of the samples showed that the TMC was homogeneously dispersed in the sample (Figure 3.b). In vitro experiments in bronchial human epithelium cells have shown that the absorption enhancing properties of TMC are preserved after supercritical fluids processing.

CONCLUSIONS

A new approach based on the use of water to modify the phase behaviour of the system DMSO-CO_2 has been developed to produce TMC microparticles. The addition of water to the DMSO shifts the mixture critical pressure of the solution-CO_2 system to higher pressures and provides a new window of operation to carry out the PCA process. It is shown that water can be used to modify particle morphology by changing the mechanism of particle formation.

Encapsulation of FITC-ovalbumine was successfully achieved under the optimised conditions for TMC precipitation. The concentration of polymer matrix is normally 10 to 1000 times the amount of pharmacologically active compound, so the morphology of the final particles will be governed by the polymer matrix and one would expect that this optimised process could be used to effectively encapsulate other proteins or plasmid DNA.

The use of water to modify the phase behaviour of the system DMSO-CO_2 overcomes the limitations of the PCA process to deal with macromolecules as proteins or highly polar polymers. The use of cosolvents to modify the phase behaviour of the system CO_2-solvent offers new windows of operation to perform the PCA process.

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