MICROPARTICLES PREPARED IN SUPERCRITICAL FLUIDS AS INHALABLE DRUG DELIVERY SYSTEMS

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ABSTRACT

Pharmaceutical processing and products are a major new area in the field of supercritical fluid technology. The inter-disciplinary co-operation of LACDR and FeyeCon initiates a general platform in the field of pharmaceutical particle formation technology using supercritical fluids.

The aim of the present project is the development of a process for the production of chitosan particles (<5 µm) with encapsulated active compounds, which are suitable for pulmonary administration. The chitosan acts as a carrier for the active compounds, as well as a compound that mediates the enhancement of absorption at epithelial surfaces. The particles are produced by expanding a chitosan/organic solvent mixture in a supercritical carbon dioxide environment. The carbon dioxide acts as an anti-solvent. This is the so-called Solvent/Anti-Solvent (SAS) technology. Chitosan and active compounds are produced at LACDR. The pharmacokinetic properties of the produced particles are studied at LACDR.

We envision the final product to be a dry chitosan microparticle formulation at a narrow particle size range. The inclusion or loading of macromolecular compounds into the microparticles during a one-step synthesis, avoiding further processing such as freeze-drying.

INTRODUCTION

Pharmaceutical processing and products are a major new research area in the field of supercritical technology. The efforts in this area have both been academic and industrial. FeyeCon is one of the independent players in the field and has developed an intellectual property position regarding supercritical technology in general and pharmaceutical particle formation in particular. The Leiden/Amsterdam Center for Drug Research (LACDR) is worldwide acknowledged as a major organization in the area of drug development, delivery and targeting in general. The Division of Pharmaceutical Technology at LACDR has gathered expertise on the use of chitosan and its derivatives for drug delivery in particular. The co-operation between FeyeCon and LACDR will initiate a general platform in the field of pharmaceutical particle formation using supercritical technology.

Recent developments in genomics, proteomics and biotechnology have made possible the large-scale production of pharmacologically active peptides and proteins, oligonucleotides and plasmid DNA (pDNA) and its application to therapy. As these compounds are degraded by enzymatic digestion when administered orally, they normally have to be applied by injection. However, this route of application bears the risk of infection and inflammation at the site of injection. It also requires the involvement of trained personnel, which increases costs in the health sector. Finally, most patients find it more convenient to inhale an aerosol or swallow a tablet than being stung by a needle.

Pharmaceutical research in academia and industry has, therefore, been focused on the identification of alternative ways to apply macromolecular compounds and the development of suitable drug carrier systems, which ensure sufficient and reproducible delivery to the site of absorption. The lung is an ideal site of absorption for macromolecular drugs. It has a large area of absorption in the deep lung, and the absence of digesting enzymes, which are present in the stomach and gut. In addition, liver uptake and metabolism is avoided, so that the dose can be decreased. This, in turn, leads to lower side effects. Another advantage of application via the lung is the fast onset of the pharmacological effect, which makes those drug delivery systems useful for application in a state of emergency (e.g., insulin).

Our aim is to produce chitosan particles with encapsulated active compounds which are suitable for pulmonary administration. Therefore, it is required to prepare un-stabilized chitosan microparticles which dissolve upon contact with the mucosal surface. The presence of the polymer close to the epithelial surface mediates the enhancement of absorption and protects the enclosed drug against enzymatic digestion. As the chitosan polymers do not form sticky gels, they are removed from the epithelial surface. The particles are produced by expanding a chitosan/organic solvent mixture in a supercritical carbon dioxide environment. The carbon dioxide acts as an anti-solvent. This is the so-called Solvent/Anti-Solvent (SAS) technology.

As a platform technology, we envision the final product to be a dry TMC microparticle formulation at a narrow particle size range. The inclusion or loading of macromolecular compounds into the microparticles during a one-step synthesis, avoiding further processing such as freeze-drying, is another goal of this project. It is the aim of this project to produce such un-stabilized chitosan particles by means of supercritical fluid technology.

Established preparation techniques for chitosan (or TMC) microparticles include spray-drying and polymer precipitation, resulting in irregular shaped particles of wide size distribution, which makes them inadequate for lung delivery. In addition, these microparticles are stabilized by cross-linking with e.g., sodium sulfate. Although these stabilized particles may serve drug carrier purposes, they lack absorption enhancement properties. Unstabilized chitosan microparticles, which are prepared by supercritical fluid technology, on the other hand, do combine both features.

During the last two decades CO_2 industrial solvent applications have been mostly developed for the extraction and fractionation of natural products, especially foods and pharmaceuticals [1]. Particle design is a spin-off of this technology receiving growing interest in the pharmaceutical industry for two reasons: increased solubility of poorly soluble molecules by micronisation and design of drug delivery systems. Preparation of microparticles by supercritical technology has been described for other biodegradable polymers, e.g., L-polylactic acid (L-PLA), ethyl cellulose, PLGA (polylactic-coglycolide acid), polycaprolactone, and soy bean lecithin.

The concept of precipitation from supercritical solutions is well established [2]. In general, an organic solution of a polymer is mixed with the supercritical fluid phase. The supercritical CO_2 is used as an antisolvent for the polymers and active compounds, but as a solvent for the organic wherein these compounds were dissolved. This procedure is called supercritical antisolvent technique (SAS). The simultaneous dissolution of the organic solvent in the supercritical fluid induces a supersaturation of the solute leading to a precipitate of small particles. The produced particles typically are of a spherical shape, with a controlled particle size and size distribution.

The residual organic solvent content is low, caused by flushing with supercritical CO_2 at high density and low viscosity, thus improving diffusion rates.

Co-precipitation with an active pharmacological compound has proven to be possible with steroids and antibiotics in matrix materials such as L-PLA and PLGA. In this technique the solvent contains both the matrix material and the drug substance; the CO_2 has to be an antisolvent for both solutes. The concentration of the matrix material is normally 10 to 100 times the amount of drug substance, so the particle morphology is governed by the behaviour of the matrix material.

EXPERIMENTS, RESULTS AND DISCUSSION

The experimental program carried out up to now comprises TMC-production and characterization, primarily carried out by LACDR and particle formation experiments, carried out in Delft by both FeyeCon and LACDR.

Chitosan, a polysaccharide derived from the deacetylation of chitin, is a glucosaminoglycan. It has been shown that chitosan enhances the absorption of peptide and protein drugs across nasal and intestinal epithelia in acidic environments. Chitosan has an apparent pK_a value of 5.60 and is only soluble in acidic solutions pH values lower than 6.0. This interferes with the biomedical application of chitosan, especially at the physiological pH value (7.40), where chitosan is insoluble and ineffective as an adsorption enhancer. *N*-trimethyl chitosan chloride (TMC) is partially quaternised derivate of chitosan with improved solubility and easy preparation. This derivative of chitosan has excellent adsorption enhancing effects across mucosal epithelia, even in neutral environments and it has also been shown that the degree of quaternisation of TMC has an important effect on its adsorption enhancing properties. TMC polymers were synthesised by reductive two-step methylation of chitosan that was accomplished by a chemical reaction between chitosan and iodomethane in the presence of sodium hydroxide. The product was precipitated from the reaction solution, washed, and dried. After ion-exchange and purification, the product was freeze-dried. By changing reaction conditions, various types of TMC were obtained (Table 1).

Table 1: Number of reaction steps for the synthesis of TMCs

TMC type (substitution degree)*	reaction step(s)	conditions
TMC 10 (10 %)	step 1	half amount of reagents
TMC 20 (16-17 %)	step 1	
TMC 50 (48 %)	step 1 & step 2	
TMC 60 (59 %)	step 1 & step 2	high MW** chitosan
TMC 80 (79 %)	step 1 & step 2	increased reaction time

*as determined by NMR analysis (see below); **MW: molecular weight

NMR analysis was performed to determine the degree of trimethylation of the polymers. The degree of quaternization was calculated with the data obtained from the ^{1}H-NMR spectra according to the following equation:

$$\%DQ = \left[\frac{[(CH_3)_3]}{[H]} \times \frac{1}{9} \right] \times 100$$

in which %DQ is the degree of quaternization as a percentage, $[(CH_3)_3]$ is the integral of the trimethyl amino group at 3.1ppm an [H] is the integral of the ^{1}H peaks between 4.7 and 5.7 ppm of the NMR spectrum.

Depending on the synthesis method, TMC's of substitution degrees in range of 10 to 79% were obtained. These polymers will further be employed in SAS preparation of particles.

Particle formation activities of FeyeCon aim at the establishment of process parameters for particle formation of TMC alone and together with macromolecules. Different strategies are followed to obtain dry and un-agglomerated particles of the different TMC varieties. A schematic representation of the experimental setup is shown in Figure 1.

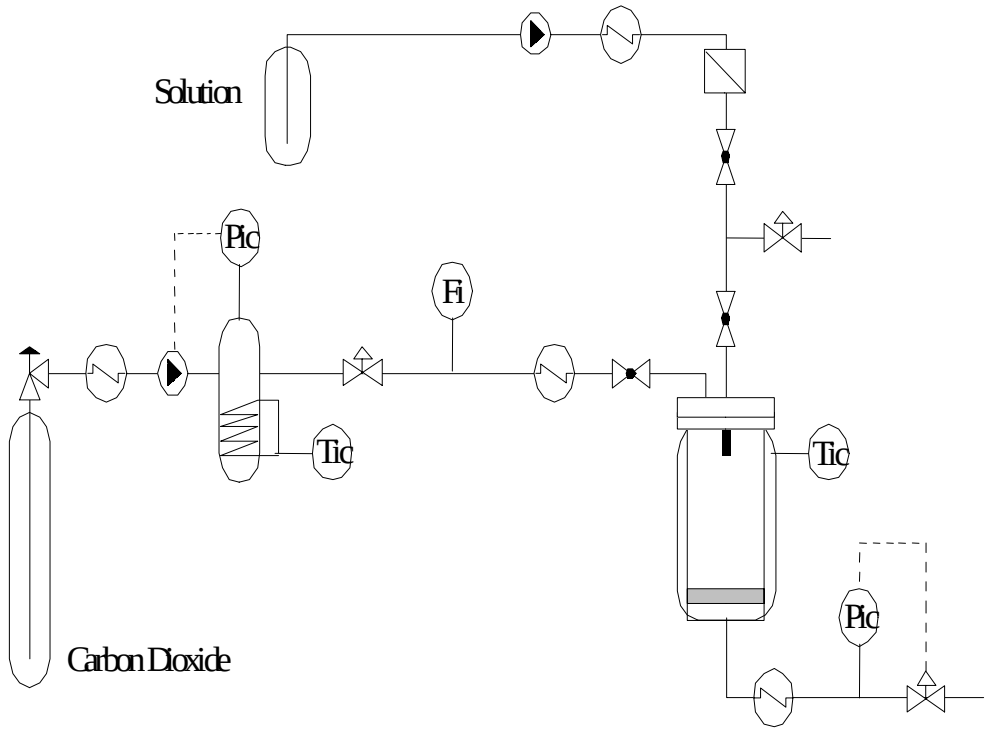

Figure 1: Schematical representation of experimental set-up for particle formation

In the first experiments we observed that the TMC type had a significant influence on particle formation and morphology. The degree of substitution, as well as the molecular weight, were identified as important factors. Significant efforts have been expended in order to measure both substitution degree and molecular weight by NMR as well as solubility in DMSO/CO_2 mixtures.

The influence of the TMC concentration in the solution on particle formation has been studied. Two areas were determined, in which particles with distinctively different morphologies were produced. The TMC concentration was found to have little influence on particle formation characteristics.

The influence of mixing energy on particle morphology has been established at various process conditions. Generally, a higher mixing energy lead to smaller particles of spherical and hollow morphology. Above a certain value, however, the morphology switches to agglomerated solid nanoparticles.

The influence of supersaturation on particle morphology has been established. Here it was expected that a lower supersaturation (i.e. a low concentration of antisolvent CO_2) would yield larger spherical particles. However, this effect was not observed using TMC. As a strategy to deal with this we decided to co-precipitate TMC with dextran, of which the particle formation is known in great detail. Examples of particles produced are depicted in the following photographs.

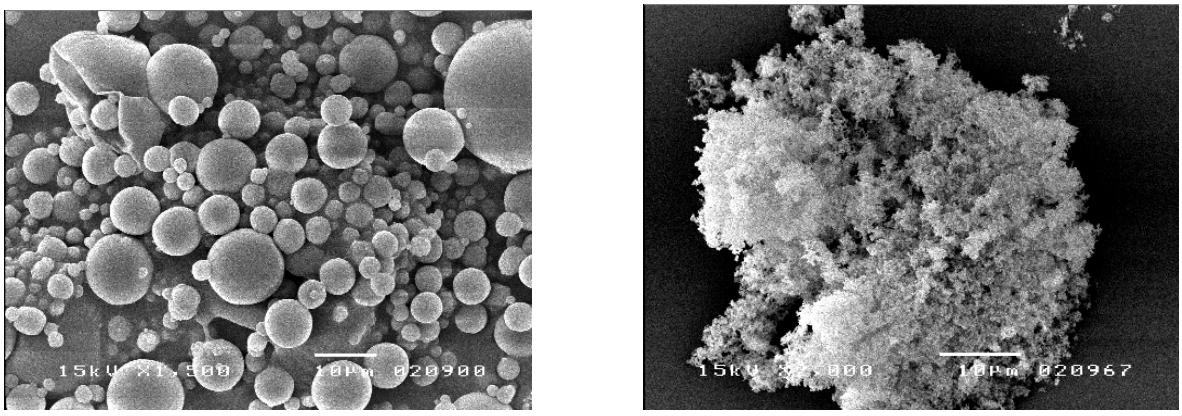

Figure. 2: Co-precipitated TMC/dextran particles

Higher mixing energies allowed the supercritical CO_2 to diffuse into the drug/polymer solution uniformly and rapidly. We observed a decrease in TMC particle size with increasing mixing energy of the polymer/drug solution and supercritical CO_2. Higher trimethylation degrees were shown to increase the absorption enhancement effect of TMC's. However, a higher trimethylation degree tended to increase solvent solubility of TMC's during SAS preparation; the precipitation of particles was therefore retarded compared to a lower degree of trimethylation. With increasing trimethylation degree of TMC, the size of particle prepared by SAS therefore increased significantly. At a methylation degree of 60%, large hollow particles were observed. We found that a trimethylation degree of 48% was most suitable for ensuing drug loading studies.

As shown in Figure 3, spherical TMC-48 particles loaded with the peptide drug buserelin acetate (LHRH agonist used in prostate cancer therapy) prepared by SAS method were in the size range of 0.2 to 5 µm. HPLC analysis of dissolved particles showed a loading efficiency of 100% (drug recovery 102.6% on average), no drug degradation products were detected. Presence of DMSO in the final product could not be detected.

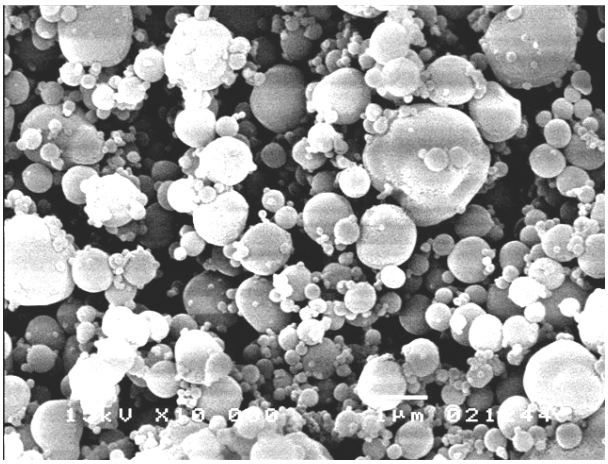

Figure 3: SEM photo of TMC particles loaded with buserelin acetate

CONCLUSIONS

In this study, we showed that the preparation of peptide drug loaded TMC particles by the SAS method is feasible. Since the preparation is carried out under mild conditions, the conformation of the peptide drug was not affected. The resulting product was a dry powder containing particles in the lower micrometer range. TMC particles prepared by the described SAS method represents an innovative delivery platform for a wide range of pharmacologically active agents [3].

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