CYCLODEXTRINS MICRONIZATION BY SUPERCRITICAL ASSISTED ATOMIZATION

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ABSTRACT

Supercritical Assisted Atomization (SAA) has been used to micronize α-cyclodextrin (α-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD). The effect of some process parameters such as precipitation temperature and solute concentration in the liquid solution has been studied to evaluate their influence on morphology and size of precipitated particles.
Cyclodextrins micronization has been obtained with sharp particle size distributions. Well-defined spherical microparticles of α-CD and HP-β-CD have been produced, ranging between 0.1 and 30 μm and between 0.5 and 20 μm, respectively.
X-ray and DSC analyses have been also performed to investigate cyclodextrins modifications after SAA processing: amorphous particles have been obtained in both cases, whereas raw α-CD was crystalline and raw HP-β-CD was amorphous.

INTRODUCTION

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides of α-D-gluco-pyranose containing a relatively hydrophobic central cavity and an hydrophilic outer surface. As a result of their molecular structure and shape, they possess the ability to act as molecular containers entrapping guest molecules in their internal cavity. Hydrophobic molecules can be included in the CD cavity in presence of water, if their molecular dimensions are compatible with the cavity. The formed inclusion complexes are relatively stable and rapidly separate from the solution. Molecules of poorly soluble drugs, volatile fragrances, explosives or toxic pesticides can be encapsulated. Therefore, CDs are widely used in many industrial products and analytical methods [1, 2]. CDs have also been largely exploited in the pharmaceutical field. They can be used in drugs either for complexation or as auxiliary additives such as carriers, diluents, solubilizers and tablet excipients. Inclusion complex formation usually results in advantageous modification of the physico-chemical properties of the complexed drug: improvement of physical and chemical stability, enhancement of the bioavailability of poorly soluble drugs, enhancing drug permeation in nasal and topical delivery, lowering the incidence of side effects and promoting a faster therapeutic action [3, 4].
The production of CD microparticles is a relevant aspect for many applications, especially for pharmaceutical ones. For example, the formulation of drug/CD micronized complexes could improve current administration routes and exploit novel delivery systems. In particular, CD particle size reduction may enhance the interaction between drug and CD molecules, thus accelerating the complex formation even in physical mixtures. Conventional methods to obtain CD microparticles are grinding, spray-drying, freeze-drying and co-evaporation. However, they do not assure an efficient control of the particle size, can cause thermal or chemical degradation and low reproducibility among different batches.
Several supercritical fluids (SCF) based techniques have been proposed for the production of micrometric and nanometric particles of pharmaceutical compounds to overcome some

drawbacks of the conventional micronization processes [5, 9]. Recently, SCFs have been applied to CD micronization and CD/drug complexes formation. For example, impregnation of CDs microparticles using supercritical CO_2 (SC-CO_2), or SC-CO_2 modified with organic solvents, has been proposed to produce naproxen/β-CD complexes [10, 11]; ibuprofen/β-CD microcomposite particles have been obtained using the Rapid Expansion of Supercritical Solutions (RESS) impregnation technique [12]; Supercritical Antisolvent precipitation (SAS)-based processes have been investigated to precipitate complexes of celecoxib [13] and naproxen [14] with hydroxypropyl- and methyl-β-CDs. However, the impregnation-based techniques showed a low efficiency and, although CDs are soluble in water, this solvent is difficult to be employed in SAS-based processes.

Recently, *Supercritical Assisted Atomization* (SAA) has been proposed [15, 16] as an efficient process based on the solubilization of controlled quantities of SC-CO_2 in liquid solutions containing a solid solute and on the subsequent atomization of the ternary solution through a nozzle. Therefore, SC-CO_2 plays both as *co-solute* being miscible with the solution to be treated, as well as pneumatic agent to atomize the solution in fine droplets. The solubilization is obtained in a packed bed saturator characterized by a high specific surface and large residence times. The solution formed in the saturator is, then, sent to a thin wall injector and sprayed into the precipitator. A two steps atomization is obtained: the primary droplets produced at the outlet of the injector (*pneumatic atomization*) are further divided in secondary droplets by CO_2 expansion from the inside of the primary ones (*decompressive atomization*). This technique can provide a good control over particle size and distribution; microparticles sizing between 0.1 and 5 μm can be easily produced [17, 18]. Moreover, one of the most important aspects of SAA with respect to other SCF-based processes is that not only organic solvents, but also water and aqueous solutions can be used.

The aim of this work is, therefore, to investigate the potential of SAA in the production of cyclodextrin microparticles. In particular, SAA processability of α-cyclodextrin (α-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD), two of the most employed CDs, will be evaluated using water as solvent. Indeed, α-CD and HP-β-CD show high solubility in water. The effect of the solute concentration in the liquid solution on CDs particles morphology, size and distribution will be studied to obtain particle size tailoring. Solid state characterization of untreated and SAA processed cyclodextrins will be performed by X-ray and calorimetric analyses.

SAA EXPERIMENTAL APPARATUS

SAA laboratory apparatus consists of two high-pressure pumps (mod. 305, Gilson) delivering the liquid solution and liquid CO_2 to a heated bath (Forlab mod. TR12, Carlo Erba, Italy) and then to the saturator. The saturator is a high pressure vessel (I.V. 25 cm^3) loaded with stainless steel perforated saddles which assure a large contact surface between liquid solution and CO_2, thus favouring the dissolution of the gaseous stream in the liquid solution. The use of long residence times in the saturator assure the attainment of near equilibrium conditions. The solution obtained in the saturator is sprayed through a thin wall 80 μm diameter injection nozzle into the precipitator.

A controlled flow of N_2 is taken from a cylinder, heated in an electric heat exchanger (mod. CBEN 24G6, Watlow, USA) and sent to the precipitator to facilitate liquid droplets evaporation. The precipitator is a stainless steel vessel (I. V. 3 dm^3) operating at atmospheric pressure. The saturator and the precipitator are electrically heated using thin band heaters. A stainless steel filter located at the bottom of the precipitator allows the powder collection and

the gaseous stream flow out. SAA apparatus layout and further details on the experimental procedures were published elsewhere [15,17-19].

MATERIALS AND METHODS

α-cyclodextrin and hydroxypropyl-β-cyclodextrin were supplied by Carlo Erba Reagenti (Milan, Italy). Water (HPLC grade) was supplied by Sigma-Aldrich (Milan, Italy). Carbon dioxide and Nitrogen (CO_2, N_2) were purchased from SON (Naples, Italy). Untreated α-CD and HP-β-CD consisted of irregularly shaped particles ranging between 20 and 500 μm. α-CD and HP-β-CD showed a solubility in water (at 25°C) of 145 mg/mL and larger than 2000 mg/mL, respectively.

Powder morphology by Scanning Electron Microscopy (SEM)

Morphological characteristics of α-CD and HP-β-CD particles were analysed by a Scanning Electron Microscope (SEM, mod. 420, LEO, Germany). Powders were dispersed on a carbon tab previously stuck to an aluminum stub (Agar Scientific, UK). Samples were coated with gold-palladium (layer thickness 250Å) using a sputter coater (mod. 108A, Agar Scientific, UK). At least 10 SEM images were taken at various positions in the precipitator for each run to verify the powder uniformity.

Particle size distribution

Particle size analysis was performed on α-CD and HP-β-CD microparticles using Malvern Mastersizer S laser diffractometer (Alfatest s.r.l., Rome, Italy). α-CD and HP-β-CD microparticles were suspended in paraffin oil. The samples were sonicated for 10 min before analysis.

Solid state characterization

Diffraction patterns of CD powders were obtained using an X-ray diffractometer (mod. D8 Discover, Bruker, USA) with a Cu sealed tube source. Samples were placed in the holder and flattened with a glass slide to assure a good surface texture. The measuring conditions were as follows: Ni-filtered CuKα radiation, λ=1.54 Å, 2θ angle ranging between 20° and 70° with a scan rate of 3 seconds/step and a step size of 0.2°.

Thermograms of CD samples were obtained using a Differential Scanning Calorimeter (DSC mod. TC11, Mettler, USA). Temperature and fusion enthalpy were calibrated with an indium standard (melting point 156.6°C). 10 mg of α-CD and HP-β-CD samples were accurately weighed, crimped in an aluminium pan and heated from to 25 to 300°C under a nitrogen purge at 10°C min^{-1}. X-ray and DSC analyses were performed in 3 replicates for each batch of material.

RESULTS AND DISCUSSION

Selection of the saturator operating parameters

The solubilization of SC-CO_2 in the liquid solution inside the saturator is one of the key steps controlling the efficiency of the SAA process [20]. The solubility of SC-CO_2 in liquids depends on the chemical structure of the liquid chosen and on temperature and pressure in the saturator, since it is related to high pressure Vapor Liquid Equilibria (VLEs) characteristic of the selected liquid-CO_2 system. Moreover, it can be also influenced by the presence of solute dissolved in the liquid.

The selection of adequate gas and liquid flow rates is also relevant for the achievement of residence times in the saturator to assure gas saturation in the liquid solution. Moreover, the mass flow ratio between CO_2 and liquid solution influences the equilibrium in the saturator of the ternary system CO_2/solvent/solute. In this work water was chosen as solvent for CDs; the

use of water is particularly advantageous when pharmaceutical compounds are processed, because it is not toxic (unlike the organic solvents) and, thus, there is no problem of solvent residues in the precipitate. Data reported in the literature give information about the binary system CO_2/water [21]; however, no data is available for the ternary systems CO_2/water/α-CD and CO_2/water/HP-β-CD. Therefore, in the selection of the mass feed ratio, we relied on our previous experience in this process. Thus, a mass feed ratio (R) between CO_2 and the liquid solution of 1.8 was used in all the experiments performed in this work, since it showed to be the most appropriate for aqueous solutions in our previous works performed on SAA [19, 22]. According to these considerations, some preliminary tests were performed setting the saturator conditions in the pressure range from 7.5 to 15 MPa and in the temperature range between 70 and 90 °C. The best results in terms of stability of the process and of CDs precipitated particles were observed operating at 8.5 MPa and 85°C. Therefore, these pressure and temperature conditions were used in all the subsequent SAA experiments proposed in this work.

Effect of precipitator temperature

Temperature optimization in the precipitator is required to assist droplets evaporation, minimizing the stress on the treated compound. Preheated N_2 at a flow rate of 800 Ndm^3/h has been used, coupled to electrical heating of the precipitator walls, to set the chamber at the desired temperature.

The effect of temperature on particle morphology was observed performing experiments on α-CD at temperatures ranging between 100 and 186°C, at a α-CD concentration in the liquid solution of 50 mg/mL. In all experiments performed at temperatures higher than 118°C well-defined spherical microparticles were produced; whereas, at 108°C or lower, coalescing particles were recovered in the precipitator due to a lower efficiency of solvent evaporation. To illustrate this effect, SEM images showing SAA processed α-CD precipitated at 108°C and at 118 °C are reported in figure 1.

Experiments performed on HP-β-CD at precipitation temperatures ranging between 118 and 160°C confirmed that well-defined spherical microparticles can be obtained also for this CD. Therefore, 118°C was set as the operating temperature in the precipitator in all the following experiments on α-CD and HP-β-CD.

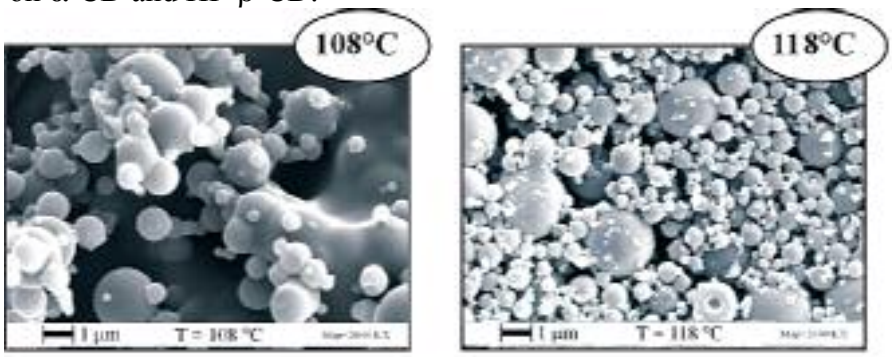

Figure 1: *SEM images of α-CD microparticles precipitated by SAA at different temperatures in the precipitator (C_{sol} =50 mg/mL).*

Effect of solute concentration

Previous works on SAA showed that solute concentration in the liquid solution is a relevant parameter in the control of particle size and particle size distribution [15, 17, 18]. Therefore, systematic experiments were performed operating at different α-CD and HP-β-CD concentrations (C) in water to explore the effect of this parameter on the materials object of

this work. α-CD concentrations between 20 and 130 mg/mL were explored. The powder recovered in these experiments was always formed by well-defined non-coalescing microparticles. Examples of the particles collected at different C are shown in SEM images reported in figure 2.

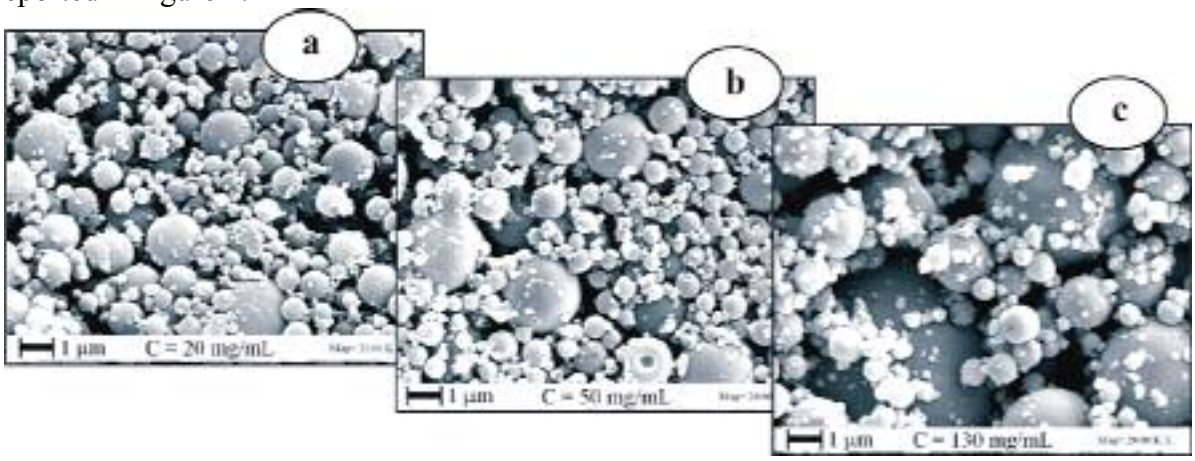

Figure 2: *SEM images of α-CD microparticles precipitated by SAA at: (a) C=20 mg/mL; (b) C=50 mg/mL; (c) C=130 mg/mL in the liquid solution (T=118°C).*

These images have been produced at the same enlargement (20K); therefore, a qualitative evaluation of the increase in particle size with solute concentration is possible. A quantitative measurement of PSDs was also performed by laser scattering analysis at C = 20, 50 and 130 mg/mL.

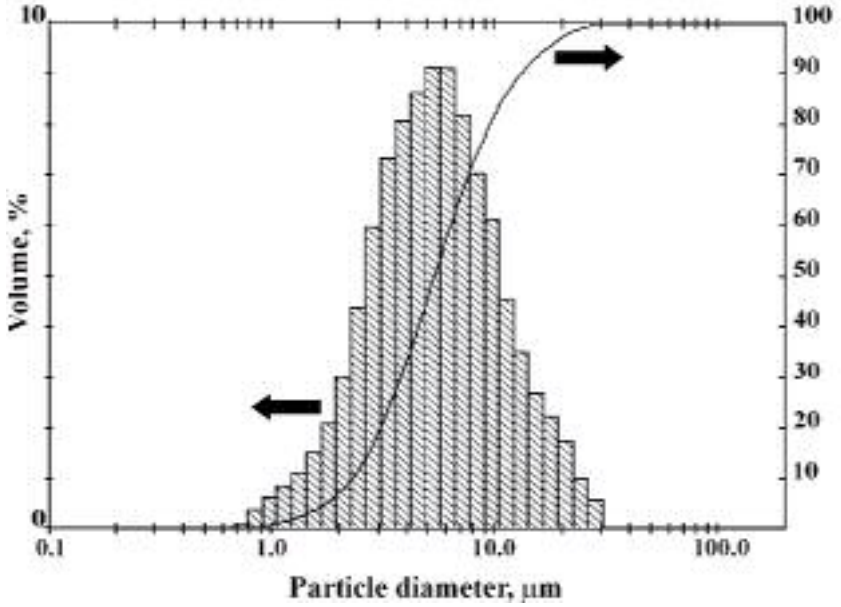

Figure 3: *PSD of micronized α-CD obtained by laser scattering at C=20 mg/mL.*

An example of PSD, obtained by laser analyser, is reported in terms of volumetric histograms and a cumulative curve at 20 mg/mL in figure 3. Volumetric distribution percentages and the mode (the most frequent value in non-symmetric distributions) obtained at different α-CD concentrations are reported in Table 1. They confirm the particles size increase and the broadening of particle distribution with the solute concentration: 90% of the total volume of α-CD powder is due to particles with diameters up to 13.1 μm at C=20 mg/mL and up to 18.3 μm at C=130 mg/mL, respectively.

α-CD Concentration	*Volumetric Distribution Percentages* [μm]					*Mode* [μm]
	10%	**20%**	**50%**	**80%**	**90%**	
20 mg/mL	2.30	3.08	5.39	9.53	13.11	5.72
50 mg/mL	2.99	3.96	6.86	12.95	17.59	5.91
130 mg/mL	2.69	3.60	7.00	13.55	18.35	6.71

Table 1: *PSDs data of micronized α-CD at different C values.*

The effect of solute concentration was investigated also in the case of HP-β-CD. C values ranging between 20 and 100 mg/mL were explored and in all the experiments well-defined spherical microparticles were precipitated. In figure 4, SEM images of particles collected at different C are reported.

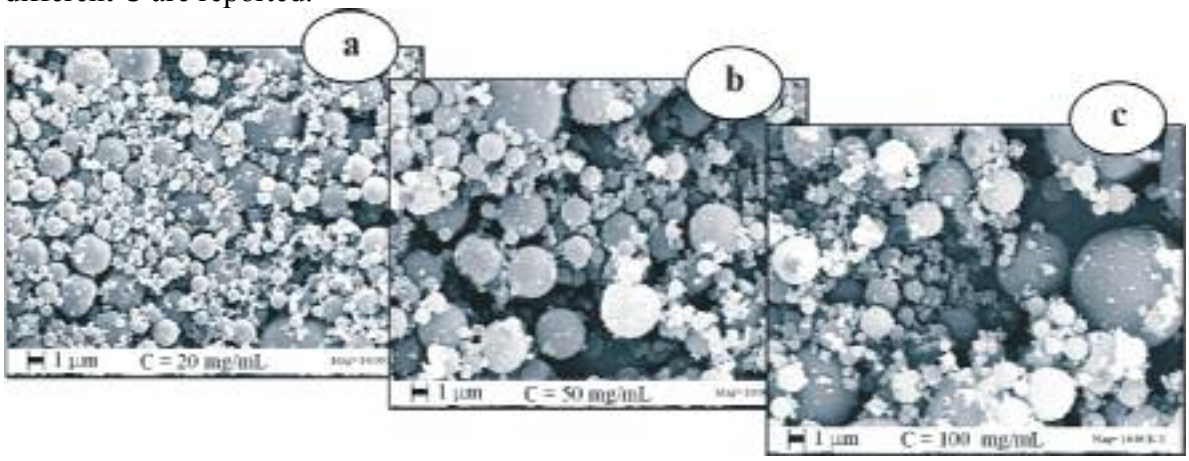

Figure 4: *SEM images of HP-β-CD microparticles precipitated by SAA at: (a) C=20 mg/mL, (b) C=50 mg/mL, (c) C=100 mg/mL in the liquid solution (T=118°C).*

Also in this case, images were produced at the same magnification (10K), allowing the qualitative evaluation of the increase in particle size with solute concentration. Indeed, laser scattering produced volumetric PSDs at C = 20, 50 and 100 mg/mL reported in Table 2 show that 90% of the total volume occupied by micronized HP-β-CD is relative to particles with diameters up to 8.45 μm at C=20 mg/mL and up to 12.04 μm at C=100 mg/mL, respectively.

HP-β-CD Concentration	*Volumetric Distribution Percentages* [μm]					*Mode* [μm]
	10%	**20%**	**50%**	**80%**	**90%**	
20 mg/mL	1.55	2.14	3.85	6.60	8.45	3.31
50 mg/mL	2.02	2.68	4.80	7.32	9.15	4.26
100 mg/mL	2.24	3.37	6.14	9.85	12.04	6.84

Table 2: *PSDs data of micronized HP-β-CDs at different C values.*

The effect of the solute concentration on particles size can be explained considering some physical characteristics of the solution, such as viscosity and surface tension. An increase of C causes an increase of viscosity and surface tension of the liquid solution, resulting in the formation of larger primary droplets and influencing also the formation and the dimensions of the secondary droplets.

Solid state characterization

X-ray and DSC analyses were performed on untreated and SAA-processed α-CD and HP-β-CD to evaluate the effect of SAA process on the solid state of these compounds.

Diffraction patterns of untreated and SAA-processed α-CD and HP-β-CD are reported in figure 5a. X-ray analysis reveals that SAA micronized α-CD and HP-β-CD are amorphous; whereas, the raw material was crystalline in the case of α-CD and amorphous in the case of HP-β-CD. This result is confirmed by DSC thermograms (figure 5b). Indeed, only untreated α-CD shows definite fusion peaks characteristic of crystalline structures. SAA processed α-CD, untreated and SAA micronized HP-β-CD show an endothermic effect in the range of 90-160°C that can be attributed to a dehydration process.

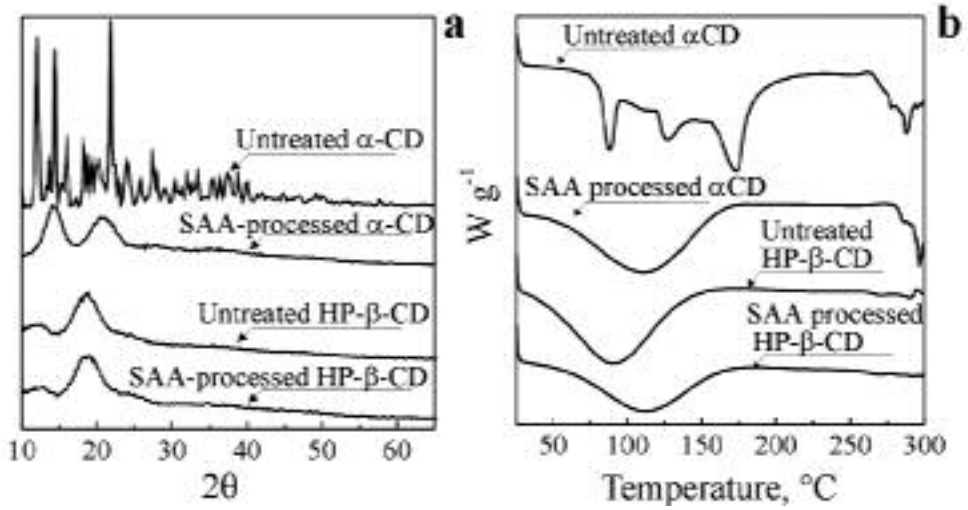

Figure 5: *X-Ray diffraction patterns (a) and DSC thermograms (b) of untreated and SAA processed α-CD and HP-β-CD.*

CONCLUSIONS AND PERSPECTIVES

SAA can be used for cyclodextrins micronization using water as solvent. It has been successfully tested on α-CD and HP-β-CD and well-defined spherical and amorphous microparticles have been produced. The results suggest that cyclodextrin microparticles can be easily produced by SAA with granulometric and solid-state characteristics useful for their pharmaceutical applications. Moreover, new prospects for the direct production of micronized drug/CD complexes using SAA technique are opened by this experimentation.

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