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Supercritical fluid micronization techniques for gastroresistant insulin formulations

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ABSTRACT

The application of three supercritical processes to the micronization of gastroresistant insulin formulations is presented. As pharmaceutical applications of the supercritical anti-solvent micronization processes are becoming established at the bench scale, it is timely that they are compared in terms of product characteristics and scalability aspects. The formulation of insulin with the gastroresistant coating hydroxypropyl methyl cellulose phthalate was conducted by the Gas Anti-Solvent (GAS), the Aerosol Solvent Extraction System (ASES) and the Atomized Rapid Injection for Solvent Extraction (ARISE) processes and results have been compared in terms of insulin content of the microparticles, product morphology and recovery. The insulin content in products was in the range of 9–47%. The particles produced by the GAS process were flake-like in the 1–2 μm range with bi-modal distribution, while particles produced by the ASES and ARISE processes were spherical in the 100–300 nm particle size range with unimodal size distribution. Compared to GAS and ASES, the ARISE process produced the highest recoveries and insulin loadings: 77% and 47%, respectively. Particle characteristics, process recoveries and operational aspects indicate that ARISE should be the process of choice for scale-up.

1. Introduction

Substances in conditions beyond their critical point exist as homogeneous systems known as supercritical fluids (SCFs). Supercritical fluids possess liquid-like solvation power and gas-like diffusivities. The high compressibility of SCFs allows high degrees of tunability of physical properties such as density and viscosity through changes in operating pressure and/or temperature. The unique properties and versatility of SCFs have been the focus of research in material processing and process unit operations including extractions, chemical reactions and particle precipitations.

Supercritical particle precipitation processes have been widely applied in the pharmaceutical area [1–7]. The clinical effectiveness of active pharmaceutical ingredients (APIs) can be limited by their low solubility and dissolution rates in biological fluids. Micronization, the reduction of the particle size in the micron range, is one of the essential steps in pharmaceutical processing and has the potential to improve the dissolution rate in biological environments and the bioavailability of APIs, especially for substances with low water solubility and high permeability through cell tissues. Particle precipitation processes based on the SCF technology have also been applied to the preparation of microencapsulated pharmaceutical formulations to protect APIs from degradation, to improve formulation characteristics such as taste [7,8] and to provide effective treatments with fewer side effects.

Various SCF anti-solvent particle processing techniques such as Gas Anti-Solvent (GAS), Aerosol Solvent Extraction System (ASES), and Atomised Rapid Injection for Solvent Extraction (ARISE) have been developed. In SCF anti-solvent techniques, carbon dioxide (CO_2) is commonly used to create an anti-solvent effect for the precipitation of the solutes from organic solutions. In the GAS process, CO_2 is added to the system and dissolves into the liquid phase, which determines an expansion of the liquid phase. During the volume expansion the solute separates from the liquid phase as a result of the reduction in solvation power. The ASES is a semi-continuous process, in which the organic solution is atomized into a continuous stream of CO_2 through a capillary nozzle or micro-orifice. Atomization through the nozzle enhances mass transfer between the solvent and the anti-solvent and solute precipitates as the result of organic solvent expansion and extraction into the CO_2-rich phase. The ARISE process has recently been developed to create uniform solution dispersion and intensified mass transfer

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capabilities through a combination of SCF anti-solvent and rapid solution injection principles. The ARISE process ensures efficient solvent/antisolvent mixing through rapid solution injection driven by pressure differential trough a relatively large orifice rather than using micro-orifices as in the ASES process. The rapid and instantaneous delivery of the organic solution improves phase homogeneity and hence decreases the nucleation density without relying on the use of spraying device such as nozzles [5]. Similar to ASES, the solute will precipitate out from the solution as the result of solvation power reduction of the organic solvent upon contact with CO_2. In all processes, the expanded organic solvent is flushed out of the system by pumping CO_2 at isobaric conditions through the precipitation vessel. Dry precipitates are collected upon depressurization [5].

The aim of this study is to provide a comparison of SCF anti-solvent processes (GAS, ASES and ARISE) in the co-processing of a composite pharmaceutical formulation. Based on the product characteristics, the effect of mass transfer and the contact between solutions and SCF media in those processes will be discussed. The model system selected consists of insulin and pH responsive polymer, hydroxypropyl methyl cellulose phthalate (HPMCP).

Insulin was selected as the model compound as its processing by SC anti-solvent precipitation has been studied in detail [9,10]. Various insulin formulations for the treatment of diabetes mellitus have been developed. Insulin is available in suspension forms, and mainly administered by injection. Oral delivery has several advantages over injection especially in the treatment of widespread long term medical conditions such as diabetes. The oral delivery of insulin presents multiple challenges including degradation in the acidic environment in the stomach and the development of oral insulin formulations would be a major step forward in the pharmaceutical industry [9–12]. In this work, the formulation of insulin with an enteric coating agent is investigated.

Hydroxypropyl methyl cellulose phthalate (HPMCP) is a cellulose derived pH-sensitive polymer and is used as an enteric coating agent to protect APIs from degradation by gastric acid or to prevent them from causing side effects in the stomach. As an enteric coating agent, HPMCP can be used for a range of applications such as sustained and targeted release preparations. Cetin investigated the in-vivo and in-vitro performance of HPMCP capsules using gamma-scintigraphy [6]. The results showed that the capsule remained intact in the stomach, confirming that HPMCP has a gastro-resistant ability. Furthermore, the data from volunteers dosed with HPMCP capsules containing ^{99m}Tc-DTPA as a tracer demonstrated that HPMCP capsules only disintegrated in the intestinal zone, showing that HPMCP is suitable to manufacture intestine targeting formulations [6]. Xu et al. prepared pH-sensitive famotidine-HPMCP formulation and reported excellent pH-dependent drug release profiles. The HPMCP coated formulation showed no famotidine release in simulated gastric fluid (pH < 3) but showed delayed famotidine release in simulated intestinal fluid (pH > 6.5) [7]. Tamsulosin hydrochloride was also successfully coated by HPMCP to create a controlled release formulation with 20% HPMCP content that showed a zero-order release in simulated intestinal fluid without pancreatin [8].

2. Materials

Hydroxypropyl methyl cellulose phthalate (HP55—MW 45,600) was purchased from Shin-Etsu, Japan and Recombinant Human Insulin (USP grade) was purchased from Sigma-Aldrich Chemicals. Acetone (99.5% purity) and dimethylsulfoxide (DMSO) (99% purity) were obtained from Sigma Aldrich. Carbon dioxide (Grade 2.5) and argon (Ar) (Grade 4.2) were obtained from Coregas. Concentrated hydrochloric acid (HCl) (32% purity) was purchased from Ajax Chemical and sodium hydroxide (NaOH) (>98% purity) was purchased from Sigma-Aldrich.

3. Methods

3.1. Gas anti-solvent

The GAS process utilizes a high pressure fluid as an anti-solvent in the separation of solutes from solutions of conventional solvents. The anti-solvent, usually CO_2, is soluble in the conventional solvent of choice. By dissolving into the starting solution, CO_2 expands the solution causing supersaturation and consequent precipitation of the solutes. The process is conducted by inserting the starting solution in a high pressure vessel and then gradually adding the anti-solvent by bubbling the anti-solvent trough a porous frit at the bottom of the vessel. As the pressure in the system gradually increases, so does the volume of the gas expanded solution. Once product separation is observed and deemed complete, the gas expanded medium is purged from the system by pumping the anti-solvent from the top of the vessel whilst the solid product is retained in the vessel by the porous frit. Additional anti-solvent is passed through the system to extract residual solvent prior to depressurization and product collection. A schematic of the GAS process is presented in Fig. 1A.

In this work, the apparatus was immersed in a temperature-controlled (Thermoline Unistat) water bath to maintain the desired operating temperature. The precipitation vessel was a high pressure sight gauge (Jerguson Series 32) with the capacity of 60 ml. A sintered filter with 0.5 μm pore size was fitted at the bottom of the vessel. The system was pressurized by delivering CO_2 by a syringe pump (Teledyne ISCO 500D). The volume of feed solution processed in each experiment was 10 ml and the process was conducted at 40 °C with a pressurisation rate of 0.1–0.2 bar/min. The slow addition of CO_2 to the solution caused the expansion of the starting solution and created a mixing effect in the vessel. Carbon dioxide was gradually added to the solution until the system reached the pressure of 95 bar, at which point the volumetric expansion of the solution was approximately 460%. A more detailed description of the GAS process is provided elsewhere [4].

3.2. Aerosol solvent extraction system

In the ASES, as in the GAS process, particle precipitation occurs when a solution is contacted with an anti-solvent that is miscible with the solution and causes reduction in the solvating power of the solvent. In the ASES process, the solution and the anti-solvent are concomitantly pumped into the precipitation vessel and mass transfer between the two streams is enhanced by solution atomization trough a nozzle. Once enough product has been generated, the solution flux is stopped and the gas expanded medium and residual solvent are extracted by passing additional anti-solvent. Dry precipitates are retained in the vessel by a porous frit and collected upon depressurization. A schematic of the process is presented in Fig. 1B and a more detailed description of the ASES process is provided elsewhere [4].

In this work, the ASES process was conducted at 40 °C and 120 bar. Temperature control was obtained by the rig in a temperature-controlled water bath. A high pressure sight gauge (Jerguson Series 32) with the capacity of 60 ml was used as the precipitation vessel. A sintered filter with 0.5 μm pore size was fitted at the bottom of the vessel to retain the precipitate. A constant flow of CO_2 was established equal to 12 ml/min measured by a Teledyne ISCO 500D syringe pump at the operating pressure and 4 °C. Organic solutions containing insulin and HPMCP were introduced into the precipitation vessel through a stainless steel nozzle (Grace, 150-mm length and 100-μm i.d.) at 0.2 ml/min using an HPLC pump (Agilent 1100 series). In each experiment, 10 ml of feed solution were processed.